PROCEEDINGS

BBEST 2017

Brazilian BioEnergy Science and Technology Conference

DESIGNING A SUSTAINABLE BIOECONOMY

Campos do Jordão, 17 a 19 de Outubro de 2017

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PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

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The Brazilian Bioenergy Science and Technology Conference (BBEST2017)

BBEST is an international conference on bioenergy and for the 2017 edition we considered within the broader field of bioeconomy how bioenergy emerges as a possible leverage and the synergies and opportunities ahead. In this sense, BBEST 2017 devoted a policy day for discussions on the bioeconomy and its links to bio-products, biotechnology, industrial innovation and sustainable development. Bioeconomy develops towards a significant and robust contributor to sustainable economic development in Brazil, Latin America and worldwide. Significant steps forward have been made since BBEST 2014, such as the start-up of the first lignocellulosic ethanol plants in Brazil and USA as well as the market introduction of 1st and 2nd generation bioplastics.

The large-scale implementation of biobased concepts also starts to direct attention of the scientific and business community to the technological challenges associated with the unprecedented scale of the first-of-a-kind plants. It underlines the need to redefine the integral refinery concepts that underpinned the economic success of the petrochemical industry clusters towards the logistic, economic and market constraints of the bioeconomy in so-called REDEFINERY-programs.

At BBEST 2017, we placed the Brazilian bioenergy & bioeconomy development and its scientific progress in the context of this new reality. In a number of plenary and parallel sessions, we brought together Sao Paulo, Brazilian and internationally leading scientists and the business community to present progress within FAPESP's BIOEN program and its international partnerships on key topics such as responsible land use, crop productivity and resilient multifunctional landscapes, harvesting technology, impact of logistics and scale, integral biorefinery development, and emerging advanced materials and energy carriers for aviation, marine and road transport. The continued progress in thinking around the sustainable bioeconomy development to benefit both developing and developed countries were discussed in the framework of an update of the SCOPE Bioenergy & Sustainability report. BBEST 2017 was also be the forum to present the latest innovation roadmaps of agro-forestry re-industrialisation programs.

We received 385 participants from 14 different countries including the Americas, Europe, Asia, Oceania and Africa regions. We had colleagues from 8 different regions of Brazil, that contributed with scientific work at BBEST2017. The profile of the participants included mostly senior researchers, graduate and undergraduate students and professionals from

several different companies. At the BBEST2017, a total of 267 different works was presented, either during oral presentations or posters. The event had 54 invited speakers that brought to Brazil the discussions about the upmost cutting-edge knowledge in the field of Bioenergy and Bioeconomics.

BBEST 2017 was also the platform for the future generations. Therefore, we hosted –among others - the finals of the 2017 Biobased Design Competition for game-changing biobusiness plans by teams of young scientists and engineers from Brazil, Europe and USA; career events for young scientists, and meet-the-CEO sessions for students and young scientists. We gave special attention to starters and other SME's since they are an essential and fast track complement to the larger industries that slowly change to biorenewables.

Bioeconomy opportunities for Brazilian science and business should be seen in the international context. At BBEST this is reflected not only in the international composition of our Chair(wo)manship and the various Scientific and Advisory Committees, but throughout the proposed program and the alternating scheme that we are developing with the ECOBIO-series in close collaboration with the Elsevier Group.

BBEST 2017 Organizing Committee

BBEST Awards

Among the posters presented during BBEST2017, 9 were chosen to receive the "BBEST Award" in the categories Master Student, PhD Student and Post Doctor. The winners received a certificate and a copy of the book "SCOPE Bioenergy & Sustainability (Souza, G. M., Victoria, R., Joly, C., & Verdade, L. (Eds.). (2015). Bioenergy & Sustainability: Bridging the gaps (Vol. 72, p. 779). Paris: SCOPE. ISBN 978-2-9545557-0-6)".

Category Master Student

1st place

[B41] Transcriptional and fermentative analysis of *Clostridium saccharoperbutylacetonicum* during optimized fermentation process using cellulosic pulp

Nakagawa, BTG[1]; Carazzolle, MF[1]; Filho, PMT[1]; Pereira, GAG[1,2]; Grassi, MCB[2]

[1] Laboratório de Genômica e Expressão - LGE, Departamento de Genética, Evolução e Bioagentes, Unicamp, Campinas, SP, Brazil; [2] Laboratório Nacional de Ciência e Tecnologia do Bioetanol - CTBE, CNPEM, Campinas, SP, Brazil.

2nd place

[A51] High nutrient demand and sustainable production of energy cane

Pinto, LRN[1]; Cantarella, H[1]; Montezano, ZF[1]; Andrade, CA[2]; Landell, MGA[3]; Xavier MA[3]

[1] IAC - Agronomic Institute of Campinas, Soil and Environmental Resources Center, Campinas, SP, Brazil; [2] EMBRAPA Meio Ambiente, Jaguariuna, SP, Brazil. [3] IAC - Agronomic Institute of Campinas, Sugarcane Research Center, Ribeirão Preto, SP, Brazil.

3rd place

[E32] Straw removal and nitrification inhibitor as mitigation strategies to N2O emission in sugarcane fields

Gonzaga, LC[1]; Carvalho, JLN[2]; Soares, JR[3]; Oliveira, BG[1]; Cantarella, H[1]

[1]Agronomic Institute of Campinas, Soils and Environmental Resources Center, IAC, Sao Paulo, Brazil; [2]Brazilian Bioethanol Science and Technology Laboratory, CTBE/CNPEM, Sao Paulo, Brazil; [3]State University of Campinas, Faculty of Agriculture Engineering, FEAGRI/UNICAMP, São Paulo, Brazil

Category PhD Student

1st place

[C02] Sucrose hydrolysis has been completely abolished in *Saccharomyces cerevisiae* using a single CRISPR/Cas9 transformation step

Marques, WL[1,2]; Mans, R[1]; Marella, ER[1]; Cordeiro, RL[2]; van den Broek, M[1]; Daran, J-MG[1]; Pronk, JT[1]; Gombert, AK[2]; van Maris, AJA[1]

[1]Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, the Netherlands and [2]School of Food Engineering, University of Campinas, Campinas, SP 13083-862, Brazil

2nd place

[A14] Identifying potential new genes related to lignocellulose degradation using transcriptome and gene co-expression network analysis in *Trichoderma reesei* RUT-C30

Borin, GP[1,2]; Carazzolle, MF[1,3]; Riaño-Pachón, DM[1,4]; Oliveira, JVC[1,2]

[1]Current address: Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas, São Paulo, Brazil; [2]Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil; [3]Centro Nacional de Processamento de Alto Desempenho em São Paulo (CENAPAD-SP), Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil; [4]Current address: Laboratório de Biologia de Sistemas Regulatórios, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

3rd place

[B12] Automated high-throughput method for *Saccharomyces cerevisiae* growth analysis

De Mello, FSB[1]; Coradini, ALV[1]; Tizei, PAG[1]; Carazzolle, MF[1,2]; Pereira, GAG[1,3]; Teixeira, GS[1,4]

[1]Departamento de Genética, Evolução e Bioagentes. UNICAMP, Campinas, SP; [2]Centro Nacional de Processamento de Alto Desempenho – CENAPAD. UNICAMP, Campinas, SP.; [3]Laboratório Nacional de Ciência e Tecnologia do Bioetanol – CTBE. CNPEM. Campinas, SP.; [4]Departamento de Engenharia de Alimentos. UNICAMP, Campinas, SP.

Category Post Doctor

1st place

[A50] Structural and functional characterization of SUGARWINS and their role in plant defense

Franco, FP[1]; Dias, RO[1]; Santiago, AC[2]; Henrique-Silva, F[2]; Moura, DS[1]; Silva-Filho, MC[1]

[1]Escola Superior de Agricultura Luiz de Queiroz, USP, São Paulo, SP; [2]Universidade Federal de São Carlos, UFSCar, São Carlos, SP

2nd place

[B59] Bioinformatics applications in biotechnology: bioenergy production

Carazzolle, MF[1,2]; Mofatto, LS[1]; Grassi, MC[1,3], Jose, J[1]; Carvalho, LM[1]; Nagamatsu, ST[1,3]; Nascimento, LC[1,4]; Tokimatu, P[1]; Silva, NV[1]; Pereira, GAG[1,3]

[1]Laboratório de Genômica e Expressão (LGE)-Instituto de Biologia-UNICAMP; [2]Centro Nacional de Processamento de Alto Desempenho (CENAPAD-SP)-UNICAMP; [3]Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE)-CNPEM; [4]Laboratório Central de Tecnologias de Alto Desempenho (LACTAD)-UNICAMP

3rd place

[E08] Assessment of the microbial diversity associated with CH_4 production from vinasse

Oliveira, BG[1,2]; Mendes, LW[2]; Cantarella, H[1]; Tsai, SM[2]; Feigl, BJ[2]; Mackie, RI[3]

[1]Agronomic Institute of Campinas, IAC, Campinas, São Paulo, Brazil; [2]Center for Nuclear Energy in Agriculture, USP, Piracicaba, São Paulo, Brazil; [4]University of Illinois, Urbana-Champaign, Illinois, USA.

G-BIB competition and award

The BBEST2017 was also the scenario of the overall final of the Global Biobased Business competition (G-BiB), an initiative of the BioInnovation Growth mega-Cluster (BIG-Cluster).

The jury selected the Brazilian team from Taubaté with their SANergya proposal as the winner of the G-BiB competition. The competition aimed to stimulate entrepreneurship and innovation. The challenge for all the teams was to write an innovative business plan based on a design for sustainable production of biorenewable products such as biofuels and biomaterials or partial solutions that will support developing those products.

N-Chroma from Wageningen University, SANergya from the University of Taubaté and Bicomer from the University of Bielefeld competed in the overall final after they won the semi-finals in respectively the Netherlands, Brazil and Germany. They were chosen from a total of 14 teams, existing of Master and PhD students.

The winning team members Arcione Ferreira Viagi, Fabricio Miguel Farinassi and Ederaldo Godoy from the University of Taubaté, Brazil, received their prize during a very competitive final at the BBEST2017.

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Biomass, agronomy, breeding, biotechnological aspects of energy plants

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Oral presentations

Genomic and transcriptomic overview of SHINE (SHN) regulatory grid and its role as a master regulator of plant secondary cell wall biosynthesis

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Plants with high contents of lignocellulose biomass with reduced or altered lignin composition are being selected for lignocellulosic ethanol production alternatively to fossil fuels. Transcription factors (TFs) are considered the most promising class of genes to modify secondary cell wall (SCW) composition. Transgenic rice lines overexpressing Arabidopsis SHINE (AtSHN) had high cellulose and reduced lignin content while in overexpression Arabidopsis SHN lines authors observed an effect on wax/cutin synthesis, suggesting that SHN has different roles in these two plants. In order to unravel SHN function in grass (plants with high lignocellulosic biomass content) and also comparing with functions previously described for SHN in eudicot plants, we used three different approaches. I – First of all, SHN expression was determined in grass species. Later, SHN was cloned from maize, sugarcane and sorghum, and their subcellular localization analyzed. Peptide sequence was used to design a specific antibody antiSHN. To seek for SHN target genes it was used Chromatin Immunoprecipitation assay (ChIP). As a result, SHN antibody recognized SHN protein from maize, sugarcane and sorghum. All these three protein were localized in the nucleus, as expected for TFs. Besides, it was identified SHN target genes from maize, sorghum and sugarcane-related t o lignin/cellulose pathway. Libraries containing SHN targets in maize and sugarcane were submitted to large-scale sequencing and data are still on bioinformatics analyses. II – It was obtained transgenic rice overexpressing SHN from sugarcane (ShSHN). Transgenic plants showed an increase of biomass and decrease of lignin resulting in a better sugar release (saccharification) comparing with wild-type (WT) plants. This plant material was used for a global transcriptomic data analysis using RNAseq approach showing that different SCW related genes were up or down regulated in these transgenic rice comparing to WT plants. III – To determine effects of ShSHN in crop plants transgenic sugarcane overexpressing ShSHN were recently obtained. It was obtained around 300 transgenic plants later transferred to the greenhouse. Plant material was collected for molecular analysis. Until now few plants were analyzed indicating 100% of transgenic confirmation using genomic DNA amplification by qPCR and ShSHN gene accumulation were 40x higher in transgenic compared to WT plants. Preliminary biometric analysis indicates an increase of culm and root diameter in transgenic sugarcanes suggesting that, as seen in rice, overexpression of ShSHN could impact on sugarcane biomass. Further other plants will be analyzed for alterations in gene expression, as well as for metabolic changes related SCW contents. From a long-term perspective, information regarding the function of SHN regulators across the plant kingdom could be used as a powerful tool for the metabolic engineering of phenolic and lignin compounds by breeding or transgenic approaches, with

subsequent impact on the production of second generation bioethanol.

Keywords: ChIPSeq, RNASeq, transgenic plants
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Silencing of a single cell wall-related gene decreases cell wall feruloylation and increases digestibility of biomass in grasses

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Plant cell walls digestibility is an important economic target for the production of liquid biofuels, efficiency of digestion by animals and the production of an array of value-added products derived from the biomass. Cell wall feruloylation decreases biomass digestibility due to the covalent linkages between ferulic acid (FA), the matrix polysaccharide and lignin components, which increases cell wall recalcitrance. Therefore, a diminished level of cross-linked ferulic acid in cell walls is thought to improve the saccharification of the biomass, facilitating the access of enzymes responsible for glucose release from cellulose. Here, we describe that RNAi silencing of a single cell wall-related gene (CWR1) from Setaria viridis was able to drastically decrease the levels of cross-linked FA (~60%) in the cell walls of this C4 model plant, increasing the saccharification levels of its biomass. Analysis of cell walls by 2D-NMR showed that the monolignol relative proportion of transgenic S. viridis cell wall did not change significantly, and the plants presented normal growth phenotype and biomass, compared to non-transformed (NT) control plants. LC-MS analysis suggested that CWR1 is involved in th e incorporation of FA into arabinose side chains of the xylan backbone. After the proof of concept in S. viridis, we have identified the CWR1 ortholog in sugarcane, and its silencing through RNAi was also responsible for the decreased levels of cross-linked FA and increased biomass saccharification in this important economic crop. We demonstrated that CWR1 is a suitable target for manipulation of grasses to improve biomass digestibility.

Keywords: Biomass, cell wall digestibility, biofuel
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Genome-wide association study of oil and fatty acids in diverse soybean genotypes

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Soybean, *Glycine max* L. Merr., is an important grain and oil seed crop that is widely grown throughout the world. The oil fraction of soybean represents 20% of the seed dry mass and is primarily (95%) used for edible oils, being the remaining oil soybean fractions used to create a variety of industrial products, such as fatty acids, soaps and biodiesel. To identify quantitative trait loci (QTL) controlling soybean oil and fatty acids components, a genome wide association study (GWAS) was performed. A collection of 96 diverse soybean genotypes was grown in two years in Brazilian field conditions and was characterized for palmitic (16:0), stearic (18:0), oleic (18:0), linoleic (18:2), and linolenic (18:3) acid, respectively. The same accessions were also genotyped by

BARCSoySNP6k with 6000 single nucleotide polymorphism (SNPs). A total of 5450 SNPs with minor allele frequency >0.05 were used to estimate linkage disequilibrium (LD) and population structure. The mean level of LD measured by r2 declined very rapidly to half its maximum value few hundred kb. The overall population structure was approximately coincident with the geographic origin. The GWAS results identified 69 SNPs in different genomic regions significantly associated with total oil and palmitic, oleic and linoleic acids content. Of these, several SNPs were localized in previously mapped QTL intervals. The loci and trait-associated SNPs identified in this study can be used to identification of genes associated with oil traits besides for developing soybean cultivars with different levels of fatty acids components.

Keywords: SNP, Glycine max, fatty acids
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Organ specific rhythms of transcription in field-grown sugarcane

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The circadian clock is an adaptation for living in a rhythmic environment. In sugarcane under constant light and temperature, more than 30% of the assayed transcripts were regulated by this regulatory network. To further our knowledge about the sugarcane circadian clock, we have measured transcript levels in the leaf +1, the internode 1/2, and the internode 5 of plants grown in a field every 2 h during 26 h. More than 50% of the expressed transcripts were rhythmic in leaves +1, which is a source organ, and about 30% of the expressed transcripts were rhythmic in the internodes, which are sink organs. More than 9,000 transcripts were considered expressed in the three organs. Of these, 2,388 transcripts were only rhythmic in the leaves, 673 were only rhythmic in the internode 1/2, and 517 were only rhythmic in the internode 5. A number of transcripts associated with Su crose Metabolism, Sugar Transport, Cell Wall Synthesis, Amino Acid Metabolism, and Chromatin Remodelling were differentially regulated in the three organs. These results suggest a great specialization of the circadian-controlled genes in each organ. We also have evidence that shade regulates the dynamics of the sugarcane circadian clocks in the field.

Keywords: Sugarcane, circadian rhythms, transcriptome
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Genetic modification of sugarcane and rice to improve biomass for biofuels

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Sugarcane supplies national demands for biofuels and it is a strategic crop as a bioenergy source. The production of ethanol from sugarcane can be increased using biomass as feedstock, e.g. straw and bagasse, complementary to sucrose. One of the main obstacles to implement a commercial scale of second generation ethanol (E2G) is the biomass recalcitrance, which is caused mainly by lignin interactions with the cellulose and hemicellulose in secondary cell wall (SCW) matrix. In attempt to contribute to technological enforcement and knowledge, it was used genetically modified rice and sugarcane to study four sugarcane genes relate

to lignin and SCW formation, i.e. SHINE transcript factor (SHN), dirigent protein (DIR), F5H and HCT. SHN and DIR were overexpressed in rice while F5H and HCT in sugarcane. SHN raised as a great potential to improve the biomass since its ectopic expression in rice promotes an increase of 340% in biomass (dry weight), a reduction of 34% in lignin content and an increase of 52.5% in saccharification efficiency in relation to the wild-type (WT). The DIR overexpression in rice did not modify the lignin amount and further studies should be lead to address its biological role in sugarcane, once previous results have shown its relationship with secondary cell wall formation. F5H overexpression in transgenic sugarcane increased the expression of the transcripts over 160-fold in relation to the WT endogenous gene, which results in an increase around 100% of syringyl/guaiacyl ratio in lignin composition. Our next step will be to test the saccharification efficiency. Transgenic sugarcane overexpressing HCT were obtained and the transcripts accumulation was over 150-fold change in relation to WT endogenous gene. Further, we will investigate the intermediary compounds of the phenylpropanoid pathway to better understanding the action of these enzymes, F5H and HCT, on the lignin biosynthesis and SCW modification in sugarcane. Taken together, knowledge and technology were generated that could be applied in a short term to obtain sugarcane with improved biomass for E2G production

Keywords: Functional genomics, transgenic sugarcane, etanol 2G

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Total recoverable sugar prediction through agronomic variables and canopy sensing

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As a seasonal crop, sugarcane products prices are affected by market supply. Therefore, growers aim to improve s easonal management and planning by knowing sugar yield before harvesting. Since precision agriculture is getting more popular among Brazilian sugarcane mills and it demands different sources of data, total recoverable sugar can be spatial predicted during the season. Thus, the aim of this research is to evaluate if agronomic variables (soil and plant properties) associated to vegetation indices are able to predict total recoverable sugar per hectare (TRS.ha $^{-1}$). For that, forty targeted plots were allocated within two sugarcane-producing fields (“Santa Luzia” farm with RB855156 variety and “Boa Vista” farm with RB867515), according to soil apparent electrical conductivity, soil types and NDVI LandSat8 satellite imagery to provide variable data. Every plot contained four sampling spots consisting of 3m-long sugarcane row each representing evaluations on three different stalk height and harvest period. Sampling spot data consisted on manual biometric evaluation of number of tillers, stalk diameter and height; moreover, Leaf Area Index (LAI), leaf chlorophyll and canopy reflectance data (NDVI and NDRE indices) where obtained by proximal sensing techniques. After each evaluation, sugarcane aboveground biomass in the sampling spot was cut manually. Data from each field was treated separately and the variables that show correlation with biomass ($p < 0.1$) were selected. These variables were submitted to stepwise regression in order to identify which variables can assist on total recoverable sugar prediction. Boa Vista farm model used the attributes stalk number from the first evaluation, NDVI and stalk diameter from the second evaluation and soil base saturation at 20-40cm depth. Santa Luzia farm model used stalk diameter from the second evaluation and clay content at 0-20 and 20-40 cm depth. Regression model for Boa Vista farm was more efficient (R^2 =0.83) than Santa Luzia’s (R^2 =0.50). We identified that agronomic

variables can be used to predict total recoverable sugar per hectare (TRS.ha $^{-1}$) by multiple linear regression and also that different field characteristics influence model accuracy, being mandatory field specific modelling.

Keywords: Precision agriculture, Proximal sensing, Sugarcane biometry
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Physiological responses of *Saccharomyces cerevisiae* strains in the presence of furans and organic acids from lignocellulosic residues

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Expanding the global production of lignocellulosic ethanol requires microorganisms with increasing resistance to pretreatment process inhibitors. Understanding how these molecules impact on the performance of *Saccharomyces cerevisiae* is of paramount importance to implement strategies to increase its robustness toward lignocellulosic inhibitory compounds, such as evolutionary engineering. In this sense, we hypothesized that the physiological effects of furans (HMF and furfural) and organic acids (acetic and levulinic acid) on *S. cerevisiae* would be unique and would have phenotypic traits demonstrated in the growth pattern of *S. cerevisiae*. For that purpose, tree strains, including a laboratory (CEN.PK113-7D) and two industrial yeast strains (SA-1 and JAY270, a derivative of PE-2), were evaluated in the presence of theses inhibitors. The impact of these compounds on quantitative physiological parameters, such as maximum specific growth rate and conversion yields were assessed in defined medium. Concentration thresholds were based on previous studies performed by our group as well as on literature data. In cultures containing 20 mM of furfural, CEN.PK113-7D presented the largest extension of the lag phase in relation to the oth er yeasts. In this condition, SA-1 exhibited the lowest lag phase elongation (67% lower than CEN.PK113-7D and 56% lower than JAY270). A similar trend was observed with 30 mM of HMF, in which SA-1 stood out as compared to the other strains. At 40 mM of furfural and 200 mM of levulinic none strain could grow. 50 mM acetic acid in non-buffered medium exhibited an elevated toxic effect on cell growth, and SA-1 was the only strain able to grow under these conditions. Overall, SA-1 is a promising platform yeast strain for second generation ethanol production and for future metabolic and evolutionary engineering strategies, and for strain robustness understanding.

Keywords: Saccharomyces cerevisiae, lignocellulosic inhibitors, industrial strains
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Heat integration in a combined 1st and 2nd generation bioethanol production process: targeting the cost reduction, environmental impact, and energy consumption

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This paper provides a renewability assessment of Bioethanol Production 1st and 2nd Generation Combined Process based on the exergy concept and the heat integration approach. The sustainable development should be guided by appropriate methods and metrics that are able to qualitatively and quantitatively measure the performance of combined first (1G) and second (2G) generation bioethanol production technology. The production process involves several steps, including the modeling and operation settings for biomass Cleaning, Preparation and Extraction unit, Clarification unit, Juice concentration unit, Fermentation unit, Utility plant, Distillation, Dehydration unit

and Pre-treatment and Hydrolysis unit. The simulation process was carried out using Aspen Plus® software. Reducing the energy used in bioethanol biorefineries is an important step towards reducing both the cost and environmental impact of the process. Heat recovery via Pinch technology approach was used to analyze the energy utilization and to investigate possible energy savings in an integrated 1G and 2G bioethanol production plant. The results indicated the global exergy efficiency (38.78%) of the process. Consequently, the average unitary exergy cost was 2.57. Concerning, the environmental impact results, it was found the CO2 equivalent index in exergetic base (130.28 gCO2/MJ products), that is the relation between the estimated global CO2 emissions emitted in the atmosphere and the exergy of the products for this integrated baseline configuration, when both, bioethanol and surplus electricity, are considered as products. Furthermore, the environmental exergy indicator "λ" was applied to quantified the renewability processes for the analyzed technological pathway. It was shown that the renewability exergy index (λ=0.62) was environmentally unfavorable (λ < 1), indicating that the exergy of the products could not be used to restore the environment to the prior conditions to the occurrence of the process. Lastly, it is observed that heating costs could be reduced by 20-30% and cooling costs by 12-24% via heat integration.

Keywords: Exergy analysis, Heat integration, Global CO2 emissions

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Development of a controller based on metabolic fluxes for micro-aerated conditions: maximization of ethanol production

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The production of chemicals and energy from renewable resources using bio-based processes has received attention as an alternative route to the petrochemical processes. Besides ethanol, several industrially relevant products such as 2-3 butanediol, acetone and isobutanol, among others, are produced und er limited oxygen conditions, which are difficult to monitor and control using dissolved oxygen measurements. In this work, an innovative control strategy based on metabolic fluxes was proposed and applied to maintain micro-aerated conditions during ethanol fermentation by Baker's yeast Saccharomyces cerevisiae. The experiments were carried out in a 5-L stirred tank bioreactor, operated in fed-batch mode, using defined medium (5.0 g.L $^{-1}$ KH_2PO_4, 2.0 g.L $^{-1}$ $MgSO_4.7H_2O$, 1.5 g.L $^{-1}$ urea) with glucose as carbon source (30 g.L $^{-1}$ for batch and 300 g.L $^{-1}$ for the feeding media). Simulations of the genome-scale metabolic model iND750 for *Saccharomyces cerevisiae* with the free software Optflux were performed to identify the ranges of oxygen and substrate fluxes that maximize ethanol fluxes. During the micro-aerated controlled fermentation (MF), oxygen supply and feed flow rate were manipulated to control oxygen and substrate fluxes as well as the respiratory quotient (RQ) at the identified levels. For comparison, an additional fed-batch culture mimicking the conventional "Brazilian fuel-ethanol plant" fermentation strategy (EPF), where no inlet gas stream is supplied, was carried with the same substrate feeding profile implemented by the controller in the MF. During the experiments, cell mass concentration in dry weight - C_X (gDCW.L $^{-1}$) - was estimated as function of optical density (OD) readings at λ=600 nm. The OD readings were used to update C_X data at-line. Glucose

concentration quick measurements from a commercial glucometer were also employed to update the derivate terms present at the substrate mass balance equation. Metabolites concentrations were measured by HPLC using an Aminex HPX-87H column (Bio-Rad), with 5 mM sulfuric acid solution at a flow rate of 0.6 mL.min $^{-1}$ as mobile phase, at 60°C. Ethanol, glycerol, and glucose concentrations were measured with a refraction index detector (Waters 410), while organic acids were detected at 210 nm (Waters 486 UV-detector). The MF cultivation showed a high ethanol yield (0.48 gethanol.gsubstrate $^{-1}$) and productivity (6.0 $g_{ethanol}.L^{-1}.h^{-1}$). Glycerol productivity for MF was low (0.6 $g_{glycerol}.L^{-1}.h^{-1}$), which resulted in a final concentration of 7 $g.L^{-1}$. EPF cultivation had an ethanol yield of 0.26 $g_{ethanol}$.gsubstrate $^{-1}$, and productivity of 3.4 $g_{ethanol}.L^{-1}.h^{-1}$. The final glycerol concentration was about 8 $g.L^{-1}$ for EPF. The superior performance of micro-aerated culture reflects the important role played by the oxygen supply during fermentation processes. The new control strategy showed that it is possible to maintain micro-aeration conditions without expensive and fragile dissolved oxygen probes.

Keywords: Saccharomyces cerevisiae, Bioethanol production, Metabolic models
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Multiscale architecture of sugarcane bagasse and its importance for cellulosic ethanol

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Lignocellulosic biomass such as sugarcane bagasse have multiscale architecture, ranging from coarse particles (centimeters) down to phenolic and sugar units (sub-nanometer). In the production of cellulosic ethanol, relevant phenomena occur across all these length scales. In this work, we present our understanding of the most critical phenomena and discuss remaining unknowns. At the millimeter to micrometer scales, we employed synchrotron X-ray microtomography to localize and characterize mineral particles trappe d in bagasse. Mineral particles have been implicated as the main cause of erosion in pretreatment equipment, increasing capital cost and downtime of industrial cellulosic ethanol facilities. In addition, most pioneer cellulosic ethanol facilities employ thermochemical pretreatments performed in aqueous acidic conditions at elevated temperature (140-200°C). We investigated the nanostructural changes promoted by such hydrothermal conditions. Gains in nanoscale porosity, which enhance enzyme accessibility, are demonstrated by calorimetric thermoporometry. However, high temperature also induces cohesive phenomena, namely cellulose co-crystallization (observed by X-ray diffraction) and lignin aggregation (fingerprinted in calorimetric thermoporometry). Mechanical action on biomass has also gained considerable attention in the recent years, especially because disk refining is an effective technology proven at industrial scale. We show mechanical refining of pretreated bagasse decreases particle size and increases nanoscale porosity, thus enhancing enzyme accessibility and saccharification conversion. We also investigated bagasse pretreatment in mild alkali (deacetylation), which has been proposed as an atmospheric chemical step alternative to pressurized hydrothermal pretreatments. We show mild alkali does not promote the cohesive phenomena (cellulose co-crystallization and lignin aggregation) induced by hydrothermal conditions. Against this background of results on biomass architecture, we discuss the prospects of pretreatments in cellulosic ethanol routes.

Keywords: Bagasse, pretreatment, structure
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Distillation with parallel streams applied to biorefinery mixtures

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Separation of low volatility and thermolabile mixtures by distillation requires the use of high reflux ratios, vacuum, and high number of trays. Mixtures of importance for biorefineries, such as potable bioethanol and biodiesel, are included in this case. These problems can be solved using distillation with parallel streams, such as para- and metastillation. In parastillation, the vapor flow is divided into two or more ascending flows that contact alternately with one descendent liquid flow, making possible the allocation of a larger number of trays per column height, with a smaller total pressure loss. In the case of metastillation, the division occurs in the liquid flow into one or more descendent streams that alternately contact with the total vapor stream, an advantage for high liquid loads. In this study, simplified calculation methodologies were developed and exergy analysis were implemented in order to highlight the potential advantages of distillation with parallel streams. A McCabe-Thiele-like procedure was developed for the calculation of para- and metastillation columns with any integer number of divisions of the corresponding stream. The thermal analyses were conducted via the exergy balance applied to the whole column and to each stage. The last one provides the exergy loss profile, which can be used as a basis for column optimizations and highlights the increase of the thermal efficiency of new configurations. It can be concluded that: i) the minimum number of trays increases with the number of phase divisions, while the minimum reflux ratio is the same for the different process; ii) in the case of parastillation, a smaller column height can be used to obtain the same separation, or by maintaining the height of the equipment, it allows the decrease of the reflux ratio; iii) the exergy loss profiles are different, according to the process type, being the parastillation the best alternative from a thermal point of view.

Keywords: Bioethanol, Distillation, Exergy
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Retro-techno-economic analysis applied to a 1G-2G ethanol biorefinery

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The implementation of bioethanol industrial plants, such as 1G-2G biorefineries using sugarcane bagasse, is still not widespread, despite their potential for decreasing the use of fossil fuels. At this point of their technical development, probably the overcoming of bottlenecks will strongly rely on the techno-economic analysis (TEA), which should provide insights concerning its feasibility. This kind of assessment strongly depends on the simulation of the industrial unit within acceptable levels of accuracy. Nevertheless, phenomenological models of complex processes might be quite specific, limited to a more or less narrow operational region, close to conditions tested experimentally. Since the technology is still not consolidated, the solutions that might be used industrially may be quickly outdated by new developments. For instance, this is the case for enzymatic hydrolysis of lignocellulosic materials. The performance of the hydrolysis reaction is strongly affected by new developments on enzyme technology and on reactor design. An alternative approach to solve this problem is to reverse the focus of TEA: rather than evaluating the economic feasibility

of a specific operational condition, TEA may be used to provide target values for key process metrics, such as cellulose conversion, biocatalyst yield, and reactor productivity. Through this approach, a set of goals to be pursued in order to achieve a minimal economic performance (zero net present value, NPV, for example) can be fed back to the R&D teams (Furlan et al., 2016). By using these simplified models which incorporate the process metrics, instead of phenomenological ones, this methodology is able to find general targets values for the main process metrics. The methodology was applied to a case study consisting of a 2G bioethanol process, integrated to a 1G plant, using both sugarcane juice and bagasse for feedstock. In this case study, liquid hot water pretreatment was used. Xylose from the hemicellulose fraction was either fermented or biodigested along with vinasse. For both cases, the methodology was able to identify the most influential process metrics, their threshold values and unfeasible regions, where economic feasibility cannot be achieved.

Keywords: 1G-2G bioethanol production, Biorefinery simulation, Techno-Economic Analysis
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Scale-up of microbial oil production by oleaginous filamentous fungi for advanced biofuels

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Microbial oils have important applications and can be used to produce nutraceuticals as well as biofuels. Conversion of microbial oils to advanced biofuels like aviation fuels has attracted increasing interest compared to conventional biodiesel because of the higher value of advanced biofuels. Oleaginous filamentous fungi are attractive microbial oil producers. However, many previous studies on microbial oil production by filamentous fungi were only conducted at laboratory scale. In this study, scale-up of microbial oil production process from laboratory scale to pilot scale by a model filamentous fungus - Mucor plumbeus using sugarcane molasses as carbon source was investigated and demonstrated. It was found that inoculation of fungal pellets led to reduced biomass production due to the increase in pellet size during scale-up process. Instead, inoculation of crushed fungal biomass increased the yields of microbial biomass and oils. Scale-up of microbial oil production process from 250 mL shake flasks to a 1000 reactor by inoculation of crushed fungal biomass enhanced microbial oil content from 15% to 26%, microbial oil concentration from 1.4 g/L to 1.9 g/L and microbial oil yield from 0.06 g/g to 0.11 g/g consumed sugars at initial sugar concentrations of 28 g/L - 29 g/L. The improvement in microbial oil production was attributed to improved mass and oxygen transfer. It is expected that much higher microbial oil yields will be achieved with a highly efficient microbial oil producer of filamentous fungi using the same inoculation strategy. Furthermore, hydrothermal liquefaction (HTL) of fungal biomass to bio-oils – precursors for advanced fuels was investigated. The liquid/solid ratio, temperature and catalyst affected the yields of bio-oils. HTL of fungal biomass with a liquid/solid mass ratio of 30 and a reaction temperature of 340°C for 60 min led to a total oil yield of 30.5%. At the same conditions, HTL of fungal biomass in the presence of alkaline and acid catalysts further increased the total bio-oil yields to 34%-35%, 8%-9% higher than the microbial oil content (26%). Degradation of phenolics-containing polymers in fungal biomass possibly contributed to the higher bio-oil yields. Furthermore, the bio-oils were fractionated using polar and non-polar solvents. The results showed that non-polar extracts (hydrocarbons and fatty acids)

accounted for 58% – 76% of the total amounts of bio-oils.

Keywords: Microbial oils, filamentous fungi, scale-up, hdrothermal liquefaction, biooils

Aeronautical Brazilian prodiesel – synergistic analysis of energy, environmental and socio-economic aspects and the perspective of use in the current days

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Vital to the global economy nowadays, the transport sector stands out for its significant contribution to the acceleration of environmental degradation, mainly through the burning of fossil fuels with increasing rates of carbon dioxide emissions. Since the early 1990s, some of the highest growth rates for these emissions were caused by air transport. In 2015, closer 3.2 billion passengers a year and around 33% of world trade traveled by aircraft operation. This sector responds for around 3.5% of the global GDP. In the "other hand", the aviation sector, according 5AR of the IPCC, published in 2013, also produce circa de 2% of the world's human-induced CO2 emission. In emerging economies, like China, India, Brazil, Russia or South Africa (the BRICS, in case), it's opportune to improve the understanding about the balance of the usually high economic growth of this sector with the global goal, clearly expressed by the United Nations Framework Climate Change Convention (UNFCCC), to reduce the CO2 emission, helping the stabilization of the concentration of GHG in the atmosphere and mitigating the impacts of the climate change. Ever since the Kyoto Protocol, there has been a strong recommendation for the aviation and marine transportations sectors to lower their GHG emissions. In this context, the global aviation sector announced, in COP 21 (Paris, France, 2015), their global goal to reduce, until 2050, their CO2 emissions by half, in comparison with their own in 2005. The plan for this goal takes in consideration measures of mitigation in short and medium term with technologic and economic instruments for decarbonizing. For example: new alternatives in short distance travels (like high speed train), retrofit planning, incentives to use biofuels as fuel source, reduction of the fossil fuels subsidies and adding cap and trade taxes for their use. In such context, the present study describes and analyzes the "Prodiesel" or "Prosene," the Brazilian biofuel for aviation developed in the late 1970s, in its energy, environmental, social and economic aspects and its use prospects today. This plant fuel can help Brazil meet its Nationally Determined Contribution - NDC – for reducing the Brazilian emission of GHG, which were included in the Paris Agreement.

Keywords: Biofuel, Aviation Sector in Brazil, GHG emission

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Ethanol conversion to gasoline, diesel, and jet fuel blend stocks and higher value chemicals (BTX)

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Vertimass novel catalyst technology converts ethanol and other alcohols into jet, diesel, and gasoline blend stocks that are compatible with the current transportation fuel infrastructure as well as higher value BTX chemical coproducts. This new technology allows ethanol producers to 1) switch between ethanol or hydrocarbon products to take advantage of market conditions to maximize profits, 2) possibly increase throughput with little other costs by debottlenecking the plant, and 3) sustainably provide these renewable petroleum products from domestic sources of cane sugar ethanol. Other benefits include the ability to lower plant water usage, reduce overall energy consumption, and drop GHG emissions to levels

required for the RFS Advanced Biofuel category. Vertimass has assembled a synergistic team of business executives, engineers, scientists, and consultants to rapidly move this technology to commercial scale. Vertimass received funds from the U.S. Department of Energy to accelerate commercialization of this groundbreaking technology and has engaged TechnipFMC Corporation to scale-up the process. Vertimass plans to partner with ethanol producers to integrate this technology into existing ethanol plants as rapidly as possible to overcome the blend wall for light duty vehicles. In addition, this novel catalyst will open up new ethanol markets for heavy-duty vehicles and air travel, with the latter helping airlines achieve sustainable aviation fuel targets. Overall, Vertimass technology has the potential to expand opportunities to use ethanol from corn in the United States, cane sugar in Brazil, and cellulosic biomass worldwide.

Keywords: Jet fuel blend stocks, higher value chemicals, BTX

Fuel and green cement from sugarcane straw

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Sugarcane industry in Peru generates around 2 million metric tons per year of sugarcane straw which do not have any use being burned in the fields. This study investigated the feasibility to produce sugarcane straw briquettes as an energy source and pozzolan bottom ashes for partial replacement of ordinary Portland cement. To reduce the high ash, chlorine, sulfur and alkali oxides contents of sugarcane straw that may cause slagging, fouling and corrosion in thermal conversion systems, the samples were chopped to 2-6 mm particle size, and washed in 3 liters of distilled water at 80°C during 30 minutes. Washed samples were dried and ground, the resulting powder was mixed with corn starch and clay. The proportions of these components were 75%, 15% and 10% respectively, measure on dry weight. The mixture was densified at 100 MPa using a hydraulic piston press. The briquettes had cylindrical shape, diameter was 36 mm, height, 40 mm, density 400 kg/m3 and high heating value was 16 MJ/kg on average. In order to obtain energy and, at the same time, reactive bottom ashes that can be used as a pozzolan material, the briquettes were burned under a controlled temperature, below 800°C, in a fixed bed reactor during 16 minutes. The resulting ashes were collected, allowed to cool fast and ground. Then the ashes were evaluated by X-ray Fluorescence and X-ray Diffraction Analysis to determine their chemical composition and the presence of amorphous substances. The results showed a high content of oxides of silicon, aluminum and iron around 88% satisfying the ASTM C618-15 standard requirements for pozzoland material, and a significant amount of amorphous material (45%). The pozzolanic activity of the ashes evaluated by the Strength Activity Index at 7 and 28 days was found to be 91% and 101% respectively which showed that the ashes qualify as a pozzolan. Cementitious mixing specimens containing 20% of sugarcane straw ashes were made and evaluated by autoclave expansion, air contend of mortar, time of setting, fineness, chemical composition and compressive sugarcane straw strength satisfying in both cases the requirements for Portlad pozzolan cements. The results showed that is possible to produce energy from combustion of sugarcane straw, and the resulting ashes are suitable for partial replacement of ordinary Portland cement.

Keywords: Biomass briquettes, portland cement, sugarcane straw

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Evolution of customized yeasts for ethanol production

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The customized yeasts for ethanol production are those that arise in industrial fermentations and have several advantages such as dominance and persistence compared to traditional strains (PE2, CAT1, FT858L, Fermel®) and baker's yeast. The customized strains are more adapted to specific industrial conditions from distilleries where they were selected (process-driven selection). These yeasts are more robust, resistant to stressful conditions and compete better with contaminants when compared to traditional strains. Consequently, the fermentation process remains more stable throughout the sugar cane harvesting season. Since 2008, the number of distilleries benefited for this innovation reached 18 units and 26 strains, which were responsible for the production of 2.27 billion liters of ethanol, representing 8.10% of Brazilian ethanol production in 2016. There are distilleries that have two, three and even four customized yeasts to start the fermentation process. These strains have been selected through a continuous program of monitoring of yeast populations in industrial fermentations by nuclear and mitochondrial DNA analyses. In addition, distilleries whose fermentation processes are carried out with customized yeast strains have obtained the highest industrial yields (CTY) due to stability of fermentations and the lowest rates of contamination by wild yeast in comparison with other distilleries without customized strains.

Keywords: Customized yeast, alcoholic fermentation, karyotyping

Hydraulic and organic rates applied to pilot scale UASB reactors for sugar cane vinasse degradation and biogas generation

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The anaerobic process is a suitable option for reducing both the organic load and the waste of energetic potential of vinasse. Anaerobic reactors at various scales are used for vinasse degradation and biogas generation. However, the difficulties of maintaining the high rate anaerobic process for vinasse degradation indicate that many steps have to be investigated to enable projects of efficient anaerobic reactors. The current research assesses the hydraulic rates associated with organic loads applied to anaerobic reactors and the biomass to obtain data for reactor designs that will provide better anaerobic degradation processes of vinasse and biogas production. The sludge blanket reactors with a capacity of 63L in the reaction area (2 m height) and total volume of 126 L (4 m total height) were constructed using PVC pipes with 20 cm diameter. They are equipped with gas-solid separators and provided with recirculation devices. Granular sludge (60L with 37 g/L volatile suspended solids-VSS) seeded the reactors. Four pilot-scale UASB reactors submitted to effluent recirculation rates from 50% (R1), 100 (R2), 200% (R3) and 400% (R4) were fed with sugarcane vinasse (COD of 19400 ± 5675 mgL-1), aiming at the production of biogas. The reactors were subjected to increasing organic volumetric loads (OLR) of 0.6 to 33 kg COD.m-3.day-1. The average COD removal efficiency was above 87% for raw COD and above 90% for COD soluble in all reactors. The total volatile acids in reactor effluent were below 450 mg.L-1 in all reactors. No accumulation of organic acids was observed in reactors effluent. The highest

methane productivity (9 LCH4. Lreactor-1.d-1) was obtained in R1 under 33 kg COD.m-3.day-1. This result is very promising for power generation in the sugar and ethanol industry, complementing the energy supplied by the ethanol and by the bagasse. As an example, vinasse generation at the industry that supplied vinasse for this research reached 1820000 m3 during the 2015/2016 crop. Using anaerobic reactors with 7000 m3 at reaction zone under 32 kg COD.m-3.day-1 this plant could supply electric energy for 40000 houses (157 kwh/month). These results are very promising for reactor design for electricity generation in the sugar and alcohol industry, complementing the energy supply by ethanol and bagasse.

Keywords: Biogas, renewable energy, vinasse
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Making the ABE fermentation economically viable: adaptive evolution and optimized fermentation process using cellulosic pulp

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Economic and environmental concerns regarding oil use have renewed the interest in fermentative production of biofuels and biochemicals. The ABE fermentation, performed by some species of the genus *Clostridium*, is a process that results in the production of acetone, ethanol and n-butanol, the latter being a four-carbon alcohol of wide industrial use and considerable added value. Although the ABE fermentation was applied industrially at the beginning of 20th century for solvent production, n-butanol is currently produced through petrochemical routes, with a global market of US$ 7.86 billion in 2014 and estimated to be US$ 9.9 billion in 2020. In order to make the fermentative process economically viable, an efficient microorganism able to produce high titers of n-butanol from a cheap substrate are required. Therefore, a viable strategy to make feasible the n-butanol fermentative production is combining microorganism selection through adaptive laboratory evolution with the development of second-generation technology, which converts the cheap and abundant lignocellulosic biomass into fermentative sugars. Low solvent yield and productivity due to toxicity of high n-butanol concentration is one of the main challenges of the ABE fermentation, with the tolerant phenotype being an outcome involving multiple genes. By performing an adaptive laboratory evolution approach in *Clostridium saccharoperbutylacetonicum* DSM14923, we were able to obtain a strain with n-butanol yield 15% higher than the wild type. The organosolv pretreatment of lignocellulosic materials separates cellulose from lignin and hemicellulose and reduces the formation of toxic compounds for microorganism. The cellulosic pulp then undergoes a hydrolysis process, originating a glucose-rich hydrolysate with low inhibitor concentration. The use of this hydrolysate for ABE fermentation, however, still does not result in a n-butanol production effective enough to make this process industrially feasible. However, by adding cellulosic pulp to the culture medium, even in small concentrations (0.05% w/v), together with enzymatic complex (cellulases and hemicellulases), we developed an optimized process for solvent production, achieving a n-butanol titer and productivity 42% higher than the control and an improvement of 340% in acetone production. Moreover, by cultivating the *C. saccharoperbutylacetonicum* evolved strain with cellulosic pulp and enzyme supplementation, we obtained an improvement in n-butanol productivity of 72%. Therefore, this study demonstrated a successful use of adaptive evolution techniques for strain development and the use of cheap raw-

materials as carbon source. Moreover, medium supplementation with cellulosic pulp and enzymatic complex resulted in unexpected metabolic changes, which has considerably increased the production of solvents. Therefore, the technology developed at this study can be the base for fermentation scaling at industrial level and can drive studies regarding *Clostridium* metabolism in order to improve ABE fermentation through genetic manipulation.

Keywords: Clostridium saccharoperbutylacetonicum, n-butanol, cellulosic pulp
Supported by: CNPq

Correlations between the concentrations of biomass inhibitors and effects on cells mass, ethanol and their productivities

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An important problem in the conversion of lignocellulose to ethanol is the severe inhibitory effects exerted by the products of the lignocellulose degradation on yeast cells. In the present work, five weak acids selected based on abundance in biomass hydrolysates were added to a synthetic medium supplemented with yeast extract to minimize the negative effects of inhibitors on growth and fermentation. High cell density fermentations were performed to produce ethanol and cells for periods of 8h at 35°C. The results obtained showed that the values of ethanol and biomass produced were less affected in the presence of levulinic acid than in formic acid. The effects of the inhibitors on ethanol production were high, while values of viability were null at concentrations of formic acid above 75 mmol.l-1. On the other hand, concentrations of formic acid showed higher negative effect on biomass productivity (Qx) than on ethanol productivity (Qp). Degrees of inhibition were greatly depended on concentration and nature of inhibitors, as well as the resistance capacity of the yeast. The 25-2 fractional factorial and the 23 full central composite designs were applied to determine the effects of main inhibitors on biomass and ethanol production. Response optimizer was used to statistically adjust the cocktail of inhibitors with minimal inhibitory effects on yeast cells during growth (13.2 ± 0.1 mg/ml biomass) and fermentation (72.6 ± 0.1 g/l ethanol).

Keywords: Second-generation ethanol, biomass inhibitors, optimization and validation of responses
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Systems biology applied to the secretion of enzymes by filamentous fungi

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Only one-third of the biomass of sugarcane is processed into ethanol, while the other two-thirds are discarded as bagasse and straw. Cellulose and hemicellulose are the most abundant polymers in sugarcane bagasse and are organized in a very complex way in the cell wall. Cellulases and hemicellulases are glycoside hydrolases responsible for the complete degradation of these polysaccharides from the plant cell wall. Filamentous fungi are the major source for the prospection of enzymes related to the plant biomass degradation, highlighting Aspergillus and Trichoderma. The attraction of filamentous fungi as machinery for expression and secretion of target proteins is based on their natural ability to secrete large amounts of proteins into the extracellular medium. Compared with other systems available for expression of heterologous proteins, in liquid cultures, filamentous fungi show an excellent performance, high yield and a low-cost option, in relation to nutritional requirements.

However, I would like to present some results obtained from studies to consolidate Aspergillus nidulans as a model for expression and heterologous secretion of cellulases and hemicellulases. The central objective was to develop a collection of lines capable of expressing various enzymatic activities involved in the degradation of polysaccharides from the cell wall of plants. In addition to studying the functional properties of these enzymes, with broad biotechnological applications including strategies for bioethanol production, we have developed super-expressing strains concomitantly with two or more synergistic enzymatic activities of cellulases and hemicellulases. Currently, through systems biology studies, we search for target genes involved in the protein secretion machinery in A. nidulans, for genetic manipulation and obtaining superior strains.

Keywords: Enzymes, Biomass conversion, Filamentous fungi
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Comparative analysis of two endoglucanases from GH45 family with potential biotechnological application

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Lignocellulosic materials are abundant and renewable, appearing as valuable substrates for many industrial applications such as second generation biofuels, green chemicals and pharmaceuticals. However, the recalcitrance of cell walls and the complexity of cell wall polysaccharides require multiple enzymes for their complete conversion to monosaccharides. Some enzymes produced by lignocellulolytic microorganisms are capable of processing these kind of materials and used in several industrial applications. Among these enzymes, GH45 endoglucanases are already used on the treatment of cotton fibers, representing a small and poorly studied group of enzymes. The present study reports the cloning, heterologous expression, biochemical characterization and comparison of two GH45 endoglucanses from thermophilic and mesophilic fungi Myceliophthora thermophila (MtGH45) and Gloeophyllum trabeum (GtGH45), respectively. The optimal pH for MtGH45 and GtGH45 was 5.0 and 5.5, respectively and circular dichroism experiments demonstrate that enzymes show a melting temperature (Tm) of 80 C for MtGH45 and 60 C for GtGH45. The recombinant proteins demonstrated different mode of action when incubated with oligosaccharides ranging from cellotriose to cellohexaose, generating mainly cellobiose and cellotriose for MtGH45 and glucose and cellobiose for GtGH45. The MtGH45 didn't show activity in oligossacharides smaller than cellopentaose while the enzyme GtGH45 was able to depolymerize cellotriose, however with less efficiency when compared with its capacity to depolymerize larger oligossacharides. Also, the microscopic analysis of pulp fibers after incubation with GH45s indicated the presence of swollen fibers, which suggested improved access of treated substrates to other enzymes. Thus, the use of these GHs 45 seems to aid subsequent hydrolysis of biomass by cellobiohydrolases.

Keywords: Endoglucanases, Filamentous fungi, Cellulose
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Can the yeast *Kluyveromyces marxianus* grow under fully anaerobic conditions?

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The development of a thermotolerant yeast strain for fuel ethanol production holds interest due to the possible economic and enviromental benefits of decreasing contamination and water usage. Nevertheless, such a strain must perform in a similar way to the current well-known *Saccharomyces cerevisiae* strains, in the first-generation non-aseptic bioprocess. One of the key characteristics required is the capacity to grow under full anaerobiosis. The thermotolerant yeast *Kluyveromyces marxianus* is a species that presents important features for ethanol production, such as: capacity to produce and excrete ethanol, an oxygen-dependent respiro-fermentative metabolism, and capacity to assimilate a wide variety of sugars. However, most results from previews studies show that *K. marxianus* is not capable of growing under anaerobiosis and no previous studies have investigated this issue thoroughly in the laboratory. Here, we investigated the behavior of *K. marxianus* under extreme oxygen-limited continuous cultivation (less than 15 ppb in the sparging gas), in chemostat cultivation with a dilution rate of 0.1 h^{-1}. After reaching a steady-state under aerobiosis (with a biomass titer of 5.81 g /L), an anaerobic condition was applied. Surprisingly, the yeast strain did not wash-out of the bioreactor under such condition. In fact, a steady-state condition was achieved after 54 h of cultivation, with a biomass concentration of 0.46 g/L. Under this condition, the major metabolic products were ethanol, biomass and glycerol, with an ethanol yield of 0.37 g g $glucose^{-1}$ and a biomass yield of 0.08 g DW g $glucose^{-1}$, suggesting a fully fermentative metabolism. However residual sugar at this steady-state was quite high (4.05 g $l\ glucose^{-1}$). This study suggests that *K. marxianus* has the potential to be employed for anaerobic bioprocesses, such as fuel ethanol production. We aim at applying evolutionary engineering to improve this strain's growth under anaerobiosis.

Keywords: Fuel ethanol, yeast physiology, industrial biotechnology
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Bio-oil and phenolic resin production from forest products wastes: a biomass cascade approach

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The present study was carried out under demand of Duratex aiming to develop a product for its own consumption based on pyrolysis, mainly biomethanol and phenolic resin. The present study tested two processes and several variables aiming the maximization of the feedstock production. It was tested both slow and fast pyrolysis route. Fast pyrolysis is a technique that utilizes biomass to produce a product that is used both as an energy source and a feedstock for chemical production. Considerable efforts have been made to convert wood biomass to liquid fuels and chemicals since the oil crisis in mid-1970s. In the pyrolysis process the main products are bio-oils and bio-char mentioning that bio-oils, are the main products of fast pyrolysis. Virtually any form of biomass can be considered for fast pyrolysis. Most work has been performed on wood, because of its consistency andr eplicability between tests. However, 2 types of biomass were tested, the Eucalyptus bark and MDF waste. The pyrolysis experiments were carried out in a batch pyrolysis fluidized reactor, which is considered the most efficient for biorefinery. The initial moisture content was 7.42%, which is almost the EMC (Equilibrium Moisture Content) for the natural wood. A complete mas balance was done and the char represents just about half of its composition (~51.4%). The second most important fraction, the bio-oil (26.4%), responsible for the phenolics structures compounds and the gases

which are a mixing of combustible and non-combustible gases. Therefore two alternatives can be considered: resol phenolic resin and bio-oil polymerization cross-linked with formaldehyde. The increase of pyrolysis temperature from 450°C to 550°C increased the proportion of medium fraction, according to previous research, where the content of water reduces while the phenolic structures content increase. Thus, the performances of phenolic resin improved when using high phenolic structure containing bark oils, but less water After the bio-oil production and characterization, boards were made using different levels of bio-oil based resin. According to the lap shear results, the PF binders produced are acceptable for panel production, meeting the standards requirements. Considering that the commercial resin has a viscosity of about 450 mPa s, a value between 8.5 and 19.8% could be used as replacement for the commercial resin. The shear varied from 2.09 to 2.49 MPa. In conclusion, the phenolic resin produced could be used to replace partially the fossil based resin representing a savings of about 30% in resin acquisition for the company.

Keywords: Biophenol, eucalyptus bark, bioresin
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Flexible production of butanol and ethanol integrated to a kraft pulp mill

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This research aimed to develop a flexible biorefinery annexed to a state-of-the-art eucalyptus kraft pulp mill. Following the traditional oil refinery model, the flexible biorefinery produces chemicals (n-butanol and its co-product acetone), as a high-value portion of "the barrel" with better margins, combined with a fuel (ethanol), which provides volume for economies of scale. The biorefinery processes 1000 dry ton eucalyptus chips/day and three pretreatment technologies were assessed: dilute acid, steam explosion, and organosolv. The fibers are converted to glucose by enzymatic hydrolysis and the sugars are fermented in either conventional batch tanks or in continuous fermentors with in-situ product recovery. Two operating regimes were defined for the flexible plant according to the ability of the microorganisms to ferment glucose (either for ethanol or butanol production) and xylose (only for butanol production). Changes in operating regime take place on quarterly basis based on the comparison of EBITDA in each quarter of the year. To calculate the switching criterion, selling prices varied randomly in Monte Carlo simulations according to normal distributions built on the basis of historical prices. The flexible production of butanol/ethanol brings economic advantages over either butanol or ethanol dedicated plants. Notably, the Net Present Value of the projects increased between 20-40% depending on the pretreatment technology. The relatively low capital intensity of steam explosion was key for its economic advantage (NPV = 184 MMUSD; IRR = 29%), and lignin should be priced at 800-900 USD/ton in order for the organosolv pretreatment to secure similar economic performance. As for the advanced fermentation, it can improve the energy efficiency of the process only if the enzymatic hydrolysis step has solids loading higher than 20% and cellulose conversion above 75%. Moreover, attractiveness of such fermentation technologies is expected to increase as the capital cost of pretreatment technologies advance in the learning curve.

Keywords: Flexibility, biorefinery, fermentation
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Challenges and dilemmas in product innovations in the biobased industry

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The biobased industry, among other challenges and opportunities, brings a wide range of possibilities regarding product innovation. Apart from problems related to cost/price competitiveness versus fossil alternatives, innovators have challenges related to the identification, the development and the market adoption of new biobased products. Many critical factors should be considered and analyzed in order to better understand innovation dynamics in this emerging industry. In this context, the following questions have to be taken into account concerning new products: drop in or non drop in? final or intermediaries? commodities or specialties? platform chemicals? biomass utilization efficiency? These dilemmas are not on-off decisions. In many cases, there are grey zones between them which need to be better understood. Besides these points, the market development and the final adoption by brand owners are critical steps which have to be faced by innovators in the biobased industry. Some of these points were previously analyzed (Bozell and Petersen, 2010, Oroski, Bomtempo, Alves, 2014; Iffland et al., 2015, for example) but there is not a comprehensive framework which could put together the technical and strategic factors. We propose to discuss these interrelated dimensions in order to shed some light in their role in biobased product innovations. We suggest, based on our research on biobased industry dynamics and on the lessons from economy and management studies on innovation adoption and diffusion, an analytical framework to explore, in an integrated way, the critical factors involved in biobased product innovations. These critical factors should be taken in consideration by innovators as early as possible in order to orientate product development strategies.

Keywords: Biobased chemicals, product innovation, innovation strategy

Sugarcane syrup and molasses as nutrients for xylitol production from hemicellulosic hydrolysate of mixture of bagasse and straw

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High-added value bioproducts can contribute to diversification and valorization of the sugar-and-alcohol agroindustry in Brazil. The existing agro-industrial by-products can be used as substrates and/or nutrients in bioprocesses that can be integrated to current productive chain and represents a vast carbon resource, which is as yet largely unexploited. This strategy may improve the efficiency of the use of energy and materials, and consequently the sustainability of this agroindustry within the concept of biorefinery. One of the bioprocess that can be considered for be integrated on a future sugarcane biorefinery is the biotechnological production of xylitol. This compound belongs to the special sweetener category owing to features like its non-cariogenicity and non-dependence on insulin to be metabolized. In Brazil, it is mainly employed in the food market. Important advances in different stages of xylitol bioprocess have been achieved at “Escola de Engenharia de Lorena – USP”, using the yeast Candida guilliermondii FTI 20037 and various agro industrial by-products, mainly sugarcane bagasse and straw. A recent research of our group showed that supplementation of sucrose (10 gL-1) to the sugarcane straw hemicellulosic

hydrolysate favored the capacity of C. guilliermondii FTI 20037 to consume xylose and produce xylitol. Thus, it was proposed the use of sugarcane molasses and syrup, by-products of the sugar-and-alcohol processes and composed of sucrose, nitrogenous compounds, vitamins and minerals, as nutrients for formulation of the fermentation medium for xylitol production from the hemicellulosic hydrolysate of the mixture of sugarcane bagasse and straw. The effect of the supplementation of sugarcane syrup or molasses to the fermentation medium on xylose-to-xylitol bioconversion and the possibility of replacing nutrients conventionally employed in this bioprocess have been evaluated separately using factorial designs. A 1:1 mixture of sugarcane bagasse and straw was submitted to dilute-acid hydrolysis, the hemicellulosic hydrolysate obtained was vacuum concentrated to increase xylose concentration and then detoxified by pH adjustment and activated charcoal adsorption. Batch fermentations with C. guilliermondii FTI 20037 were carried out at 30 ºC, initial pH 5.5, for 72h and on 125 mL Erlenmeyer flasks with 50 mL of fermentation medium, which corresponded to hemicellulosic hydrolysate supplemented or not with sugarcane molasses or syrup, and the conventional nutrients solution of rice bran extract, (NH4)2SO4 and CaCl2·2H2O, according to factorial designs. Statistic analysis of data showed that supplementation of conventional nutrients did not have significant effects on xylose consumption and xylitol production, differently from sugarcane molasses addition. Furthermore, improvements on xylose consumption and xylitol volumetric productivity were achieved when conventional nutrients were partially or completely substituted by supplementation of sugarcane molasses or syrup, which leads to the possibility of reducing costs of biotechnological production of xylitol integrated on a sugarcane biorefinery.

Keywords: Sugarcane molasses, Sugarcane bagasse and straw, Xylitol

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Mitigating the cold flow problems of biodiesel by ethyl esters blends

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The composition of esters of a biodiesel, and the alcohol used in transesterification, defines its physical properties. This is of utmost importance considering the design of engines. Cold flow properties are one the main limitations for biodiesel application, due to the presence of saturated esters. For mitigate the crystallization problem, blends could be considered. Despite this is a known idea, literature presents few works on it. In fact, the solid-liquid equilibrium (SLE) theory shows that when two compounds are mixed, system tends to decrease its melting temperature. This was already verified for mixtures of pure methyl and ethyl esters. In this context, 3 facts could be mentioned: 1) ethyl esters and binary mixtures of them present lower melting temperature than methylic esters; 2) ethanol are claimed as a “green” alternative for producing biofuels replacing methanol; However, 3) the ethylic route presents operational problems, which makes methylic routes most industrially applied. Considering these pros-and-cons, and trying to mitigate the cold flow problems, ethylic biodiesel blends were evaluated in this work. For this, ethylic model-biodiesels from chosen sources: soybean, cottonseed, murumuru, ucuuba, and coconut fats/oils were formulated as well as blends of them. Sources were chosen due to the different fatty acid profile, including different ratios of saturated, unsaturated, medium and long carbon chains. Model biodiesels were composed by the main 3 or 4 esters of the respective biodiesel composition, and blends were considered at the ratio 1:1. Crystallization and melting profile

were experimentally evaluated through differential scanning calorimetry (DSC), and their experimental solid fraction curves (SFC) described through the thermograms, considering the partial areas of the peak of the heating cycle. Also, SFC curves were simulated by the SLE theory, and deepest thermodynamic information about the blends could be accessed. Melting profile of ethylic biodiesels followed the same trend from binary pure compounds. It means that the melting temperatures of the biodiesel blends are always lower than the melting profile of the pure biodiesels. The final melting temperature of the blends decreased up to 20°C approximately, from the pure biodiesel, and the initial melting temperature decreased in all cases. The ratio saturated:unsaturated esters were significant in the profiles, as well as the percentage of medium carbon chains. These two factors altered the ideal thermodynamic behavior of the mixture, promoting a higher or a lower decreasing in the melting temperatures. One should considers that most of the biodiesels presented a final melting temperature lower than the soybean oil ethylic biodiesel, one of the most common vegetable oil source. This is highly significant in order to promote perspectives for production of other vegetable fats/oils matrices.

Keywords: Solid Fraction Curve, Vegetable oils, Ethanol

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New proposal for the use of sugarcane filter cake by supercritical technology integrated to a first and second generation biorefinery: thermo-economic evaluation

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In the last thirty years, the sugarcane sector in Brazil has undergone a major modernization. Embracing the biorefinery concept, this sector produces ethanol and electricity but it is also has been investigating product diversification strategies. In this context, the investigation of different high added-value products from process wastes is opportune. This study evaluates the integration of supercritical fluid extraction process to obtain sugarcane wax extract from the filter cake residue. This cake is eliminated from the decantation process of sugarcane juice and it is generally used as a fertilizer. From this cake a lipophilic material, containing long-chain fatty alcohols and phytosterols, can be selectively recovered by means of the use of supercritical CO2 as extracting solvent and further be used as nutraceuticals or pharmaceuticals. Apen Plus® software was used to simulate the sugarcane biorefinery producing electricity, conventional and cellulosic ethanol and wax extract. A thermal-economic model was developed in Matlab software to perform energy integration and the economic analysis of this novel biorefinery concept. It was investigated different supercritical CO2 extraction conditions based on the experimental data of laboratory and pilot scale experiments performed at LASEFI/UNICAMP. The results showed that by increasing temperature and pressure of the extraction process it is possible to produce more wax extract at an overall lower investment as lower extraction time is necessary, decreasing the number of extractors working in parallel. The integration of the extraction process to the sugarcane biorefinery had no impact on the overall ethanol production (conventional and cellulosic) and had small impact on the electricity available for sale to the grid, decreasing only around 3% the net electricity. Payback time is strongly reduced by the integration of the extraction process decreasing 48% when the worst extraction condition is used and 74% when the best condition is used. The selling price for the wax extract strongly influences on the economic

viability of this process. The integration of the extraction process to the sugarcane biorefinery is only economic attractive if the wax extract selling price is higher than 26.5 USD/kg.

Keywords: Biorefinering, process simulation, supercritical fluid extraction

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Experimental characterization of combustion processes in a SI engine fuelled with Syngas and Methane

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A wide variety of bio-residues can be converted into gaseous fuel (syngas) via gasification; this intermediate product can be directly used in spark ignition (SI) engines, making biomass gasification a very interesting way to exploit the energy content of industrial secondary streams and agricultural wastes. The main problem of variable feedstock and production methods is that the syngas composition can also differ significantly, changing the optimal operational condition of the engine and creating combustion variability. In order to avoid these disadvantages, control methods based on the tuning of spark timing, engine load and on the addition of supplement fuels can be used. Nevertheless the optimization of SI engines fuelled with syngas requires deeper understanding of the related thermal mechanism in engine-like conditions. Therefore, to improve current knowledge and provide reference data for modelling and simulation of internal combustion engines, combustion was investigated in an optically accessible PFI-SI single cylinder engine fueled with three different gas fuels. This fuels are equivalent syngas mixtures with different proportion of H2, CO and CH4. Also the dilution content was vary to a wide fuel study. Methane, as the main constituent of natural gas, was used here as reference fuel. Stoichiometric and lean operation was studied in detail through thermodynamic and optical approaches. The engine was operated at fixed rotational speed (900 rpm) at wide open throttle; excess air ratio was raised from 1.0 to 1.4 (close to the flammability limit of methane) and spark timing was adopted according to the maximum brake torque of the baseline fuel to compare the different cases in the same fluid dynamic conditions at ignition. Cycle resolved digital imaging was applied and flame front propagation speed, morphology parameters and centroid motion were evaluated through image processing. The optical results were correlated to in-cylinder pressure data.

Keywords: Biomass power, Syngas, Optical investigations SI engines

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The balance of greenhouse gases for a sugarcane plantation in Brazil

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Fluxes of carbon dioxide (CO2), nitrous oxide (N2O) and methane (CH4) were measured nearly continuously using the eddy covariance method (sonic anemometer, infrared gas

analyzer and quantum cascade laser spectrometer) above a sugarcane plantation in Pirassununga municipality (São Paulo State, Brazil) over 367 days. The period of measurements started 156 days after planting (October 2015) and covered the first harvest (November 2016) without trash removal and the application of ammonium fertilizer (8 g N m-2 of NH4). The yearly integrated net ecosystem exchange (NEE) indicated that the plantation was a carbon sink removing -5852.9±198.6 g CO2 eq. m-2 from the atmosphere. The NEE of CO2 fluxes dominated the balance (-5928.3±198.6 g CO2 m-2) with the N2O and CH4 fluxes, being minor sources to the atmosphere of 63.7±1.3 and 11.7±1.7 g CO2 eq. m-2, respectively. The cumulative flux of N2O observed before the harvest (242 days) was 163.8±4.8 mg N2O-N m-2; the effect of the N fertilization persisted for approximately 84 days resulting in 140.6 ± 4.6 mg N2O-N m-2. Assuming the background flux of N2O can be represented by the flux measured during the 84 days after the fertilization effect ceased (67±2.9 mg N2O-N m-2) then the emission factor was estimated as 0.92±0.07% of the N applied. In contrast, the CH4 fluxes were small and did not show any peak through the cycle. Accounting for the harvest removal of stalks biomass accumulated during the period of measurements (3.7±1.2 kg CO2 m-2) and the decay of the trash remaining on the soil surface after the first harvest (1.1±0.1 kg CO2 m-2), the net ecosystem carbon balance (NECB) was -1.0±1.2 kg CO2 m−2. Therefore, during this typical year when the yield in stalk fresh weight (SFW) was 12.2±2.1 kg SFW m−2 the sugarcane system was approximately neutral, or a carbon sink based on the lower limit of the uncertainty.

Keywords: Sugarcane, eddy covariance, greenhouse gases

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On the impact of the agro-environment: the new profile of the sugar alcohol occupation

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In 2007, the Agro-Environmental Protocol of the Sugarcane Section was signed by private and public sectors. The Protocol formally established the preference for mechanical harvest rather than slash-and-burn. Labor and environmental laws, the market, management issues, and financial reasons motivated the change, which was led by sugarcane entrepreneurs. Furthermore, mechanical planting and harvesting enhanced the administration of the agricultural process. This study analyzes the consequences of such technological and managerial changes in sugar cane plantations. In particular, this research focuses on how this change in the process of planting and harvesting affected the physical arrangement of workers in this technological configuration. The study was conducted in the state of São Paulo between 2007 and 2014 and involved sugar alcohol companies that work with planting and manufacturing of crude and refined sugar and alcohol. Data collected at the Ministry of Labor and Employment provided information about the number of people involved in different kinds of occupation in these businesses. After data collection, the study classifies different occupations into groups and sub-groups. As a result, the study finds that in 2007 the mechanical harvesting was present in 42% of all sugar cane plantations located in the state of São Paulo. In 2013, this number was much higher (85%). Consequently, between 2007 and 2014, the number of workers in the sugar-alcohol plantations decreased 16% (52,000 people). The reduction was caused mostly by a decrease of the group classified as “People Employed in Sugar Cane Plantations,” which shrank by 41% (85,000

people). More specifically, there was a decline of 59% (105,000) in the subgroup classified as "Cane Workers," which encompasses people who manually harvest sugar cane. However, in turn, the number of people working with agricultural mechanization increased by 74%. Also, the group "People Employed in Manufacturing" (mills and distilleries) increased by 14% and the group "Transport, Administration, and Support," by 48%. Among the latter, the subgroup "Transport and Maintenance" climbed 81% (26,000 people). This was because of a higher number of machines employed and the decision made by companies not to outsource this service. In the same group, another subgroup called "Administration" also saw an elevation in 33% increase in the number of employees (5,000 people), since more control was necessary to manage the agricultural work and business process. In conclusion, the profile of the sugar-alcohol occupation has gone through an intense change due to the rise of mechanization and the decrease of manual labor.

Keywords: Sugar alchol ocuupation, agro-enviromnent, mechanical harvest
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Sustainability of intensified pastureland: land cover monitoring

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Although there is demand for biofuels from industry, the global enthusiasm for bioenergy often face problems with food production or land clearing. In this regard, pastureland appears to be a promising source, in which has great potential to intensify food production making land available for bioenergy, however, the main challenge in this context is to identify pasturelands areas. Remote sensing is an appropriate tool to map and monitor this type of land cover mainly at the national/the regional level, and the variables involved in this process must be understood. Extension of remote sensing techniques to quantify pasture and the degree of intensification would be extremely valuable. In this context, the aim of this study was to build a spectral library for crop-livestock with different managements and afterward use it to detect pastureland intensification. The study area was the west of Sao Paulo state which is known to have traditional pastureland areas. Sample points were chosen and their characteristics were studied based on a survey of farmers related to land management. These data were spatialized to help the understanding of the spectral behavior of the intensification and to allow a further land classification to areas with the same pattern. These points were analyzed using the Vegetation Temporal Analysis System - SATVeg a web tool developed by Embrapa Agricultural Informatics in which allow verify the temporal profile of Normalized Vegetation Index - NDVI based on Moderate Resolution Imaging Spectroradiometer - MODIS data. For all farms visited the NDVI profile presented variation before and after intensification. Most of the farmers in the region reported doing some management in order to intensify the areas. This intensification was made in different ways, such as, on rotational (crop-livestock), intercropping, succession systems in the same area or applying fertilizers. Thereafter, the NDVI of these areas significantly increased after the management, moreover, the spectral behavior showed different characteristics from those where there was no intensification. This is an indication that the remote sensing is an important tool to identify the intensification on pastureland because it is necessary to

understand the temporal and spectral behavior of this process for monitoring purposes.

Keywords: Geospatial Analysis, Land Availability, Pastureland Intensification

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Linking soil microbiome with sustainability

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Sustainable agriculture depends on healthy soil and soil microbial diversity and thus must focus on managing soil microbes to deliver more efficient ecosystem services to crops. Moreover, intensified crop production demands integrated nutrient management systems to maintain agricultural productivity and protect the environment. Using beneficial native microbes that promote plant health and quality, and recycling crop residues with low environmental impact are ultimate practices for sustainable food and energy production. Here I will present our studies on using biological native resources (beneficial bacteria and fungi) and recycling organic residues for sustainable crop production. Our studies have shown that sugarcane endophyte bacteria and fungi when inoculated in plantlets promote sugarcane plants growth, quality and health (microorganisms antagonistic to sugarcane pathogens). Subsequently following the crop cycling production, our studies on recycling of crop residue as a sustainable practice, showed that the combination of sugarcane residue (vinasse), rich in carbon, nitrogen and potassium when applied together with inorganic fertilizer, emits more nitrous oxide than inorganic fertilizers through nitrification process carried out by bacteria. In order to mitigate N2O emissions we showed that the use of inhibitors of nitrification is a practical solution and, more importantly these compounds do not affect soil-borne microbial community diversity.

Keywords: GHG, nitrification, vinasse

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Do land use and agricultural development drive food security? National-level data suggest not

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Food insecurity is a bottleneck to human progress and its reduction is a top sustainable development goal. Though food security has been thoroughly defined and measured, understanding of its causation is often driven by intuition rather than empirical inquiry. We employed a data-driven approach to identify the causal factors responsible for food security. Using the Economist Intelligence Unit's Global Food Security Index (GFSI) as a proxy, we examined correlations between the GFSI and a dataset of socioeconomic and agricultural variables characterizing 113 countries. Guided by these correlations, we developed multivariable linear regression models to quantitatively represent the relationship between GFSI scores and groups of explanatory variables. These models and statistics are limited in resolution to the national level, allowing identification of broad trends but not specific causative models for food security at the local level. Results show that amount of land in agricultural use in a nation is not correlated with food security with any statistical significance. The amount of land under agricultural use per capita explains just 0.02% of the variation in food security scores among the countries examined. Measures of agricultural development range from very weak to moderate predictors of food security, with

cereal grain yield per capita and fertilizer use per hectare explaining 10% and 41% of the variation in GFSI, respectively. Contrarily, measures of economic development such as GDP per capita, energy use, and logistics performance were strongly correlated with and predictive of a nation's food security score. GDP per capita alone explains 88% of the variation in GFSI across all 113 countries. Multivariable regression models further substantiated these results. The inclusion and exclusion of agricultural development and land use metrics in the model had virtually no influence on its ability to predict GFSI scores. A full-factor model including arable land, fertilizer use, cereal yield, logistics performance, energy use, and GDP as explanatory variables achieved an R-squared value of 0.92. This is only 4.5% higher than the R-squared achieved by GDP alone. The data suggest that contrary to conventional wisdom, land availability is not an important causal factor in national food security. Nor are crop yields and measures of agricultural input influential in models predicting a nation's GFSI status. Though GDP per capita is an imperfect measure of economic development, it was the best predictor of food security among the explanatory variables studied. In the context of the Food vs. Fuel argument, the data suggest that the biofuel industry – though it uses land – may be a lever to improve national food security by accelerating economic growth.

Keywords: Agricultural development, food security, social impact

Supported by: National Science Foundation Graduate Research Fellowship Program

Effects of straw removal rates on sugarcane yields, soil carbon stock and its quality in south-central Brazil

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Understanding the impacts of straw removal and regional issues on changes in quantity and quality of soil organic carbon (SOC) is crucial for improving soil functions while sustaining high crop yield in intensive sugarcane (*Saccharum officinarum* L.) cropping systems. Field experiments were set up for scientific purposes within commercial farms to assess the effects of straw removal rates on sugarcane yields, SOC stocks and humification levels of soil organic matter (SOM) under diverse edaphoclimatic conditions (Quirinópolis-GO, Chapadão do Céu-GO and Quatá-SP) in the most dense cultivated sugarcane region in Brazil. The complimentary goal was to assess the effects of crop rotation on sugarcane yields during two harvest seasons. The treatments with three straw removal rates (0, 50, and 100%) were established in a randomized block design with four replications within two paired areas, one sowed with *Crotalaria spectabilis* (crop rotation; CR) and the other kept under bare fallow (BF) during the sugarcane-replanting period. Sugarcane yields were accounted through an instrumented truck equipped with a loading cell, and soil samples were collected to a 40-cm depth after a 2-year period of the trials establishment, with SOC stock being computed for the equivalent layers of 0-10 and 0-40 cm. The short-term effects of straw removal showed a clear trend of the adverse impacts on SOC storage for all assessed locations. In fine-textured soils (Quirinópolis-GO), complete straw removal significantly depleted SOC stock at a rate of 1.45 and 2.8 Mg ha $^{-1}$ yr $^{-1}$ in the 0-40 layer within the areas under CR and BF, respectively. Similar responses to straw removal were observed in sandy soils (Quatá-SP), in which SOC decreased at a rate of 0.25 Mg ha $^{-1}$ yr $^{-1}$ in the 0-10 cm when 100% straw was removed from soils in the CR area. Laser-Induced Fluorescence Spectroscopy showed high stages of SOM humification with increase in the magnitude of depletion of soil C stock, which suggests that

the straw removal is degrading soil quality with a less labile C present in the SOM. Over two years, avoiding straw removal led to cumulative yield gains of up to 28 Mg ha $^{-1}$ in Chapadão do Céu-GO, and from 34 to 61 Mg ha $^{-1}$ in Quirinópolis-GO. Similarly, the inclusion of crop rotation within sugarcane cropping cycle increased sugarcane yields by up to 8.8% (25 Mg ha $^{-1}$) in Chapadão do Céu-GO, and by 9.2% (27 Mg ha $^{-1}$) in Quirinópolis-GO. These results suggest that sugarcane-legume rotation cropping systems could be agronomically advantageous and provide additional revenue for sugarcane industry. Our findings also support the conclusion that the straw removal for other purposes, even in a short-term assessment, already begun to modify the quantity and quality of soil carbon as well as adversely impact sugarcane yields.

Keywords: Straw management, Soil organic matter, Ethanol production

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Development pathways for biofuels initiatives: lessons from developing countries

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Since the first climate change report, in 1995, global interest in bioenergy has been remarkably high. Liquid biofuels gained attention due to its acknowledged ability to reduce GHG emissions, increase energy security and catalyse foreign investment, which is essential to foster novel value chains and spur agriculture-related jobs and income. Many biofuel initiatives converged to regions such as Sub-Saharan Africa and South America where land is available and climate suitable for biomass production. Over the years, scientist and policy makers realised that making biofuels work in poor and developing regions was far from trivial, where success experiences contrasts with a mounting number of failed initiatives. Our objective, therefore, is to underline key aspects of decision making in existing sugarcane projects, which may lead to success or failure of biofuel initiatives. In august 2015, we visited two remarkably different sugarcane projects, in Coruripe (Brazil) and in Xinavane (Mozambique). In both regions, we interviewed farmers, local experts and technicians guided by a semi-structured questionnaire. Our approach focused on the relationship between the sugarcane industry and local communities, as well as the policy environment for biofuel production. Coruripe is a family farm cooperative formed by farmers with 20-30 ha of land. Supported by land reform and ethanol policies (e.g. Proalcool), it developed as an agroindustrial cooperative of sugar, ethanol and a number of different products, mainly fruit-based (e.g. candies and juice) and dairy. Through value added strategies and diversification, farmers in Coruripe can maximize economic gains and reduce economic risks. The cooperative also maintains a number of education and capacity building programs for farmers and their families. Xinavane, on the other hand, is one of the largest and most updated sugarcane mills in Mozambique, responding for nearly 50% of the national sugar output in 2013. With colonial roots, Xinavane is a known sugarcane estate that is also conspicuous for its leading role in the engagement of smallholder farmers' associations (1-2 ha of land per farmer) for sugarcane production under outgrower schemes. Despite technologically advanced, ethanol production did not take-off in Xinavane due to lack of proper policies for biofuels and economic competitiveness. Moreover, there is debate over smallholders' contracts, with concerns related to information asymmetry,

insufficient revenues and food insecurity risk. The relative success of Pindorama Cooperative (Coruripe) in promoting ethanol and rural development contrasts with the decision making environment in Xinavane, where progress on both biofuels and rural development has being rather sluggish. Long way to development vs. Short way to development). Local consultation and participation, value added products (diversification) associated with food security strategies and the right combination of policies seems to be key to avoid pitfalls and reap the opportunities associated with biofuel initiatives in the developing regions.

Keywords: Biofuels, Cooperatives, Developing countries

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Co-processing of pyrolysis oil in conventional refinery operations to produce biomass-derived gasoline and diesel fuel blendstocks

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The fast pyrolysis of lignocellulosic biomass has been gaining relevance along last years due to its ability to convert raw lignocellulosic biomass into a liquid product, frequently referred as bio-oil. The use of lignocellulosic wastes, such as wood chips and corn cobs, brings the advantage of not impairing food security, since they are not used for human consumption. Additionally, agriculture economics may even increase by using these agriculture residues. Fluid Catalytic Cracking (FCC) is one of the main processes in a petroleum refinery and used worldwide to converting heavy petroleum molecules into valuable products such as gasoline, LPG and LCO (diesel range). The cracker naphtha produced from the FCC is usually the predominant stream in the gasoline pool in most of the refineries in Brazil and USA. Nevertheless, literature has frequently mentioned limits for a direct introduction of raw bio-oils, the product of the fast pyrolysis of biomass, in a commercial FCC unit. Bio-oils characteristics, such as low miscibility with hydrocarbons, high acidity, high tendency to form coke and poor chemical stability are commonly cited as limitations or even as impediments for its direct use. On the other hand, many tests carried out at FCC lab scales units with heavy feeds showed that their results do not necessarily reflect bio-oil behavior obtained at larger scales, especially in respect to coke formation tendency and results obtained in a circulating FCC pilot riser are frequently superior to those observed at lab scales. The use of larger scales offers advantages to a better understanding of bio-oil co-processing. Petrobras demonstration unit in São Mateus do Sul (State of Paraná, Brazil) has been used intensively for many years to develop its FCC technology. Among its features is the possibility of using one or more of multiple feed nozzle injection points positioned along the riser reactor. Therefore, bio-oil and the regular FCC streams are segregated, dispensing the use of any dispersant agent, and can be introduced separately into the riser reactor. In the present work, raw bio-oils from pine woodchips were co-processed with a standard Brazilian gasoil and tested in a 200 $kg.h^{-1}$ FCC demonstration-scale unit using a commercial FCC equilibrium catalyst. Two different bio-oil/VGO weight ratios were used: 5/95 and 10/90, running at a conventional reaction temperature and other operating FCC conditions. The influences on conversion, product yields and gasoline were investigated. A longer test run was also carried out at 5 % bio-oil (uninterrupted for 70 hours). The liquid effluent was then distilled to produce 400 gallons of gasoline and 400 gallons of diesel materials for additional studies of the quality of

the biofuels produced using this route. The co-processing of raw pine bio-oil in the FCC unit operating at 200 $kg.h^{-1}$ flow rate with vacuum gas oil and 5 or 10 wt. % bio-oil reached a cumulative time of 400 hours.

Keywords: biofuels, pyrolysis, gasoline, diesel
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A complex region of the sugarcane genome syntenic to a soghum QTL linked to sugar accumulation

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The *Saccharum* hybrid is derived from hybridization between S. *officinarum* (2n = 80) and S. *spontaneum* (2n = 40 – 128). This unusual condition results in a genome highly polyploidy and aneuploidy, high genome size (around 10Gb) and high content of repetitive regions. Was used the synteny between sugarcane and sorghum with the aim to transfer a QTL identified in *Sorghum bicolor* to a *Saccharum hybrid* and understand the genome structure and the complex architecture of a genomic region related to sugar accumulation. The QTL for Brix was identified in a specific position on sorghum genome chromosome 3 (SB-03). The sorghum genes from QTL region were used as a reference to determine homologous genes in sugarcane transcriptome. A total of 61 genes shared between sorghum and sugarcane were used to screen an SP80-3280 BAC library. Only BAC clones that were positive for at least two genes were selected. With this strategy, sixty-eight individual BAC clones were selected, and it was believed that many duplicated genes, pseudogenes and repeats regions containing partial genes were avoided. These BACs were pooled, sequenced using PacBio producing a deep sequencing, individually assembled and its genes and repetitive elements were annotated. A total of 51% of the BACs sequences was identified as repetitive elements, and it can reinforce the reason for sugarcane genome expansion. To cover almost totally the QTL region was necessary at least 1,25 Mb represented by nine BAC sequences. Some BAC sequences showed overlapping possibility and the formation of four syntenic blocks. The selection BAC approach was very successful and based on synteny between sorghum and sugarcane almost all genes were recuperated in sugarcane.

Keywords: *Sorghum bicolor* (L.) Moench, *Saccharum officinarum*, BAC library, polyploid, PacBio sequencing
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The Centre for Solar Biotechnology: Developing next-generation solar powered microalgae systems for the production of high value products, foods and renewable solar fuels

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The global economy is valued at ~$119 Tn and is expected to grow significantly as our population rises from 7.5 billion towards 9.6 billion people by 2050. This increase in population is forecast to require approximately 70% more food (UN), 50% more water (OECD), 50% more fuel (International Energy Agency) and 80-100% reductions in CO_2 emissions (UN Paris Climate Change agreement) to maintain political, social, fuel, water, food and climate security. While, these challenges highlight important growth opportunities, they must be achieved sustainably for a secure future. Microalgae are positioned at the nexus of these challenge as they tap into the huge energy resource of the sun (~2300x global energy demand), capture CO_2 and can expand photosynthetic capacity into the oceans or onto non-arable land using

saline water. The captured solar energy and CO_2 are used to produce a broad range of biomolecules which collectively form biomass. Through bio-refinery processes these natural or engineered biomolecules can be separated into high value products (e.g. recombinant proteins >$1000 kg^{-1}), nutraceuticals (e.g. anti-oxidants, unsaturated fatty acids), foods/feeds and CO_2 neutral renewable fuel (e.g. crude oil, biodiesel, methane, ethanol and hydrogen, <$300 Ton^{-1}). From a climate change perspective, CO_2 neutral solar fuels are critically important as 80% of global energy demand is provided as fuels and only 20% as electricity. Despite the fact that we must achieve CO_2 emissions reduction of ~50% by 2030 to stay within global warming 'safe zone', to date there are only a few demonstrated large scale renewable fuel solutions (e.g. Brazilian sugarcane). In 2017, we launched the Centre for Solar Biotechnology (https://imb.uq.edu.au/solar) which has now grown to include ~ 30 international teams focused on the development of advanced microalgae/cyanobacterial systems that can use fresh, saline or wastewater and support the development of future bioinspired solar technologies. The talk will briefly summarize the centres work on the development of a range of solar powered industries including the development of *Protein Therapeutics*, *Functional Foods*, *Aquaculture and Livestock feeds*, *Clean Water*, *Solar Fuels*, the *Integrated Bioeconomy Project*, *GreenSmart Cities* and *Growing Roads* and how this work intersects and helps to advance the bioenergy sector. Our process pipeline for the development of next generation microalgae systems will also be presented. In broad terms this pipeline includes molecular, structural and cell biology and process engineering. Specifically, it incorporates high-throughput processes for the purification of native microalgae strains, efficient cryo-preservation protocols, robotic nutrient and light optimization screens, nuclear and chloroplast engineering strategies, recombinant fusion protein production, pilot scale systems design as well as techno-economic and life-cycle analyses to guide next-generation system development and scale up.

Development of renewable ionic liquids for biomass conversion

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Ionic liquids (ILs), solvents composed entirely of paired ions, have been used in a wide variety of process chemistry and renewable energy applications. Imidazolium-based ILs show remarkable abilities to dissolve biomass, and are thus an ideal media for biomass pretreatment and depolymerization. Although very efficient, imidazolium cations are currently expensive and therefore their large scale use and industrial deployment, e.g. in biorefineries, is limited. In an attempt to replace imidazolium-based ILs with ILs derived from renewable sources that retain their efficiency for biomass pretreatment, we synthesized a series of tertiary amine based ILs from aromatic aldehydes derived from lignin and hemicellulose, the major byproducts of lignocellulosic biofuel production. A comprehensive analysis of extractable cell wall carbohydrates and sugar yields from switchgrass and switchgrass pretreated with tertiary amine based ILs derived from vanillin ($[Van][H_2PO_4]$), *p*-anisaldehyde ($[p\text{-}AnisEt_2NH][H_2PO_4]$) and furfural ($[FurEt_2NH][H_2PO_4]$) confirmed their effectiveness for biomass pretreatment. The amounts of sugar released by enzymatic hydrolysis of the cellulose present in switchgrass was comparable to that obtained after pretreatment with 1-ethyl-3-methylimidazolium acetate ($[C_2mim][OAc]$). Enzymatic saccharification with $[FurEt_2NH][H_2PO_4]$ and $[p\text{-}AnisEt_2NH][H_2PO_4]$

provided 90% and 96% of total possible glucose and 70% and 76% of total possible xylose, respectively, after biomass pretreatment. Computationally, [Van][H_2PO_4] showed the lowest net basicity, and poor lignin removal efficiency and low sugar yields were observed experimentally. We found that [$FurEt_2NH$][H_2PO_4] and [*p*-$AnisEt_2NH$][H_2PO_4] had higher □ values and higher net basicity than [C_2mim][OAc]. Though [$FurEt_2NH$][H_2PO_4] and [*p*-$AnisEt_2NH$][H_2PO_4] were slightly less effective towards lignin removal, sugar yields from SG pretreated with these compounds were nearly equivalent to yields from SG pretreated with [C_2mim][OAc]. Glycome profiling experiments suggest that the biomass derived ILs [$FurEt_2NH$][H_2PO_4] and [*p*-$AnisEt_2NH$][H_2PO_4] act on plant cell walls in a mechanism distinct from [C_2mim][OAc], and studies are underway to understand these process implications in terms of lignin and hemicellulose depolymerization and IL recycling. These results indicate that biomass derived renewable ILs are very effective in pretreating biomass, and establish an important foundation for the further study of these unique compounds in other industrial applications. Our concept of deriving ILs from lignocellulosic biomass and/or other renewable sources shows significant potential for the realization of a "closed-loop" process for future lignocellulosic biorefineries, and has far-reaching economic impacts for other IL based process technology currently using ILs synthesized from non-renewable sources.

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A new model for sustainable expansion of biofuel production in Brazil: sugarcane biomass to biofuels and animal feed

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Bioenergy production, particularly liquid biofuels, has been promoted as a means to enhance energy security and reduce climate change impacts. However, the expansion of biofuels production based on food crops and its impact on land use have given rise to the so-called "food vs. bioenergy" debate. Livestock production, which includes grazing land and cropland dedicated to animal feed production, is by far the largest anthropic use of land resources worldwide, accounting for approximately 80% of all human use of land. Brazilian livestock production has low average productivity because of extensive management, low inputs and adoption of technology. In this context, integration of cellulosic biofuels and livestock production can improve land use in Brazil, since more intensive systems can maintain, or even increase, food production while reducing land use. As a result, it is possible to expand cropland for biofuels production without displacing food crops or livestock production, which might otherwise take place in forested or other environmentally-sensitive areas. This presentation focuses on optimizing the integrated system of biofuel production and animal feed production, aiming at the sustainable production of both food and bioenergy and considering the synergies between the production chains of sugarcane (considering both first and second generation processes), livestock (cattle) and other crops (e.g. corn, energy cane and soybean). Biomass pretreatment processes such as Ammonia Fiber Expansion (AFEX) and steam explosion (StEx) can generate enhanced cattle feeds as well as improved feedstocks for cellulosic ethanol production. These two pretreatment processes are compared using the Virtual Sugarcane Biorefinery (VSB) – a computer framework that

simulates the entire production chain and assesses the sustainability impacts of different biorefinery alternatives/routes. Representative computational models for each system, based on experimental and literature data, and for sustainability assessment methodologies are being developed and integrated for optimization of economic, environmental and social impacts of sugarcane-livestock-bioenergy production.

Keywords: Cellulosic biomass, biofuels, animal feeds, Virtual Sugarcane Biorefinery, AFEX, steam explosion, sustainability

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The challenge of not exceeding the 1.5 C guardrail and the role of bioenergy in Brazil

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The historic Paris Agreement set an upper limit of global GHG emissions to avoid exceeding limits of global warming considered to be dangerous to human wellbeing and to life on the Planet. The upper limit was set at 2 C of warming, keeping in mind that the global mean temperature at the surface has risen by about 1 C since the Industrial Revolution. It also set a more challenging limit of 1.5 C warming, which would cause considerable less global and regional impacts. Reaching this more stringent limit imposes a cap on emissions, that is, virtually decarbonizing the global economy by mid-century and removing CO2 from the atmosphere in the second part of the century and beyond, aiming at stabilizing CO2 concentrations at about 350 ppm, which is consistent with a long term warming of less than 1.5 C, in addition to reducing substantially the emissions of other key GHG gases. It has been suggested a role for bioenergy coupled to carbon capture and storage, the so-called BECCS as one possibility for removing up to 15 GtonCO2/year from the atmosphere towards the end of the century. Additionally, restauration of ecosystems and a sustainable agriculture would represent substantial carbon sinks in the future. Brazil has a role to play in leading the bioenergy-based mitigation efforts. Productivity of biomass production has to increase substantially if this drive to use bioenergy is not to cause further deforestation and land use change. The ambitious Brazilian NDCs for 2030 timeframe will be an adequate platform to develop a sustainable bioeconomy which supports the ambitious goals of the Paris Accord, while at the same time gives rise to in-country technological innovations and new bioindustries.

Keywords: climate change mitigation; bioenergy; Brazilian NDCs; Paris Accord

Progress in understanding smut disease affecting sugarcane

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Among the relevant issues impacting sugarcane agricultural practices are fungal diseases that constitute a worldwide threat to the cultivation. Smut is one of these diseases maintained in crop fields since its identification and that re-emerges in the international scenario mostly due to climate changes and modern green harvesting practices. *Sporisorium scitamineum* is the the causal agent of sugarcane smut. The disease is characterized by the development of a whip-like structure from the shoot apical meristem (SAM) of sugarcane. Infected SAM produces the whip instead of leaves, flowers, or floral organs. The most effective way to control smut is the selection of resistant varieties. However, the pathogen displays genomic variability and, thereby, potential to adapt to a changing environment and to

overcome resistance. Hence, a deep understanding of the pathogen and of the sugarcane biology is essential. Smut has been the focus of our studies aiming at decipher the molecular cross-talking between host and pathogen. For the past few years we have been studying the molecular mechanisms related to the interaction between sugarcane and *Sporisorium scitamineum* using global approaches (functional genomics, transcriptomics, proteomics and metabolomics) to delineate a comprehensive overview of this very particular pathosystem (Taniguti ei al., 2015; Schaker et al., 2016; 2017). We uncovered some of the molecular mechanisms behind smut resistance (Peters et al., 2016) as well as related to symptoms development in susceptible genotypes (Peters, et al., 2016; Schaker et al., 2016; 2017). We revisited issues such as those of the infection and tissue colonization (Carvalho et al., 2016), whip morpho-anatomical structure (Marques et al, 2017) and pathogen genetic variability among South American isolates (Benevenuto et al., 2016). We also compared genomes of nine species of smut fungi isolated from eight crop and non-crop hosts: maize, barley, sugarcane, wheat, oats, *Zizania latifolia* (Manchurian wild rice), *Echinocloa colona* (a wild grass), and *Persicaria* sp. (a wild dicot plant) (Benevenuto et al., submitted) to identify species-specific genes potentially related to host specificity. An integrative view of the obtained data produced hypotheses and candidate genes to be further investigated. So far we know that sugarcane undergoes an extensive transcriptional change very early after inoculation. This early phase of infection in more resistant genotypes is characterized by a strong oxidative burst and changes related to the antioxidant systems to delay or impair fungal proliferation. ROS probably signs to increase chitinases, glucanases, thaumatin activities, and other antifungal molecules, tissue lignification and hormonal changes via MAPK signaling. Whilst, for whip development in susceptible genotypes changes occur in carbon partitioning, tissue lignification via PTAL overexpression, breakdown of starch and hormonal changes, in which auxin is the most relevant. We also proposed that whip development and flowering probably share some of the molecular mechanisms involving the MADS-box family of transcriptional factors. Our focus now is to use the hypothesis raised in functional experiments to uncover useful candidates for disease management and potentially to breeding programs.

Keywords: *Sporisorium scitamineum*, metabolomics
Supported by: FAPESP, CNPq, CAPES

Development of techniques to study the phenomenological separation process and pneumatic transport of multiparticulate solids for the improvement of bioenergy production

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The phenomenology of the pneumatic interaction between particulate mixtures is still little known, despite the great potential for practical applications in the industrial and agricultural sectors. In this line, the objective of this study is to conduct an extensive study of the phenomena involved in the aerodynamic transport of multicomponent particulate aiming at developing a high-performance low-consumption pneumatic separator for agricultural products. In particular the effects of particulate composition in saltation phenomena and aerodynamic entrainment are investigated. To this end, a horizontal pipe transport is under construction where various particulate compositions can be subjected to different velocities of the carrier phase. Image processing techniques were developed allowing determination of particulate velocity, concentration, velocity profile and flow regime in order to characterize the multiphase flow pattern. The results serve as input data to the

development of an empirical model to predict, among other parameters, the specific energy consumption (kJ/kg/m) in function of the separation efficiency.

Keywords: multiphase flow transport, flow regime, bioenergy, sugarcane
Supported by: FAPESP

Fibria initiatives towards biobased materials production

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Planted forests are part of the solution to challenges associated with climate change and contribute to a low-carbon economy. It is part of Fibria strategy to develop products and services with high added value that go beyond the concept of commodity and replace non-renewables derivatives such fossil fuels and chemicals as a source of raw material. Through the Rapid Themal Pyrolysys (RTP) process, in which biomass is subjected to a thermochemical treatment, bio-oil or Renewable Fuel Oil (RFO) is obtained which can replace fossil fuels in generation of energy or be co-refined with oil. Bio-oil can also be upgraded using different catalysts and ex-situ hydrogenation creating a high quality liquid fuel. Besides that, bio-oil fractionation can generate an array of biochemicals to be used immediately in industrial processes. By means of a partnership with Ensyn Corporation (Canada), Fibria will build an operational unit in Brazil for the development and marketing of bio-oil. Another initiative in Fibria biorefinery platform is lignin production. Lignin is a complex natural polymer that occurs in plants and made up of three primary phenylpropane units (syringyl, guaiacyl and p-hydroxyphenyl) of varying amounts and inter-unit linkages Lignin is derived when pulping black liquor (mill side-stream) is precipitated and neutralized by acid washing. In the pulp mill, lignin can be used as biofuel in the lime kiln for replacement of fossil fuels. Lignin can also be used as a green alternative to many petroleum-derived substances, resins, rubber additives, thermoplastic blends, binders, precursor to carbon fiber and pharmaceuticals. It is also known to be the only renewable source for industrial aromatics production. In 2015, Fibria acquired the Canadian company Lignol, today called Fibria Innovations, expanding its patent bank of lignin-related processes and products. Recently Fibria demonstrated in pilot scale that lignin can replace some of the active principles of fossil origin currently used in wood panels. Next step includes the construction of a production unit in Aracruz, Brazil, for the commercial development of lignin applications, with environmental, cost, and performance benefits.

Keywords: Biorefinery, Lignin, Bio-oil, Biomaterials, Pulp Mill

Developing markets for biofuels and bioproducts: challenges and opportunities for the EU industry

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The Advanced Biofuels sector is very quickly evolving towards industrialisation and commercialisation thanks to massive public and private funding of research and technology development. Both thermochemical and biochemical routes are generating new opportunities, and becomes more and more integrated, in many cases. However, the large scale development of these sustainable solutions for decarbonising transports depends on the policy in place, which varies in different world regions. A wide debate is being held in the EU about the European Commission proposal for a new Directive, named REDII, covering also the biofuel sector. This stimulated the EU industry to discuss and elaborate their

priorities and needs, and to bring this to the attention of the policy makers. In this framework, the Alternative and Renewable Fuel Forum (ART Fuel Forum, AFF) brings together many of the major EU Industrial players on Advanced Biofuels and Renewable Transport Fuels into a single room, which aims at discussing and elaborating analysis, and providing recommendations on low carbon transport fuels policy and makets from the view of the participating industries. ART Fuel, established on the initiative of the European Commission DG Energy through a dedicated tender, is coordinated by Exergia (Greece) and RE-CORD (Italy), and covers all transport biofuels sectors, with a special focus on Aviation, Maritime and Heavy Duty. ART Fuel has recently developed a set of key messages, that represents the main priorities and needs as regards policy development. The messages outline what is needed, according to the industries' opinion, to develop markets for Advanced Renewable transport fuels.

Keywords: Advanced Biofuels, European Directives
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Miscanthus genetic resources for improving biomass yield and their potential to also improve sugarcane

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Miscanthus is a close relative of sugarcane that is naturally distributed throughout East Asia and Oceania from ~50 °N in eastern Russia to ~20 °S in New Caledonia. To date, biomass production of *Miscanthus* in North America and Europe has focussed primarily on a single sterile triploid clone of *M.* ×*giganteus* that was derived from a natural cross in Japan between a tetraploid *M. sacchariflorus* and a diploid *M. sinensis*; this clone was imported to Denmark in the 1930s. To guide efforts at breeding *Miscanthus* for higher yield, we have conducted studies of *M. sinensis* and *M. sacchariflorus* population structure based on region-wide sampling and thousands of RAD-Seq SNPs. We subsequently phenotyped large germplasm panels of >600 individuals from each species at multiple field trial locations in Asia, North America and Europe, and conducted genome-wide association studies. Within *M. sinensis* we identified seven geographically distinct diploid genetic groups. For *M. sacchariflorus*, we found three tetraploid and three diploid genetic groups; moreover one of the tetraploid groups was derived from a polyploidization event that was distinct from the others, which represents an important new opportunity for developing improved triploid *M.* ×*giganteus*. Individuals and genetic groups with high yields over many locations or at specific locations were identified. Exceptionally high dry-biomass yields, similar to maximum yields reported for sugarcane, were observed for some tropical and subtropical accessions of *M. sinensis* grown at our trial in Zhuji, China (~30 °N). At northern trial sites, diploid interspecific hybrids between *M. sacchariflorus* and *M. sinensis* typically outyielded *M. sinensis* accesssions, indicating that the interspecific combination is advantageous accross a range of ploidies. New natural triploid *M.* ×*giganteus* genotypes were found in Japan and Korea. Additionally, we have obtained intergeneric hybrids between sugarcane and *Miscanthus*, which will facilitate

introgression of genes for resistance to diseases of sugarcane and for tolerance to low temperture.

Keywords: *Miscanthus sinensis*, *Miscanthus sacchariflorus*, population structure, miscane

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Microalgae biomass accumulation and lipid production under the lens of omics approaches

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The socio-economic and environmental problems associated with the use of fossil fuels such as increased air and water pollutants and the concomitant increase on global energy demand imposes a big challenge to be solved by our current society. These challenges can be faced through the development of sustainable production of bioenergy and chemicals. The integration of microalgae-based bioremediation with the production of renewable microalgae biomass and high-value compounds appears as a promising sustainable path for overcoming these challenges. Despite the advances in microalgae cultivation, the generation of biomass for massive production of fuels and chemicals is still limiting. Therefore, a better understanding on how changes on carbon concentration, light and nutrients availability affect microalgae biomass production and composition and how we could modify such phenotypes is urgently needed. Therefore, we analyzed the alterations of the biomass accumulation and composition of cells under photoautotrophic conditions in response to changes on carbon dioxide and nitrogen concentration using omics approaches including proteomics, metabolomics, transcriptomics and genomics approaches. Omics data was applied for modeling biological networks and contributed to the identification and selection of candidate genes and networks for future biotechnological applications such as transcriptional engineering of microalgae strains.

Keywords: Systems biology, networks, lipids, metabolism

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A systems biology approach to study lignification in C4 grasses

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Secondary cell walls account for the majority of total plant biomass and, as mostly composed of polysaccharides, constitute a promising source of fermentable sugars for the production of biofuels and biomaterials. However, the presence of the aromatic polymer lignin largely precludes the release of fermentable sugars during enzymatic hydrolysis of cell wall polysaccharides in the biorefinery. Therefore, it is essential to unraveling the molecular mechanisms underlying lignin metabolism in order to better exploit the potential of lignocellulosic biomass. In the context of the bioeconomy, grasses emerge as a prominent lignocellulosic feedstock due to their high yield potential for biomass production. Still, many aspects of lignin metabolism in grasses, including transcriptional regulation, biosynthesis and polymerization, remain poorly understood, in contrast to eudicots species. Moreover, grasses differ considerably from eudicots in vascular patterning and cell wall composition, suggesting the presence of many grass-specific molecular and biochemical mechanisms that are not found in eudicots and

whose knowledge cannot be extrapolated from data obtained with eudicot model plants. Our lab is applying a systems biology approach based on transcriptomics and metabolomics to correlate changes in cell wall composition with changes in gene expression in *Sorghum bicolor*, with a major focus on lignin and phenolic metabolism. Accordingly, cell wall analyses, phenolic profiling and RNAseq were performed along an elongating sorghum internode, which represents a powerful system to study cell wall deposition because it is formed by different developmental zones with increasing amounts of secondary cell walls. Because pathway perturbations can affect the expression of several other genes of the same biosynthetic pathway, allowing the identification of novel genes with hitherto unknown functions, a lignin mutant was included in our analyses: the *bmr6* mutant, with a nonsense mutation in a *CINNAMYL ALCOHOL DEHYDROGENASE* gene. Along the elongating internode, lignin and cellulose amounts increased from the bottom, which includes the basal intercalary meristem zone and the elongation zone, to the top, which comprehends the maturation zone, depicting a gradient of secondary cell wall deposition in both genotypes. Ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC-MS)-based phenolic profiling revealed the major metabolic shifts in the phenolic metabolism throughout the development (along the elongating internode) and between the genotypes. In addition, RNAseq revealed clusters of genes with similar expression patterns along the developmental gradient and in response to *CAD* loss-of-function. Currently, the transcriptomic data is being analyzed to generate a co-expression network and a database of co-expressed genes to allow the selection of candidate genes that potentially play a role in secondary cell wall formation and lignin biosynthesis in C4 grasses. The obtained knowledge might be very helpful for future biotechnological strategies to reduce biomass recalcitrance in bioenergy crops, such as sugarcane.

Keywords: lignin, transcriptomics, phenolic profiling, C4 grasses, sorghum
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Advances in mechanical preprocessing of high moisture biomass: Impact on quality, cost, and performance

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More than 50% of the herbaceous biomass and most woody biomass available in the U.S. has moisture contents >30 % (w.b.). High moisture content in the biomass creates challenges in storage (resulting in dry matter loss), transportation (limiting payloads), preprocessing (increasing energy requirements for size reduction and drying), and handling and feeding (plugging in hoppers and feeders). Currently, biorefineries are not ready to use high moisture biomass mainly due to high preprocessing cost. Conventional methods require about $62/dry ton to preprocess herbaceous biomass at 30% (w.b.) moisture content to densify into pellets. Developing cost-effective preprocessing solutions is critical to utilize this high moisture biomass for biofuel production. Idaho National Laboratory (INL) is working on developing new technologies that can reduce pelleting costs by 50% compared to conventional pelleting process. Techno-economic analysis of the conventional pelleting process indicated that efficient moisture management is critical for reducing the cost. In this study, fractional muilling, high mositure pelleting, and low temeprature drying were tested to reduce pellet production costs. In fractional milling, larger screen openings are used in the stage-1 grinder, while a separator is inserted between stage-1 and stage-2 grinders to bypass the fraction that has met the specification. The major advantages of using a fracional milling include a) avoiding redundant

preprocessing and thereby saving energy; and b) tighter particle size distribution with reduced fines. In high moisture pelleting, biomass is pelleted at moistures >20%. The major advantage of this process is biomass loses some moisture due to frictional heat generated in the pellet die due to compression and extrusion. Studies have indicated that there is about 5-10% (w.b.) moisture loss during high moisture pelleting. For example, when biomass is pelleted at 30% (w.b.) moisture content, there is about 10% mositure loss when it is conveted to pellets. The final pellets have moisture content of about 20% (w.b.). The high mositure pelleting process makes drying of pellets optional; for example, pellets can be dried only when highly durable and aerobically stable pellets are needed. Further, the high moisture pellets produced can be dried using a grain dryer, which is less capital and energy intensive compared to rotary dryer which is typically used in conventional method. At the INL, studies on high moisture pelleting were conducted on lab and pilot scale pellet mills resulting in pellets with a bulk density of about 608 kg/m^3 and 98% durability. Techno-economic analysis of the advanced preprocessing system developed at INL indicated that pelleting costs are reduced to about $29/dry ton as compared to $62/dry ton—more than a 50% reduction. In terms of sugar yields, the pellets have performed better than milled biomass.

Keywords: biomass, mechncial preprocessing, size reduction, drying, densification, quality, techno-economic analysis

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Biomass preprocessing to produce conversion ready feedstocks

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Currently biorefineries are not able to operate at the desired capacity due to biomass variability and specification limitations. Raw biomass resources do not meet conversion specifications due to the diversity, variability, and uncertainty associated with large-scale biomass resources. As such, there is a need to fully recognize the magnitude of biomass variability and uncertainty, as well as the cost of failing to design feedstock supply systems that can mitigate biomass variability and uncertainty. A paradigm shift is needed, from biorefinery designs using raw biomass feedstocks to advanced feedstock supply systems that deliver conversion-ready feedstocks. The conventional feedstock supply systems, reduces the operating costs but fail to meet the physical properties and chemical composition desirable by the biorefineries. Direct coupling of conventional feedstock supply system with sophisticated biorefineries are limiting the biorefineries to operate at the desired capacity. Research has indicated that most of the biorefineries are currently operating at less than 50% of the desired capacity. Engineering the feedstock to meet the physical properties and chemical composition will help the biomass to meet the specifications desirable for conversion. There are various mechanical chemical and thermal preprocessing methods which can help to improve the biomass physical and chemical properties and make it a conversion ready feedstock. A conversion-ready feedstock is an industrial-scale feedstock resource for which the chemical and physical properties of that resource are within the engineered performance parameters of biorefinery handling and conversion systems; in other words, all preprocessing, blending, sorting, leaching, drying or anything else necessary to make a feedstock ready for conversion/utilization is done prior to that feedstock being delivered to the biorefinery conversion systems. Integrating active biomass preprocessing controls (e.g., drying, sorting, sizing, fractionating, leaching, densifying, etc.) can mitigate off-spec performance deviations of large-scale biomass resources in directly coupled downstream conversion systems. In this presentation we will discuss the chemical,

mechanical and thermal preprocessing methods and further instrumentation of the preprocessing systems to improve the process efficiency and meet the desired biomass specification for conversion.

Keywords: Biomass, Physical Properites and Chemical Composition, Mechanical Chemical and Thermal Preprocessing

Bioenergy and bioeconomy - carbon value

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In their recent report to the G20, IRENA and IEA have shown that bioenergy supply should expand to constitute about three-eighths of all renewable energy produced in the year 2050. But investment in bioenergy, particularly in plants to demonstrate the production of liquid biofuels from wood and grasses at scale, has been lagging behind what is needed. This is largely due to low oil prices and low carbon values in the market place, which make it difficult for liquid biofuels to compete with petroleum-based diesel and gasoline in the transport sector, even though such biofuels are is likely to remain the only practical alternative for renewable supply of aviation, marine shipping, and heavy freight transport. However, there are several technologies for advanced liquid biofuels which offer real potential to compete within the next two decades, assuming a substantial carbon value is put in place to meet the goals of Paris to keep global warming well below two degrees Celsius. IRENA's presentation will show how the prospects for these advanced technologies, which make possible the use of a wide range of lignocellulosic resources – including rapidly growing grasses like energy cane and short rotation coppice wood from agroforestry – can be firmly supported by a realistic value of carbon in the marketplace.

Keywords: carbon, transport, lignocellulosic, biofuel

Beginning to 'design' biomass lignins for the biorefinery

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Lignin remains one of the most significant barriers to the efficient utilization of lignocellulosic substrates in the current focus on biofuels production. Inspired largely by the recalcitrance of lignin to biomass processing, plant engineering has routinely sought to alter lignin quantity, composition, and structure by exploiting the inherent plasticity of lignin biosynthesis. More recently, researchers are attempting to strategically *design* lignins for increased degradability. One such method, via the so-called 'zip-lignin' approach, is showing particular promise. Poplar has been engineered to incorporate monolignol ferulate conjugates into the lignification process, by using an exotic transferase gene, *FMT*, and a xylem-specific promoter. This results in the introduction of readily cleavable ester linkages into the backbone of the polymer, and delivers significantly improved processing. Various applications for which these altered trees appear superior are emerging. In attempting to modify monocot lignins, another transferase appears to be useful for improving cell wall digestibility. This involves *p*-coumaroylation that occurs naturally in monocots, but is not evident in dicots – providing dicots with that pathway has interesting implications. Nature continues to inspire us with new avenues toward lignin modifications that provide value-added coproduct potential. For example, the ramifications of finding that grasses are using tricin, a flavonoid from beyond the monolignol

biosynthetic pathway, to start lignin chains are intriguing; tricin itself is valuable, and plants with tricin knocked out have higher lignin and lower CW digestibility. New classes of authentic lignin monomers continue to be discovered, portending even more wide-ranging approaches to lignin valorization.

Keywords: Poplar, lignin, transgenics, 'clip-offs', pretreatment, digestibility, valorization
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Using genomic sequencing to understand the sugarcane genome structure

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Sugarcane has a complex polyploid hybrid genome of 10 Gbp which makes sequencing and assembling the genome a major challenge. We present here the Australian contribution to the R570 sugarcane genome sequence. We present data on sequence generated from over 1000 R570 BAC clones, these were selected using a number of approaches and were in part targeted to important QTL identified in Australian germplasm. In a parallel approach, we have also generated large amounts of whole genome shotgun (WGS) sequence from variety R570 and assembled this sequence into 3670594 scaffolds. This WGS data was generated from a range of DNA fragment sizes between 180 bp and 32,000 bp, which should enable even complex regions of the genome to be ordered. In addition to this PacBio long read technology has been used to generate 31.7 Gbp of data with an average read length of 7282 bp. This has been used to help resolve repeats and increase scaffold lengths. In total the data covers the complete genome sequence to a level of 73-fold, representing roughly 1000-fold monoploid genome coverage, which highlights sequence variation at each locus. We present here the analysis of the multiple alleles of genes that are present in the polyploid genome of sugarcane. Analysis of this data has identified large numbers of single nucleotide polymorphisms (SNPs), which are currently being tested for association with desirable traits amongst a population of plant lines. A defined genome sequence will be used by many researchers to identify the basis of traits and to capitalise on knowledge of traits from related crops such as sorghum. Previous work has identified quantitative trait loci (QTL) for traits such as biomass in sugarcane. Bioinformatic tools can now identify the underlying gene sequences from the sugarcane genome sequence. In a similar way, the sugarcane homologues of genes that are known to enhance productivity in other species can now be identified. In addition to revealing underlying biological mechanisms, these genes will be valuable as targets for selection or genetic modification to enhance variety development.

Keywords: NGS, Single nucleotide polymorphism, QTL

Thermophilic consolidated bioprocessing with cotreatment: a potentially disruptive paradigm for low-cost second generation ethanol production

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Data will be presented from a comprehensive recent study aimed at evaluating the cumulative and relative impact of "multiple levers" to overcome the lignocellulose recalcitrance barrier, including choice of biocatalyst and feedstock, genetically modified plants and less recalcitrant natural variants, and several non-biological approaches to augment the deconstruction process. Anaerobic thermophilic bacteria are found to be decisively more

effective than industry-standard fungal cellulase at solubilizing cellulosic biomass under a broad range of conditions. However even the best plant cell wall-solubilizing biocatalysts require some assistance in order for lignocellulose to be processed with high yields in a reasonable amount of time. As an alternative to thermochemical pretreatment, we are investigating physical disruption once fermentation is initiated – termed cotreatment. Results presented include: a) demonstration of fermentation in the presence of physical disruption at an intensity sufficient to substantially increase lignocellulose solubilization, b) high extents of solubilization comparable to conventional pretreatment, c) lignin residues with less modification than result from thermochemical pretreatment. Taking advantage of the outstanding capability of thermophilic anaerobic bacteria to ferment cellulosic biomass without added enzymes requires that metabolic engineering tools be developed and applied to these organisms in order to bring product yields and titers to industrially acceptable levels. Recent progress will be described involving the cellulose-fermenting *Clostridium thermocellum* as well as hemicellulose-utilizing thermophiles such as *Thermoanaerobacterium saccharolyticum.* Thermophilic consolidated bioprocessing with cotreatment represents a nascent alternative to the conventional processing paradigm involving thermochemical pretreatment and added fungal enzymes. Recently-published technoeconomic analysis indicates potential for an 8-fold shorter payback period and feasibility at 10-fold smaller scale as compared to technology based on the current processing paradigm.

Keywords: Second generation, ethanol, low-cost, cotreatment, consolidated bioprocessing

Supported by: US DOE, US Department of Agriculture, Enchi Corp.

Towards regional re(de)finery models for materials, fuels and energy

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Biobased production of chemicals, fuels and energy –when done right- can make significant positive contributions to abating emissions and other climate impacts often associated to fossil alternatives, to food security and poverty, can provide (impact) investment alternatives, and overall facilitate sustainable development. Key requirements are robust financial feasibility, also in case of unfavourable economic environments such as in case of (todays) low prices of fossil alternatives, and (near) complete utilisation of the feedstock to avoid wastes and emissions. With the first lignocellulosic biorefineries (for ethanol) coming on line, and technological maturity is emerging, it is timely to investigate how they blend in (or disrupt) a landscape of chemicals and fuels industry that is mostly organised in port-industry clusters (Rotterdam, Singapore, Sjanghai, others) worldwide, connected to networks of downstream industries (packaging, coating, car, others). The biobased opportunity requires a redefinition of the refinery concept – the REDEFINERY-, to the characteristics of biobased and other renewable feedstocks to allow as much as possible full utilisation of all bio-mass of the feedstock, and not only its energy content as is the case in current biofuels plants. This is particularly important for products that have no alternatives such as chemicals, materials and liquid fuels for aviation, marine and long-distance/heavy duty applications.

During the presentation, we will discuss: overall yield and efficiency requirements, how specific technology/feedstock /product combinations contribute to these objectives and how new applications and technologies open new opportunities such as bioconcrete and other

materials. Attractive and current examples will be presented, in the framework of a rough innovation roadmap; we also discuss how and which REDEFINERY structures also financially enable the robust large scale development of a biobased economy. This also deals with establishing specific (intermediate) platforms that can be converted efficiently into a wide range of chemical and fuel products; a last item is the role of logistics. Since biobased feedstocks need to be collected from large areas, the infrastructural requirements are fairly different from those of the fossil industry where extraction is in general from a single or a few wells, or from the agro-food/feed industry that has already some large scale food-oriented processing complexes operational. All of this should be seen in the framework of the local opportunities that regional implementation provides. These are different in the Brazilian, and European context (Netherlands, Ireland), allowing specific and differential developments. Although this is work-in-progress, we plan to discuss some thoughts for further discussion.

Sustainable biobased value chains: business challenges

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Now, in the transition phase from the fossil- into the bio-based economy, biobased value chains are facing 3 business challenges: i) cost, ii) market pull and iii) business framework. Fossil carbon sources still dominate because of easy production, logistics and transformation. Processing of fossil feedstock has been optimized since 100 years, resulting in cost-efficient refineries, chemical plants and processes. On the other hand biobased feedstock are costly to grow and ship and their transformation to chemicals, fuel and energy has by far not reached the level of efficiency of fossil-based methods. Reducing investment- and running cost of production facilities is therefore a priority. In the last 2-3- decades science has provided a cornucopia of plant materials suitable for industrial purposes and of bio-based materials such as polymers, composites, adhesives, lubricants and more. Start-ups and big industries picked up the ball with tremendous success in some examples. But the final breakthrough is still outstanding. It needs a critical market pull and the engagement of brand owners to achieve broad market penetration. Biobased value chains comprise materials, chemicals, fuel, energy and heat. These value chains need to establish specific industrial networks and these networks need to merge and interlace. This time-consuming process depends on a supportive general legislative and administrative framework.

Commercial and technical partnerships: pivotal to realize a sustainable bioeconomy

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Corbion is the global market leader in lactic acid, lactic acid derivatives and a leading company in functional blends containing enzymes, emulsifiers, minerals, and vitamins. Corbion products are made from carbohydrates and other renewable resources and the company is based on fermentation and enzymes in process and product development. Corbion, in close conjunction with its partners and customers, develops sustainable solutions for a variety of markets ranging from bakery ingredients to high-end biomedical materials. Corbion's business activities are organized in two main units: biobased ingredients (running business and innovations in food, feed and bio-chemicals) and biobased innovations (break through innovations in materials and chemicals). As part of biobased innovations, Corbion has been expanding its range of products into renewable materials and

chemicals as illustrated by the recently announced joint ventures with BASF (Succinity GmbH, succinic acid) and TOTAL (Total-Corbion JV, PLA or polylactic acid). In order to meet our sustainability development goals, there is a necessity to move from the current linear economy models to a circular more sustainable model. In a circular economy, waste is minimized and used as a resource and products are re-used and recycled as much as possible. Nevertheless, losses will occur also in a circular economy model (e.g. by incineration and landfill) and these have to be replenished, ideally from a renewable resource. The challenge to realize such a circular economy is huge and Corbion realizes that such challenge cannot be met by a single company. In contrast, many stakeholders in the value chain, including knowledge institutes, companies, governments and the end consumer, are needed to establish a functioning circular, biobased economy.

Keywords: Circular Economy, Lactic Acid Tree, Biobased Economy, Corbion.

Engineering sugarcane cell wall hydrolysis

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One of the most important barriers for development of 2G bioethanol technologies (the cellulosic ethanol) is the efficient use of pretreatments and enzyme cocktails during the process. Although some success has been achieved, the production costs are still high. Despite the fact that the search for enzymes capable to hydrolyze plant cell walls has been quite intensive, relatively little effort has been made towards solving the difficulties associated with recalcitrance to hydrolysis, which is a phenomenon related to the complexity of the cell walls that are encrypted into a Glycomic Code. I will report some of our findings related to the chemical structure and biophysical properties of sugarcane cell walls that are leading us to understand how polymers interact within the walls, conferring recalcitrance to sugarcane cell walls. On the basis of the chemical structure and polymers properties, we proposed a conceptual model for the architecture of the sugarcane cell wall. To hydrolyze them enzymatically, we proposed the hypothesis that the wall should be subjected to a sequential attack of domain-targeted enzyme consortia until complete hydrolysis could be achieved. This hypothesis was corroborated by the discovery that the enzyme complexes produced by *Aspergillus niger* and *Trichoderma reesei* follow, in different ways, the expected sequence of hydrolases production when these fungi were grown on sugarcane biomass and bagasse. The same has been observed when we studied an endogenous mechanism extant in sugarcane that alters its own walls during root development. Using RNAseq, we detected more than 1,000 cell wall related genes, among them, 49 were hydrolases. With these results, it became possible to define strategies to induce controlled endogenous hydrolysis of the cell walls in planta. We have fully characterized 4 genes related to the disassembly of sugarcane cell walls. They belong to the classes of transcription factors (RAV and ERF) and hydrolases (endopoligalacturonase – EPG) and an alpha-arabinofuranosidase – alpha-Ara). scRAV1 directly controls scEPG1 expression by repressing it. Plants overexpressing scRAV1 have been produced and are under analysis at the moment. This is the first step for bioengineering sugarcane cell wall hydrolysis using the knowledge about an endogenous process used only in specific parts of the plant´s body.

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The Brazilian agribusiness in the emerging bioeconomy

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Brazil is one of the largest countries in the world, with an extensive surface of continuous land, a large supply of fresh water, abundant solar energy, and a rich biodiversity. The wide range of climatic conditions, from temperate to tropical, together with advanced capacity in technology development, allowed considerable diversification of agricultural systems in the country, which has become one of the world's largest producers of food, feed, fibers and renewable fuels. The substantial modernization of the Brazilian agriculture, observed after the 1970s was a result of a strong government will, translated as coordinated policies that led to increased R&D capacity and increased volume of credit, improved infrastructure, tied to support policies of stock management, improved distribution and commercialization of food and agroindustrial products. These coordinated policies and support mechanisms led to a better allocation of resources, increased productivity, improved product quality and reducing food prices. Responding to increasing concerns over agriculture´s footprint on the natural resource base, the agricultural research system in Brazil has also taken important leaps, in a short period of time, towards development of innovations for increasingly safer and sustainable agricultural systems. Brazil has set challenging targets to reduce the agricultural sector's carbon dioxide emissions and, as a result, the country has launched a Low Carbon Emission Agriculture Program to stimulate agronomic practices that help environmental preservation and productivity enhancement. Mixed farming systems combining crop, livestock and forest production integrated in the same area and with efficient use of inputs are also becoming a reality in close connection with the nascent bioeconomy. The growth of the bio-based economy in Brazil, in connection with the agribusiness sector can generate multiple opportunities for economic development and creation of new jobs, including in rural areas. Among the major routes considered are the metabolic processes of organisms (plants, animals and micro-organisms) with focus on the production of substances and materials of high value, targeted to multiple uses (chemical and biochemical, medical, pharmaceutical, nutritional, energy, etc.). Biomass and bio-refinery technologies are being developed and special attention is being given to research in molecular biology, multiple "omics" sciences, nanotechnology, information technology, among others, to meet the demands for production of sustainable energy, chemicals and new bio-based materials. These and many other developments indicate that the Brazilian agriculture is already evolving in sync with the emerging bioeconomy, an innovative revolution based on the utilization of biological resources and biological processes to sustainably provide goods and services in food and agriculture and across many other economic sectors.

Keywords: Agriculture, Agribusiness, Biomass, Bioeconomy, Research and Innovation.

Sustainable biobased value chains: business challenges

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Innovation for biobased economy is rapidly emerging providing various, often partial solutions for enhancing production from biobased materials. Examples include better yeasts, improved biocatalysts or downstream processing methods. These are expected to be embedded in improved multi-product biorefineries, contributing to more viable business options for large existing industries. However, many smart ideas do not get to full exploitation which could be a consequence of a

lack of entrepeneurship or (financial and mangement) support to young bright scientists and engineers. In order to boost the required etrepeneurship further the Global Biobased Business Competition (G-Bib) was launched, as an initiative of BE-Basic in The Netherlands, BBEST in Brazil and Clib2021 in Germany. Teams of young scientists and engineers in the three countries were supported by masterclasses in business development and by business experts mentors to develop their business idea. In this session the finalists of each country will present their idea and compete for the G-Bib award provided by Corbion. The teams will be reviewed by experts in biobased business development who will each introduce their key messages on success and fail factors in entrepeneurship. Many such factors relate to challenges in non-technological areas, including for example: 1) feedstocks availability & quality, 2) overall sustainability, including soil and agricultural practice, and 3) value sharing & investments. These issues are related to both stakeholder collaboration and governance as well as environmental assessment and monitoring. BE-Basic has been exemplary in providing further insights in these issues and methods on how to overcome these. The results show that reconciling targets for biobased innovation requires integration of these methods early in the innovation process. This presentation will discuss the integral sustainable design of biobased value chains including the key enablers to become economically robust and secure long-term, sustainable supply of bioresources, improve agricultural management and steer for social benefits.

Keywords: biobased production chains, innovation, business development

Supported by: BE-Basic, TKI Biobased Economy, NWO

Bioenergy and bioeconomy: markets, strategy and GHG emissions reduction

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Canada as well as other governments are increasingly recognizing the importance of the cluster concept to accelerate BioEconomy development. The cluster concept as it applies to BioEconomy value chains is unique in many ways, related for example to developing markets for the gamut of diverse bioproducts for which innovation – at various levels of development – is emerging. Biomass raw materials are in the regions, are bulky, and are expensive to harvest and deliver in sufficient quantities for commodity production. At the same time, market pull comes from facilities and consumers in urban areas. Government must play an important role to support investments in rural green infrastructure, break-through research, innovation, technology transfer – and the effective management of these activities in poles of excellence. Regional, and in the case of the BioEconomy trans-regional, clusters will undoubtedly be critical to accelerate BioEconomy value chain development and create the associated benefits related to job creation, the application of clean technology, and the opportunity for radical GHG emissions reduction. The initial part of this presentation will link the cluster concept with markets, strategy and GHG emissions reduction in the forest products sector. Forestry companies are seeking to define their unique biorefinery strategies for implementation, and decision-making regarding the most promising route is not obvious especially at the early stage of design. To be successful, a focus on strategic planning at the corporate level is essential considering not only technology opportunities assessment, but also the assessment of market and value chain strategies for competitive position in the longer-term. Systematic

approaches are needed for defining transformative strategies considering critical factors such as biorefinery technologies, proven production scales, emerging market opportunities, market competitive advantages, market value proposal associated with product portfolios, the integration potential of the biorefinery strategy at the corporate and mill level, the identification of the level of technology, market and business risk of each strategy at an early-design stage, and the economic viability of each biorefinery strategy option. A phased-approach for implementing the biorefinery will be presented in the second part of this presentation, where for each phase, technology and market risks are identified and addressed, and where the technology strategy serves the business strategy. A systematic tool for decision-making is introduced where not only typical economic metrics are used to assess the potential of a biorefinery strategy, but also a combination of risk-based metrics considering economic, business, environmental and financial risks. Climate policy further helps accelerating project development/deployment. The question remains: Are policy scenarios such as carbon pricing likely to change the decision of forestry companies to implement strategies with higher GHG emission reductions? A case study is used to concretize the proposed approach considering the identification of the most promising biorefinery strategy using Multi-Criteria Decision-Making (MCDM) as a tool for effective decision-making, and considering the impact of policy scenarios on the investment decision-making.

Keywords: BioEconomy, Markets, Value Chain Gaps, Strategy, Decision-Making, GHG Emissions

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Genomic analysis of transgressive segregation in autopolyploid sugarcane

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Sugarcane is a dual-purpose crop for energy and sugar production. It has the highest biomass production reaching 1.9 billion tonnes in 2014 worldwide. To improve sugarcane biomass accumulation, we developed an interspecific cross between *Saccharum officinarum* 'LA Purple' and *Saccharum robustum* 'MOL5829'. The selected F1 individuals were self-pollinated to generate a transgressive F2 population with a wide range of biomass yields. Leaf and stem internodes of fourteen high biomass and eight low biomass F2 extreme segregants were used for RNA-seq to decipher the molecular mechanism of rapid plant growth and dry weight accumulation. Gene Ontology terms involved in cell wall metabolism and carbohydrate catabolism were enriched among 3,274 differentially expressed genes between high and low biomass groups. Specifically, up-regulation of cellulose metabolism, pectin degradation and lignin biosynthesis genes were observed in the high biomass group, in conjunction with higher transcript levels of callose metabolic genes and the cell wall loosening enzyme expansin. Furthermore, UDP-glucose biosynthesis and sucrose conversion genes were differentially expressed between the two groups. A positive correlation between stem glucose, but not sucrose, levels and dry weight was detected. We thus postulated that the high biomass sugarcane plants rapidly convert sucrose to UDP-glucose, which is the building block of cell wall polymers and callose, in order to maintain the rapid plant growth required for biomass accumulation. The gene

interaction of cell wall metabolism, hexose allocation and cell division contributes to biomass yield, expanding our understanding at the molecular level required for energy cane breeding and engineering.

Keywords: Biofuel, Carbohydrate metabolism, Cell wall, *Saccharum*

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The Paris agreement and the transition to the low-carbon transport sector: the role of the biofuture platform

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Despite representing almost 25% of global emissions of CO2, the transport sector has not received adequate attention by governments and international organizations aimed at developing and implementing actions to combat climate change. Among the alternatives of decarbonization of the transport sector, advanced biofuels, such as first-generation sugarcane ethanol (E1G) and cellulosic or second-generation ethanol (E2G), are an effective solution, scalable and of rapid implementation. However, these biofuels still face industrial scheduling challenges. It is in this context that Brazil, led by Itamaraty, has succeeded in creating the Biofuture platform, an international alliance between twenty countries that seeks an increase in the flow of investment, foster policy dialogue, and promote technological exchange to develop advanced biofuels and bioeconomy.

Keywords: bioeconomy, international energy governance, Biofuture Platform

Improving sugars utilization and inhibitors tolerance in yeast via adaptive laboratory evolution

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Adaptive laboratory evolution (ALE) is an efficient scientific technique to create microorganisms more relevant for industrial applications. ALE consists of subjecting a population of microorganisms to a given environment, allowing natural selection and increasing the overall fitness of the population. This is only possible due to the ability of the microorganisms to adapt quickly to different environmental conditions. In the current scenario, where the development of a sustainable economy based on the use of renewable resources for bioprocesses has been encouraged, ALE can be an interesting alternative to overcome some process limitations. Lignocellulosic biomass usually contains 25-40% hemicellulose, a fraction whose key building block is the pentose sugar xylose. Unfortunately, xylose cannot be efficiently utilized by many microorganisms and, for the development of a bio-based economy, the conversion of xylose into industrially relevant products is of great importance. In addition, xylose is usually recovered from biomass in a liquid media containing a mixture of several components, including toxic compounds. The generation of toxic compounds is unavoidable during the biomass fractionation step, and such compounds inhibit the metabolism of the microorganism during fermentation. Although many efforts have been done to reduce the toxicity of biomass hydrolysates, this topic still deserves attention.

Recent studies have shown how some of these limitations (sugars utilization and tolerance to inhibitors) may be overcome by using ALE in microbial cell factory design. ALE was an efficient strategy to improve the resistance of

Spathaspora passalidarum to acetic acid, for example, which is one of the most important inhibitor compounds present in biomass hydrolysates. In addition, the adapted strain was able to co-ferment glucose, xylose and cellobiose under microaerobic condition without lag phase. Interesting results were also obtained for different *Kluyveromyces marxianus* strains, both in terms of improved xylose utilization and tolerance to toxic compounds. The potential of ALE to adapt biotechnologically relevant microorganisms to lignocellulosic material is huge, and the results can be reflected in terms of improved sugars utilization, tolerance to inhibitor compounds, and growth performance.

Keywords: biomass hydrolysates, fermentation, cell factory design, adaptive laboratory evolution

Production of bio-derived chemicals from lignocellulosic biomass: challenges, mitigation strategies and select success stories

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The high demand for, and impending depletion of petroleum reserves, the associated impact of fossil fuel consumption on the environment, and volatility in the energy market have elicited extensive research on alternative sources of traditional petroleum-derived products; namely biofuels and bio-chemicals. Fossil oil is largely associated with gasoline, however, approximately 6,000 petroleum-derived products currently exist in the market, with sundry applications. Ironically, while biofuels are more popular with the public, the other petroleum-derived products have not attracted similar attention despite their vast economic values. Thus, given the finite nature of petroleum, it is critical to deploy substantial resources and research efforts to the development of renewable chemicals (similar to the efforts devoted to biofuels). Theoretically, bio-production of gasoline-like fuels and the 6,000 petroleum-derived products is within the realm of possibility, since aquatic and terrestrial ecosystems harbor an abundance of diverse microorganisms, capable of catalyzing unlimited numbers of chemical reactions. Moreover, the fields of synthetic biology and metabolic engineering have evolved to the point that a wide range of microorganisms can be enticed or manipulated to catalyze foreign or vastly improve indigenous biosynthetic reactions. Therefore, development of different routes for efficient conversion of biomass to platform chemicals such as butanol, butanediol, succinic acid, fumaric acid, and malic acid will be discussed. Conversion of lignocellulosic biomass hydrolysates (LBH) to butanol and butanediol, the challenges associated with LBH utilization and mitigation strategies, and significant breakthroughs on fermentation of LBH containing lignocellulose-derived microbial inhibitory compounds (LDMICs) to fuels and chemicals will be presented. Strategic examples such as: (i) enticing fermentation microorganisms to develop defenses against LDMICs, and (ii) integrating LDMIC-detoxifying and/or catabolic genes in fermentation microorganisms via metabolic engineering to enhance LBH utilization will be presented as well, to demonstrate recent progress towards improving the production of bio-derived chemicals from lignocellulosic biomass.

Keywords: Butanol, butanediol, succinic acid, lignocellulosic biomass, LDMICs

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Natural products from sugarcane industry - would be a player in the future Brazilian bioeconomy?

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The plant chemical diversity is fantastic, and natural product molecular structures is reflected in a large variety of biochemical and genetic pathways, which is responsible for several classes of biologically active secondary metabolites. These compounds are important, as they communicate plants with the machineries of their ecosystem, the physic environment (biotope) and the living community (biocenosis), thus resulting indispensable for the survival of the species. Also, plant secondary metabolites are important supplies for the production of drugs, foods, cosmetics, fragrances, colorants, and agrochemicals, which support the vigorous bioeconomy in developed countries. Thus, increasing the acknowledgment on the function of natural products and advance analytical methods on extractable amount of these compounds so far, sugarcane is a source matrix of useful natural products.

Keywords: Natural Products, Bioproducts, Sugarcane
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Ethanol use in transportation: research needs

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Energy use in the transportation sector (road vehicles, railways, ships, airplanes) in 2015 was distributed in Brazil as follows: 44.4 % diesel, 2.3% biodiesel, 27.7% gasoline, 17.4% ethanol (anhydrous and hydrated), 4.3% kerosene, 1.8% natural gas, 0.9% fuel oil and 0.2% electricity. Energy from biofuels reached 20.7% – far beyond other countries. Based on the importance of biofuels in Brazil, an agreement among FAPESP and Peugeot-Citroën (PSA) lead to the creation of the Engineering Research Center "Prof. Urbano Ernesto Stumpf" (ERC), a "locus" where state-of-the-art and multidisciplinary research could be developed. The ERC is open for new partners and new projects, such as the evaluation of ethanol hybrid vehicles, ethanol-driven fuel cells, and other biofuels possibilities. In the long term, it must also attain a self-sustained budget. The research plan for the first four years of the ERC, entitled "Conceptual study of an advanced ethanol-fueled engine", aims to explore the specificities and positive characteristics of ethanol as fuel, and was begun in November of 2014. The expected result is a conceptual proposal to present an ethanol engine with better performance and simultaneously better efficiency. Four Laboratories in four Universities are involved in this research project: Biofuel Engine Laboratory (LMB) at UNICAMP, Laboratory of Environmental and Thermal Engineering (LETE) at USP, Laboratory of Combustion, Propulsion and Energy (LCPE) at ITA, and Division of Engines and Vehicles (DMV) at IMT. Two of them (LETE and LCPE) deal with fundamental studies on air/fuel mixture preparation and turbulent combustion of ethanol, from experimental methods and CFD models. The use of technologies not yet fully tested for ethanol (such as direct injection, turbocharging, water injection, and variable compression ratio) is the subject of the other two, combining experimental tests (DMV, LMB) and thermodynamic simulations (LMB). Some preliminary results were obtained: intensity of tumble and swirl turbulence in a cylinder with optical access, characterization of the ethanol spray by laser optical methods, experimental results for twin PFI injectors, experimental effect of the injection timing in the engine performance, simulation of the engine

performance and emissions of NOx, CO and UHC, a model for detonation forecast, friction reduction in bearings by surface texturing techniques simulations. Ethanol is a proven solution for the reduction of GHG emissions in light vehicles; the project whishes to evaluate its possibilities as a substitute for diesel engines in light trucks. On the other side, ethanol production must increase to feed the light vehicles fleet – otherwise the flex-fuel vehicles will continue running on gasoline.

Keywords: Ethanol as fuel, Internal combustion engines, Engine emissions
Supported by: FAPESP and Peugeot-Citroën do Brasil (Groupe PSA)

Increasing sugarcane gains through historical partnerships and private investments

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Increases in productivity in the last decades of various agricultural crops, such as soybeans and corn, have been often compared to that of sugarcane for the purpose of assessing genetic gains and discriminating strategies and efficiencies of breeding programs. Biological differences and agricultural practices are not always taken into account in these comparisons, and the results, often distorted, do not fulfill their purpose of generating guidelines for the productive ecosystem, universities, companies, funding entities, government and markets. Sugarcane presents contrasting characteristics from those of annual crops. Its genome is polyploid and aneuploid, its cultivation is semi-perennial and its productivity is the result of a sum of several years of harvest. In our view, the results presented by breeding programs in Brazil have been highly satisfactory and made it possible, for example, to reach nine million hectares cultivated and distributed in almost all the states of the country, with a 50% growth in the last 10 years. The private support of the mills and other players of the business to the programs has been fundamental for this success, since from one side we have the scientific knowledge and adequate methodology of the academy and from the other a clear vision of the needs. Allied to this, São Martinho has been investing in new technologies that add significant gains to the business. MPB (pre-sprouted nursery plants) is an example of a new technology that has its origin in the partnership between the private sector and the research institutions.

Keywords: Sugarcane mill, genetic improvement, private investments

Poster presentations

Biomass, agronomy, breeding, biotechnological aspects of energy plants

Sugarcane allelic dynamics in duplication region

Sforca, D.A. [1], Vautrin, S. [2], Cardoso-Silva, C.B. [1], Mancini, M.C. [1], Cruz, M.V.R. [1], Pereira, G.S. [3], Conte, M. [1], Bellec, A. [2], Dahmer, N. [1], Fourment, J. [2], Gautier, N. [2], Rodde, N. [2], Sluys, M.A.V. [4], Garcia, A.A.F. [3], Martins, E.R.F. [1], Berges, H. [2], Vincentz, M. [1], Souza, A.P. [1]

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Sugarcane modern hybrids (*Saccharum spp.*) have an unusual combination of factors resulting in a genome exhibiting high complexity: highly polyploidy, heterozygous and aneuploidy. They are derived from hybridization between *S. officinarum* (2n = 80) and *S. spontaneum* (2n = 40 – 128), in a process called 'nobilization'. The *Saccharum hybrid* SP80-3280 has a BAC library represents 2.4X the sugarcane genome. The sequence of the gene HP600 (gene linked to a QTL for sugar content in *Sorghum bicolor*) was used to explore the genetic and genomic allelic dynamics of sugarcane modern hybrid. The screened among 221,184 BAC clones of SP80-3280 BAC library showed the presence of HP600 gene sequence in 23 BACs. Each BAC clone was individually sequenced, assembled and manually annotated for genes and repeat regions. The comparison among the BAC clones has shown two different regions, indicating HP600 gene was duplicated. This duplication has around 7kb and it content two truncated genes: HP600 and the adjacent gene CEMP-C. The Region01 (not duplicated) was composed by seven haplotypes (10 BAC clones) has shown a high level of synteny and collinearity with sorghum, rice and maize. The Region02 (duplicated) was composed by nine haplotypes (13 BAC clones), had shown a low synteny and collinearity with sorghum, rice and maize. The analyses of molecular clock had shown a divergence time around 2.96 Mya (+- 0.12 Mya). Since the divergence between *S. officinarum* x *S. Spontaneum* happened 1.5-2.7 Mya, probably this duplication occur in *Saccharum* genus. In sorghum and rice genomes was not found evidence of the duplication of the HP600 and CEMP-C. In maize, a gene very similar to HP600 truncated was found, but analyses have shown that it is a genetic convergence. Using BAC-FISH was possible to observe eight markers for Region01 and ten markers for Region02. Also was found expression evidences for HP600 and CEMP-C in Region01 and Region02 mapping transcriptome sequences. Fourth-four SNPs markers are genotyped in a bi-parental population (SP80-3280 x RB835486) using mass spectrometry (Sequenon®) and analyzed using SuperMASSA software. Was possible observe two ligation groups, both with duplicated markers, showing a distortion in genetic distances. These results demonstrate (1) the low sinteny and collinearity found in Region02 could be explained by the recombination events occur during the 'nobilization' of Saccharum hybrid; (2) it was possible to find evidences of duplicated and truncate genes expression; (3) demonstrate duplicated regions can cause distortions in a genetic map; and (4) has shown the complexity involved in sugarcane genetic and genomic, what can be useful for understanding the complex polyploidy genome.

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

Economic and environmental impacts in sugarcane production to meet the Brazilian agreement signed at COP-21: the role of precision agriculture

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The Brazilian agreement signed at the Conference of the Parties (COP) on Climate Change reaffirms the vital role of Brazil with sugarcane production, the main Brazilian biomass to produce renewable fuels like ethanol. The country committed to reduce 43% of greenhouse gas emissions (GHG) by 2030, when compared to 2005 emission levels. The commitment also provides that the participation of biofuels in the national energy matrix will be 18% in 2030. To meet the established targets, estimates indicate that ethanol production in 2030 should be 54 billion liters, almost double of the current production (28 billion liters). To attempt the ethanol and sugar production targets, sugarcane production should increase from 657 million to 942 million tons. From the agronomic point of view, two alternatives are possible; increase the planted area and/or agricultural yield. The present study aims to evaluate the increased sugarcane production potential to meet COP-21 estimates. Were evaluated the economic and environmental impacts of increased production by increasing the planted area and agricultural yield of São Paulo state, the main sugarcane producer in Brazil. In this context, were evaluated how the precision farming technologies can helps sugarcane production reach the agreement. The economic and environmental assessments of production scenarios were evaluated using the Virtual Sugarcane Biorefinery (VSB). The results show that it is more feasible to invest in management techniques to increase agricultural yield. This alternative can reduce up to 25% in the total production costs. However, it is expected that average yield rise from 77.5 Mg ha^{-1} to 110 Mg ha^{-1} by 2030. On the other hand, a combined average annual growth rate of 1.5% in yield and area expansion is sufficient to reach the production target by 2030, with an increase of 0.8 million hectares and a yield of 95 Mg ha^{-1}. Achieving this yield baseline seems to be far away, but investment in technology and management can contribute significantly. The adoption of GNSS and traffic control generates gains of ~10% in total production, contributing to achieve the goal. From the environmental point of view, investing in Precision Agriculture technologies is essential. Studying the spatial and temporal variability of crops and soils will maximize production, reducing production costs and environmental impacts through the rational use of inputs. The variable rate technologies to fertilizer in the right place with the right amount can contribute significantly to reduce the greenhouse gases emissions, mainly of nitrogen sources. Investing in the sugar-energy sector for proper crop management will be essential for Brazil to meet the production and GHG reduction targets.

Technology Roadmap in New Technologies for Precision Agriculture

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Precision Agriculture (PA) is a modern farming management concept, using digital techniques to monitor and optimize agricultural production processes, to manage agricultural fields considering their spatial and temporal variability. To make feasible and promote PA adoption with the needed information detail, tools for soil and crop sensing are demanded as

well as crop monitoring techniques. PA methods promise to increase the quantity and quality of agricultural output while optimizing input usage. A wide range of enabling technologies for PA are available and the market is fully embraced by the sector and investors, but the full potential of PA has not yet been harnessed. The academy, research and development institutes, and companies show high potential for establishing partnerships aiming technology development to improve PA suitability for growers with different profiles, farms size and levels of in-field technology adoption, however many technology and non-technology gaps must be fulfilled. In order to understand the present actions, challenges, opportunities, and build strategies for development of PA innovative technologies a wide audience workshop involving 114 experts from academy, government and private sector was carried out during May 24-25, 2017 (Campinas, São Paulo). The debates focused three main topics: 1) soil spatial characterization, 2) crop monitoring, and 3) site-specific pest management. The main technological gaps/barriers pointed out were related to the lack of R&D and Investment in: i) soil sensing technologies (possible soil characterization techniques: spectroscopy, nutrient availability by ion-selective sensors, classification by sensors such as – electrical conductivity and magnetic susceptibility); ii) data communication and transfer; iii) data interpretation and modeling; iv) UAV (drone technology) for crop monitoring, variability identification and provide auxiliary data; v) intelligent insect traps and pest alerts by modeling; vi) autonomous site-specifically manage; vii) new sensors (cost effective multispectral sensors and improvement of available sensors, low cost wireless sensor networks); and viii) image (techniques for processing and analysis and availability). Related to non-technological gaps and barriers, the main notes were: i) lack of specific policy for tax incentives and financial support (local equipment development and equipment/components importation); ii) a plan to human resources formation in PA; iii) lack of a current PA scenario mapping; iv) lack of efficient regulatory tools (telecommunication, data privacy, sustainability incentives and compliance, UAV, data sharing for common good). In conclusion: i) many of PA equipment and techniques are imported and has not been developed for the specific characteristics of Brazilian growers, mainly the small ones; ii) in order to really reach the growers with new technologies, it is mandatory to improve the agricultural extension service in Brazil, and iii) to improve the PA development and adoption in Brazil a "National Precision Agricultural Program" need to be developed and implemented in short term (up to 5 years)

De novo assembly and annotation of sugarcane transcriptome infected with *Puccinia kuehnii*

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Brazil is the main producer of sugarcane in the world, which is used as raw material for sugar and ethanol production. Given the environmental impacts and global dependence on oil production, coupled with world demand increase for sugar and a need for renewable fuel, sugarcane emerges as a strong candidate to provide renewable energy worldwide. Commercial sugarcane cultivars are inter-specific hybrids originated from crosses between *Saccharum officinarum* and *S. Spontaneum*, followed by backcrosses with *S. Officinarum*. Additionally, the polyploidy and aneuploidy of this crop makes its genome very

complex. Understanding differential gene expression in contrasting conditions may provide information for the genetic improvement of this plant. For performing differential expression analyses, it is first necessary to obtain a reference sequence against which to align reads. Due to its genomic complexity, obtaining a sugarcane reference genome sequence is a challenging task, and current efforts have not yet produced a suitable reference. Nonetheless, it is possible to use the transcriptome as a reference, albeit only a few sugarcane transcriptomes are available. In this context, the aim of this work was to perform *de novo* transcriptome assembly using reads originated from an experiment in which plants from cultivar SP89-1115 were inoculated with the fungus *Puccinia kuehnii* and analyzed at different time points. In the pre-processing step, the twelve first 5' bases and read ends with Phred quality score < 20 were removed. Reads of residual rRNA from library preparation were filtered by aligning with eukaryotic rRNA sequence (18S and 28S). Remaining reads were then used to assemble the transcriptome using the Trinity software, yielding a total of 374,281 transcripts, with an N50 metric of 685 and GC content 47.76%. Next we performed gene ontology functional annotation using the program Trinotate, which identified 74,575 transcripts using the Viridiplantae Uniprot database and 55,986 transcripts with the Fungi database. Cross-comparison of both annotations revealed 4,571 Fungi exclusive transcripts, which were removed from the assembly. We also assessed the number of transcripts that appear to be full-length comparing with the *Sorghum bicolor* reference. Although many transcripts were completely assembled, some of them appeared to be fragmented, likely due to the polyploidy and genomic complexity of sugarcane, which causes contigs to break. We will also present and discuss the use of this transcriptome as a reference sequence for variant calling of a panel of sugarcane genotypes, based on a genotyping-by-sequencing strategy.

Identification, phylogeny, and expression analysis of putative coding p-hydroxycinnamates genes from *Saccharum* spp.

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Sugarcane is a very important crop for Brazilian economy. The biofuel productivity could be even higher if the cell wall sugars were converted to second generation ethanol. However, biomass recalcitrance is one of the major challenges for cost effectiveness of this process. In sugarcane and other grasses, it has been shown that hidroxycinnamic acid content, mainly ferulic acid (FA) and p-coumaric acid (pCA), is related to cell wall recalcitrance. The genes involved in hidroxycinnamic acid incorporation belong to BAHD transferases family (clade Va, genes At1 to At10), and four of them have already been functionally studied in grasses species (At1, At4, At5, and At10). Nevertheless, none of them were identified or studied in sugarcane. In this work, we have identified and annotated the genes belonging to BAHD transferase family in the grasses sorghum (96), maize (105), rice (132), brachypodium (95), setaria (130), and the eudicot Arabidopsis thaliana (63), using BLASTp and HMMER searches against their available genomes. For sugarcane, the searches were conducted against SUCEST database (75), distinct RNA-seq data (54 in internode, 58 in leaves and 23 in roots), and the draft genome sequence (183). A phylogenetic analysis using maximum likelihood method allowed identification of sugarcane

clusters of orthologs genes (COGs) for ScAt1 to ScAt10. The COGs for ScAt1, ScAt4 and ScAt9 were detected in all transcriptomes analyzed, whereas the others were present only in specific database. Besides, COGs such as ScAt6, ScAt7, and ScAt8 are representative only in transcriptome data, but not draft genome. In silico transcriptome expression analysis showed that ScAt1 and ScAt9 have similar expression pattern, which could indicate related function. Also, ScAt4 and ScAt8 are highly expressed in stems, especially in most mature internodes. ScAt6 is highly expressed in roots, while ScAt10 is expressed in stems, and ScAt7 is expressed in leaves and stems. Preliminary real-time expression analysis containing a pool of distinct stages of internode development, young leaves, and root revealed higher expression and primer functionality of ScAt1, ScAt8, ScAt9, and ScAt10 COGs. Additional COGs are under evaluation. As perspective, all COGs identified will be evaluated by RT-qPCR in sugarcane internodes developmental samples with contrasting composition of secondary cell wall to confirm the specificity or a general expression pattern.

New fungus strain isolated from decomposing straw with high potential for enzymatic hydrolysis of lignocellulosic biomass

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The utilization of the lignocellulosic biomass for ethanol production is an alternative to oil based fuels. Nevertheless, the high cost of commercial enzymes is the major bottleneck to consolidate the technology of 2G bioproducts. In the present work, a filamentous fungus with high potential on secreting lignocellulolytic enzymes was isolated from decomposing sugarcane straw. The fungus was cultivated in Mandels medium supplemented with pretreated straw (10 g/L). After 144 hours, the liquid fraction was concentrated and used for enzymatic determination and enzymatic hydrolysis of pretreated straw. When the new isolated was compared with the reference strain, Trichoderma reesei RUT C30, it showed higher endoglucanase, xylanase and β-glucosidase activities, but lower FPase activity. The enzymatic hydrolysis was performed at 50ºC, 150 rpm and pH 5.0 in 10 mL scale with 5% total solids during 72 hours. The ratio between enzyme loading and pretreated biomass was 3.0 FPU/g. The glucose and xylose resulted from the biomass hydrolysis were quantified by HPLC. The global efficiency of the hydrolysis using the enzymatic extract from new strain was 30%, while an efficiency of 22% was reached using the reference strain RUT C30 extract. The transcriptome analysis was also performed to compare the expression profiles of pretreated cane straw and cellulose. Several full-length transcripts of xylanases, endoglucanases and β-glucosidases were identified and, these sequences can be cloned into bacteria, expressed, purified and its activities verified separately. Further studies should be conducted in order to improve the performance of enzymatic hydrolysis of lignocellulosic materials.

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Sugarcane bagasse hydrolysate as potential carbon source for low-melanin Pullulan production: effect of influent variables in the fermentation performance

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Sugarcane bagasse (SCB), an interesting low-cost lignocellulosic biomass, can be used in

bioprocesses for different biomolecules production. This biomass present in its composition more than 60% of carbohydrate fraction, which can be enzymatically hydrolyzed after pretreatment process, releasing sugars as glucose, xylose, cellobiose and arabinose. These sugars can be substrate for low-melanin pigment pullulan production by wild strain of Aureobasidium pullulans, in fermentation process assisted by blue light-emitting diode (LED). Pullulan presents potential application for drug delivery, bone tissue engineering and others. Considering its potential application, low-melanin pigment production was evaluated and optimized using, as carbon source, enzymatic hydrolysate obtained from sugarcane bagasse pretreated in alkaline condition (0.3 M, 70°C and 4h). Variables as temperature (25-35°C), stirring speed (160-240 rpm) and yeast extract concentration (0.5-3 g/L) were evaluated considering pullulan production as response variable. Temperature and stirring speed were the most influent variables on the pullulan production compared to yeast extract concentration. In optimized conditions (25.3°C, 232 rpm and 1.88 g/L), 25.19 g/L of pullulan (0.48 g/g of total consumed sugars "glucose + xylose") and productivity of 0.28 g/L.h were achieved after 96h of fermentation under blue LED light. Glucose and xylose consumption in 96h of fermentation were 100% and 68.39%, respectively. The color of obtained product was white due to non-production of melanin pigment during fermentation, as a consequence of the effect of LED light incidence. The obtained product presents potential application in different areas as food, medical, polymers, pharmaceutical and other. Moreover, the use of sugarcane bagasse hydrolysate for pullulan production is attractive considering a context of an integrated biorefinery.

Technology roadmap in ingredients, functional processed food and health

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The development of new ingredients with functional properties has been expanding the functional products offer to a market which, in the last decade, grew more than food and drink market as a whole. The market for ingredients and functional foods will continue to grow in the coming years. The question is whether this growth will be similar to that observed in the last ten years. That is because the following limiting factors can interfere in this growth: conservative regulatory system, lack of skilled labor, lack of alignment with the world trends, lack of proper communication with the consumer, the cost of large-scale production of functional ingredient and the cost to prove its efficacy and safety. This growth will create new products with high aggregated value – 10% per year (2016-2020) and 5% per year (2020-2050), will increase number of formal jobs due to the growth of the functional foods market – 10% per year (2016-2020) and 5% per year (2020-2050) and will cause a GHG reduction due to the growth of industry productivity – 2,5% per year (2016-2050). To achieve these projections, the contribution of the following areas will be of great importance: new food processing technologies; new processing parameters; microencapsulation; nanoencapsulation; nutritional genomics; identification, extraction and purification of bioactive compounds; organic synthesis; fermentation, extraction and purification of bioactive compounds; and synthetic biology. The main conclusion from the Agropolo Workshop is that the most important to Brazil is the construction and implementation of a national plan for the development of ingredients and functional foods. Performing this plan will allow to organize all the existing infrastructure for networking and in addition

creating the necessary infrastructure for the development of this strategic area. Important actions that can be part of this national plan are: adequacy of Brazilian regulatory agencies in order to accelerate the development of innovation; expanding networking, investments and the degree of internationalization of R&DI system; improvement of access of small and medium-sized companies to the equipment for processing and analyzing; and development of studies focused on issues such as: identification of validated biomarkers of exposure and effective use of emerging "omics"; identification of biomarkers and molecular procedures for demonstration of efficacy and safety of bioactive compound; and human microbiome. Another important conclusion from the Agropolo Workshop is the construction of a communication plan to explain to society and consumers the real benefits of functional foods, helping them to feel less confused. That way it will be possible to overcome the technical and non-technical challenges, as well as barriers and gaps.

Use of calcium ammonium nitrate fertilizer in sugarcane production reduce NH_3 and N_2O emissions

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The definition of the best management practices of N fertilization, choice of source, dose, time, and mode of application, not only influences productivity but also the losses by ammonia volatilization (NH3) and emission of greenhouse gases, especially nitrous oxide (N2O). In this study area (Latitude: 22º64'S and Longitude: 47º61'W) an experiment was conducted with four randomized blocks on red Nitisol. Two N sources (urea - "UR" and calcium ammonium nitrate - "CAN"), in five doses (30-60-90-120-180 kg ha^{-1}) of N mineral and control (without N) were tested. Evaluations included N2O, CO2 and CH4 emissions in the three crop cycles (plant cane, 2nd and 3rd ratoons) and NH3 in the two crop cycles (2nd and 3rd ratoons). The N2O fluxes in the plant cane were lower than in the ratoon crop. The accumulated emission of N2O from UR was significantly higher in comparison with those of CAN in all three crop cycles. The emission factors expressed as percentage of fertilizer N emitted by N2O, for UR were 0.8% (plant cane), 1.1% (2nd ratoon) and 0.8% (3rd ratoon) and for CAN, 0.4%, 0.7% and 0.5% for each cycle respectively. The maximum emission factor calculated was 1.4% (UR, 120 kg N ha^{-1}, 2nd ratoon) and the lowest was 0.2% (CAN, 30 kg N ha^{-1}, plant cane). Most of the emission factors measured were less than 1% used by IPCC. Relative emission was used to compare fertilizer impact balanced by yield, UR had higher relative emission (20.3 mg N-N2O kg^{-1} cane stalk) than CAN (16.2 mg N-N2O kg^{-1} cane stalk). The NH3 volatilization losses for CAN were lower than 1% while for the UR as losses were higher varying from 11 to 16% (2nd ratoon) and 5 to 9% (3rd ratoon) of the applied N. Therefore CAN contributed to reduce both NH3 and N2O losses.

Sucrose content and lignocellulose accumulation in sugarcane stems depicted in relation to internode vegetative age and Saccharum genotypes

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Saccharum hybrids acquired several characteristics through plant breeding, including suitability for sucrose and lignocellulose production. In the context of clean-renewable energy, the sugarcane mills could integrate first and second-generation ethanol production with the synthesis of high

value chemicals from bagasse. Integrated first and second-generation ethanol production encouraged new breeding efforts, including dual-purpose hybrids, from which sucrose and biomass with low enzymatic recalcitrance can be obtained. The present study utilized one commercial (RB92759) and four experimental sugarcane hybrids (H58, H89, H140 and H321) developed by RIDESA to evaluate the dynamics of sucrose and lignocellulose accumulation at different stages of internode vegetative development. Sucrose and lignin increased with progressive maturity stages of internodes, while the content of glucans and hemicelluloses decreased. The RB92579, H89 and H140 sugarcane genotypes displayed the lowest variation in the pattern of sucrose accumulation in the stem in comparison with the other hybrids. The pattern of lignin deposition in relation to the internode vegetative age of the RB92579 and H89 hybrids were also strongly retarded in comparison with the other hybrids. The H140 hybrid exhibited the highest lignin content and the greatest rate of lignin deposition in relation to the progressive changes in internode vegetative age. The results of this research suggest that the internode vegetative development pattern along the stem determines the dynamics of sucrose and lignocelulose accumulation, and therefore, the level of biomass enzymatic recalcitrance, since lignin deposition reduces the enzymatic digestibility of the secondary cell walls. Sucrose accumulation and the occurrence of cellulose encapsulating components, including lignin and grucurono-arabinoxylans, should be considered in the context of dual-purpose sugarcane hybrids development.

Analysis of sugarcane genomic regions by the identification of BAC clones bearing genes of interest

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Sugarcane plantation bears a high economic importance, due to sugar and ethanol production, and Brazil is world's major producer of sugarcane. Modern sugarcane cultivars are interspecific hybrids (*Saccharum officinarum* x *S. spontaneum*), with polyploid and aneuploid genomes, making it difficult to construct genetic maps and to detect QTLs (Quantitative Trait Loci). The knowledge of the variations in the nucleotide sequences of different alleles enables information achievement of inter and intra genotypes variation within a species. An efficient technique to access allelic variation between homologous loci in a species is the sequencing of BAC (Bacterial Artificial Chromosomes) clones bearing specific genic loci of interest. In this work, a BAC library of the cultivar IACSP93-3046 (165,888 clones) was screened for the identification of clones bearing genes that encode for a DnaJ (possible single copy gene) and a Trehalose-6-phosphate synthase/phosphatase (TPS) (probably duplicated) proteins. The gene transcripts were obtained from sugarcane ESTs and transcriptome databases and both are upregulated under stress conditions. A BLASTn search was performed against the sorghum genome database. The sugarcane DnaJ transcript presents similarity to a sequence located at the chromosome 09 of sorghum, in a region of QTLs for stress response. A sequence in the chromosome 02 of sorghum, located in QTLs for stress response and development, is similar to the sugarcane TPS transcript. Putative exon/intron boundaries in the transcripts were established using the sorghum gene models. Primer pairs were designed for the putative

gene exon sequences, with expected amplicon sizes ranging from 70bp to 150bp. More than a hundred thousand BAC clones were screened by Real-Time PCR for each putative exon of both genes using a Pool 3D platform. Thirteen BAC clones were positive for the two exons of the DnaJ gene. For the TPS gene, nine clones were positive for the three exons and six were positive for one exon at least. All positive BAC clones were extracted and will be sequenced in the PacBio platform. The BAC sequences will be assembled and annotated, aiming to discover and analyze allelic polymorphisms among sugarcane hom(oe)ologous chromosomic regions and compare their allelic organization. The analysis of hom(oe)ologous regions will lead to a better understanding of the genetic/genomic organization of the sugarcane genome.

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Genomic and transcriptomic analysis of *Saccharum spontaneum* reveals new candidates for promoter sequences of tissue-specific and constitutive genes

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Saccharum hybrids – sugarcane and energy cane – are important sources of feedstock in Brazil. Sugarcane is mainly used in the production of sugar and ethanol, and energy cane is a prominent crop dedicated to biomass. The energy cane crop is based on the selection of Saccharum hybrids with high biomass and low sugar content, determined by crosses between commercial varieties of sugarcane and Saccharum spontaneum (a wild relative with desirable characteristics of lignocellulosic biomass). Because of these crosses, energy cane hybrids may present a complex genome with high levels of polyploidy and aneuploidy, and they are able to generate a pattern of segregation and heterosis composed by a specific combination of both parent species and a transcriptional regulation that may be unique to the hybrid. To better understand this heterosis, previous studies of our laboratory carried out analyzes of one of the parents, S. spontaneum (US85-1008), using low coverage DNA-seq, RNA-seq from different tissues (root, culm and leaves) and bioinformatics analysis. In order to create a tissue expression atlas, we developed a reference loci assembly pipeline to generate gene sequences and use them to calculate the expression profiles from different tissues of the plant. In summary, this methodology generated a large and very well constructed set of genes and transcripts sequences totalizing around 41,000 predicted genes. From these genes, 90% of them were well supported by transcriptomic data. Although this methodology cannot produce large contigs, a small part of them contains at least 1500 bp upstream of the genes, representing candidates for promoter regions. Promoters are important tools for the manipulation of the expression of endogenous genes and transgenes, since they are cis regulators elements recognized by proteins involved in the beginning of the transcription. In plants, the promoters can be classified as: constitutive (expressed in all tissues), inducible (modulated by non-endogenous factors, as environmental conditions), and temporal specific expression (specific stage of development) or spatially (tissue specific). For these reasons, the main aim of this study is analyzing the genome and the transcript atlas of Saccharum spontaneum in order to identify and characterize new promoter sequences from

constitutive and tissue specific genes. Thus, we intend to validate these promoters in Arabidopsis thaliana and Saccharum spp. using transient transformation of plants and analyze their expression profiles using quantitative real-time PCR (qRT-PCR) experiments.
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Comparing pastureland distribution across Brazil using maps with different spatial-temporal scales

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Pasturelands occupy large areas in the tropics and are potential sources to intensify food production, providing lands available for bioenergy. However, its demands an appropriate identification of spatial-temporal pasture distribution. Remote sensing techniques represent an efficient alternative to map and quantify this land cover at national and regional levels, incorporating several maps and databases already available. In this context, an integrated analysis with different land cover maps would be very interesting for quantifying and analyzing areas with pasture and different levels of intensification. The challenge remains in understand the effect of different databases and spatial-temporal scales incorporated in these maps on pasture quantification. The aim of this study was to compare two pasture maps across Brazil with different temporal and spatial scales and their potential to quantify livestock areas. We analyzed the pasture maps from the Global Agricultural Lands data for the year 2000 and the national land cover map from the Project of Conservation and Sustainable Use of Brazilian Biological Diversity - PROBIO - for the year 2002. The first map was generated based on satellite data from the Moderate Resolution Imaging Spectroradiometer (MODIS) and Satellite Pour l'Observation de la Terre (SPOT) sensors combined with agricultural inventory data, which the pasture areas are available as grid cells with different pasture fractions (from 0 to 1) and ≅ 10 km of spatial resolution. The second map was generated based, mainly, on satellite data from Landsat Enhanced Thematic Mapper Plus (ETM+) sensor (30 m of spatial resolution), which the pasture areas were classified as cultivated pasture in shapefiles format. The comparison between these maps was performed using the PROBIO map to generate a national pasture map with grid cells representing pasture fraction (same spatial resolution of the global map, ≅ 10 km). They presented a spatial autocorrelation of 0.49, considering all the pasture fraction for both maps (0 to 1). A map of differences was generated and made possible to identify the most discrepant regions. The differences could be associated to the PROBIO pasture classification, which denoted a specific class for cultivated pasture while some areas present a predominance of natural pasture. Due to the temporal and spatial differences between these maps, some discrepancies would be also related to land use change and intensification process. To address these changes, samples points were chosen and their spectral behavior will be also analyzed using the temporal profile of the Normalized Difference Vegetation Index (NDVI) from MODIS sensor for years 2000 and 2002. These results could represent an important indicative for analyzing pasturelands from different maps and to generate a concise database to identify pasture intensification and yield gap analyses across Brazil.
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Identifying potential new genes related to lignocellulose degradation using transcriptome and gene co-expression network analysis in *Trichoderma reesei* RUT-C30

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Our dependence on fossil fuel sources and the concern about environmental changes have generated a worldwide interest to explore new alternative sources of fuel and energy, such as second-generation (2G) ethanol produced from lignocellulosic biomass. In Brazil, sugarcane bagasse has been proposed as a promising residue for this biofuel production due to its abundance and low cost. However, biotechnological challenges must be overcome to make this fuel an economically viable substitute for fossil fuels. In this regard, it is necessary to reduce the cost of enzyme mixtures used to break down the lignocellulose and release the fermentable sugars. The biomass-degrading fungus *Trichoderma reesei*has been considered a model for cellulose degradation because it has the capacity to produce and secrete a large amount of enzymes which have been used in several cocktails. In spite of all genetic efforts that have been made, it is not completely clear how this fungus use its cellulolytic system to deconstruct the plant cell wall neither the fine-tuning regulatory elements involved in this process. In this work, we investigated the transcriptome of *T. reesei* RUT-C30 strain grown on sugarcane bagasse by RNA-Seq at three time points (6, 12 and 24 hours) and evaluated the co-expressed genes. Our goal was to find out which genes are activated or repressed under this condition and identify co-expressed genes that have not been characterized so far. Several differently expressed genes (DEGs) were found upregulated on bagasse, including CAZymes, sugar transporters, transcription factors and uncharacterized genes coding for proteins with signal peptide. It was possible to calculate the correlation between the DEGs using WGCNA package and group them in two subnetworks composed by up and downregulated genes. Several interesting genes were identified with high number of links (hubs) in each subnetwork, such as predicted transcription factors and genes coding hypothetical proteins. In addition, several small clusters were found within the subnetworks. GO and KOG analyses were carried out to provide more information about the classes of grouped genes. Numerous genes without any predicted function were co-expressed along with genes related to cell wall degradation. All together, the data indicate several genes which can be new targets for further studies in order to improve the cellulolytic potential of *T. reesei* strains and decrease the cost of enzymatic cocktail used for 2G ethanol production.

Physiological plasticity is important for maintaining sugarcane growth under water deficit

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The water availability at early phenological stages is critical for crop establishment and sugarcane varieties show differential performance under drought. Herein, we evaluated the relative importance of morphological and physiological plasticity of young sugarcane plants grown under water deficit, testing the hypothesis that high phenotypic plasticity is associated with drought tolerance. IACSP95-5000 is a high yielding genotype and IACSP94-2094 has good performance under water limiting environments. Plants were grown in rhizotrons for 35 days under three water regimes: high (soil water matric potential [Ψm] higher than 20 kPa at 0.15 m depth); intermediate (minimum Ψm varied between -65 and -90 kPa at 0.15 m depth) and low (Ψm reached values lower than -150 kPa at 0.15 m depth) water availability. Our data revealed that morphological and physiological responses of sugarcane to drought are dependent on genotype and intensity of water deficit. In general, IACSP95-5000 showed higher physiological plasticity given by leaf gas exchange and photochemical traits, whereas IACSP94-2094 showed higher morphological plasticity determined by changes in leaf area, specific leaf area and specific root length. As IACSP94-2094 accumulated lesser biomass than IACSP95-5000 under varying water availability, it is suggested that high morphological plasticity does not always represent an effective advantage to maintain plant growth under water deficit. In addition, our results revealed that sugarcane varieties face water deficit using distinct strategies based on physiological or morphological changes. When the effectiveness of those changes in maintaining plant growth under low water availability is taken into account, our results indicate that the physiological plasticity is more important than the morphological one in young sugarcane plants.

pGVG: a new Gateway-compatible vector for transformation of sugarcane and other monocot crops

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Genetic transformation methods are a key biotechnological tool to improve crop yield. Although *Agrobacterium*-mediated transformation is successfully used in many cereals, most vectors are specific for dicots and based on the *CaMV35S* promoter, which generates lower expression levels in monocots. In this study we describe the construction and functional validation of a vector, pGVG, for stable and strong gene overexpression and silencing in monocots. This plasmid is based in the pCAMBIA2300 backbone, and presents an expression cassette comprising the maize polyubiquitin promoter *Ubi-1*, Gateway technology recombination sites, a FLAG-tag and a CaMV35S terminator. pGVG also has a selective cassette based in the *Ubi-1* promoter and the *NPTII* resistance gene. We showed that pGVG is efficient to delivery and integrate genes with different sizes into the sugarcane genome. The functionality of pGVG was demonstrated by overexpressing the *GUS* reporter gene, producing strong GUS activity in callus and transgenic plantlets. Several transgenes were used to transform sugarcane, and high levels of expression were observed by qPCR. We also used RNAi constructions, which resulted in effective gene silencing, even when targeting multiple genes in the same expression unity. Therefore, pGVG proved to be a versatile vector to overexpress and knockdown genes in an efficient way in sugarcane. Moreover, since the *Ubi-1* promoter and the *NPTII* gene have been

used in several other monocots, we believe pGVG may also be used in many other species.

Specific heat of acid suspensions of cassava bagasse: a necessary tool for designing heat transfer in bioethanol industry

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Biomass conversion into small chain sugars has been widely studied in order to optimize the bioethanol production. An enhanced hydrolysis of the residues is a result of both raw matter characteristics and pretreatment conditions. Cassava bagasse is a renewable source with interesting composition due its high contents of residual starch from starch extraction process, besides the presence of hemicellulose and cellulose. The development of an effective acid pretreatment can make viable its use in biofuel industry. The efficiency of such processes depends on the correct design of the unit operations, which is in turn dependent of thermophysical properties. Thermal properties, as specific heat, are associated with an accurate design of heat transfer processes. In addition to heating and cooling of slurries, specific heat (cp) is also useful for determining the acoustic fields produced in pretreatments assisted by high-intensity ultrasound. In this way, specific heat of acid suspensions of powdered cassava bagasse was determined by a differential scanning calorimeter (DSC 8000, Perkin Elmer)using standard sapphire disks as reference material. Analyses were carried out varying the solids concentration (0-10% w·w-1), phosphoric acid in the dispersant (0-10% w·w-1) and temperature (5-45°C). The values ranged between 3830.0 and 4142.6 J·kg-1·K-1, whereas the higher ones were acquired in suspensions with low concentrations of biomass and phosphoric acid at higher temperatures. The reduction in specific heat was probably related to the significant contribution of the low heat capacity of biomass and phosphoric acid. Carbohydrates, such as fibers and starch, have specific heat approximately three times lower than pure water at the same temperature. Similarly as reported for different foodstuff and dried residues, thecp of the slurries was also a linear function of temperature. As the system temperature increased, the slurries seemed to have higher capacity of storing energy. A polynomial model could be fitted to experimental values with high determination coefficient R^2=0.9214 and low mean relative error MRE of 0.48%. The modeling of cpat different conditions provides easy-to-use information to be applied in the development and control of acid pretreatments of cassava bagasse.

Inter-relationship between photosynthetic efficiency, $\Delta^{13}C$, antioxidant activity and sugarcane yield under drought stress in field conditions

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The aim of the present work was to associate gas exchanges, photosynthetic efficiency, carbon isotope discrimination and antioxidant activity with the yield of sugarcane varieties submitted to drought stress under field conditions. Six sugarcane varieties were submitted to drought stress in three phenological stages: tillering, intense growth and ripening. In the tillering phenophase, all varieties were affected similarly under water stress, presenting reduced gas exchange, resulting in high leaf temperature and increased intrinsic water use efficiency. In addition, the increase in carbon isotope discrimination (Δ13C) and the bundle sheath leakiness (ϕ)

were more evident during the tillering phase in stressed plants. In the intense growth phenophase, water stress caused more intense physiological disturbance. In general, intense reduction of stomatal conductance, transpiration, increased leaf temperature, intrinsic water use efficiency, increased activity of catalase and ascorbate peroxidase antioxidant enzymes and lipid peroxidation were observed in the most sensitive to drought varieties RB72454, RB855113 and RB855536 in this phenophase. Also, the photosynthetic apparatus was also more affected, with reduced photosynthetic capacity, maximum and effective quantum efficiency of photosystem II, chlorophyll content and SPAD index. Therefore, intense growth phenophase can be considered the best to discriminate sugarcane genotypes most sensitive and most tolerant to drought. In all phenophases, the variety RB92579 maintained better physiological homeostasis during drought, with maintenance of the integrity of the photosynthetic apparatus and defense against oxidative stress, being the most productive under drought stress. This work suggests that sugarcane breeding programs should consider photosynthesis, quantum efficiency of photosystem II, total chlorophyll content, SPAD index, leaf temperature, lipid peroxidation and carbon isotope discrimination as potential physiological indicators for the selection of more tolerant sugarcane varieties to drought stress. The best phenophase to screen sugarcane genotype to drought stress should be performed during the intense growth phase.

Penicillium citrinum whole cells catalyst for treatment of lipid-rich wastewater

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Dairy industry wastewater contain high levels of lipids (oils and greases), being often dumped into watercourses without previous treatment. Due to the low solubility in water and solidification at room temperature, the lipids cause many problems to the anaerobic digestion like toxicity to acetogenic and methanogenic microorganisms, biomass flotation, foams formation in the reaction medium and decreased adenosine triphosphate (ATP) concentration, reducing the effluent biodegradability. As an alternative the enzymatic technology has been used in the recent years to the development of products and processes less aggressive in environmental terms. However, factors associated with the high cost of obtaining the enzymes, related to the extraction and purification steps, make it impossible to apply them on an industrial scale. In this context, whole cells from the filamentous fungus *Penicillium citrinum* URM 4216 was prepared and used to perform the enzymatic hydrolysis of lipid-rich wastewater. The whole cells were cultivated in a basal medium containing per litre: carbon source (30 g), nitrogen source (70 g), NaNO3 (1 g), KH2PO4 (1 g) and MgSO4.7H2O (0.5 g) in flasks under orbital agitation. After 96 h of incubation, biomass was separated from the culture broth by filtration, washed with water and acetone and dried under vacuum for 24 h. Lipase activity was measured both dry biomass (mycelium-bound lipase) and filtrate (extracellular lipase). The mycelium-bound lipase production was maximized and physicochemical parameters, such as carbon (olive, soybean, colza and sunflower oils) and nitrogen (soy peptone, bacterial peptone and yeast extract) sources, initial pH of the medium (5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0), temperature of fermentation (30; 35 and 40°C) and inoculum size (10^5; 10^6; 10^7; 10^8; 10^9 spores) were studied to determine the best conditions for mycelium-bound lipase production. Olive oil and soy peptone were found to be the best carbon and nitrogen sources, respectively, and the adjustment of the culture broth to pH 7.0 and 30°C of incubation temperature with inoculum size of 10^9 spores improved the mycelium-bound lipase activity.

Under the optimized conditions, the whole cells were prepared and showed the lipase activity of 280 ± 18 U g^{-1} against only 15.2 ± 1.4 U g^{-1} to the filtrated, indicating the retention of lipase into the mycelium. The performance of whole cells was evaluated in hydrolysis of a crude dairy wastewater. The hydrolysis assays were carried with 10% m/m of biocatalyst (6500 U) in a reactional medium containing dairy wastewater (DQO 50000 mg L^{-1}) under orbital agitation at 37°C. After 6 h of reaction, the dairy wastewater treatead showed free fatty acid of 271.2 ± 18.1 mmol L^{-1}, almost 12 times most then non-treated dairy wastewater, indicating the potential of *P. citrinum* whole cells to be used in pretreatment of lipid-rich wastewater.

Evaluation of sugarcane genotypes under a twelve-month planting cycle

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In the Center-South region of Brazil, sugarcane has three planting seasons. The first one between January and April, which is known as eighteen-month planting cycle; the second is known winter planting cycle, which is characterized by the planting carried out between May and August; and finally, the planting held between September and November which is known as twelve-month planting cycle. The aim of this study was to evaluate sugarcane genotypes under a twelve-month planting cycle. The experiment was carried out at the Agricultural Science Center of the Federal University of São Carlos, located in the Araras city, São Paulo State. Fifteen genotypes were evaluated in a randomized block design with four repetitions. The plots consisted of four 12-meter long rows, spaced at 1,4m. The variables analyzed were: number of plants per meter (from the 1st to the 8th month after planting); flowering and pithiness; height, diameter and weight of stalks; sucrose content (POL%C); fiber content (%); tons of sugarcane per hectare (TCH) and tons of sucrose per hectare (TPH). According to the results, genotypes RB005983, CTC-20, CTC-14, RB975201, RB975242 and RB965902 showed higher TCH, with values above 122. It is important to note that the better genotypes classified into TCH also presented the higher values of height. According to the results of TPH, the genotypes RB005983, CTC-20, CTC-14, RB965902 and RB975201 were the most suitable to the twelve-month planting cycle, because they presented the higher values, ranging from 20.22 to 24.36, and non-flowering. In addition, the genotype RB975375 can be an excellent option to twelve-month planting cycle, even with flowering, due to good yield in TCH (106,51) and TPH (19,84). Greater productivity can be achieved also by the correct management of genotypes and this study was important to indicate the better genotypes for twelve-month planting season.

Classification trees for delving into sugarcane production in Brazil under climate change

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While several studies have been developed for understanding the impact of climate change on Brazilian sugarcane production, the underlying mechanisms that lead to increase or decrease in production are seldom evaluated. This may be due to the complexity of analyzing the great and intricate amount of data of the outputs from the simulation models. Decision trees are powerful tools to deal with complex data for their ability of using different types of response variables, of interactive exploration, description and prediction and of providing graphical

interpretation of results involving interactions. Thus, they allow us to both quantify and better understand the impacts of the variables in each process. This approach could lead to insights when searching for improved varieties. We simulated the effects of climate change in the production using both the DSSAT/Canegro and the APSIM-Sugar models in three different locations, under two different soils, with three planting dates, two cultivars, irrigated and rainfed. As for the climate change scenarios, they were generated by two different techniques for twenty-six different General Circulation Models (GCMs) and two different Representative Concentration Pathways (RCPs), for two different periods, plus the baseline. This yields 15,048 combinations and since each is repeated 30 times, there are 451,440 results. We then analyzed them by using decision trees. From the daily outputs of the simulations, we created variables to group the effects in four quarters of the cycle. We also discretized the results considering the deviation of one standard deviation from the baseline years and created two classes for dry mass sucrose content: lower and equal or higher. Three levels of trees were developed: one referring to the high-level scenario description, one to the boundary conditions (weather and soil) and one to the growth process variables. In the high-level trees, we observed the DSSAT-Canegro model was more severe in its predictions, leading to most cases in which sugar content decrease was observed. These cases were often detected for the later planting date both in one expansion area of sugarcane and, in the cases of more rigorous scenarios, in sandy soils of the other locations evaluated. From the boundary tree, we noticed all these effects were mainly led by higher temperatures in the third quarter of the cycle associated to either higher temperatures in the beginning of the cycle or low soil evaporation in the last quarter. Finally, the low-level tree showed these conditions increased plant transpiration in the third quarter and daily biomass increase in the last quarter. This approach allowed for analyzing the growth of sugarcane for several climate change scenarios that had not been previously considered, and stablishing in more detail the potential negative effects of climate change on the crop results.

Proteomic analysis of microalgae *Chlamydomonas reinhardtii* under nitrogen deprivation in photoautotrophic conditions

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Microalgae have high potential as a feedstock for production of biofuels and chemicals. It stands out for the high capacity of lipid biosynthesis (triacylglycerol). However, it is not a commercially feasible option yet, due to the inversely correlation of high lipid concentrations and high biomass productivity in most of microalgae species. Previous studies used overexpression and inactivation approaches of specific genes found in biochemical pathways of lipids and starch biosynthesis, in order to increase triacylglycerol (TAG) production. However, most of these studies did not achieve the expected success. Furthermore, most of the previous studies about lipid accumulation in *Chlamydomonas reinhardtii*, under nitrogen (N) deprivation, used organic carbon source for cultivation (mixotrophic conditions), which do not contribute to air bioremediation and increase the costs of oil production. In this research, we aim to investigate the biological and regulatory networks involved in the accumulation of lipids in response to N deprivation in photoautotrophic condition. For this purpose, we intend to use integrative analysis of profiling gene transcripts of transcription factors and regulatory factors, the total and regulatory proteome and metabolome of cells in a time

series approach and photoautotrophic conditions. So far, we identified the proteome in a 24 h time course experiment of N deprivation. The *Chlamydomonas reinhardtii* strain CC503 were cultivated in 2L bioreactors in high-salt medium (HSM) medium at 26° C in autotrophic growth conditions with continuous light illumination (90 µE.m-2·s-1) and constant aeration (0,5 L.min-1) in 0.04% CO2. Cells were harvested in a 24h time-course design with 10 time-points (TPs) in the transition from N-replete to N-starved conditions and a proteomic shotgun analyses (Label-free LC-MS/MS) was carried out. The proteomic analysis identifies 525 proteins that were found in at least one-time point with high reproducibility and indicated the existence of clusters of proteins with similar expression patterns. Proteins ranging from regulatory phosphates to primary metabolism showed to be modulated. A study using integration of "omics" data in a systems biology approach can generate comprehensive information in order to understanding complex phenomena, such as lipids accumulation.

Allelic expression bias in sugarcane revealed by an SNP-based mapping

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Polyploidy is an inheritable condition that has more than two chromosomes set occurring in the cell. Modern cultivars of sugarcane ⟨Saccharum spp.⟩ are complex polyploids that have multiple copies of homoeologous chromosomes. Several studies have shown that allelic expression is sensitive to dosage effects and may be influenced by many regulation mechanisms. Composition and dosage impacts in the allelic expression are poorly understood in sugarcane. For this reason, an SNP-based approach was used to investigate ratio between genomic allelic dosage and allele expression through different sugarcane tissues. The RNA-Seq was obtained from leaf, root, bud and stem of SP80-3280 and IACSP93-3046 hybrids. To estimate genomic dosage a total of 290 SNP loci genotyped by Mass Spectometry ⟨Sequenom®⟩ were analyzed on SuperMASSA software. The loci sequences were used as reference for mapping RNA-Seq data using bowtie2. The variants ⟨SNPs and Indels⟩ were called using freebayes software. The vcf files were filtered using *in house* scripts to identify the SNP loci and allele frequency based on mapping results. A total of 211 loci had allele expression information distributed among tissues and genotypes. The majority of the genes have frequencies from allele expression ⟨RNA-Seq⟩ and allele dosage ⟨genotyping⟩ correlated, whereas expression is proportional to allele dosage. It was observed expression pattern tissue-specific and genotype-specific. Some genes had their expression pattern unchanged independently of the genotype or tissue observed. There are evidences that at least 10% of the loci investigated have allelic bias, whereas one allele is preferentially expressed. Based on this observation, probably some fraction of these homoeologous genes are unequally expressed. These finds are the first report on allelic expression bias in sugarcane and have a great impact on our understanding about molecular mechanisms related to allelic expression dynamic in sugarcane.

Technology roadmap in urban and agricultural wastes

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Large cities and metropolitan areas, such as Campinas, concentrate the generation of waste, most of organic origin. Among these are solid wastes, sewage sludge, and green waste. Organic waste or by-products of agriculture are more scattered, except in some vertically integrated agro-industrial activities, such as the sugarcane. In this case, large amounts of vinasse, bagasse, and filter cakes are generated in a localized manner, thus finding more feasible conditions for a proper disposal in the environment or to be treated and recycled. However, in general, the treatment and/or the transformation of urban and agricultural wastes into new products are the main goals to be met by municipal/state administrations and industries throughout the country. Most local administrations are behind schedule for that – only 10% of the municipal administrations have concluded their MSW plans. On the other hand, there is a consensus that mature commercial technology exists (in most cases), and also a good critical mass of researchers with expertise to design efficient, economically viable and environmentally sustainable waste processing chains. However, many uncertainties remain concerning desired products, technologies/processes, value chains models, and what is the level of consensus between different stakeholders from industry, academia and government from each subject. In order to understand the needs, technological capabilities, gaps and barriers, and identify solutions for the main issues a wide workshop was held on Aug 31-Sep 01, 2016 (Campinas, SP). The main opportunities for Campinas Metropolitan Region is the conversion of MSW, green residues, sewage sludge and sugarcane vinasse into bioenergy (biogas from biodigestion), biofertilizers (organic and organo-mineral fertilizers), and recycling. The main gaps and challenges are related to the i) lack of public policies, incentives and laws (mainly standards qualities for new bioproducts); ii) low quality of the MSW; iii) low interaction between municipalities and private sector to solve the problems and implement viable solutions; iv) high cost of logistics; v) lack of sustainability studies; and v) technological issues (improvements in biofertilizers production and biodigestion, scaling-up, development of new products, and other). In conclusion: i) the Metropolitan Campinas Region has the infrastructure, manpower, public scientific institutions, and private sector to implement economically viable and environmentally sustainable waste value chains; iii) MSW use is far from the set goals for bioenergy production and recycling of nutrients, which adds to the challenges; iv) vinasse is presently recycled (ferti-irrigation), however is not employed to produce bioenergy (only some cases) or new products. A good example of success is the recent partnership signed between the Campinas City Hall, Instituto Agronômico and SANASA (Campinas Water Supply and Sanitation Company) to the project “Green Recycling” to convert approximately 100 ton per day of green residues by composting into 75 ton per day of organic fertilizers.

Temporal gene expression profiles of sugarcane infected by *Puccinia kuehnii*

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Reported in Brazil less than one decade ago, orange rust is a sugarcane foliar disease caused by the biotrophic phytopathogen *Puccinia kuehnii*. Due to its increasing economic importance, understanding its mechanisms of infection is fundamental. The purpose of this

work was to evaluate, by RNA sequencing, how P. kuehnii can cause changes in the sugarcane transcriptome during disease establishment. In addition, examining the temporal changes in gene expression can contribute to provide a better overview of the infection process. The biological material used to generate the temporal libraries were sugarcane leaves from the susceptible cultivar SP89-1115 sampled at the time of P. kuehnii spores inoculation (0 h), 12 hours post infection (hpi), 24 hpi, 48 hpi, 5 days post infection (dpi) and 12 dpi. After sequencing, the reads were pre-processed and approximately 800 million of them could be mapped to a reference transcriptome obtained from six different sugarcane genotypes. We then performed differential expression analysis in three ways: i) an Analysis of Variance type test; ii) a comparison of each time after inoculation against the first time point (0 h) and iii) a comparison of adjacent times. We also conducted functional enrichment analysis to identify processes in which the differentially expressed transcripts were involved. When adjacent time points were compared, transcripts that showed significant changes in expression were detected mainly at 12, 48 hpi and 12 dpi, most of them being related with phoyosynthesis and oxidation. They could reflect the action of the pathogen in disturbing the metabolic maintenance and cellular signaling. Transcripts in the comparison between 0 h and 48 hpi showed similar levels of expression, and based on the gene expression profiles of Gene-Ontology-annotaded transcripts, we observed repression and stimulus waves between 12 hpi vs 0 h and 48 hpi vs 24 hpi. Therefore, two interpretations of the results were formulated, and one does not exclude the other. The first considers that repression of defense response pathways, like phytohormones signaling and lignin deposition in the cell wall, may have occurred by the action of P. kuehnii in the first hours of inoculation. The second takes into account that, being susceptible, the sugarcane cultivar may have failed to recognize the pathogen and/or to control the secreted effector proteins, enabling its modulation for fungal establishment. There is evidence of partial reestablishment of cellular homeostasis at 48 hpi, indication that this condition may have been important for *P. kuehnii* development.

Unraveling co-expression networks in sugarcane ancestral genotypes

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The interest in bioenergy crops, such as those belonging to the Saccharinae subtribe, in particular *Saccharum spontaneum*, has increased due to improvements in technologies related to the production of cellulosic bioethanol. Understanding the flow of biological information underlying complex traits, such as sucrose and biomass yield, is imperative for the establishment of reliable breeding strategies. Taking this into account, a gene co-expression network approach is a promising option since it captures biologically important patterns in gene expression data. Using a customized oligoarray for sugarcane (CaneRegNet - Agilent Technologies), we obtained the expression profile of immature and intermediate internodes and leaf+1 from three sugarcane ancestral genotypes: *S. officinarum* (caiana listrada), *S. spontaneum* (IM76-229) and *S. robustum* (IN84-058); and a commercial hybrid, RB867515. In order to detect clusters of highly co-expressed genes (modules), we analyzed the transcriptome data using the Weighted Gene Co-expression Network Analysis (WGCNA) R package, to the normalized, log2-transformed expression matrix. In this study, we identified 33 modules, of which 27 were significantly correlated (from 0.59 to 0.97) with at least one of the following traits: tissue, plant heigh, culm fresh weight, degrees Brix, and total soluble sugar, reducing

sugar, sucrose and lignin contents. We observed three modules that contrast in their correlation to important features of carbon partitioning: two of them showed a positive correlation with sucrose content (0.71 and 0.72) and a negative correlation with lignin content (-0.71 and -0.61), respectively; the other showed a negative correlation with sucrose content (-0.63) and a positive correlation with lignin content (0.89). These modules were further evaluated to verify their validity as targets for sugarcane and energy cane breeding. The most commonly gene ontology (GO) terms found in the modules show a variety of processes related to primary metabolism and biosynthesis. This is expected since previous analysis show sucrose content and lignin metabolism are associated to various signals and biological processes. We also identified the connection between top nodes and other genes in their respective modules. Although the annotation of these top nodes remains unknown, based on the guilt-by-association principle, we assume that genes sharing similar expression profiles may be involved in the same regulatory pathway. Given the recent sequencing of the sugarcane genome and identification of alleles, we need to update the gene probe annotation. In addition, the discovery of new transcripts by RNA-seq, in combination with the examination of allele-specific expression, may allow for a better interpretation of the obtained results. Finally, the next step to fully describe the link between genotype and phenotype, as well as to understand underlying gene regulation, is to elucidate the coordination of networks on different molecular levels (gene, protein, metabolite).

Genetic parameters estimates for sugarcane technological traits through REML/BLUP and multivariate analysis in different harvest times

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The estimation of genetic parameters is of great importance to the breeding programs contributing to the choice of the most appropriate breeding method, traits to be select at the beginning and in advanced stages of the breeding program, as well as, in the genetic gains prediction by selection. This work aimed to estimate the genetic parameters and to predict the genotypic values of 100 sugarcane genotypes using REML/BLUP (Maximum Restricted Likelihood/Best Unbiased Linear Predictor) and principal component analysis. This approach was use to select superior genotypes of two distinct agronomic groups: high fiber genotypes (group 1) and genotypes with potential to accumulate sucrose (group 2). Three experiments corresponding to three harvest times were conduct at May, July and September in a randomized complete block design with four replicates. For each experiment, five technological variables (Brix, Pol, purity, reducing sugar and fiber) were evaluated. Multivariate analysis of variance (Manova) was performed considering the five technological variables and principal component analysis was applied to discriminate genotypes for sugar and fiber production. The genotypes differed by considering all variables at one time. In the joint analysis of the three experiments for group 1 genotype IN8458 presented the highest genotypic value of fiber and IACSP043150 the highest genotypic value of Pol. Pol and Fiber heritability had high magnitude indicating the possibility of high

accuracy selection. Group 2 showed change in the ranking of genotypes between seasons and harvest times. In addition, genotypes with short to long useful industrialization periods were identified.

Decision-making of the correct period and N fertilizer rate to be applied in sugarcane field in Brazil

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The low efficiency of nitrogen fertilizers is a major concern worldwide, threatening the sustainability of sugarcane production. Improve N use efficiency (NUE) by sugarcane, which can be achieved by adopting best fertilizer management practices, can reduce environmental impacts. Therefore, our objective was to assess decision making on the time of N-fertilizer application and its rates during the crop season in the south-central region of Brazil. For this, three experiments were established with the same design (split plot) and evaluated for two years. The main plots were different N-fertilizer application time (0, 30, 60, 90, and 120 days after harvest - DAH), and in the split plot, five N rates (0, 50, 100, 150 and 200 kg N ha-1). Each experiment was harvested in a different period during the same crop season: the first at that season's beginning (April), the second in mid-season (August) and the third in end of season (October). Both the harvest season and time of N fertilizer application modified the sugarcane yield. The N-fertilizer applied until 60 DAH increased the yield in the experiment harvested at the crop-season beginning. In the area harvested mid-season, the best time to apply N-fertilizer started at 90 DAH. However, the N-fertilizer application times did not differ at the end of the crop season. Therefore, it is important to highlight that the best time to apply N in our three experiments is when the soil has moisture. The N rates that obtained the highest sugarcane yield were 130, 140 and 100 kg N ha-1, respectively, in the experiments harvested at the beginning, middle and the end of the crop season. Also, calculated in each area was the N economical rate that offsets the fertilizer cost investments. The N economical rates obtained for the beginning, middle and end crop season were respectively, 90, 110, 85 kg N ha-1, demonstrating that the gains obtained by applying considerable amounts of fertilizers (rates above 120 kg ha-1 of N) may not cover the investment in N-fertilizer.

TOR: The central hub integrating primary metabolism and growth in plants

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Plant growth is controlled by metabolic network regulation, development and carbon partitioning between sink and source organs. There are now considerable genetic and biochemical evidences that the target of rapamycin (TOR) kinase is one of the key pathways in sensing and transducing sugars to control growth. We used genetic and pharmacological approaches to further investigate the kinetics of TOR inhibition in plants under different photoperiods to identify the mode of action of this pathway in controlling C metabolism. By combining metabolic profile, biochemical assays and gene expression analyses, we showed that down-

regulation of the TOR complex (TORC) leads to accumulation of starch in the light, but this was not linked neither to allosteric regulation nor for the redox regulation of ADPG pyrophosphorylase (APGase). Surprisingly, the levels of maltose, the main starch breakdown product, were lower in plants exposed to short-term inhibition of TORC when compared to the control, suggesting an impaired starch breakdown at dawn. In addition, massive changes in organic acids from the TCA cycle clearly show that TOR influences not only C metabolism related to storage and use of sugars but also affect the energetic status of the plants. Furthermore, massive changes in the levels of transcripts and proteins involved in starch metabolism and sugar transport revealed a role of this pathway in keeping sucrose homeostasis to sustain plant growth.

A post-transcriptional regulation of NRT2.1/NAR2.1 transport system likely limits nitrate uptake in sugarcane (Saccharum spp.) roots

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Improving the efficiency of nitrogen (N) uptake in crop systems is a global challenge to reduce N losses and the consequent environmental impacts. Sugarcane (*Saccharum* spp.) is the most suitable energy crop for biofuel feedstock; however, the reduced recovery of N fertilizer by sugarcane roots challenges its sustainability and competitiveness. This low N recovery has been associated with a low nitrate uptake by sugarcane roots, which differs from the major C4 grain crops, such as sorghum and maize. To understand the regulatory control of nitrate uptake in sugarcane roots, we used RNA-seq analyses to define both sequences and expression profiles of the major nitrate transporter genes differentially expressed among contrasting N treatments. In N-sufficient sugarcane roots, a predominant transcriptional mechanism for modulation of the nitrate high-affinity transport system was shown by 15N-nitrate uptake correlation with NRT2.1 and NAR2.1 expression in roots upon N deficiency or inorganic N (ammonium or nitrate) treatments. Conversely, in N-deficient sugarcane roots, the transcription induction of NRT2.1 and NAR2.1 did not correlate with the markedly repression of nitrate uptake in response to nitrate re-supply or high N provision, indicating the existence of a post-transcriptional regulatory mechanism. These findings suggest that high-affinity nitrate uptake is regulated at transcriptional and post-transcriptional levels in relation to the physiological status of the plant and, that, post-transcriptional regulation of NRT2.1 and NAR2.1 activity is likely a determinant mechanism for discrimination against nitrate uptake observed in sugarcane roots, contributing to the low nitrate use efficiency in this species.

Effect of dose and time of application of ripeners on the raw material quality and residual in the sugarcane

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The management of the beginning of the sugarcane harvest season should be done with varieties of early maturation, but this period is extremely dependent on the climatic conditions, since under unfavorable conditions provide low sugar content, causing quantitative and qualitative losses of the raw material, making the use of ripeners of fundamental importance to achieve satisfactory yields. The objective of this study was to evaluate the efficiency of the application of three ripeners (glyphosate, trinexapaque-ethyl and sulfometurom methyl), and the changes in pre

and post-harvest technological characteristics, and the amount of residues of ripeners as a function of time of action of the products. The study was carried out with the variety RB966928, in plant cane and ratoon, in the 2014-2015 and 2015-2016 crops in Igaraçu do Tietê, SP. The treatments consisted of the application of glyphosate, at the dose of 0.45 L ha-1 of commercial product (CP); trinexapaque-ethyl, at the dose of 0.80 L ha-1 of CP; sulfometurom methyl, at the dose 0.02 kg ha-1 of CP, and control, applied in five seasons 60, 45, 30 and 15 days before harvest (DBH) (from March to May 2015). There was an increase in sucrose content at different application times, with a higher magnitude for glyphosate at 0.45 L ha-1, followed by sulfometurom methyl 0.02 kg ha-1 and trinexapaque-ethyl 0.80 L ha-1. Glyphosate impaired stalk yield (TCH), pol yield (TPH) and sugar yield (TAH) in both plant cane and ratoon, from 45 DBH. Sulfometuron methyl 0.02 kg ha-1 and trinexapaque-ethylic 0.8 L ha-1 increased TPH and TAH only with application at 60 DBH. Sulfometuron methyl maintained pre and post-harvest chlorophyll levels at 60 DBH. There was detection of residues from the ripeners in different sections of the plant, with greater concentration in the tops with applications between 15 and 45 DBH, as well as in regrowth. Despite the greater gains in raw material quality provided by glyphosate, this ripener reduced stalk and sugar yield. The responses on the ripening and raw material quality are dependent on the time of action of each ripener on the sugarcane.

Prospection and expression analysis of expansin genes involved in sugarcane cell wall formation

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Expansin plant proteins are involved in no hydrolytic cellulose modifications during several biological processes such as cell enlargement, softening of fruits and abiotic stress tolerance. Different studies have demonstrated potential biotechnological applications of these proteins in crop improvement to enhance biomass and in lignocellulose enzymatic saccharification processes. Expansin gene family is organized in three subfamilies: alfa-expansin (EXPA), beta-expansin (EXPB) and expansin-like (EXPL). EXPBs seems to be more effective on cell walls of grasses. The present study identified gene members of the Expansin superfamily in sugarcane partial genome and transcriptome databases. In order to do this, proteins sequences of rice, maize, sorghum, Brachypodium and sugarcane sequences were analyzed phylogenetically by Maximum-Likelihood method. The bioinformatic analysis provided 166 transcripts of Expansin, which could be arranged in homologous groups, yielding 24 EXPA, 27 EXPB, and 4 EXPL in sugarcane. Quantitative real time PCR analysis of six EXPB (ScEXPB4, ScEXPB6, ScEXPB12, ScEXPB25, ScEXPB27 and ScEXPB28) and 1 EXPA (ScEXPA25) transcripts was performed in different internode samples of two sugarcane contrasting genotypes. The comparative expression analysis showed that ScEXPB28 was the highest expressed gene. These results may help the selection of a specific Expansin gene acting in the structural organization of the cell walls from sugarcane stem tissues.

Characterization of ABA-activated kinases in sugarcane: cloning, biochemistry and crystal structure

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Water availability is one of the biggest challenges faced by Brazilian sugarcane producers. It impacts crop production and limits sugarcane cultivation areas. Nevertheless, the physiology of drought response in sugarcane is poorly understood. Abscisic acid (ABA) is one of the major plant hormones involved in different types of biotic and abiotic stress responses. SnRK2s proteins make up a subfamily of ABA-activated protein kinases that play a central role in ABA-mediated cellular processes such as stomata opening and closing, seed germination, root growth and abiotic stress tolerance. Knocking out the three SnRK2s genes in Arabidopsis thaliana resulted in ABA-insensitive plants that highly sensitive to drought stress. Here we combine genetic, biochemical and biophysical methods to investigate the role of sugarcane SnRK2 proteins. We identified and isolated 3 SnRK2s from the sugarcane genome with high identity levels to homologous genes from both monocotyledons and dicotyledons. Recombinant sugarcane SnRK2 proteins were purified from an E. coli heterologous expression system. Biochemical assays showed all three proteins are active kinases and capable of autophosphorylation in vitro. We also solved the crystallographic structure of one of the sugarcane SnRK2s. Together with in vitro phosphorylation assays, analysis of the crystallographic structure suggests a novel regulatory mechanism for this kinase subfamily. To evaluate the importance of SnRK2 proteins to sugarcane development and response to drought, we are currently performing studies with transgenic plants engineered to express either reduced or increased levels of these kinases. Our findings will further our understanding of how SnRK2 proteins convey ABA hormone signaling during drought response in sugarcane.

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Calorific value of urban forest wooden residues

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The pruning practices and removal of trees of urban areas are responsible for generating large amounts of wood residue. In Brazilian cities, they are commonly intended for dump areas, open-air burning or disposed in landfills. With the approval of the Law N° 12,305 - National Policy on Solid Waste, in August 2010, these practices are now prohibited and counties need to implement technological solutions that allow the valuation of these materials. One of the alternatives with great potential for solving this problem is the use of urban forestry wood waste as a source of energy, either in the simplest forms such as firewood or chips, or in energy products processed as pellets or briquettes. This study intended to make the physical characterization (bulk density and moisture content) and the determination of the high calorific value and lower calorific value of the wood residues from the branches of 7 species most commonly used in the urban forestry in the State of São Paulo, Brazil. Composite samples were obtained from the branches of three trees of each of the following species: canelinha (*Nectandra megapotamica*), chapéu-de-sol (*Terminalia catappa*), ficus-benjamin (*Ficus benjamina*), flamboyant (*Delonix regia*), oiti (*Licania tomentosa*), sibipiruna (*Caesalpinia pluviosa*) and tipuana (*Tipuana tipu*). The tests were performed according to NBR 8633 (ABNT, 1984). The bulk density of the species ranged from 0.170 to 0.185 g/cm³ and the moisture content from 13.01 to 37.75%. Although there was little variation between species high calorific value, it was possible to obtain three levels of classification. The lowest, composed of ficus, canelinha and sibipiruna ranged from 4609 to 4626 kcal/kg, the intermediate, composed of

flamboyant, oiti and tipuana varied from 4676 to 4700 kcal/kg and the highest was 4750 kcal/kg, referring to the chapéu-de-sol. The lower calorific value varied in the same way in relation to the species and classes, with values from 4305 to 4322 kcal/kg for the first class, 4372 to 4396 kcal/kg for the second and last class composed only by the chapéu-de-sol, with 4446 kcal/kg. This indicates that although it did not vary much between the species of wood, the high and lower calorific value of the chapéu-de-sol was higher than in the other species. The seven species were considered to be similar to the most used wood for energetic purposes in Brazil, such as eucalyptus, presenting great potential in the use as an energy source, a promising alternative for the counties that until now face problems for the adequate destination of these residues.

Eucalyptus for energy: assessment of long-term yield simulations to improve forest planning and management

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Eucalyptus is the most planted hardwood tree in the world. In Brazil, it is the most cultivated genus for reforestation, representing 72% of the 5.6 million ha of total planted forests area in the country. About 46% of the Eucalyptus yield is destined to energy generation (firewood and charcoal), which demonstrates the importance of this genus, as a renewable resource, in the Brazilian energy scenario. In view of the great edaphoclimatic variability of Brazil, a large variability of Eucalyptus yield is observed. In this context, the hypothesis of this study is that the use of long-term yield simulations can be an important tool to increase resilience, planning, and yield of Eucalyptus forests. Thus, the aim of this study was to carry out long-term simulations of potential and attainable yield and relative evapotranspiration (the relationship between actual and maximum forest evapotranspiration) of Eucalyptus in different producing regions of Brazil. For this, the Agroecological Zone Model – FAO (MZA – FAO) was adapted, calibrated and evaluated with observed Eucalyptus yield data and soil water holding capacity of different locations and years. The calibration process considered 103 observed Eucalyptus yield data, whereas the model performance was evaluated with 35 independent observed yield data from ten different locations in southern Brazil, from 2006 to 2013. The calibrated model was applied to the following locations: São Gabriel (RS), Telêmaco Borba (PR), Mogi Guaçu (SP), Bocaiúva (MG), Brejinho de Nazaré (TO) and Urbano Santos (MA). In these locations, simulations of potential and attainable yields were carried out from 1980 to 2016, assuming that an Eucalyptus cycle of seven years. The MZA – FAO model presented a satisfactory performance for estimating Eucalyptus yields in different locations and years of evaluation (R2 = 0.90 and 0.96; d index = 0.99 and 0.99 in the calibration and evaluation processes, respectively). The long-term simulations were able to identify the spatial and temporal climate variability in Brazil. The yield gaps by water deficit were on average 7.8% (37.3 m3 ha-1), 12.5 (61.3), 13.7 (63.0), 27.1 (154.4), 35.7 (198.0) and 35.9 (215.9) in the PR, SP, RS, TO, MG and MA, respectively. The accumulated water deficit during the seven-year cycle ranged from 605,8 mm (PR) to 4.635,0 mm (MA). The relative evapotranspiration ranged from 0.93 (PR) to 0.64 (MG). The analysis of yield gaps by the long-term simulations is a tool that can help forest companies to plan and manage the forests appropriately, according to the environmental conditions.

Different architectures of promoter region for the Scdr1 gene and its variants in sugarcane

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Changing environmental factors set a challenge for the development of agriculture. The control of these variations - in particular the availability of water - is fundamental for maintaining productivity and for increasing yields. Studies approach the water-plant relationships through the evaluation of *in vivo* experiments - physiological conditions - and *in vitro* - characterization of genes responsive to lack of water. Previous studies showed that heterologous expression of the *Scdr1* gene from Sugarcane in tobacco transgenic plants contributed to greater tolerance to water stress. The characterization of this gene, in particular of its promoter region (PR), has potential for biotechnological applications. However, the study included only the sequence of *Scdr1* gene. Sugarcane is known for its ploidy and it is likely that the expression of *Scdr1* gene is controlled by a complex regulation network. To contribute with knowledge of this system the present study aims to characterize the PR of the *Scdr1* gene using an *in silico* bioinformatics approach based on DNA sequences from the sugarcane genome draft of SP80-3280 variety and SAS DNA sequences from the SUCEST-FUN database. Initially we performed a similarity search of the *Scdr1* gene against the database using both nucleotides and amino acid sequences. Next, we used the Bedtools suite to extract the PR of the 13 genes obtained in the search, with a maximum size of 2000 nucleotides. Then we used MEME to process the set of PRs to detect motifs that could constitute potential Transcription Factor Binding Sites (TFBS). We were able to identify 6 motifs. For the 13 PRs identified, 6 presented the same set of motifs, and 2 of them, the same architecture. All TFBSs found in our analysis are responsible for anchoring transcription factors (TFs) with AP2/ERF, WRKY and Myb domains. We also observed that 4 TFBSs found in the 6 PRs showed dependent positioning. Of these motifs, two TFBSs showed conserved distances of 35 nucleotides. Furthermore, these two motifs are exclusively involved in the hybridization with TFs of AP2/ERF and WRKY domains. The other 2 TFBS showed a distance of 168 nucleotides, however each motif was associated with the TF of a different domain. One with the AP2/ERF domain TF and the other with the Myb domain TF. Despite the difference between the TFBSs in the 168 nt window, the two motifs are associated with drought-sensitive TFs. However, their responses are in independent paths of water stress signaling. This characteristic may contribute to the development of sugarcane more tolerant to drought, since this characteristic brings flexibility in the modulation of the *Scdr1* gene in front of the different environmental stimuli related to water stress.

Assessment of sugarcane breeding trials through the coefficient of variation estimate

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The sugarcane crop has great economic importance, occupying around 27 million hectares in more than 130 countries; the main technology with potential to contribute to the cane productivity and sustainability is associated with genetic improvement. Sugarcane breeding programs evaluate several genotypes in field trials under different environmental conditions seeking to identify those promising and those that should be discarded. For this, low estimates of the coefficient of variation (CV%) are desired, since they would present greater experimental precision, greater reliability in the obtained results and the materials would be properly

discriminated. The aim of this study was to evaluate the experimental quality of sugarcane breeding trials. For this, the agroindustrial traits data sucrose content (PC) and tons of sugarcane per hectare (TCH) were collected with support of the Sugarcane Breeding Program of the Federal University of São Carlos (PMGCA/UFSCar). The average value of CV% of each trait and to each parameter evaluated (growing environment, harvesting type, crop cycle, plot length, number of rows and number of repetitions) were submitted to the comparison by Tukey test at 5%. This work showed that the CV% value of TCH trait is influenced by all parameters under study, except for number of repetitions. CV% value of PC trait ranged according to the crop cycle and the growing environment. The experiments carried out by PMGCA/UFSCar have good precision and should be implanted on intermediate growing environments, with the experimental unit of five rows with 50 m in length. This study is important to guide and direct the trials of the breeding program throughout the process of selection of new cultivars.

Sugarcane data integration through SUCEST-FUN Project

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The high efficiency in sucrose storage and biomass accumulation has turned Sugarcane (*Saccharum* sp.) into a target crop for ethanol and sugar production, and more recently, bioenergy exploration. These features encouraged the investment in sugarcane breeding programs which led to the accumulation of a high amount of genetic, molecular and physiological data for many varieties in a wide range of conditions. Integrating this information is a key step to achieve a broader understanding of cane physiology and its relation with environment and climate changes, aiming the directional design of more productive varieties, also capable of growing in adverse conditions. SUCEST-FUN Regulatory Network Database (CaneRegNet) is an initiative oriented to implement different tools in an integrated manner allowing Sugarcane data analysis focused on five different aspects: i) gene annotation; ii) gene expression; iii) integration of public resources; iv) sequencing projects and v) functional genomics. The available information in the database include 43,141 putative transcripts known as Sugarcane Assembled Sequences (SAS), assembled from 237,954 high quality ESTs, and 195,765 contigs identified through full-length enriched cDNA libraries sequencing. Derived from this information the SUCEST-FUN database also contain transcripts catalogs related to signal transduction (SUCAST), metabolism (SUCAMET), cell wall production and transcription factors. The platform includes microarray experiments designed from SUCAST and SUCAMET catalogs and also a custom-designed array covering approximately 40% of the unique genes found at SUCEST-FUN database, in both, sense and antisense orientation. These resources allow the exploration of gene expression data for different cane varieties, tissues and also biotic and abiotic stimulus, allowing the user to perform several analysis, such as the identification of significant expressed genes, expression correlation, term enrichment, among others. SUCEST-FUN also incorporates a genome browser environment (Generic Genome Browse - http://www.gmod.org), that facilitates the integration of genomic information, like BACs sequences and synteny analyses, and allows the exploration of whole genome sequencing projects in a global and dynamic way. The platform is in constant development and can be accessed at http://sucest-fun.org. Future plans include the integration of pipelines oriented to gene

expression analysis for next generation sequencing (NGS) data and the improvement of available tools to handle new data sets included in the database. The use and enhancement of these resources allows the exploration of available information in a big data context, helping in the comprehension of Sugarcane biology and contributing with researches focused on yield improvement.

Soil erosion model to assess straw removal potential for bioenergy

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Straw removal map adopted in many areas managed with sugarcane has positive consequence from the perspective of renewable energy potential and soil and water conservation. The aim of this study is to model soil erosion caused by runoff and establish the straw removal availability for bioenergy proposes. Here, we use GIS (Geographic Information System) to integrate the factors of the Universal Soil Loss Equation (USLE) to model soil loss. The study area with 515 hectares, which is cultivated with sugarcane located in Piracicaba, SP, has great variability of soil types and topography. We estimate rainfall erosivity (R) using a time series data of 20 years from the Department of Water and Electricity, slope length and steepness (L and S) using the Shuttle Radar Topography Mission (SRTM) digital elevation model. We use the data from the literature for sugarcane crop to obtain soil cover management (C) and control practices (P). We use soil texture from soil samples analysis to model soil erodibility (K) and used a soil map (scale 1:25.000) to spatialize K factor. We define soil loss tolerance as the potential limiting factors for straw removal which indicate the highest value of erosion rates that should have. If erosion rates are higher than the average tolerance limit considered in the literature (12 Mg ha-1 year-1), they are considered non-acceptable and better management practices should be taken such as leave the straw to cover the soil against erosion. However, if the erosion rates are lower than tolerance limit, they are acceptable to remove straw. Although, we understand that a minimum mass of straw left at the field is necessary to ensure the ecosystem services (i.e., water storage, soil protection, nutrient cycling and biomass production). Thus, sugarcane fields under different soil types and climate conditions produce variable straw amounts ranging from 8 to 30 Mg ha-1. The optimal quantity of straw left adopt here is greater than 7 Mg ha-1 of dry straw to maintain the agronomic and environmental benefits. We found that the variance of soil loss across the area ranging from 0.3 to 73.5 Mg ha-1 year-1. The potential straw removal which represents lower than the tolerance limit is 54% of the area. If we consider an average of 14 Mg ha-1 of straw production in the area, the sustainable straw removal of the study area would represent 1,946.7 Mg. This study provides information based on soil erosion to guide on sustainable decision-making of straw removal for bioenergy production.

Genetic transformation of sugarcane for improving bioenergy

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Genetic transformation is an efficient technique to improve agronomic and biological traits in sugarcane plants. A methodology using immature leaves and embryogenic callus has been established and improved in our group in order to provide transgenic sugarcane to

functional studies of genes related to sugar content, drought stress, CO2 fixation and lignin biosynthesis. Immature leaves are used as explant to initiate cell and tissue culture on MS basic medium supplied with growth regulators NAA and 2,4-D. After 3 to 4 months embryogenic calli are suitable for biolistic or Agrobacterium mediated transformation with putative genes to be studied. With the goal to obtain sugarcane plants with reduced lignin content aiming to improve biofuel production, the vector pAHC17 was modified introducing a 535bp COMT antisense (COMT-AS) fragment inserted between the maize Ubi1 intron and nopaline synthases 3' end untranslated sequence (NOS) as terminator. It was used with vector pHA9, which carries nptII selective marker to cotransform sugarcane explants by biolistic methodology. Among 24 transgenic lines, 4 displayed a simple integration pattern: P9, P16, P20 and P32 from SP803280 and one line, P4 and P9 from RB835486 displayed two copies. Clones of primary transformants were vegetative propagated and grown in the green house. Quantitative RT-qPCR showed significantly reduced expression level of COMT gene in leaves, 13% in P9-SP80, 14% in P20 and P32-SP80, 14% in P4-RB83 and 12% in P9-RB83. All transgenic sugarcane displayed a thin unlignified-stretch around de wholly peripheral culm area between the epidermis and the first vascular bundles. The neutral monosaccharide composition of the cell wall from alcohol insoluble residue (AIR) of the COMT-AS sugarcane stalks displayed significantly increase of arabinose and presence of rhamnose only observed in the transgenic plants. A microarray of four cDNA lines (P9-SP30, P32-SP30, P4-RB83, P9-RB83) chosen based on lignin content and the controls WT-SP803280 and WT-RB835486 were used for transcriptomic analysis. The hybridization resulted in 143 differentially expressed genes with 22 contigs present simultaneously in P32SP.P9RB vs WT.SP.RB + P9SP.P4RB vs WT.SP.RB within them, 17 with up and 5 with down expression. These results show that manipulation of specific genes, as COMT in the lignin biosynthesis resulted in decrease of lignin content and release of sugars with higher potential to increase saccharification and biofuel production.

Gene expression analysis highlights pathways preferentially expressed on sugarcane leaves and roots drought responses

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The undesirable effects of fossil fuels use has turned sugarcane into an attractive crop for not only sugar but also biofuels production, increasing its economic and environmental importance. Brazil needs to expand the planted area to soil with less favorable conditions which makes the study of drought, one of the abiotic stresses that most affects this crop's yield, essential. Drought impairs normal growth and leads to major losses for the agroindustry. Thus, this work aims to provide a comprehensive analysis of sugarcane drought responses in the physiological and molecular levels for two plant parts, leaves and roots. In order to do that we performed the analysis of physiology and transcriptome (microarray) of drought stressed SP80-3280 sugarcane plants in three time points (4 days of stress, 6 days of stress and re-watering) of a greenhouse experiment. Preliminary results identified more than 10,000 significantly expressed genes and more than 7,000 differentially expressed genes, with most of the genes altered when plants were kept without irrigation for 6 days, condition in which

physiological parameters such as photosynthesis, transpiration and stomatal conductance decreased more than 90%. Microarray data allowed us to observe that leaves show mainly up-regulated genes and that after 4 days of stress, the plant is mostly transducing the signal from the environment, while after 6 days and after rehydration there is a more functional response of the plant, exemplified by the down-regulation of photosynthesis related genes, with re-watering leading the metabolism back to homeostasis on leaves. In the case of roots, most of the genes were down-regulated, but after re-watering roots take longer to go back to the initial condition, with its differentially expressed genes still being down-regulated. The differentially expressed genes were plotted against KEGG pathways using Pathview (Bioconductor). The results highlight the importance of phenylpropanoid and lignin biosynthesis pathways for the leaves response to drought stress. This polymer as a highly hydrophobic one could be acting to avoid the plant's loss of water to the atmosphere. Furthermore, the up-regulation of fatty acid biosynthesis may demonstrate that on leaves there is a higher need to stress adaptive reorganization of membranes and maintenance of cellular energy supply under water stress. Opposing to that, on roots there is an up-regulation, even when stress is moderate, of genes belonging to the galactose pathway, which involve the metabolism of the galactinol, raffinose and stachyose family of oligosaccharides. This pathway may have an important role on osmoprotection and the protection of plant cells from oxidative damage against abiotic stresses on sugarcane roots. Altogether, these results will give us insight for defining better suited candidate genes for plant breeding.

Selection strategies for energy cane breeding

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With the increasing interest in renewable energy sources, the energy cane has emerged as an excellent alternative, because it has a significant potential of expansion and high biomass production. However, it is a type of sugarcane poorly explored by the genetic improvement and it still needs studies and strategies that contribute to obtaining higher fiber content cultivars as well as higher biomass yielding, in addition to maintaining suitable levels of sugar which is desired by the sugarcane industry. Some sugarcane breeding programs are demanding efforts to develop this new type of sugarcane, among them, is the Interuniversity Network for the Development of the Sugarcane/Energy sector - RIDESA. The aim of this study is to present the strategy adopted by the Sugarcane Breeding Program of the Federal University of Alagoas to select energy cane cultivars. Genetic crosses are performed at the Serra do Ouro Flowering and Crossing Station, located in Murici-AL (09º13'S, 35º50'W; 450 m), using commercial hybrids with access of *S. spontaneum* and *S. robustum* species. Individuals from the segregating populations are selected in the first breeding stages taking into account characteristics such as higher number of stalks, biomass yield, average stalk diameter, fast vegetative growth, resistance to pests and diseases, among others. In the later stages, agroindustrial analyses are performed to select clones with higher yield of dry matter. This strategy, has made it possible to obtain energy cane clones with suitable sugar content and high fiber content (20 to 40% higher) compared to the current cultivars. This new raw

material, in addition to providing juice for the production of sugar and first generation ethanol, it will contribute with more fiber for greater production of electricity and lignocellulosic ethanol. Despite the advances obtained in a short time, there are still some challenges to be overcome by the research to obtain non-flowering, disease resistance (smut, orange rust, among others), and higher yielding biomass cultivars.

Carbon metabolism in four varieties of sugarcane and its accumulation in sink organs

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Bioenergy stands out as an important substitute for fossil fuels and biofuels has been gaining attention in recent years, as well as technologies for the transformation of biomass. In Brazil, the most commonly used biofuels are ethanol and biodiesel from plant tissues. The demand for ethanol has increased in lately and the production of ethanol in the last years reached about 27 billion liters. However, this production is still low against the demand for renewable fuels. Ethanol produced from sugarcane comes from the fermentation process of the accumulated sucrose in the stalks. The production of sugars in plant bodies occurs by means of complex biochemical processes involving different subcellular components. These processes are a set of reactions that coordinate the transfer and metabolism of nutrients and assimilates among the different plant organs. These biochemical processes are dynamic and can be affected in their different ways by various interferences such as abiotic and biotic stresses, among others. In addition to sucrose, other sugars are important for carbon accumulation in plants, which are used in different metabolic pathways. In this study, mature stalks of four varieties of sugarcane (RB72454, RB855156, RB867515 and RB92579) were divided into upper, middle and lower part, analyzed using a metabolic profiling approach, and their total soluble sugars (TSS) and lignin quantified in order to assess the relationship of TSS accumulation and other metabolites in sugarcane. The results show positive correlations among some key compounds of primary metabolism in sugarcane stalks and also suggests the importance of the study of sugar alcohols in these plants in order to assess the contribution of other sugars, such as oligosaccharides of the raffinose series, in the stem carbon storage. These results provide a new perspective for future strategies to obtain plants with higher productivity.

Quality of sugarcane biomass for electricity production

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Sugarcane mills are using straw from mechanical sugarcane harvesting for electricity production. However, this biomass affects the combustion process in boilers. The aim of this study was to evaluate the biomass quality used in sugarcane mills. Were tested samples of bagasse, straw, and mix (bagasse and straw) collected in three different times in a single day. Therefore, we characterized the samples by moisture content, granulometry and mineral impurities. Moisture was determined by gravimetric after oven drying. The granulometry analysis was performed by fractionation using sieves of mesh size between 0.15 and 90 mm. The mineral impurities were determined by the difference between constitutive and total ashes. Dried and pulverized samples was burned in muffle furnace for total ashes content determination. For constitutive ashes, samples

were washed and then were subjected to the procedure described above. The moisture (wt%, wet basis) for bagasse, straw, and mix were 49.6%, 24.4% and 43.7%, respectively. A decrease in bagasse moisture was observed along the sampling times, however the same behavior was not observed in straw. The addition of straw decreased the mix moisture content. The biomass boilers are designed to operate with bagasse as a fuel, which has moisture around 50% (wt). The change in moisture fuel can provide fluctuations in boiler operation. The bagasse granulometry showed similar behavior in all samples. The highest percentage of bagasse was retained in sieves with mesh sizes of 0.50 mm, 0.85 mm and 1.70 mm. Thus, the mean size of bagasse particles was 1.00 mm. For straw, the mean size was 6.82 mm. In mix samples, with a straw rate of 5% in bagasse (dry basis), the mean size was 3.9 mm. The size of straw differs from the bagasse, which significantly influences the mean particle size of the mix. Boilers operation can be compromised using large size and low-density particles; consequently, this biomass can be dragged by airflow during combustion. Therefore, straw size can be a limit factor for its use combined with bagasse. The mineral impurities for bagasse, straw, and mix were, respectively, 7.4%, 8.5% and 7.7%. Thus, we observed that the straw incorporation causes an increase in mineral impurities content of the mix in relation to bagasse, which may result in heterogeneous material. The mineral impurity is an undesirable characteristic in biomass used to burn in mills. Since, it can contribute to damage the equipment by fouling, slagging, corrosion, and increased amount of maintenance in the boiler. The results allow inferring that straw use requires adjustments from recovery stage to its mix with the bagasse in mills. The improvement of these stages is important to allow the use of straw mixed to bagasse as a fuel in biomass boilers for steam and electricity generation.

Transcriptomic and metabolomic analyses of sugarcane hybrid, SP80_3280, throughout growth and development

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Current global fossil fuel consumption stands at 80% on the world energy matrix, and its use has led to severe and potentially irreversible environmental threats. The improvement of bioenergy crops, such as sugarcane, is of immense importance in order to ensure a sustainable future. Genetic and metabolic engineering attempts have not been entirely successful due to the lack of information available with regards to sucrose metabolism, transport and accumulation. In order to aid in the understanding of these processes, integrated omics approaches were taken, specifically by coupling transcriptomics and metabolomics. Field experiments were conducted to study sugarcane variety SP80_3280, a hybrid of Saccharum officinarum and S. spontaneum, planted in two different seasons (one-year and one-and-a-half-year cane). Transcriptomics studies were conducted using a custom microarray (Agilent) and data analysis was performed using the SUCEST-FUN Cane Transcriptome platform. Liquid chromatography mass spectrometry, specifically HPLC-ESI-MS/MS (Agilent & AB Sciex) and UPLC-ESI-MS (Shimadzu & Bruker), was used for targeted and untargeted analyses. Sucrose, fructose, glucose and trehalose in +1 leaves and internodes 1, 5 and 9 were quantified and correlated with gene expression information. A total of 8,540 and 10,336 differentially expressed transcripts were identified in the +1 leaves and internodes, respectively, of which 33 and 37 were isolated due to their direct association with starch and

sucrose metabolism (KEGG map 00500). Leaf sucrose content in the sugarcane plants from both experimental fields showed the same metabolic pattern, high-low-high-low. This might be due to the negative feedback inhibition of sucrose and/or hexoses on photosynthesis. A positive correlation between sucrose contents in the +1 leaves and the transcripts associated with cell wall invertases (CWI) in the immature internodes (I1) were observed, which might also explain this sucrose metabolic pattern in the leaves. Transcripts associated with CWIs in the I1 were highly expressed, thus providing the rapidly growing culm with the necessary resources for growth and development. The expression of these transcripts decreased along the maturation of the culm, however a specific transcript associated with trehalose-6-phosphate synthase (TPS) was found to be highly expressed in the maturing internodes (I5), indicating that this precursor compound and/or trehalose itself might serve as signaling molecules which will initiate the sucrose accumulation process. Since metabolites are the end products of gene expression and regulation, protein translation and enzymatic activities, they can provide us with valuable information concerning the biochemical status of a cell, tissue, organ or organism. By integrating this information with transcriptomics data, and thus taking a systems biology approach, we aim to elucidate plant growth, sucrose accumulation and biomass production, specifically focusing on carbon partitioning and metabolic regulation.

Is *Saccharum* a natural group? Phylogenomic analysis of the chloroplast genome sequences of species from the *Saccharum* complex, including the native Brazilian ones

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The commercial release of transgenic cultivars requires information about the potential risk of gene flow to endemic wild relatives. Modern sugarcane cultivars derive from a series of hybridization and backcrossing among *Saccharum* species. The so-called *Saccharum* complex holds around 40 species from a few genera, including *Erianthus*, found in various tropical regions. In Brazil, three native species originally considered *Erianthus* were reclassified as *S. angustifolium* (Nees) Trin., *S. asperum* (Nees) Steud., and *S. villosum* Steud., based on inflorescence morphology. Here, we used Next Generation Sequencing to obtain the full chloroplast genome sequence to obtain a phylogenomic reconstruction of the *Saccharum* complex, including these 3 Brazilian species, and to develop specific SNP markers to follow potential hybridization and gene flow in these species. We ran low coverage Illumina genomic sequence of the commercial cultivar 'SP80-3280', the parental *S. officinarum* 'Muntok Java' and *S. spontaneum* 'SES 208', the Brazilian species (*S. angustifolium; S. asperum* and *S. villosum*), *Erianthus bengalensis* ('US4714') and *Miscanthus nepalensis* ('IND82318'), plus sequences available at the NCBI. The chloroplast genome size ranged from 141,182 (*S. asperum*) to 141,869 bp (*E. bengalensis* 'US4714'), and all circular genomes were correctly assembled. In relation to the published sugarcane chloroplast genome 'NCo310', the number of SNPs ranged from 3 (*E. bengalensis* 'US4714') to 355 bases (*M. nepalensis* 'IND82318'). For the native Brazilian species, the number of SNPs was 96 bases for *S. angustifolium*, 197 for *S. villosum* and 207 bases for *S. asperum*, when compared to NCo310. A limited variation in number of genes was observed among species (from 199 to 204). The phylogenomic reconstruction based on chloroplast DNA does not allow the separation of the *Saccharum* and *Erianthus* specimens

(polyphyletic), corroborating the existence of a *Saccharum* complex, which included the 3 Brazilian species. The occurrence of gene flow between commercial sugarcane and the endemic Brazilian species is under investigation using markers described here. However, differences in phenology and a strong tendency for self-pollination appear to predominate among the Brazilian native species.

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Sucrose related molecular markers used to population structure analysis of sugarcane

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Sugarcane is predominant an autopolyploid plant with a variable ploidy level, frequent aneuploidy and a large genome. Genetic improvement is extremely important to generate productive and resistant cultivars. Molecular markers are a powerful tool to aid the selection process and drive parental choices. However, due to the genetic complexity, the practical applications of molecular markers have been notably delayed in sugarcane, in contrast to other crops that have already advanced to marker assisted selection and genomic selection. The objectives of this study were to (i) estimate genetic distances between 134 access of a Brazilian Panel of Sugarcane Genotypes (BPSG) and also to biparental progeny of sugarcane (BP), originated from cross between cultivars SP80-3280 and RB835486, using functional molecular markers (EST-SSR) to sucrose trait, (ii) perform principal component analysis (PCA) with the genetic distances matrices through R software, (iii) population structure analysis using STRUCTURE software, and (iv) compare phenotypic averages of sucrose content (POL%C) between subpopulations determined by STRUCTURE. The package vegan, implemented in R software, was used to estimate genetic distances with Jaccard's coefficient. The genetic distances ranged from 0.09 to 0.95 and from 0.16 to 0.93 to BPSG and BP, respectively. The PC1 explained 63.16% and 38.52% of variation showed by the genetic distances matrices to BPSG and BP, respectively. The STRUCTURE software showed two and three subpopulations in BPSG and BP, respectively. The phenotypic averages of the two and three subpopulations obtained were slightly different between them: 14.91 (± 1.91) and 15.01 (± 0.98) to BPSG and, 15.34 (± 0.61), 15.57 (± 0.62) and 15.65 (± 0.68) to BP. These results showed that the functional markers were able to identify subpopulations, with different phenotypic averages, within a diversity panel and biparental progeny of sugarcane. Therefore, others populations should be evaluated to verify the accuracy of these molecular markers for assisted selection.

Impact of straw removal in soil fertility, carbon storage, and sugarcane yield

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Renewable energy sources from sugarcane straw have gained increasing importance in the current energy scenario, making the sustainable management of the soil indispensable not only for the feedstock production but also for reducing greenhouse gases emissions. Adoption of crop rotation during renovation period and

adequate management of plant residues are appropriate strategies for soil conservation in sugarcane areas. The hypothesis of this study is that straw removal for bioenergy production will affect soil fertility and carbon storage in the medium term, resulting in yield losses of sugarcane fields. This study aimed to evaluate soil fertility, carbon (C) storage, and sugarcane yield as a function of crop rotation and removal of sugarcane straw. The research was developed in two sites, one with clay texture (site 1) and another with sandy texture (site 2). Prior to sugarcane establishment, the field was divided into two areas, one with crop rotation (*Crotalaria spectabilis*, "sunn hemp") and another left fallow (without crop rotation). Both fields were conducted similarly during cane-plant crop cycle. The experimental design, installed sequentially after first and second years, was a randomized block design with three levels of straw removal (0, 50 and 100%) and four repetitions. Sugarcane yield was monitored during two years, while soil fertility and soil C storage were evaluated at the end of second year. In site 1, crop rotation improved soil fertility by increasing the levels of available P, K, and cation exchange capacity. This is possible the reason to the 8.0 Mg ha-1 yield gains promoted by crop rotation on the average of two years on this site. In site 2, crop rotation did not increased yield or soil fertility. Maintenance of 50 or 100% of straw resulted in yield gains in both sites, varying from 14.0 to 4.4 Mg ha-1 in sites 1 and 2, respectively. Maintenance of 100% of straw increased soil C storage (<0.4m soil depth) in site 2. Crop rotation has potential to improve soil fertility and sugarcane yield, while maintenance of 50% (> 8.0 Mg ha-1) of straw is required to increase sugarcane yield.

Genetic structure analysis of the IAC Sugarcane Germplasm Bank assessed by molecular markers

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Sugarcane is among the most important bioenergy crops word wide especially in Brazil which is the world largest sugarcane producer. In the last years, sugarcane breeding programs has focus on the development of high biomass genotypes mainly by exploring *Saccharum spontaneum* accessions as parent in crosses with commercial varieties. Therefore the knowledge of the genetic structure of sugarcane germplasm banks not only promotes the rational use of the genetic variability available in the breeder's germplasm collection but also its efficient management. In the present work, the genetic structure of a group of 593 accessions (genotypes) encompassing sugarcane basic germplasm and breeding genotypes from the Campinas Agronomic Institute (IAC) Sugarcane Genetic Breeding Program was assessed by microsatellite markers (SSRs) derived from a set of 12 SSR primer pairs. The 12 SSR primer pairs used to assess the genetic variability was promising to discriminate the 593 accessions and capture the genetic variability of the Sugarcane IAC Germplasm bank as also to check for probable mislabeled accessions in a routine manner during new accession acquisitions. The genetic structure analysis divided the germplasm bank accessions into two subgroups (K = 2; ΔK = 1454.96) corresponding to the basic germplasm and breeding genotypes which underwent selection process. The Molecular Variance Analysis (AMOVA) of these two sub-groups revealed a genetic differentiation value of 14.4% (P <0.001). The phylogenetic analysis clustered the genotypes according to their pedigree and maintained the breeding genotypes closer to the *Saccharum officinarum* accessions confirming their close genetic relationship. The *S. spontaneum* accessions had greater dissimilarity with the breeding genotypes allowing to identify potential divergent parent

combinations that can maximize genetic variability of the progenies. A core collection with 156 genotypes (approximately 30% of the total accessions evaluated) was established based on the molecular data. The results obtained can be applied in the management of the IAC germplasm bank directing the importation of new accessions, as well as in crosses for conventional or bioenergy sugarcane development.

Structural and functional characterization of SUGARWINS and their role in plant defense

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In sugarcane fields, colonization of the stalk by opportunistic fungi usually occurs after *Diatraea saccharalis* caterpillar attacks sugarcane. Plants respond to insect attack by inducing and accumulating a large set of defense proteins. We identified two homologues of a barley wound-inducible protein (BARWIN) in sugarcane, which were designated SUGARWIN1 and 2 (sugarcane wound-inducible proteins). Although BARWIN function has not been fully established, antifungal properties have been described for a number of homologues. SUGARWIN1 and 2 gene expression are induced in response to wound and *Diatraea saccharalis* damage. Although the recombinant SUGARWIN protein does not affect insect development, it promotes significant morphological and physiological changes in *Fusarium verticillioides* and *Colletotrichum falcatum*, which lead to fungal cell death via apoptosis. In this study, we deepen our understanding of the role of SUGARWINs in plant defense and the molecular mechanisms by which these proteins affect fungi by elucidating their molecular targets. Our results show that SUGARWINs play an important role in plant defense against opportunistic pathogens. We demonstrated that *SUGARWINs* are induced by *C. falcatum*, and the induction of *SUGARWINs* can vary among sugarcane varieties. The sugarcane variety exhibiting the highest level of *SUGARWIN* induction exhibited a considerable reduction in *C. falcatum* infection. Furthermore, SUGARWIN1 exhibited ribonuclease and chitinase activity, whereas SUGARWIN2 exhibited only chitinase activity. This variable enzymatic specificity seems to be the result of divergent amino acid composition within the substrate-binding site that was demonstrated by protein modeling. Our results show that SUGARWINs play an important role in plant defense against opportunistic pathogens and can be important to red rot disease control.

High nutrient demand and sustainable production of energy cane

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Despite the increasing interest in energy cane to produce second generation ethanol, thermal energy, and bio-based materials, varieties available are few and there is little information on their actual yields and nutrient needs and how this affects sustainability. Energy cane is a crop in the making: the clones have different contributions of *Saccharum spontaneum*, which confer more tillers but with less sugar, generating plants with high biomass and low sugar. The aim of this study was to evaluate nutrient accumulation, dry matter production and characteristics of 28 clones of energy cane of IAC's breeding program in two regions in

Brazil with contrasting climates. The field experiments were set up in Pradópolis, the main sugarcane region in São Paulo state (mild winter and small water deficit), and in Goianésia, (warm and a long dry winter) in Central Brazil, in randomized blocks with 28 treatments and 3 replications. Dry matter yields and nutrient accumulation were measured at the end of the plant cane cycle and again, 2, 6, 8, and 12 months after sprouting in the ratoon cycle. In the latter cycle tillering was also evaluated. The clones were divided in four groups according to the yields ($P < 0.05$). Our results demonstrated that in Goianésia the average of the dry matter yields, were 41, 34, 31 and 29 Mg ha^{-1}, for groups 1, 2, 3, and 4, respectively. There was no clear relation between the number of tillers and yields (average tiller per meter: 24.9, 23.1, 22.2 and 22.9 for groups 1, 2, 3, and 4, respectively). The higher yielding group in Goianésia had clones varying from 16.3 to 32 tiller m^{-1}, suggesting that higher contribution of *S. spontaneum* is not responsible for high biomass yield. There was some degree of coincidence between the higher yielding clones in both sites: 5 of the 7 best clones in Pradópolis were either in group 1 or group 2 in Goianésia, but one clone of the leading group in both sites was at the bottom group (4) in the other indicating differences in regional adaptation. Nutrient accumulated in the above-ground plant was 171 kg ha^{-1} N (ranging from 137 to 209), 23 kg ha^{-1} P (ranging from 18 to 33) and 364 kg ha^{-1} K (ranging from 276 to 458). These are grossly the amounts of nutrients exported from the field and are much higher than the usual rates of nutrients applied to sugarcane ratoons in Brazil: 110, 18, and 117 kg ha^{-1} of N, P, and K, respectively. In addition to higher nutrient requirement to sustain yields and preserve soil fertility compared to sugarcane, heavy fertilization is an issue that must be considered for the energy and greenhouse gas balances of energy cane.

Study on morpho-physiological differences between sugarcane and energy cane

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Sugarcane (*Saccharum spp.*) is one of the most economically important grasses in the world. It is produce sugar and also bioethanol and bioelectricity. Sugarcane is a C4 plant, of high photosynthetic efficiency, since that synthesis and accumulation of sucrose occur during a maturation phase. On the other hand, genotypes with low content of sucrose, high fiber content and high accumulation of biomass are referred as energy cane and have as main ancestors representatives of *S. spontaneum*. The leaf is responsible for photosynthesis and production of carbohydrates. The leaf area (LA) can be correlated to several morpho-physiological parameters of agronomic traits. Knowledge of leaf area in sugarcane varieties can be useful to correlate with their productive potential, either in dry matter or in sugar. In this way, the objective of this study was to characterize the variables (leaf area index – LAI, assimilation rate (A) and intercelular CO_2 concentration (C*i*)) in contrasting genotypes for sucrose content and fiber content. The genotypes evaluated were SP80-3280, RB855156 and IN84-58. The experimental design was in a randomized block with five replicates. The LAI was calculated according to the formula LAI = CxLx0.75x (N + 2) / area, where C corresponds to leaf + 3 length, L to leaf + 3 width, and N is the number of green leaves. A and C*i* were determined in 5 and 7 months after planting (MAP) on sunny days between 08:30 and 13:00h from third visible dewlap (leaf + 3) leaves of five individual plants. A and C*i* were measured using a Infra-Red Gas Analyser (IRGA) LCPro+ portable photosynthesis system

(ADC, England). The photosynthetically active radiation (RFA) in the leaf chamber, provided by light source, was established for 1500 µmol m^{-2} s^{-1}, and the CO_2 concentration of the leaf chamber was adjusted to 380 mL L^{-1}. For the morphological (LAI) and physiological (chlorophyll a, chlorophyll b) traits a significant difference (p-value ≤ 0.05) was observed between genotypes. Genotype IN84-58 presented lower LAI (7.74) at 5 MAP and (18.25) at 7 MAP. However, no significant difference was found in the photosynthetic rate and internal CO_2 concentration between the genotypes.

Metagenomic link between enhanced bio-methane production and water hyacinth (*Eichornia crassipes*) pre-treatment

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Water hyacinth (*Eichornia crassipes*) is a fast growing water weed that threatens the aquatic ecosystem and human activities hence it is essential that the plant be controlled. One such control method is the utilisation of the plant as a substrate for anaerobic digestion (AD). This study was aimed at establishing the link between microbial diversity, pre-treatment method and biogas yield when using water hyacinth as a substrate. Physical and biological pre-treatment methods were tested. All pre-treatment methods were tested with and without the addition of an inoculum. Microbial community shifts were monitored using denaturing gradient gel electrophoresis (DGGE) and biogas yield and composition were monitored using a digital manometer and gas chromatography respectively. The pre-treatment methods that included inoculum showed microbial community stability and early production of bio-methane whereas cultures that lacked inoculum showed distinct community structure variations in response to the method of pre-treatment. While biogas was produced by cultures lacking inoculum it was initially primarily composed of carbon dioxide. However, as digestion proceeded an increase in bio-methane was observed concomitant with a shift in the microbial community structure and increased diversity. Canonical correspondence analysis (CCA) showed the dynamic changes of microbial populations and biogas production were strongly correlative. In cultures lacking inoculum, all mechanical pre-treatment methods resulted in maximal biogas yield with the exception of the homogenization treatment. This may be attributed to the homogenization process resulting in the damage of fragile microorganisms that are essential for biogas production or the release of inhibitors during the homogenization process. This study proves that the method of water hyacinth pre-treatment influences microbial community structure and concomitant biogas production in cultures lacking the addition of an inoculum. Furthermore, a resilient biogas producing consortium was found to be associated with the collected water hyacinth. This has implications for downstream bio-augmention studies.

Biofuel Technologies, including biomass process engineering and biofuels production

High-efficient CRISPR/Cas9 system-based approach for the industrial *Saccharomyces cerevisiae* genome editing

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Saccharomyces cerevisiae yeasts adapted to industrial conditions – industrial strains – are largely employed on the production of chemicals, such as renewable fuels. Even though S. cerevisiae yeasts are known as organisms easily susceptible to genetic manipulation, industrial strains are usually diploid and prototrophic, for this reason, require the development or adaptation of efficient genetic manipulation tools. In this context, the CRISPR/Cas9 system figures as the best genomic manipulation alternative due to its simplicity, efficiency and non-necessity of previous handling and co-integration of selection markers. In order to achieve a simple, cost-effective and less time-consuming method, we developed an approach based on the yeast co-transformation with an easily synthesized donor template and a single-DNA plasmid, the pGS004, that carries the dominant selection marker and the regulatory elements to drive the expression of both components required to DNA edition – pTEF1-Cas9-tCYC1; pSNR52-sgRNA-tSUP4. In addition, a unique enzyme restriction site strategy has been implemented to induce a single cut and ligation event that allows the quick shift of the sgRNA sequence. As for the donor template, it comprises a 90-base pair (bp) double-stranded oligonucleotide (dsOligo), it is homologous to the target DNA region, but contains an in-frame STOP codon replacing the PAM sequence and can be easily synthetized by 5-20 cycles of the standard high-fidelity amplification, using two 55-bp primers containing 20 overlapping nucleotides. The high concentration synthesis of dsOligo takes less than one hour, and the sgRNA shift consumes around two days and had its high efficiency proved by colony PCR and sequencing. To evaluate the DNA edition efficiency a pGS004 plasmid containing the specific URA3 locus sgRNA and the appropriated dsOligo have been co-transformed into industrial diploid yeast JAY270 using small-scale LiAc transformation procedures. Seven concentrations of pGS004 combined with five concentrations of dsOligo were tested in order to establish an optimal transformation condition with satisfactory edition efficiency. We quantified the number of colonies obtained in all our transformation events and checked for uracil auxotrophy, seven of our trials presented more than 88 percent of URA3 locus editing efficiency and this frequency is independent of the number of colonies obtained. For the best pGS004-dsOligo combination, we were able to reach 81 colonies with 96 percent edition efficiency. In conclusion, the transient expression of Cas9 and sgRNA on a single vector effectively resulted in a marker-free permanent modification. This CRISPR/Cas9 method is easily applicable, which can be quickly adapted and tested for any genome edition in S. cerevisiae and facilitates the metabolic engineering of industrial strains. Additionally, preliminary tests presented similar DNA edition efficiency for other industrially relevant strains, and suggest appropriate efficiency when applied to genes integration or simultaneous editing of two loci using the gap-repair-based multiplex approach.

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

Technology roadmap in advanced biofuels

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Economic development is inherently flanked by increased transport, and thus economic development requires sustainable transport of people, resources and products, where biofuels can play an important role. In Brazil, a substantial part of people and freight transport relies on light vehicles and trucks, which are already sustainably supplied by the mature 1st generation bio-ethanol and biodiesel industry with a well-recorded history of technical, market and financial development, as well as by an emerging 2nd generation bio-ethanol industry. However, transport sectors have less alternatives to address the aviation (passengers) and marine (freight). Among the main requirements are: sustainable biofuels with high energy densities, low sulfur content, good availability and competitive pricing, and especially for aviation, more critical specifications (ASTM and others). Take into account the aviation sector commitments (ICAO timeframe 2016-2050) and the emerging maritime legislation (IMO legislation), a sustainable integral biofuels value chains will need to be deployed in Brazil and worldwide. Aiming to develop a roadmap for advanced biofuels, and a concrete set of next steps (innovation and investment programs), a wide audience workshop involving 102 experts from academy, government and private sector was held during October 17-18, 2016 (Campinas, São Paulo). The discussions focused three inter-twined objectives: 1) provide criteria/measures for a conducive environment for (future) competitive and sustainable integral biofuels value chains for the projected volumes in time; 2) understand the societal dimension; 3) have a clear insight on how the advanced biofuels implementation in SP and Brazil impacts the Brazilian and global environment ambitions. The main technological gaps and barriers pointed out were related to the i) decision support tools/scenario analyses; ii) data availability/management; iii) optimisation of existing technologies; iv) croptimisation; v) integral biorefinery development; vi) flanking technologies; vii) engineering (design) of non-technological topics (market, legal issues, production/trade/consumption, financial issues, education and manpower skill training, and infrastructure demands and development); and ix) breakthrough / alternatives /disruptive development. Related non-technological gaps and barriers the main notes were: i) socio-economic and sustainability study; ii) national strategy; iii) logistics; iv) international agreements; v) R&D agenda; vi) education and training, and v) communication plan. In conclusion: i) the period up to 2030 allows for testing advanced biofuels value and supply chains at relevant industrial volumes, from field to distribution, and technically as well as non-technical; ii) the period after 2030 is predicted to be focused at scale-up in an emerging integrated biorefinery context. The next step agenda needs to consider: i) Champion deployment (e.g. Bioport Brazil, a professional platform in line with Plataforma Brasileira de Bioquerosene); ii) quantitative scenario analysis & data collection; iii) National Strategy, iv) Strategic Communication Plan and Policy Briefs; and v) integration programs (pilot/demo plants).

Hydroesterification of chicken oil under subcritical pressurized conditions for biodiesel production

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Brazil stands out as the second largest producer of chicken on the world, where in 2015 it produced about 13 million tons of chicken meat. So a large amount of waste is generated annually in the slaughterhouses, in special, chicken oil. Chicken oil is obtained by processing the non-edible parts of the chicken which correspond to approximately 7.3% of the chicken mass. In addition, some industries also process feathers, blood and offal that do not go into the food trade. Such waste, when incorrectly disposed, its contributed to environment polution. Thus, the use of this raw material brings environmental gains, adds value to a product without commercialization, reduces to biofuel production costs and allows a diversification of the oil crops used for biodiesel production in Brazil. However, the chicken oil has high acidity and water content and can not be used as raw material for biodiesel production by conventional method (basic transesterification), whereas it can cause secondary reactions decreasing the reactional yield. Hydroesterification is an alternative process to conventional biodisel production methods, as it can be carried out using low quality raw materials such as crude or residual oils that have a high acidity (acidity above 3 mg NaOH/g) and water content (0,1% w/w). The aim of this study was to produce biodiesel by hydroesterification from chicken oil. This reaction occurs in two steps, hydrolysis of the triglycerides with water that produces FFAs and glycerol followed by esterification of FFAs with a alcohol to obtain biodiesel. The hydrolysis reactions were performed under subcritical pressurized conditions based in factorial design 2^3 with central point. The influence of the molar ratio (oil:water), temperature and reaction time were evaluated watching the FFAs yield. In the hidrolysis reaction, the best result (90.4% of FFAs) was attained with 250 °C and 2 hours. Molar ratio showed no significant influence. After evaluation of the model, the equation of the regression with R^2 of 0.9488 was obtained. In order to optimize the results, a new reaction was carried out at 263°C for one hour, obtained 91,6% of FFAs, without increasing the energy costs of the reaction. The FFAs produced in the optimum condition were esterified with methanol and ethanol using 1.0% acid catalyst (H2SO4) at 65 ± 5°C for 1 h. The ester conversion using the methyl route was 73.5% (w/w) and for ethyl route 47.4% (w/w). The water content was 331.4 mg/kg and 326.9 mg/kg for the methyl and ethyl ester respectively.

Optimization of energy and water consumption in the bioethanol integrated production process from molasses and cane bagasse: Colombian case.

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The bioethanol production in Colombia is carried out in distilleries annexed to sugar mills, where there are obtained ethanol and sugar as main products and in some cases, heat and electricity from the cogeneration systems. In 2015, about 24 million tons of sugarcane were milled, producing approximately 2 million tons of sugar and 456 million liters of ethanol. In addition, about 5 million tons of bagasse were produced for the paper industry and cogeneration systems, which contributed about 1,380 GWh/year. In the present paper, an integrated scheme of first and second

generation bioethanol production is proposed with the mainly purpose of increasing the yield and decreasing the thermal energy and fresh water requirements. In this sense, first, the individual schemes of first and second generation were modeled using Aspen Plus ® software and were evaluated using the exergetic analysis methodology in order to identify the irreversibility and efficiencies at each stage of the process. Then the energy consumption reduction of the process was evaluated through energy integration (network of heat exchangers) applying mathematical programming and, finally, the use of streams with water disposal for re-use in the process was evaluated reducing the fresh water consumption, formulating the problem of the optimal allocation of water as a nonlinear mixed-integer programming problem (MINLP). The bioethanol production from the integrated scheme of first and second generation increased at least 40% compared it with sugar-bioethanol production scheme. The identification of process stages which presented inefficiencies and irreversibility of each scheme analyzed was possible by exergy analysis. The heat exchanger network with the minimum annual cost was identified through energy integration applying mathematical programming. Around of 20% of fresh water consumption was minimized using mathematical programming in the integrated scheme. The integration of second generation bioethanol to a sugar-bioethanol process could increase, not just, the bioethanol production in Colombia, also, it allowed the diversification of products and decreases energy and water requirements.

Pretreatment of switchgrass by steam explosion in a semi continuous pre-pilot plant

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Switchgrass (*Panicum virgatum*) is a perennial warm season grass highly valued as an energy crop resource for the production of bioethanol due to its high carbohydrate content, fast growth, and ability to grow in lands that cannot support crop or food production. One of the key steps in the overall process for the production of bioethanol from lignocellulosic materials like switchgrass is the pretreatment, which is essential to overcome the feedstock recalcitrance making the cellulose fibers more susceptible to enzymatic hydrolysis. Several technologies have been proposed for biomass pretreatment, among of which, steam explosion has been demonstrated to be efficient for a variety of feedstocks. However, the efficiency and the selectivity of this process is highly dependent on the feedstock and conditions applied, being the temperature and residence time the two main parameters affecting the results. Therefore, the present study evaluated the impact of the temperature and residence time on the pretreatment of switchgrass by steam explosion in a semi-continuous pre-pilot plant able to generate between 3 and 7 kg of pretreated solid material. For the assays, different combinations of temperature (170, 185 and 200 °C) and residence time (5, 10 and 15 min) were evaluated, which were combined through a 2^2 central composite design leading to 11 experiments. The severity factor (an empiric factor calculated by considering the combination between temperature and time) was also calculated to each reaction condition in order to help explain the influence of process severity on the removal of hemicellulose and lignin fractions during pretreatment. Chemical composition of the residual solid material after pretreatment was determined to each

condition assessed. The content of carbohydrates and inhibitor compounds (including the sugar degradation products, furfural and hydroxymethylfurfural) in the liquid fraction after pretreatment was also determined. As a whole, increasing the severity factor favored the hemicellulose removal from the feedstock. The optimal pretreatment condition was established in order to obtain maximum hemicellulose solubilization in the liquor (with minimal generation of inhibitory compounds) and maximum amount of cellulose retained in the residual solid material. The obtained results, already in a pre-pilot scale, are very promising and contribute to the development of an ethanol biorefinery using switchgrass as a feedstock.

Sugarcane straw as a carbon source for fungal xylanases and cellulases production in submerged culture

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Sugarcane straw was evaluated as substrate to produce fibrolytic enzymes by different cultures: *Trichoderma reesei* QM9414, *T. reesei* 2768, *T. Harzianum N51*, *T. harzianum* FS09, *Aspergillus fumigatus* M51 (CCT 7732), and *A. fumigatus* U2370. The screening of these microorganisms in monocultures, using sugarcane straw as a carbon source, was performed in Erlenmeyer flasks to select the best producer of xylanases and cellulases. *T. reesei QM9414* culture reached the maximum enzyme production in submerged fermentation, in pH 4.5, at 28ºC, and 180 rpm, and after was scaled up to 3L bioreactor, showing the same production yield. Mixed cultures with these fungi are also tested, and produced less enzymes than pure ones. The xylanase enzyme produced was characterized by the optimum pH (3-11) and temperature (20-70ºC) and the effect of these factors in its activity. Some ions: Cu^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{3+}, Ag^{1+} and EDTA (Ethylenediamine tetraacetic acid), were applied to determine the xylanase stability on these conditions. Thermal stability was not observed at temperatures above 50°C and pH 5-6 was established as the enzyme stability range. The optimal xylanase activity was stablished at 50ºC and pH 5. These results indicate that sugarcane straw is a good substrate for fibrolytic enzymes production and that it is possible to employ the produced enzymatic complex in some industrial processes which require mild conditions of pH and temperature. Besides this, Cu+2 and Ag1+ resulted in a strong inhibition of xylanase, while Mg2+ has a stimulatory effect on it.

Fermentative H_2 production from carbohydrates constituents of lignocellulosic biomass

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Hydrogen (H2) is a highly energetic and alternative fuel when compared to derivate fossil fuels. Moreover, H2 is a clean energy source and its combustion produces only water, which makes it even more advantageous. From this perspective, the interest in technological development studies and processes has widely increased in order to obtain this biofuel from renewable sources. One of the H2 production routes is the fermentation via carbohydrates rich compounds, such as lignocellulosic biomass, since the main monomeric components comprehend glucose and xylose. This study evaluated the biohydrogen production from xylose and glucose fermentation, using the recently isolated *Clostridium beijerinckii* Br21 strain. The batch fermentation assays were performed in 100 mL

flasks containing 60 mL of culture medium with xylose or glucose at 15 g L^{-1} as carbon sources. The flasks were incubated at 35°C for 45 hours and periodic samples were collected in order to evaluate cell growth through absorbance analysis at 600 nm. The substrate consumption was evaluated using the DNS method and the hydrogen production using a system consisting of an inverted flask containing NaOH 5% (w/v) solution and a measuring cylinder, which helped to determine the volume variation due to gas production. The gas composition was analyzed using gas chromatography. The data of H2 accumulated volume along the assay was added to the *Statistica 7* software and modeled according to the modified Gompertz model in order to obtain the kinetic parameters: maximum H2 production rate (*Rm*), maximum H2 production (*Hmax*), and lag phase (*λ*). The results showed that the total substrate consumption was similar for both carbohydrates (around 9 g L^{-1}) whereas cell growth was improved in the presence of glucose when compared with xylose`s. The kinect parameters presented higher H2 production (*Hmax*) in the presence of glucose (104 mL) compared to xylose (51 mL). On the other hand, the *Rm* was higher when xylose was fermented, 7 mL h^{-1}, compared with glucose 5 mL h^{-1}. In addition, it can be observed that the lag phase (*λ*) of *C. beijerinckii* Br21 in the presence of xylose as a substrate was higher when compared to the fermentation of glucose, being 35 and 21 hours, respectively. In summary, this study showed the microorganism ability to ferment and produce hydrogen from the two main carbohydrates constituents of lignocellulosic biomass.

Multi-objective optimization in sugarcane diffusers for obtaining maximum sucrose extraction efficiencies

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Brazil is the second biggest ethanol producer in the world, being this biofuel an important commodity for the Brazilian economy. The extraction of juice is one of the first and main stages of sugarcane processing, and obtaining high sucrose extraction efficiencies is essential for maximizing ethanol and sugar production. Maximum sucrose extraction efficiencies are achieved when two desired objectives are reached: (1) lowest sugar concentration in the megasse that leaves the equipment and (2) highest sugar concentration in the final percolating liquid. However, the increase of one objective compromise the other, i.e. an increase in the imbibition water flow rate that is added to the equipment is capable of decreasing the final concentration of sugar in the megasse but also decreases the final concentration of sugar in the percolating fluid due to dilution. Therefore, it can be concluded that for different imbibition water flow rates, the objectives being optimized are intrinsically antagonistic. The main objective of this work is to perform a multi-objective optimization that enables the choice of optimum operational conditions for the design and operation of industrial sugarcane diffusers. The operational conditions that can be set by the operator are the imbibition water flow rate and the percolating liquid flow rates that enter in each stage of the diffuser. Results show that an increase in 8% or higher in the imbibition water flow rate favour the system optimality, being maximum imbibition flow rates, limited by the occurrence of flooding, always desired in the diffuser. Furthermore, the recirculation of part of the percolating liquid that enters in at least 8 out of 10 diffuser's stages to the same stage increase the probability of achieving operational conditions that are close to optimum.

Immobilization of *Cutaneotrichosporon mucoides* in calcium alginate gel aiming the production of biosurfactant from sugarcane bagasse hemicellulosic hydrolysate

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Surfactants are synthetic products with tensoactives and emulsifiers properties of fundamental importance for industry. To supply the industrial demand, surfactants are produced by chemical reactions using petroleum derivatives, which represent a risk for the environment due to the harmful substances generated in the process. In addition, the petroleum is a non-renewable raw material and its price is dependent on the often oscillating trade balance. Faced with this problem, for the sustainable development of society, in the last decades non-petroleum processes and products have been studied and created. The biosurfactants (BS) present themselves as potential substitutes for synthetic surfactants because they have the same or superior emulsifying and tensoactive properties as well as ecofriendly products due to their high biodegradability and low or non-toxicity. Also as an advantage, BS can be produced from agroindustrial by-products such as waste oils and lignocellulosic biomass reducing production costs and making these products more accessible. Brazil is agricultural rich country and one of the largest producers of sugar cane in the world. Bagasse generated during sugarcane processing is used for energy generation, however, its excess can be used as a raw material for bioprocesses due its composition present lignin, cellulose and hemicellulose. This study has as main objective the use of the sugarcane bagasse hemicellulosic hydrolyzate for the production of BS by immobilized *Cutaneotrichosporon mucoides* cells. SCB was hydrolysed using concentrated sulfuric acid (1 mg SBC : 10 mL sulfuric acid). After acid hydrolysed was concentrated around 5-fold by vacuum evaporation until a final xylose concentration of 60 g/L. This concentrated hydrolysate was detoxified by liming method being first neutralizated with calcium oxide followed by activated charcoal adsorption. Detoxified hydrolysed was supplemented with mineral nutrient solution and the concentration of xylose and pH were adjusted at 40 g/L and 5.5 respectively. The cells of *C. mucoides* (1 g/L) were immobilized on spheres of calcium alginate and fermentation was performed in Erlenmeyer flasks at 30°C and 72 h of cultivation. In parallel, fermentations were carried out in free-cells of *C. mucoides* in same experimental conditions and the results were compared. Both fermentations (free and immobilized) the evaluated parameters were: consumption of xylose and emulsification index (EI24) as an indicator of BS production. The results showed that in fermentation with free-cells in Erlenmeyer flasks presented a total consumption of xylose of 95% and EI24 of 42% and fermentation with immobilized cells, the total sugar consumption was 86% and EI24 of 54%. These results show the potential of sugarcane bagasse hemicellulosic hydrolyzate as carbon source in BS production and the viability of the use of immobilized cells in the fermentations to obtain this biomolecule.

Simulation of syngas production from sugarcane bagasse gasification in a steam-blown bubbling fluidized bed using Aspen PlusTM

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Renewable energy sources is a topic that has gained great attention due to aspects such as the worldwide dependence on fossil reserves, the environmental issues related to their use,

and the planet's growing energy demands. In this context, biomass thermoconversion processes, particularly gasification, have become an option for bioenergy production. Gasification is a thermochemical process that converts a wide range of carbonaceous resources into synthesis gas, a gaseous mixture, which mainly contains H2, CO, CO2, CH4 and light hydrocarbons, and can be applied in the generation of heat, power, and fine chemicals. In the Brazilian energy matrix, sugarcane bagasse, an agricultural residue derived from the sugar-alcohol industry, is a promising feedstock for gasification due to its abundance. However, there is a lack of data in the literature of sugarcane bagasse gasification in fluidized beds, especially when it comes to the effects of operating conditions. In order to address this subject, this work aims at studying the influence of some operational conditions on syngas production via sugarcane bagasse gasification in a steam-blown bubbling fluidized bed reactor. Thus, a simulation model based on Gibbs free energy minimization was proposed in the Aspen Plus™ software in order to assess the effects of gasification temperature, pressure, biomass moisture content, and steam to biomass ratio (S/B) on syngas composition and gasifier performance using a 2^4 experimental design. As results, higher temperatures led to higher H2 and CO contents, and lower CO2 and CH4 contents because, according to the Le Châtelier principle, higher temperatures favor endothermic reactions. Raising temperature also increased syngas lower heating values, as well as carbon conversion and cold gas efficiencies. Higher pressures, on the other hand, caused a decrease in H2 and CO concentrations, and an increase in CH4 concentrations due to the fact that higher pressures shift reactions chemical equilibrium to the side of lower volumes, resulting in the consumption of H2 and CO and production of CH4. Biomass moisture content and steam to biomass ratio had similar effects on syngas composition: when higher water contents and S/B were applied, H2 and CO2 concentrations increased, whereas CO and CH4 concentrations decreased. However, in the case of gasifier performance, different optimum values were obtained for carbon conversion and cold gas efficiencies since S/B has a higher reactivity than water content. The present work has shown that sugarcane bagasse gasification is a potential and feasible process for clean energy production, contributing as an alternative to diversify the world's energy matrix, and reduce the current dependence on fossil reserves. Moreover, it can be shown how powerful the simulation platform in modern process engineering is.

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Quality applied to biodiesel production

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The oil and residual fat from the process of the food frying process is an important raw material used for the production of biodiesel. The evaluation of this raw material for the production of high quality biodiesel demands time and the use of precise technical analysis with sophisticated equipments. This work had as objective to produce biodiesel from waste cooking oils (WCOs) and to correlate its quality with the analysis of an olfactory profile with an electronic nose. First, 12 samples of waste cooking oils were collected from three different sources (residences, university restaurant (UR) and common restaurants). The samples of waste frying oils were characterized by level of acidity (LA) and peroxide (LP), viscosity, density and electronic nose. The recognition of the patterns of the electronic nose signal was done through the statistical techniques of Grouping Analysis, Association between Principal Components and Grouping Analysis and Neural Networks. All the oil samples were submitted to

the transesterification reaction for biodiesel in a jacketed reactor with mechanical agitation at 500 rpm, using ethanol in the proportion of 6:1 molar in relation to the oil, NaOH as catalyst at 1.3% by weight relative to the amount of WCO. For each sample, 3 reactions were performed at temperatures of 45, 55 and 65°C for 1 hour. The conversion to transesterification esters were analyzed by Nuclear Magnetic Resonance (1H NMR). Analyzing the results, all samples of WCO had level of acidity lower than 1.00 mgKOH / goil, making the WFO suitable for biodiesel production. The Residence source presented higher level of peroxide. The kinematic viscosity values found are between 35.346 and 37.3789 mm²/s. The parameters adopted for the transesterification reaction resulted in almost total conversion of the initial glycerides from WCO into ethyl esters. The temperature variation in the adopted range was not a relevant parameter to obtain higher conversion rates, since reactions at 65°C and 45°C resulted in similar conversions, the lowest temperature being more interesting in economic terms, aiming at a pilot or industrial scale. None statistical techniques used was able to correlate the electronic nose data with the parameters of the waste cooking oils, evidencing the need to use more complex technique, such as stochastic modeling.

Automated high-throughput method for *Saccharomyces cerevisiae* growth analysis

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The need to replace fossil fuels has motivated research into renewable energy sources. In Brazil, second generation ethanol is an important alternative to fuels derived from oil. In this context, the yeast *Saccharomyces cerevisiae* has been demonstrated as a microorganism with commercial potential. However, by-products with inhibitory characteristics, derived from biomass pretreatment, are a barrier to yeasts metabolism. A step towards solving this problem is the growth analysis of different yeast strains under stress conditions, such as in hemicellulosic hydrolysates. Therefore, in this work an automated method for this purpose was created to give robust and fast results. The method developed for high-throughput *S. cerevisiae* growth analysis includes two steps: (i) acquiring data related to microbial growth, using optical density values as a function of time, and; (ii) processing the acquired data. The software HiTGA (High-Throughput Growth Analysis) controls the automatic pipetting platform Microlab StarLet® (Hamilton Co.), coupled to a Cytomat® (Thermo Scientific) plate incubator and a 96-well plate optical density reader Spectramax Plus 384® (Molecular Devices). The system "robot + incubator + reader" allows the automated assembly of 96-wells plates with the capacity of 360 µL of growth culture that can be stored in controlled temperature and analyzed according to the optical density of each well. The incubator can store up to 31 plates, representing the screening possibility of up to 2.976 organisms and/or growth conditions in one experiment. At the end of step one, the software outputs the optical density of each well related to its respective time of reading. In the second step, these data are used to analyze microbial growth. The software OCHT (One-Click High-Throughput) is able to automatically (i) correct the OD values; (ii) calculate the average and standard deviation between replicates; (iii)

build curves of microbial growth through the approximation of the corrected OD (using non-linear fitting with a sigmoidal function) and; (iv) calculate growth kinetic parameters from the fitted data: maximum growth velocity (μmax), biomass production (CDWfinal) and adaptation time (Tlag). It is also possible to export graphics of individual or multiple growth curves and histograms of the growth kinetic parameters results. As proof of concept, this method was used to evaluate the growth of four different yeast strains in different oxidative stress conditions – 8 hydroxymethylfurfural (HMF), 7 furfural and 3 vanilin concentrations –, totaling approximately 1.000 microfermentations, for which analysis would typically be very laborious. The growth of 110 haploids – obtained from the manual dissection of the FMY001 strain – was also analyzed in oxidative stress induced by 80 mM of HMF. The results showed this method to be robust and reproducible, allowing the screening of tolerant strains to industrial inhibitors and haploids phenotypically equivalent to the parental FMY001.

Modeling of anaerobic biodigestion of vinasse in sequencing batch reactors operated with immobilized biomass (AnSBBR) using ADM1 model

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In recent years the demand for mathematical models is constant, because than can make simulations and optimizations of the processes of production, since they provide an overview of the production process without requiring time-consuming and/or expensive experimental tests. In the same time, there has been a growing in search for alternative energy sources, since there will be a shortage of fossil fuels in the future and also because the use of these fossil fuels causes problems of environmental pollution, a fact that aggravates the greenhouse effect. In this context, the sugarcane vinasse, residue from the production of ethanol, is being used for the production of methane. The vinasse presents as main physical-chemical characteristics the presence of high concentrations of organic matter, high concentrations of nutrients, such as potassium and sulfate, and low pH. Due to these characteristics, this wastewater is mainly destined for fertirrigation, but significant volumes are produced per year (10 to 15 L of vinasse per liter of produced ethanol), and environmental impacts are reported with this practice. Therefore, anaerobic biodigestion of sugarcane vinasse can be an alternative to mitigate this environmental problem and also to recovery energy from this wastewater. This paper intends, based on data already published in the literature, to propose the modeling of vinasse's biodigestion in order to provide the best understanding of the process, aiming to give tools to enable the application of such technology in full scale. Experiments of anaerobic biodigestion of vinasse was carried out by Albanez *et. al.* (2015) in an anaerobic sequencing batch reactor with immobilized biomass (AnSBBR), aiming at the production of methane. This reactor was submitted to increasing organic loads of vinasse, in five phases (1.000, 2.000, 3.000, 4.000 and 5.000 mg COD L^{-1}, phases I, II, III, IV and V, respectively). After that, the modeling of the data using the Anaerobic Digestion Model Number 1 (ADM1) was performed using Matlab software, as mathematical simulation platform, aiming to elucidate the methane production, the production and consumption of the organic

acids and to evaluate the COD removal for the anaerobic treatment of vinasse. The ADM1 model adequately described the dynamic behavior of the organic matter concentration over time, as well as the accumulation of volatile acids, making possible to do predictions and optimizations about the anaerobic treatment of vinasse in AnSBBR reactors.

Evaluation of corncob hemicellulosic hydrolysate for the second generation production of ethanol by Scheffersomyces stipitis CBS 6054

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A renewable primary energy source as lignocellulosic materials (nonpetroleum-based source) appears to be suitable to produce alternative liquid transportation fuels as ethanol. Brazil is the third largest producer of corn in the world, with a production volume expected for 2017 of 88 million tons. Lignocellulosic materials, such as corncob could be another interesting alternative for the second-generation production of ethanol due to its large-scale availability, low cost, lower fossil fuel inputs etc. The main composition of these materials is cellulose, hemicellulose and lignin. Therefore, pretreatment is required to reduce the recalcitrance of lignocellulosic biomass by opening or partially breaking up the recalcitrant structure, while releasing the xylose from hemicellulose as a major sugar and minimizing the formation of inhibitors for further fermentation step. A large number of proposed pretreatment methods are usually divided into physical, chemical, physical-chemical and biological. Diluted acid pretreatment is the most used pretreatment to obtain a biomass hemicellulosic hydrolysate. The aim of this work was to use the response surface methodology to improve corncob diluted sulfuric acid pretreatment to obtain a hemicellulosic hydrolysate rich in D-xylose with low concentration of toxic compounds to produce a second generation ethanol by the yeast Scheffersomyces stipitis CBS 6054. The experiments were conducted in AISI 316 stainless steel jacketed reactor with a total volumetric capacity of 50 liters. The pretreatment conditions of the corncob (solid/liquid ratio of 1:10) were according to factorial design 22 with 4 repetitions at the central point and trials corresponding to the centered phase by using different concentrations of diluted sulfuric acid as a catalyst (50 to 200 mgH2SO4/gdry matter) and temperature (110 to 150°C). The corncob hemicellulosic hydrolysate obtained at optimized condition (50 mg H2SO4/g dry matter and 133°C) was vacuum evaporated to increase the D-xylose to 72 g/L, detoxificated by pH alteration and subsequent treatment with active charcoal adsorption (3%w/v). After, it was autoclavated and supplemented with nutrients (yeast extract 5 g/L, MgSO4.7H2O 0.5 g/L, (NH4)2SO4 3.0 g/L). The Scheffersomyces stipitis CBS 6054 inoculum (1.5 g/L) was freshly harvested (24 h) after cultivation in Erlenmeyer flasks (1000 mL) containing 400 ml YEPD with 30 g/L of glucose under 200 rpm at 30°C. The fermentation conditions were conducted at 150 rpm and 30°C. This yeast fermented efficiently the xylose (96.38%) to ethanol (yield of 0.28 gethanol/gxylose) and volumetric productivity (0.20 gethanol/L.h), with a cell yield of 0.48 gcell/gxylose at high specific cell growth rate (0.14 h-1). The corncob hemicellulosic hydrolysate was suitable for the second generation production of ethanol by Scheffersomyces stipitis CBS 6054.

Kluyveromyces marxianus as a robust systems and synthetic biology platform through the establishment of a CRISPR/Cas9 system

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Among "non-standard" yeast, there is increasing interest in studying Klyuveromyces marxianus (Km) given its combination of industry-appealing traits: broad range of carbon source utilization, ability to grow at temperatures up to 52°C and ferment at 45°C and one of the highest growth rates among eukaryotes. It is not surprising, however, that most of the studies published on Km up to date exploits its general biotechnological potential rather than studying the basic biology underlying its interesting traits. The lack of genetic tools has hampered in-depth genetics and biochemistry focused studies in Km and in many other non-model organisms. Our goal is to tackle the lack of genetic tools and develop K. marxianus into a robust platform for systems biology studies and synthetic biology applications in industrial microbiology. With our CRISPR/Cas9 system, we aim to investigate the molecular basis of thermotolerance and engineer top-producer strains for value-added chemicals from alternative carbon sources. We established a CRISPR-Cas9 genome editing in Km and used this system to engineer several strains to use in classical genetics. We are successfully using CRISPR-Cas9 to knockout genes of interest and identify ones that might have an important role in thermotolerance. We constructed a library of mutants and screened for loss of thermotolerance, finding a few strong hits that are now being investigated in detail. Our synthetic biology tools are also being used to explore the potential of Km to produce renewable fuels and chemicals. We have laid the synthetic biology foundation for using K. marxianus in industrial applications, creating genetic tools and identifying strains with promising lipid production, high-temperature fermentation, and alternative sugar consumption.

Enzymatic activity of a recombinant β-1,4-endoglucanase from sugarcane giant borer (*Telchin licus licus*) aiming second generation ethanol production

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The sustainable development requires sources of renewable fuels to production of clean energy. In the last 35 years Brazil presented a substantial production and utilization of ethanol fuel, however until few years ago its production was limited to fermentation of sugarcane molasses. Since 2015 this scenario begins to change by introduction of second generation ethanol in the Brazilian energetic matrix. The technology to production of ethanol fuel in Brazil is consolidate and have been used in the last decades, however Brazil remains behind countries like USA in relation of using others sugar sources, like amide or lignocellulosic plant material, to ethanol fuel production. This research comprise a search by genes coding enzymes to plant biomass degradation, the cloning of this genes, its heterologous expression and enzymatic activity characterization, having in mind the possibility of using such enzymes in biotechnological processes to second generation ethanol production. A cDNA databank of Sugarcane Giant Borer was used as source of genes coding enzymes to plant biomass degradation. Three

genes (coding to a xylose reductase, a β-1,4-Endoglucanase and a β-glucosidase) were cloned and expressed in Pichia pastoris. The Sugarcane Giant Borer is a Lepidoptera insect which, in its larval stage, feeds only on sugarcane biomass. Once this insect larvae feeds only on sugarcane biomass, its transcriptome is a good source of enzymes to sugarcane biomass degradation. Until now only the recombinant β-1,4-Endoglucanase activity could be detected in a assay to carboxymethylcellulose degradation. Assays with the others recombinant enzymes are in progress. Preliminary data suggest that a recombinant β-1,4-Endoglucanase is active when expressed in heterologous system. Others assays to the characterization of recombinant Sugarcane Giant Borer enzymes are in progress aiming to select enzymes to be used in biotechnological process to second generation ethanol production.

Hydrodynamic cavitation assisted alkaline hydrogen peroxide pretreatment of sugarcane bagasse

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The recalcitrant structure of lignocellulosic biomass impairs its use as a raw material for biofuels and chemicals production. Thus, previous steps are required in order to improve the enzyme access into carbohydrate fraction for releasing of fermentable sugars. Pretreatment processes produce changes in composition by removal of lignin or hemicellulose, and in the structure of the material by the reduction of crystallinity and increase in the porosity of the material. Among some new alternatives, hydrodynamic cavitation (HC) was recently reported as a promising pretreatment method, resulting in higher enzymatic digestibility in low process time. In this context, HC was used for intensification of alkaline hydrogen peroxide pretreatment of sugarcane bagasse in order to reduce the pretreatment time under mild conditions (60°C). A Box-Behnken design was used in order to optimize the variables pretreatment time (2-10 min), H2O2 concentration (0.2-1.0%v/v) and NaOH concentration (0.1-0.3 M). Optimization was carried out in terms of total sugars released (TSR) after enzymatic hydrolysis steps. The highest cellulose (94.96%) and hemicellulose (93.62%) hydrolysis yield were achieved in the following conditions: 10 min of process time, 0.6% v/v of H2O2 and 0.3 M of NaOH. In those conditions, 63.58% and 28.02% of lignin and hemicellulose were removed during pretreatment; moreover, the solid composition after pretreatment corresponded to 61.24%, 27.62% and 14.33% of cellulose, hemicellulose and lignin, respectively. HC-assisted process presents potential to accelerate the reaction time under milder condition of temperature and to improve the enzymatic hydrolysis yield. This behavior could be attributed to mechanical (microjets and shockwaves) and chemical (release potential oxidative radical) effect of HC.

Scale-up of packed bed bioreactor for cellulase production: criterion for flow rate determination

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One of the main drawbacks of the enzymatic route for the second-generation ethanol (SGE) production is the cost of the hydrolytic enzymes, which represents the third highest cost in the whole process. Solid-state fermentation (SSF) has been proved to be an effective technique to produce cellulases and hemicellulases from solid by-products of the agro-industries. Therefore, SSF is a promising alternative to reduce the costs of SGE and to mitigate environmental problems of inadequate disposal of organic solid wastes. Nevertheless,

no SSF bioreactors is commercially available, resulting in little industrial application of SSF-based processes. Therefore, this work aimed to develop a criterion to determine the flow rate for packed-bed SSF bioreactors as a step to scale-up this equipment. Regardless submerged fermentation, for which the criterion of volume of air per bioreactor volume is usual, no criterion is well defined for the air flow rate range for SSF bioreactors. In recent work of our group, the Damköhler (Dam) number has been used to determine the operational air flow rate; however, Dam is based solely on thermal aspects, and it was observed that the oxygen concentration is also an important variable to be considered. In this work, a packed-bed bioreactor was instrumented to sample gaseous data (O2 and CO2) in a system composed by sugarcane bagasse and wheat bran (weight proportion 7:3) for the grown of Myceliophthora thermophila I-1D3b at 45°C and 75% moisture content. The bioreactor was a jacketed tube made of stainless steel with 7.62 cm internal diameter and variable height. The wall temperature was kept constant flowing water throughout the jacket. An online system of gas sampling was implemented to sample data from several axial positions of the bioreactor along the cultivation period. The production of endoglucanase was used as the response variable and the minimum airflow rate was determined, as well as a new non-dimensional number based on the total mass of substrate. Two alternatives of introducing the air in the system were used: introducing the air totally by the bottom of the column; splitting the air flow rate in two streams, one by the bottom and another by the middle of the 80 cm height column. The second option has proved to be more effective. Form the results here presented, the important operational condition air flow rate can now be estimated for every packed-bed SSF bioreactor.

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Integrated high-efficient tools for the study and development of *Saccharomyces cerevisiae* commercial strains

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The greater awareness of the environmental consequences resulting from fossil fuels usage leads to an increased interest in the development of renewable energy sources. In this scenario, the production of hemicellulosic ethanol stands out due to the possibility of more efficient utilization of natural resources. However, the economic viability of industrial processes is multifactorial and depends, among other things, on the performance of the yeast used. For this reason, *Saccharomyces cerevisiae* yeasts adapted to industrial conditions – industrial strains – have been considered ideal platforms for the development of commercial yeasts, as well as candidates for the study of genes which provide tolerance to stresses observed under industrial conditions. In this context, the main goal of this work was to develop and to integrate high-efficient tools for the study of the strains naturally adapted to industrial environment, optimization of commercial strains, and development of new ones. This integrated approach comprises: (i) automated high-throughput methods for the *S. cerevisiae* growth analysis; (ii) flow cytometry-

based procedure for high-throughput isolation of recombinant haploids; (iii) bioinformatics tools for genomes analysis; and (iv) CRISPR/Cas9 system-based approaches for precise genome edition. Applying the developed software HiTGA (High-Throughput Growth Analysis) and OCHT (One-Click High-Throughput) enables the automatic quantification of the optical density-based growth of thousands *S. cerevisiae* microcultures and calculation of the main kinetic growth parameters – lag phase, growth rate and final biomass produced. Among other applications, HiTGA and OCHT have been used to test the xylose consumption, the growth of different strains on variable stress conditions, and the hemicellulosic hydrolysates toxicity. Using the mating-type-specific promoters driving the appropriate fluorescent proteins expression and flow cytometer, an unlimited number of individual recombinant haploids can be obtained with very high purity, requiring minimal and transient strain engineering. Segregants presenting equivalent parental robustness may be used to map quantitative trait loci (QTLs) and, additionally, more industrially relevant haploids may be continually obtained and combined by crosses, allowing the application of laboratory evolution based on sexual hybridization, a powerful complementary approach to metabolic engineering. From DNA sequencing data, single-nucleotide polymorphism, copy-number variation, QTLs and other genome variations may be identified using validated bioinformatic pipelines. Finally, an easily applicable tool for the genome editing based on CRISPR/Cas9 system has been developed and enables high-efficient genetic manipulations of any *S. cerevisiae* strain. This facilitates the metabolic engineering of industrial strains and could be applied in the validation of candidate alleles related to the industrially relevant phenotypes.

Bioethanol and flex-fuel vehicle technologies: a patent citation network

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Bioethanol fuel produced from renewable energy sources becomes an alternative product or complementary additive to the non-renewable energy sources fuel, aiming at reducing the effects of greenhouse gas emissions and the direct dependency on petroleum as a fuel (Babcock et al., 2017). In order to understand the dynamics of ethanol fuel technology diffusion and the changes in local and global market structure, besides the directions of the area P&D, patent citation networks were elaborated. The current study uses this organized base of information for developing patent identification indicators more important from the point of view of technological trajectories and, as a consequence, of economic interest. Network analysis and construction methods were developed in Odysseýs software using USPTO (United States Patent and Trademark Office) query structure “ABST/((Bioethanol OR Ethanol) AND Fuel) AND ACLM/((Bioethanol OR Ethanol) AND Fuel)”. That is, the software return all patents from USPTO database containing the terms "bioethanol fuel" or "ethanol fuel" in the patent fields “abstract” and “claims”. The analysis period comprehends all patents filed between the years 1976 until July 2017. The obtained patent citation network consists of 2124 patents, of these, 45 are isolated nodes type components. The main structure with the largest number of connected patents - Giant Component - is formed by 1811 patents. From these patents, 202 patents have the search terms, and 87% of them belong to American patent holders. Other patents belong to countries such as Japan, Germany, France, Netherlands and Canada, 17% of the 202 total patents. By the inclusion of the keyword "fuel"

made it possible to identify emergency in technological trajectories in the field of combustion engines improvement that use ethanol, reflecting the competition effect ahead between this engine and electrical engines. Trajectory identification was performed by Search Path Link Count (SPLC) calculation which revealed the research importance in flex vehicle engines. That is the novelty of the this work, as the use of the query "fuel" allows to dismember the Giant Component on subnetworks that contain distinct technological trajectories. When combine words relating to biofuel and biofuel use in automobiles, notice that the importance of biofuel obtaining technologies reduces due to the search for improvements in flex fuel vehicles.

Purification and biochemical characterization of a halotolerant and thermostable ß-xylosidase from *Colletotrichum graminicola*

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In order to ensure the economic viability of the production of second-generation ethanol, it is necessary the development of efficient technologies for the enzymatic hydrolysis of lignocellulosic materials. In addition, the large consumption of water by biorefineries has attracted great attention for the use of non-potable water resources, such as seawater. Therefore, enzymes tolerant to high salt concentrations and the by-products generated or accumulated in the biomass pretreatment steps are widely studied. This study aimed the purification and biochemical characterization of a ß-xylosidase produced by *Colletotrichum graminicola* under solid-state fermentation. The enzyme was purified by three sequential chromatographic steps with a yield of 24.2% and purification factor of 23.8-fold. The pure enzyme (Bxcg) showed a total carbohydrate content of 54% (w/w), isoelectric point of 4.2 and an apparent molecular weight of 128 kDa. The enzyme showed good halotolerance, retaining approximately 63% of the control activity in the presence of 2.5 mol L^{-1} NaCl. pH and temperature optimum were 4.5 and 65°C, respectively. NaCl did not affect the optimum temperature of Bxcg, but the optimum pH rose from 4.5 to pH 5.0 in presence of 2.5 mol L^{-1} NaCl. Bxcg retained stable over a wide pH range (4.0 - 7.5) both in the absence and in presence of salt. The enzyme showed excellent thermal stability with a half-life of 30 minutes at 70°C and the presence of high NaCl concentration (2.5 mol L^{-1}) resulted in an increase in the thermostability of the enzyme. In the absence of salt, Bxcg hydrolyzed *p*-nitrophenyl-ß-D-xylopyranoside with Vmax of 348.8 ± 11.5 U mg^{-1}, KM of 0.52 ± 0.02 mmol L^{-1} and high catalytic efficiency kcat/KM= 1432.7 ± 47.3 L $mmol^{-1}$ s^{-1}). In the presence of salt, the kinetic parameters were slightly lower, resulting in catalytic efficiency 1.5 fold lower. Bxcg was able to hydrolyze xylooligosaccharides from xylohexaose, including xylotriose with 4-O-methyl-glucuronic acid branch. Bxcg and a purified endo-xylanase from the same microorganism had a strong synergistic effect (3.1 fold) for hydrolysis of xylan beechwood. The enzyme was tolerant to various organic solvents and surfactants at concentration of 5% (v/v). In summary, Bxcg has attractive properties for application in saccharification processes of the lignocellulosic biomass, particularly under high salinity and/or in the presence of residues of biomass pretreatment steps.

To be or not to be: can *Saccharomyces cerevisiae* grow under full anaerobiosis without lipid supplementation or is full anaerobicity not achievable in the lab?

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The budding yeast *Saccharomyces cerevisiae* plays an important role in biotechnological applications, ranging from fuel ethanol to recombinant protein production. It is also used as a model organism to understand eukaryotic gene regulation and cellular physiology. *S. cerevisiae*'s ability to grow under anaerobic conditions is of particular interest for some industrial applications, in order to maximize product formation and decrease costs related to mixing and aeration. Despite the abundant amount of information and the industrial relevance, it is still not conclusive whether *S. cerevisiae* can grow under complete anaerobiosis as the biosynthesis of lipids requires molecular O2. Thus, we revisited the much-studied fermentative metabolism in *S. cerevisiae* using a set of well-defined experiments to clarify if it can indeed grow under complete anaerobiosis. We used an O2 trap (OTC-2, Agilent) coupled to a N2 stream (Certified; 5.2, Air Products, < 3 ppm O2), to provide an inlet N2 gas with less than 15 ppb O2. Under such severe O2 limitation in a chemostat without lipid supplementation, the biomass concentration dropped to 0.31 gDCM L^{-1}. We also noticed that the fraction of unsaturated fatty acids in cells dropped to 40.5% (w/w) as compared to 70% when cultivated with oleic acid/ergosterol. The altered lipid profile under anaerobic conditions could give a hint as to why certain yeast species persist in fermentation vats possibly by offering more tolerance to certain stress factors.

Organosolv pretreatment produces an inhibitor free hydrolysate with superior fermentability at high-solids loadings

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Never has the issue of sustainability garnered so much importance than now. The fifth assessment report by the Intergovernmental Panel on Climate Change necessitates us to take drastic actions to combat the emissions of greenhouse gases. A rising population, an urban lifestyle and increased economic growth would place enormous pressure on the global energy demand and food production. Thus, targeting industrial chemicals – valued at 3 trillion USD per year, with bio-based processes will enable the production of these chemicals from a non-petrochemical feedstock. Biomass is a renewable feedstock that is available abundantly. However, it needs to be processed, to release the sugars that can be utilised by microorganisms to produce various products of interest. Several pretreatment methods are currently available for biomass deconstruction, but inevitably they produce compounds, such as hydroxy methyl furfural and furfural, that are toxic to the microorganisms. Organosolv (with ethanol as a solvent and sulphuric acid as a catalyst) pretreatment has shown much promise, as it yields three distinct and clean streams — cellulose, hemicellulose and lignin, that are less toxic to the microorganisms. The enriched cellulose fraction can be hydrolysed using a cocktail of enzymes to release the glucose monomers and subsequently be fermented to ethanol using native yeasts. In this

study, we report the sugar yields during the hydrolysis of organosolv pretreated birch and spruce biomass and the superior fermentability of birch biomass over spruce, in an SSF process using Ethanol Red yeast strain. Ethanol yields up to 95% of theoretical maximum at 5% solids loading could be achieved in a small-scale set-up. Large-scale studies, including LCA analysis, would provide a conclusive evidence on the efficacy of this pretreatment method over others.

Understanding the metabolism of hemicellulosic sugars from omics of the yeast-like fungus *Kalmanozyma brasiliensis* GHG001 and the comparative genomics with Ustilaginaceae (Basidiomycetes)

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The production of Second Generation (2G) Bioethanol is strongly dependent on pretreatment of plant biomass (e.g. sugarcane bagasse) followed by fermentation of monomers present in the cell wall polymers (cellulose and hemicellulose). Xylan and its pentose monomer xylose are the main sugarcane hemicellulosic components. Biotechnological improvements for biomass hydrolysis in the context of bioethanol production are only possible with a deeper understanding of how these sugars are metabolized by native organisms. In this work, we explore transcriptomic and genomic data of *Kalmanozyma brasiliensis* GHG001, a yeast-like basidiomycetous fungus known to grow in xylan and xylose as sole carbon sources. RNAseq experiments were performed aiming to improve the genome structural annotation ("pool" experiment) and study the metabolism of xylan and xylose through differential gene expression (DGE) analysis comparatively with growth in glucose ("sugar metabolism" experiment). To have insights on sugar metabolism and possibly on regulation of gene expression, we employed methods for functional annotation of Carbohydrate-Active Enzymes (CAZymes) and Transcription-Associated Proteins (TAPs) on the improved proteome of *K. brasiliensis* GH001, as well on other 15 Ustilaginaceae members, then identified correlation, expansions and contractions among CAZyme and TAP family sizes in the fungal family. As main results, we observed an improvement of the structural annotation of K. brasiliensis protein-coding genes after inclusion of transcriptome data by an increased number of proteins and annotated features (particularly CAZymes and TAPs). DGE revealed that at least two previously characterized enzymes were identified as highly expressed in xylose and xylan: an extracellular endo-xylanase involved in xylan hydrolysis and a xylitol dehydrogenase, involved in xylose metabolism. The identification of other GHs following the same expression patterns may indicate a synergistic action on xylan deconstruction or xylose metabolism; additionally, identification of TAPs in gene expression analysis and evolutionary insights using comparative genomics methods indicate possible roles of these elements on the regulation of genes involved in biomass deconstruction. Our work provides a platform for functional experiments in *K. brasiliensis* GHG001 that can help in the biotechnological improvement of 2G bioethanol.

Fluorescence-based approach for the high-throughput isolation of industrial *Saccharomyces cerevisiae* recombinant haploids

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Saccharomyces cerevisiae yeasts adapted to industrial conditions – industrial strains – are considered appropriate platforms for the development of commercial strains for production of fuels and other chemicals derived from renewable materials. In this context, the laboratory evolution based on sexual hybridization is considered a powerful complementary approach to metabolic engineering. However, combine industrially relevant features by crosses require the isolation of a large number of recombinant haploids, typically obtained by manual dissection of tetrads. This is an extremely laborious procedure depending on the number of segregants required. Then, the development of high-throughput approaches for tetrad dissection and haploid isolation has great impact on the study of the strains naturally adapted to industrial environment, optimization of commercial strains, and development of new ones. For this reason, the main goal of this study was to develop a very simple and efficient approach to isolate recombinant haploids using flow cytometry. This method includes the transient expression of two fluorescent proteins controlled by the mating-type specific promoters, random digestion of *ascii*, flow cytometry sorting and isolation of individual haploids. To simply the analysis and to allow the use of equipment containing the usual lasers configuration, we decided to use an orange fluorescent protein with a large stokes shift that can be excited simultaneously with but detected separately from EGFP (Ex. 488 nm; Em. 600 and 505 nm respectively). For that, the coding sequences for both fluorescent proteins were fused respectively to *MATα*-specific *STE3* and *MATa*-specific *STE2* promoters by the Gap-Repair method, resulting in the pMF002 vector containing a dominant selection marker. To confirm the functionality of this plasmid the industrial strain JAY270 (*MATa/α*) and its derived haploids JAY289 (*MATa*) and JAY290 (*MATα*) were transformed with pMF002 by LiAc procedures. Then, the functional analyzes were performed using epifluorescence microscopy (Olympus BX51 FLIII) and flow cytometry (Beckman Coulter Gallios). The random digestion of *ascii* procedures were achieved using optimized concentration of Lyticase (Sigma-Aldrich), after induction of the sporulation in appropriated condition. Spores isolation was performed using FACSAria III (BD Bioscience) and ploidy was confirmed by PCR using mating-type specific primers. In the visual inspection analysis, transformed haploid cells *MATa* and *MATα* showed green and orange fluorescence, respectively. As expected, the diploid cells showed no fluorescence. Using optimized cytometry parameters was possible to detect and distinguish the both fluorescence signals. As a proof of concept, cell-pool carrying pMF002 obtained from the random lysis of JAY270 tetrads were analyzed. One thousand cells expressing green or orange fluorescence were collected and, after individual colony mating-type verification, 100% of purity was observed for that and for two other industrially relevant yeasts. In conclusion, this approach enables the rapid isolation of specific mating-type haploids, requiring minimal and transient strain engineering.

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

Evolutionary approach for searching and describing pentose transporters from yeast *Spathaspora passalidarum*

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Aiming the reduction of dependence on petroleum derived energy sources, biofuels, especially bioethanol, appear as an alternative. While ethanol acquisition by yeast fermentation from sugarcane juice and syrup has already been optimized, second generation ethanol production, by fermenting pentose sugars existent on the plant´s fibres, remains a challenge for researchers. *Spathaspora passalidarum* is a yeast species known for its xylose fermentation capabilities in anaerobic conditions, however most of its transporters are still uncharacterized. Since available information of specific pentose transporters is scarce and most known transporters have a higher affinity to hexose than to pentose sugars, lowering 2G ethanol specific productivity, obtaining a sugar transporter with high xylose uptake is essential for constructing a robust industrial yeast strain. We used an evolutionary approach to compare, rank and choose 10 *Spathaspora passalidarum* uncharacterized genes from a set of 255 predicted sugar transporters of 18 yeasts genomes. Using these genomes, we assigned all predicted proteins into gene families and chose the most efficient described pentose transporters from literature, such as GXF1, as baits to obtain candidate sequences from the families. A BLAST search for sequences similar to the baits retrieved 10 different homolog families and the phylogenetic relationships for each family were reconstructed by maximum likelihood method. Subsequently, we compared the ratio for non-synonymous substitutions and synonymous substitutions (ω=dN/dS) between each branch from the gene tree and chose the genes with highest ω as candidates, based on the premise that genes that have accumulated more non-synonymous substitutions from their ancestors have a higher chance to carry new adaptations for their sugar transport. For future inquiries, we aim to isolate the chosen candidates, one from each family, characterize them functionally and transform in an industrial yeast strain to test sugar uptake and consequently ethanol production. Besides yet uncommon for biotechnological purposes, our work methodology had implemented an evolutionary approach to search for the best gene candidates for industrial applications based on the evolutionary forces shaping them. Our findings include novel putative pentose transporters from an important organism in ethanol field that will be further explored.

Genomic and transcriptomic analysis of a yeast strain capable to depolymerize lignin

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The research for renewable sources of energy is constant due to the fact that fossil fuels can negatively affect the environment, the economy and the society. In this scenario, lignocellulosic biomass has several advantages, this material consists of cellulose and hemicellulose, that can be used for ethanol production, and lignin, which can be converted into high value chemical compounds. Despite having a high recalcitrance, in virtue of its complex polymeric structure, lignin can be degraded by some

oxidative enzymes of bacteria and fungi. The first enzyme described was the LiP, a lignin peroxidase that was isolated from white-rot fungi, *Phanerochaete chrysosporium*. After that, several types of extracellular oxidative enzymes were subsequently identified and characterized, and these can be assigned to major classes: heme peroxidases and laccases. Besides these enzymes, different studies show that microbial metagenomes from sources where biomass is rapidly degraded, for instance active soils, could reveal novel pathways and bioconversion routes for lignin degradation, which is not always possible by traditional lab cultures. In this work, using genomic and transcriptomic strategies, we were able to characterize a yeast strain capable to depolymerize lignin from the genus *Rhodosporidium* sp. which is known to produce lipids and carotenoids. The genome contains 50 Mb with 17,245 predicted proteins, including lignin depolymerizing enzymes, such as DyP (dye-decolorizing peroxidases), transcribed in response to growth on lignin fragments. The high biosynthetic capacity of *R. fluviale*, in addition to its unusual strategy for lignin degradation, attests this yeast's potential for further biotechnological applications.

Adaptive evolution of *Saccharomyces cerevisiae* PE-2 to treatments of high ethanol content

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One important trait sought in industrial yeasts is their tolerance to high ethanol titers present at the end of very high gravity fermentations, such as for the production of ethanol as biofuel. Even a slight increase in ethanol tolerance by fermenting yeasts might have a huge impact on large-scale productivity by bioethanol powerplants. Here we used adaptive laboratory evolution as a valuable tool to select for ethanol tolerant yeast strains. Basically, we conducted an ethanol survival experiment in which four haploid Saccharomyces cerevisiae PE-2 populations were submitted to harsh ethanol treatments for two hours at 32°C, followed by a recovery period in an ethanol-free medium (2-4 days). Such cycles of shock/recovery were reinterred with increasing ethanol content. The ethanol treatment started from an initial shock of 19% (v/v) ethanol (during which most of the cells died) and progressed through about 70-80 cycles of shock/recovery, until the four populations were well adapted to shocks of 28-30% (v/v) ethanol. Competition assays between the evolved populations and the ancestor show a clear pattern of antagonistic pleiotropy, in which the evolved strains achieve higher fitness than the progenitor to tolerate ethanol shocks, however they are largely outcompeted by the ancestor under normal growth conditions and even during propagation on liquid medium containing 8% (v/v) ethanol. Whole genome sequencing (Illumina MiSeq platform) recovered 67 point mutations across the four final populations. Functional analysis of the affected genes suggests a prominent role of trehalose accumulation and inhibition of the RAS/PKA pathway in improving survival rates to ethanol shocks. Molecular genetic analysis of key mutations found during whole genome sequencing are currently underway and will allow a fine understanding of the evolution process.

Mineral particle identification in sugarcane bagasse by image analysis of X-ray microtomography data

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Sugarcane bagasse is a lignocellulosic biomass with potential to be used as raw material for

applications involving renewable fuel production, more specifically cellulosic ethanol. A challenge for these applications is the presence of mineral particles in the biomass, which causes problems such as corrosion, sintering, and vitrification in boilers, gasifiers, and combustors because of the presence of inorganic constituents. On the other hand, X-ray microtomography is a non-invasive technique able to capture three-dimensional images of solid samples with a resolution up to ~1 µm. At this scale, it is possible to visualize the cellular structure of the sugarcane bagasse, allowing quantification and morphological analysis of its different cells and mineral particles. In this work, image processing and analysis were employed to quantify 3D morphological features of the mineral particles trapped in the sugarcane bagasse. The 3D images of the bagasse were obtained by x-ray computed microtomography at the LNLS IMX beamline of the Brazilian Synchrotron Light Laboratory (LNLS). Results show the sizes, shapes and preferred location of mineral particles within the bagasse structure and demonstrate the potential use of the microtomography to support the development of novel biomass cleaning technologies. Likewise, the obtained results provide valuable information for the future development of new techniques for removal of sugarcane bagasse impurities in the bioenergy industry.

Organic fertilizer and bio-products from aviary waste oxidative pyrolysis

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New technologies for the use of chicken litter as a feedstock for higher value-added products are of interest to all segments of agribusiness. Thus, pyrolysis can solve the problem of waste disposal (chicken litter) and generate a new revenue stream in aviculture. Through the process of fast pyrolysis of chicken litter in auto-thermal reactor fluidized can get the products with economic potential. Samples of product of pyrolysis, biochar were characterized, as were the bio-oil and gas effluents from the process. The Fast Pyrolysis Plant with capacity of 20 kg / h biomass aims to pilot production of biochar, bio-oil and extract acid. The fluidization air was heated at 400°C, 450°C and 500°C and injected into the bed. Chicken litter undergoes where it is removed biochar pyrolysis in the reactor the reaction products undergo a cyclone separation from where it is removed biochar, with a flow rate of 8.5 kg / h (42.5% w / w), the flow goes to the centrifugal recuperator, where it is separated bio-oil and the acid extract, the remaining gases are burned in the combustion chamber. The chicken litter biochar produced in fast pyrolysis plant of auto-thermal was characterized and compared to the standards required by Brazilian law. He attended the standards contained in IN SDA No. 25 and was framed in the category of organic fertilizer compound class A. The results of the analytical characterization and physico-chemical products of pyrolysis products show that the 3 has the potential to be used for up-grades to products with higher added value. The yield of bio-charcoal is about 5% higher than the pyrolysis temperature is 400°C above the temperature of 450°C. Compared with the literature, the gain was approximately 7%. At 400°C the production of bio-oil is impaired. Although the yield of condensable be 6% greater than the pyrolysis at 450°C the formation of acid extract is accentuated at the temperature of 400°C. Reaching 30% of 44%; In tests of auto-thermal pyrolysis of chicken litter with a temperature of 400 ° C the amount of organic matter in biochar was significant.

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A novel thermo-halo-solvent tolerant xylanase from *Colletotrichum graminicola*: Purification and biochemical characterization

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Agro-industrial residues are highly convenient sources for the production of lignocellulosic ethanol. The economic viability for this process is highly dependent on an efficient hydrolysis of xylan, raising a great interest in xylanases with attractive properties for industrial applications. Furthermore, different pretreatment steps are used to reduce the recalcitrance of the lignocellulosic biomass, enabling the action of hydrolytic enzymes on polysaccharides and increasing the efficiency of hydrolysis. However, residual solvents, as well as salts in high concentrations and some byproducts of the alkaline pretreatment are potential inhibitors of enzymes in subsequent steps. Thus, in this study a novel thermo-halo-solvent tolerant xylanase (Excg1) from *Colletotrichum graminicola* produced under solid-state fermentation was purified and biochemically characterized. Excg1 as purified by a procedure which involved hydrophobic interaction chromatography in Phenyl-Sepharose CL-4B and ion exchange chromatography in DEAE-Fractogel. Similar apparent molecular masses were estimated by gel filtration (17.3±1.9 kDa) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (20.0±2.4 kDa), suggesting that Excg1 is monomeric. The enzyme showed good halotolerance, retaining about 85% and 50% of the control activity in the presence of 0.5 mol L^{-1} and 3.0 mol L^{-1} NaCl, respectively. The optimum temperature of Excg1 (65°C) was not affected by NaCl, but the optimum pH rose from 5.5 in the absence and presence of 0.5 mol L^{-1} NaCl to 6.0, in 2.5 mol L^{-1} NaCl. Excg1 was highly thermostable at 50°C, with half-lives around 48 h in either water or 0.5 mol L^{-1} NaCl and a residual activity of 75% at 2.5 mol L^{-1} NaCl. Excg1 was fully stable at pH 3.0–10.0 in the absence of salt, and from pH 4.0–10.0 in the presence of 0.5 mol L^{-1} and 2.5 mol L^{-1} NaCl. The enzyme hydrolyzed beechwood xylan with maximal velocity and apparent affinity constant of 481.3±34.0 U mg^{-1} and 3.7±0.3 mg mL^{-1}, respectively. Similar kinetic parameters were obtained in the presence of 0.5 mol L^{-1} NaCl, but a maximum velocity about 34% lower was determined in 2.5 mol L^{-1} NaCl. Excg1 was tolerant to various organic solvents at a concentration of 5% (w/v) and also to sodium acetate up to 200 mmol L^{-1}. Xylobiose and xylotriose with a 4-O-methylglucuronic acid branching were the main products of beechwood xylan hydrolysis, and the time course of hydrolysis was not affected by NaCl 0.5 mol L^{-1} or sea water. To the best of our knowledge, this is the first report of a halotolerant endoxylanase from fungal origin. The properties of Excg1 suggested good potential for use in lignocellulose saccharification processes particularly using sea water or under high salt conditions, or in the presence of residues and/or byproducts of pretreatment steps, contributing to improve the economic viability of 2G ethanol production.

AZF1 transcription factor affects expression of genes related to plant biomass degradation in *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is widely studied due to its ability to produce biomass-degrading enzymes that can be use in industry to produce second-generation ethanol and other related products. Different approaches have been made throughout the years regarding the improvement of *T. reesei* strains, and the most effective one seems to be the understanding of its regulatory elements so that we can manipulate it to produce higher enzymatic yields. Here, we analyzed differently expressed genes to mine novel *cis*-regulatory elements controlling cellulase expression. Using a series of bioinformatics approaches, we identified an AZF1 homologue in *T. reesei* with potential role in the regulation of cellulase coding genes. In order to validate its function, a deletion strain was constructed using a deletion *cassette* and homologous recombination. The parental strain was Tu6_Δtku70 containing auxotrophy for uridine. Differential expression of 22 genes related to plant biomass degradation (*cel6a, cel3a, cel7a, cel11a, cel61a, cel45a, cel7b, cel1b, cel3b, cel3c, cel5a, cel5b, cel12a, cel61b, cel3d, cel3e, cel74a, xyn1, xyn2, xyn3, xyn4, swo*) was analyzed by Real-Time Quantitative PCR using sugarcane bagasse or cellulose as carbon sources. Notably, in cellulose, at least 9 cellulases genes (*cel1a, cel61a, cel3b, cel5a, cel12a, cel61b, cel6a, cel7a, cel3a*) were down-regulated in the mutant Δ*azf1*. The culture with sugarcane bagasse was also affected by this regulator, since at least 6 genes (*cel61a, cel5a, cel6a, cel7a, cel3a, cel7b*) were down-regulated in the mutant when compared to the parental strain. Furthermore, the gene encoding for a swollenin showed a very significant differential expression in both culture media. Xylanases, on the other hand, did not seem to be influenced by azf1. Still, these results show that the transcription factor AZF1 is possibly playing a role and helping other transcription factors in the regulation of biomass degradation. This finding could lead to novel strategies for genetic manipulation of *T. reesei* towards the pursuit of optimized enzyme production.

Acid pretreatment with high solid loads for mixture: straw plus sugar cane bagasse

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The use of agroindustrials residues about lignocellulosics sources to obtain many chemicals product it is an alternative to contribute for the valuation of these subproducts. Thus, the ethanol produced by lignocellulosic materials it is an interesting option for increase the production of this fuel without increase the agriculture area for production of biofuels, since the ethanol demand has increasing even more in the last years, with the objective of substitute the oil and his derivates, contribute for reduce of negative impacts for environment. Lignocellulosic biomass intended for production of biofuels may be pretreated in an acid thermochemical process stage (i.e. H2SO4-catalyzed hydrothermal pretreatment carried out in either concentrated/diluted acid system). The process produces a solid material named water-insoluble solid (WIS), which is characterized for presenting amorphous and crystalline regions. WIS is constituted mainly by cellulose plus residual hemicellulosic and lignin fractions, which is susceptible of conversion into fermentable sugars through enzymatic hydrolysis. In this work the raw material used was the mixture straw plus sugar cane bagasse (50% for each material), were done 27 samples. The experimental conditions were carried out; the temperature was 80, 100 e 120 ±°C, the H2SO4 was ranged in concentrations of 1.0, 2.0 and 3.0% w/v and time 60, 80 and 120 min. The pretreatments were performed in high solid loading of 20%. After the characterization of the samples bagasse plus sugarcane was concluded that the pretreatment process applied in this

work was efficient, since the values of cellulose were high, varying between 54 and 70%, Hemicelluloses values between 13 to 20% and lignin between 17 and 25%, in relation to 100 g of in-nature sample. As well as obtaining excellent values for the yield of the process, varying between 63 and 92%. It can be concluded that with the application of hydrothermal pretreatment catalyzed with dilute sulfuric acid there is an efficient removal of hemicelluloses for the worked material, this means that most of the hemicellulosic fraction is hydrolysed in sugars (xylose, arabinose, among others).

Algae biomass hydrolysis-derived inhibitors: impact on the production of H_2, consumption of substrate, and growth of the recently isolated *Clostridium beijerinckii* Br21

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Hydrogen, a clean fuel, can be generated by fermentation of renewable raw materials, such as biomasses. Algal biomass is a promising substrate to produce biofuels: it sequesters high amounts of CO2, grows at high rate, and does not require arable land. Although algal biomass does not contain lignin, it still has a complex chemical structure that demands a hydrolysis step before fermentation. Hydrolysis of algal biomass releases monosaccharides, but it can also produce substances that inhibit fermentation. This study evaluates how the three main inhibitors of fermentation derived from hydrolysis of algal biomass hydrolysis, namely 5-hydroxymethylfurfural (HMF), levulinic acid (LA), and formic acid (FA), affect the process of fermentation. We evaluated the kinetics of fermentation by using a H2-producing microorganism recently isolated in our laboratory, *Clostridium beijerinckii* Br21; we used glucose, as the source of carbon, and different concentrations of each inhibitor. We examined the effect of HMF, LA, and FA at concentrations ranging from 0.5 to 2.5 g/L, 1.0 to 4.0 g/L, and 0.5 to 2.0 g/L, respectively. During fermentation, we analyzed the composition of the gas by gas chromatography, the concentration of glucose in the liquid by the 3,5-dinitrosalicylic acid (DNS) method, and cellular growth at 600 nm. We determined the specific rates at which H2 was produced, glucose was consumed, and cells were grown in the presence and in the absence (control) of the inhibitors. The results of the fermentations provided a mathematical model to estimate the concentration of the compounds that inhibited 50% of the specific H2 production rate (EC50). In general, the latency phase increased, and the H2 specific production rate decreased when the concentration of the inhibitors increased. The EC50 for the production of H2 in the presence of HMF, LA, and FA was 0.89, 2.50, and 1.15 g/L, respectively. At their EC50, HMF, LA, and FA affected the cellular growth of *C. beijerinckii* Br21 by 13.9, 55.5, and 41.65%, respectively. On the other hand, HMF, LA, and FA at their EC50 affected the rate at which glucose was consumed by 22.36, 31.69, and 20.67%, respectively. The results indicated that HMF acted more specifically in the metabolic pathways involved in the production of H2, whereas LA and FA affected, besides H2 production, bacterial growth. These results have helped us to estimate threshold concentrations of HMF, LA, and FA in algae hydrolysates and have paved the way to employ algal biomass as a substrate to produce H2 by *Clostridium* in the most appropriate way.

Engineering of new *Saccharomyces cerevisiae* strains capable of using xylose for the production of bioethanol

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One of the most effective and used organisms in the production of bioethanol is the yeast *Saccharomyces cerevisiae*. This yeast is well-known in terms of genetics and physiology, having its metabolic pathways and bioengineering methods well described. *S. cerevisiae* have been used in industrial processes for centuries, however, there are still some challenges that should be targeted, such as its inability to ferment pentoses, like xylose. Xylose is one of the most abundant sugars in lignocellulosic biomass, which is an important alternative to fossil energy sources. There are two main xylose catabolism pathways, the Xylose Reductase - Xylitol Dehydrogenase (XR - XDH) pathway and the Xylose Isomerase (XI) pathway. In the XR-XDH pathway, xylose is first reduced to xylitol by XR and then XDH oxidised xylitol to xylulose. On the other hand, XI converts xylose directly to xylulose, without the usage of cofactors and xylitol formation. Aiming at the utilization of xylose for ethanol production, this work has the objective to construct recombinant *S. cerevisiae* strains using XI conversion pathway. Seven new genes encoding putatively xylose isomerases were selected based on sequence similarities to known XI. These genes were synthesized and cloned in the p424 plasmid, which were used to transform a laboratorial *S. cerevisiae* strain. In addition, two control strains were created: LC7XIPiro, containing the XI-*Piromyces* cloned in the p424 plasmid (positive control) and LC7Ø, transformed with the p424 plasmid lacking XI encoding gene (negative control). Nine recombinant strains were obtained and characterized through aerobic xylose fermentation capacity. Two different pre-cultivation conditions in minimal medium were evaluated, one with glucose and another with xylose as sole carbon source. Analysis of the fermentation performance of the 9 strains (7 expressing new XI and two controls) revealed a better performance of the strain transformed with the *Piromyces* gene (positive control) in both conditions, with and without pre-cultivation in xylose. In addition, it showed that the positive control had a higher growth rate when fermented after a pre-cultivation in xylose. Among the new genes, strain LC7MUCI showed slightly better growth than the other transformants. In this work, genes encoding putatively new xylose isomerase were identified and transformed in a *S. cerevisiae* strain. Seven new strains were constructed and tested for growth in media containing xylose as sole carbon source. New strains showed low XI activity.

Evaluation of the cellulolytic potential of filamentous fungi from the coastal environment

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The Brazilian territory has a great biodiversity, making it an excellent hotspot for the development of bioprospecting studies of fungi producing hydrolases for biotechnological purposes. Among the Brazilian ecosystems, Restinga, due to its peculiar characteristics by the meeting of the sandy and saline coastal strip with herbaceous vegetation, represents an environment little explored in terms of filamentous fungi biodiversity. Fungi are capable of producing several hydrolytic enzymes, such as proteases, lignin peroxidases, cellulases and xylanases. The cellulases correspond to a group of enzymes of great biotechnological importance, which are able to degrade the cellulosic fraction of the vegetal biomass. Therefore, the present work aimed to evaluate the cellulolytic potential of filamentous fungi isolated from Restinga de Marambaia. Six samples of sand and mangrove were collected and the fungi strains were isolated by serial dilution technique in salt mineral medium containing 1.0% (w/v) microcrystalline cellulose. The system was

incubated at 28°C for 10 days and after that period, strains with fungal characteristics were selected, purified and stored in sterile water at 10°C. The strains were grown in salt mineral medium containing 1.0% (w/v) filter paper and 0.1% (w/v) yeast extract at 28°C / 150 rpm/ 7 days and the activity of CMCase and FPase was determined by the DNS method. 32 fungi strains isolated were tested for cellulase production by submerged fermentation. The highest production of CMCase (1088.47 U/L) was observed by strain M6-23 and FPase (431.41 U/L) by strain M6-40, after 7 days of fermentation. The results suggest that Restinga de Marambaia, that belongs to the Atlantic forest, could be considered as hotspot for isolation of cellulolytic filamentous fungi with great potential for application in biotechnological processes.

Hydrolyzed algal biomass as feedstock for production of hydrogen by a β-glucosidase-producing *Clostridium beijerinckii*

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Currently, the production of hydrogen relies on fossil fuel-based methods, but this fuel can also originate from the fermentation of carbohydrate-rich materials, such as biomasses, a sustainable and economically viable process. In this context, macroalgal biomass is a potential feedstock for the fermentative production of hydrogen. Compared to land plants, algae grow faster and have higher ability to fix CO2. In addition, algae do not contain lignin, which facilitates their hydrolysis. Here, we hydrolyzed the macroalgae *Kappaphycus alvarezii* with diluted HCl, to obtain fermentable carbohydrates and low concentration of inhibitors of fermentation, and we used the hydrolysate as substrate to produce H2 by fermentation. We had optimized the conditions for hydrolysis of the algal biomass as follows: HCl at 0.0335 mol/L, 0.225 g of algal biomass, temperature = 90°C, hydrolysis time = 8 h. The hydrolysate presented 1.67 ± 0.2 g/L of TRS, and 175.53 ± 40.13 mg/L of the inhibitor 5-Hydroxymethylfurfural (5-HMF). We employed the algal hydrolysate as substrate in fermentative assays by the H2-producing strain *Clostridium beijerinckii* Br21, grown at 35°C for 48 h. We conducted the fermentative assays in three different conditions. Assay 1 used a pre-inoculum of *C. beijerinckii* Br21 cultivated in medium containing galactose as the source of carbon. In these conditions, the pre-inoculum did not present β-glucosidase activity. The second assay involved the same pre-inoculum, but the fermentation flask also contained 9.37 U of recombinant β-glucosidase from *Humicola insolens*. For the third assay, we grew the pre-inoculum of *C. beijerinckii* Br21 on cellobiose as the carbon source to induce the intracellular production of β-glucosidases (0.33U/mL). We monitored the volume of hydrogen as a function of time for 60 h. The assay induced with intracellular β-glucosidases (assay 3) afforded the highest volume of H2, 0.98 mmol of H2/L, whereas assays 1 and 2 yielded 0.83 and 0.77 mmol of H2/L, respectively. H2 also evolved faster in assay 3, 0.11 mmol of H2/L.h; assays 1 and 2 gave very similar production rates for this fuel, 0.06 and 0.07 mmol of H2/L.h, respectively. Assays 1, 2, and 3 provided conversion ratios (Y) of 4.35, 3.7, and 3.42 mmol of H2/g of algae biomass, respectively. Therefore, induction of intracellular β-glucosidases of *C. beijerinckii* Br21 in the presence of cellobiose favors conversion of algal biomass to hydrogen.

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- DESIGNING A SUSTAINABLE BIOECONOMY -

Inhibition of pure and mixed biomass hydrolysis byproducts on H_2 production by *Clostridium beijerinckii* Br21 and *Clostridium acetobutylicum* ATCC 824

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Hydrogen production by biological pathways, known as biohydrogen production, has gained prominence in recent years because it enables the use of renewable materials as raw material. Lignocellulosic or algal biomasses are potential substrates for H2 production by fermentation. However, most of the methods available for hydrolysis of such biomasses result in carbohydrate degradation products, which are known to inhibit fermentation. This study evaluates how three different inhibitors derived from biomass hydrolysis, namely 5-hydroxymethylfurfural (HMF), levulinic acid (LA), and formic acid (FA) affect H2 production by two microorganisms of the genus *Clostridium* sp. We conducted fermentation assays with *Clostridium beijerinckii* Br21 and *Clostridium acetobutylicum* ATCC 824 and measured H2 volume as a function of time to calculate the H2 production rate. We added HMF (0.9 g/L), AL (2.50 g/L), and AF (1.15 g/L) concentrations previously estimated to be the concentrations that inhibited H2 production by *C. beijerinckii* Br21 by 50% (IC50) to the fermentation tests. We also performed assays involving a mixture of inhibitors by adding the previously estimated IC25 to *C. beijerinckii* Br 21, namely 0.66, 2.15, and 0.9 g/L HMF, AL, and AF, respectively, which were mixed as follows: HMF+AL, HMF+AF, AL+AF, and HMF+AL+AF. Addition of the HMF, AL, and AF IC50 concentrations to the assays with *C. acetobutylicum* ATCC 824 inhibited H2 production by 45.6, 61.3, and 59.7%, respectively. Assays with the mixture of inhibitors HMF+AL, HMF+AF, AL+AF, and HMF+AL+AF inhibited H2 production by *C. beijerinckii* Br21 by 58.9, 57.4, 47.5, and 86%, respectively, and H2 production by *C. acetobutylicum* ATCC 824 by 68.1, 66.7, 54.7, and 87.8%, respectively. Combination of inhibitors, especially organic acids with HMF, potentiated the inhibitory effect on H2 production by the two investigated microorganisms. However, H2 production by *C. beijerinckii* Br21 was less inhibited by organic acids and by their combination with HMF, showing the potential robustness of these microorganisms during H2 production from biomass hydrolysates.

Experimental ethanol recovery from ethanolic solutions by pervaporation

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Pervaporation is a membrane separation process that promises to reduce production costs and increase productivity in bioethanol production. The organophilic pervaporation process is strongly focused on the recovery of ethanol from both fermentation (in situ) and fermented wine. In the first case, it is hoped to increase the productivity, avoiding the inhibition of the fermentation by ethanol. In the second case, it is focused on the reduction of the energy consumption of the distillation. However, to evaluate this process it is necessary to know the behavior of the separation and the influences of the main variables of operation. In order to facilitate the understanding of the ethanol recovery during the fermentation or from fermented wine, usual conditions were used with only ethanol-water mixtures. In this work, the recovery of ethanol from ethanol-water mixtures was studied using a commercially polydimethylsiloxane (PDMS) membrane. As operating variables, the ethanol concentration (1 – 9%wt) in the solution and

the feed temperature (34 – 50°C) in the pervaporation process were evaluated. In the results, it was observed that the fluxes of ethanol and water in the permeate increased following a linear behavior. When the feed temperature was studied, the fluxes in the permeate increased following an exponential relationship. In general, the fluxes in the permeate presented Arrhenius behavior, varying between 12 g/(m2h) and 540 g/(m2h) for ethanol and between 277 g/(m2h) and 1034 g/(m2h) for water; while the separations factor varies between 2.3 and 6.4.

Improvement of industrial yeast strains by breeding and adaptive evolution for second-generation ethanol

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Lignocellulosic residues, such as sugarcane bagasse, are considered a promising feedstock for second generation ethanol. The nutritional deficiencies of sugarcane bagasse hydrolysate could be circumvented by molasses supplementation, which might function as a nutrient source for the fermenting organism and also as an ethanol booster for distillation. Addition of nutrient rich molasses is expected to allow yeast cells to maintain high viability, which is of paramount importance in a cell recycling process. In this scenario, yeast tolerance toward inhibitors coming from both substrates is therefore essential for the deployment of this process. The objective of the present work was to generate multi-tolerant yeast strains by adaptive evolution of hybrids coming from industrial strains. For this purpose, Saccharomyces cerevisiae strains widely used in Brazilian sugarcane mills (PE-2, CAT-1 and SA-1) were sporulated and the tetrads of these strains were dissected resulting in ca. 200 haploids cultures. These haploids were used for poly-crossings by mixing 7 different pools of all haploids. Hybrids from these crossings were subjected to an adaptive evolution during 100 generations in media with increasing stress conditions (sugar, ethanol, acetic acid, acidic pH), and 105 evolved colonies were isolated. In parallel, 11 haploids were UV mutagenized and the resulting irradiated colonies were screened again in increasing stress conditions. From this screening, 20 irradiated tolerant haploids were crossed resulting in 191 hybrids. These 191 hybrids from UV mutagenesis together with 105 evolved colonies were selected for hydrolysate and molasses tolerance and also for high gravity fermentations. Twenty-four hybrids and the parental strains were ultimately evaluated in fermentation with cell recycle using hydrolysate and molasses as substrate. Several industrially-relevant fermentation parameters were evaluated, including yeast growth, ethanol yield, cell viability, and byproduct formation. Some of these strains and their derived haploids have also showed excellent growth performance in Miscanthus hydrolyzate. Thus, they were transformed with a cassette containing three genes for xylose metabolism (xylose reductase, xylitol dehydrogenase, xylulokinase) and evaluated for xylose fermentation. This strategy of hybridization followed by adaptive evolution allowed us to obtain more tolerant strains for industrial hydrolysate fermentation. These tolerant hybrids could be an important source for background strains in second-generation ethanol production.

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- DESIGNING A SUSTAINABLE BIOECONOMY -

Transcriptional and fermentative analysis of *Clostridium saccharoperbutylacetonicum* during optimized fermentation process using cellulosic pulp

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Environmental, geopolitical and economic problems related to oil and other fossil fuels led to an increased interest for the renewable production of fuels and chemicals. n-Butanol (C4H9OH), a chemical of extensive use in the industry, has the potential to be produced from lignocellulosic biomass by bacteria from the genus *Clostridium*, through the so-called ABE fermentation (Acetone, n-Butanol and Ethanol). Several strategies on genetic engineering, culture medium and process optimization are dedicated to improve the fermentation and yield high solvent concentrations. These strategies, however, can bring better results from in-depth knowledge on the microorganism's metabolism. By adding cellulosic pulp derived from pretreatment of lignocellulosic materials and an enzymatic complex of cellulases and hemicellulases to the culture medium, we developed an optimized solvent production process for the bacteria *Clostridium saccharoperbutylacetonicum* DSM14923, improving the bacteria's acetone and n-butanol yield and productivity. Moreover, this fermentation condition also enhanced *C. saccharoperbutylacetonicum* tolerance to oxidative stress. Therefore, this study had as main objective the transcriptome analysis combined with the fermentative profile of *C. saccharoperbutylacetonicum* to understand the microorganism's metabolism in this favorable condition for high solvents production. The data acquired identified several changes in gene expression that suggests a quick re-assimilation of acetic acid and its conversion into acetone, due to an anticipation of solventogenic phase and a change in redox balance. We propose that these changes in bacterial metabolism are related to variations in expression of genes involved with solventogenesis regulation (*abrB*), stress resistance (*groES* and *groEL*), energy metabolism (ATP synthase *operon*) and redox balance (NAD synthase *operon*). We highlighted these genes as potential targets for genetic engineering in order to further improve a strain for industrial application.

Flash point measurements and prediction for binary mixtures of ethanol and hydrocarbons

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The global demand for energy has been increased and the necessity to find renewable energy sources make a good alternative the use of biofuel and fossil fuel blends as it has been done in Brazil with the addition of 27% of ethanol into gasoline. This possibility brings the interest of investigating the addition of biofuels, ethanol into aviation kerosene. Some studies report the use of bio-kerosene obtained from sugarcane, but considering the specifications required for aeronautical use, a subsequent stage of purification and separation of the most suitable fractions for use in gas turbines is required and is feasible for use at commercial scale. The use of hydrated ethanol as an aeronautical fuel is a common reality in Brazilian country town, however, the use of ethanol is limited to light airplanes with piston engines, a relatively small market. Embraer is the Brazil's largest aircraft manufacturers and it has been developing flex-fuel engine systems which allow the use of aviation fuel mixtures and ethanol for aeronautical engines. It is necessary to determine physicochemical properties to check the feasibility of use fuel blends in aviation fuel business. Among these

properties stand out the flash point (FP) that is one of the most important parameters to characterize the potential risks of fire and explosion of flammable liquids. Experimental FP data have clearly become important to ensure safe storage of flammable materials and for this reason a series of studies for predicting the FP of pure substances and their mixtures can be encountered in the literature. The aim of this work was the determination of the FPs of hydrocarbon binary mixtures with ethanol to correlate these data using a thermodynamic approach and to test this approach to predict FP values of multicomponent mixtures. The equipment used for the experimental determination of FP was the FLPH Miniflash from Grabner Instruments (Austria) operated according to ASTM methodology (D6450.05, 2010) recommended for biofuels using 1 mL of sample. All samples were tested in triplicate. In this study, flash points of binary mixtures of ethanol and hydrocarbons were measured by using the closed cup apparatus. The obtained experimental data were employed to develop simple and accurate models for predicting the flash points of binary miscible mixtures.

Carbon catabolite repression on cellulase production: mitigation by a novel batch-feeding induction system

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The ascomycete Trichoderma harzianum have been suggested with a potential candidate for the formulation optimized enzyme mixtures for sugarcane bagasse saccharification. The carbon catabolite repression (CCR) mechanism adopted by Trichoderma sp. is mediated by the transcription factor CRE1. CCR represses genes related to cellulase production when a carbon source is readily available in the medium. In previous work the T. harzianum recombinant with overexpression of the main positive regulator of cellulase and hemicellulase gene expression (XYR1) showed elevated FPAse production when cultivated on batch induction system using hydrothermal pretreatment followed by NaOH delignification (BHD) sugarcane bagasse on bioreactor but notably the cre1 expression was upregulated. In this work we investigated CCR during the synthesis of cellulases using different strategies of batch-feeding induction using a qPCR analysis of cre1. The mathematical model describes the cell growth, variation of substrate concentration and production of FPase, in different sugarcane bagasse concentrations and batch-feeding induction. The model was used to predict substrate feed profiles for maximization of FPases production. FPases production rate was considered dependent on cell concentration and two inhibition terms were added to restrict the enzymes production. First is dependent on substrate concentration inside the reactor and the second depends on the enzymes already synthesized. Batch experiments (10 g/L and 30 g/L of BHD) indicated inhibition of enzyme biosynthesis and carbon catabolite repression. A pulsed fed-batch protocol with 15 g/L initial BHD concentration followed by 7.5 g/L BHD pulse additions at 24, 48 and 72 hours. The proposed model for cellulase production, achieved enzymatic activities up to 1.73 ± 0.37 FPU/mL, 332.64 ± 8.87 IU/mL, and 14.60 ± 0.39 IU/mL of cellulase, xylanase, and β-glucosidase, respectively. This model contributed to the non accumulation of glucose and cellobiose during the cultivation resulting in carbon catabolite repression (CRE1) mitigation.

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- DESIGNING A SUSTAINABLE BIOECONOMY -

Selection of tolerant *Saccharomyces cerevisiae* strains for second-generation ethanol production

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Brazilian distilleries harbor advantageous conditions for the second-generation ethanol production, since available feedstock, fermenters, distilling equipment and qualified professionals are available. Nevertheless the fermentation of this novel substrate poses to yeast a new challenge due to the presence of several inhibitors (acetic acid, aldehydes and phenolic compounds) generated during pre-treatment of the lignocellulosic substrate, namely sugar-cane bagasse. Bagasse hydrolysate is devoid of mineral and organic nutrients necessary for yeast fermentation and addition of cane molasses supplis not only the required nutrients but also contributes for a desirable buffer capacity of this new resulting substrate. Molasses also allows for a higher ethanol titer, reducing the energy cost of the distillation step. Nevertheless molasses also contains some yeast inhibitors (excessive amounts of Ca, K, SO2 and other products from sugar thermal degradation), and new strains of *Saccharomyces cerevisiae* would be required to cope with inhibitors from both sugar sources. The fed-batch fermentation process with cell reuse is widely used in Brazilian distilleries, with very high cell density resulting in very short fermentation time and low sugar deviation for yeast biomass production. As a result, high ethanol yield are achieved, although the yeast recycling procedure intensifies the stressing action of the inhibitors (since the same yeast cell is subjected to many rounds of fermentation). The aim of the present work was the selection of S. cerevisiae strains tolerant to a cell recycling fed-batch fermentation process using a mixture of molasses and bagasse hydrolysate as substrate. Initially was observed that the most tolerant available industrial strains (PE-2, CAT-1 and SA-1), were not robust enough for the fermentation of the proposed substrate in a cell recycling protocol. The mentioned industrial strains were subjected to a breeding program: they were sporulated and isolated haploid cells used in mass and directed crossings. Hybrids were selected for tolerance in molasses-hydrolysate substrate, using high throughput microplate assays. Meanwhile ca. 600 indigenous strains, isolated from Brazilian distilleries, were screened for tolerance in the same substrate and the more promising strains were also used in the breeding program. Several resulting hybrids showed superior traits when compared with industrial stains (PE-2 and SA-1) in fermentation trials using cell recycling fed-batch protocol. Physiological and technological parameters (ethanol yield, biomass gain, glycerol formation, residual sugar, cell viability, fermentation rate and cellular glycogen and trehalose content) were used to distinguish the superior hybrids. It is proposed that these hybrids would be promising backgrounds for insertion of metabolic traits aiming xylose fermentation for a complete consumption of sugars from the lignocellulosic substrate.

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Study of microalgae growth and lipid accumulation for biodiesel production

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Microalgae have been recently considered to be used as feedstock for biodiesel production. Many of them present high growth rate, high biomass productivity and can use carbon dioxide to grow, accumulating different products (protein, carbohydrates, lipids, etc.). Some parameters have to be considered for the strain selection with a potential to be applied in

biodiesel industry, including productivity of lipids, growth rate and fatty acid profile. The present study focuses on the evaluation of four potential strains for biodiesel production by comparing biomass concentration and lipid accumulation: *Chlorella* sp., *Chlorella vulgaris*, *Desmodesmus* sp. and *Desmodesmus brasiliensis*. The experiments were performed in 250 mL Erlenmeyer flask, BG-11 media, light flux of 61 µE m^{-2} s^{-1}, photoperiod of 24 h, mean temperature of 26°C and shaker agitation for a period of 21 days. All cultivations were carried out in duplicate. Biomass concentration was monitored once a day with UV spectrophotometer at a wavelength of 682 and 680 nm for *Chlorella vulgaris* and *Chlorella* sp., respectively, and 684 nm for *Desmodesmus* sp. and *brasiliensis*. After 21 days of cultivation, the biomass was recovered by centrifugation. Cell disruption was executed in wet biomass by autoclaving under 125°C for 5 min. Finally, it was performed lipid extraction of lyophilized biomass using a procedure based on Bligh & Dyer modified method. As results, growth curves of the four strains were obtained to identify the phases of the S-shaped curve. Lag phase presented a short duration of 1 day, and exponential phase was achieved for all strains. Exceptionally *Chlorella* sp. reached a stationary phase, at approximately the 8th day. During the growth period of three weeks, it was not observed death phase. *C. vulgaris*, *C.* sp.,*D.* sp., and *D. brasiliensis* achieved an average biomass concentration of 0.89, 0.84, 0.78, and 0.68 g L^{-1}, and lipid content of 21.9, 21.8, 19.4, and 14.5%, respectively. In conclusion, the studied strains showed potential for biodiesel production once they presented high growth rate and lipid accumulation

Influence of the carbon sources on the fatty acid profile and biodiesel properties from microbial oil obtained by *Mucor circinelloides*

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Production of biodiesel using single cell oils (SCOs) has recently been highlighted as a potential source of renewable energy and compatible with the biorefinery concept. The wild strain of *Mucor circinelloides* URM 4182 from Brazilian culture collection was previously selected as potential oleaginous microorganism supplied SCO with suitable properties for biodiesel synthesis. Here, we are interested in assessing the accumulation of storage lipid by this strain in various carbon sources, such as sucrose, xylose, fructose, starch, ethanol and glycerol. These sources were selected because they are available in large amounts in agroindustrial residues. The goal was to establish a low cost feedstock and investigate the influence of the carbon source on the fatty acid profile and the biodiesel properties obtained by direct transesterification of the microbial biomass. When using as biodiesel feedstock the object of production is not only the amount of SCO but also its lower concentration in polyunsaturated fatty acids (PUFAs), in order to increase the biofuel oxidative stability. Under the used conditions, with the exception of ethanol, *M. circinelloides* could germinate and grow on all tested carbon sources in liquid media and the microbial lipids produced contain significant amount of TAGs compared with growth elaborated on glucose-based substrates. Additionally, the oil accumulated in other carbon sources has lower degree of unsaturation. Although, all substrates produced high percentages of C18 unsaturated fatty acids. Oleic acid ($^{\Delta 9}$C18:1) is the principal

fatty acid accumulated by the microbial cells (amounts sometimes higher than 20% w/w), while linoleic ($^{\Delta 9,12}$ C18:2) is found in the second position (ranging from 3.7% to 15.4%). It was also evident that culture grown using soluble starch and glucose produced the highest percentages of eighteen carbon PUFAs (around 29.5%), whereas sucrose gave the lowest concentration (19.5%). Direct transesterification using solid acid catalyst (12-molybdophosphoric acid support on alumina) under conditions previously set up (200°C for 4 h) has shown to be effective at converting the triacylglycerol into alkyl esters, reaching high FAEE yield (96.1% to 98.5%) and low byproducts levels. The properties of biodiesel derived from sucrose based feedstock allow selecting this carbon source as the most suitable one to obtain SCO having properties that attend the quality required by standard agencies.

Biotransformation of a phenylpropanoid by ligninolytic fungal and bacterial strains

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Lignans or phenylpropanoids constitute the molecular skeleton of lignin. Filamentous fungi and actinomycetes are known about their wide production of bioactive compounds, including enzymes, which can be employed in various biotechnological applications. Therefore, the aim of this study was to analyze the biotransformation of phenylpropanoid molecules as models of lignin compounds. For this, these compounds were offered as substrates to known ligninolytic fungi and actinomycete strains, previously selected by our research group. This goal is related to the application of these ligninolytic systems, looking for their use in the improvement of lignin digestibility for bioethanol production. In this sense, the isolate 63.1 (an endophytic fungus), from Bertioga mangrove, and one actinomycete named as B6V2-14F, from the caatinga rhizosphere, were cultured in appropriated culture media, during 72 hours, at 28°C, 150 rpm. The cultures were centrifuged and the biomass (pellet) dissolved in phosphate buffer 1 mM (pH 7.0), added by the lignan substrate (2 mg/mL). The biorreactions were incubated and monitored during 72 h at 28°C, 150 rpm. Aliquots of 2 mL were sampled at different times (0, 24, 48 and 72 hours), following by GC-MS (gas chromatography coupled to mass spectrometry) analysis. Preliminary obtained results showed that both microbial isolates were able to biotransform the substrate, in the following product: pregn-5-en-20-one, 3-(acetyloxy)-6,16-dimethyl-, (3β, 16α), among other minority compounds. These results could showed some ability of the strains to oxidize the compound, which motivated more specific analysis about the molecular structure of the product. The next steps of this study are also the confirmation about the ligninolytic enzymes under the lignan, elucidating reaction mechanisms that can allow the application of these in processes to improve lignin digestibility, using sugar cane bagasse as substrate.

Economic, environmental and social impacts of different sugarcane production systems

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Sugarcane is largely cultivated in tropical countries, representing a main agricultural product and relevant feedstock for agroindustry. Its cost typically means around 50 to 60% of final cost of sugar or ethanol production. Brazilian sugarcane sector has experienced several changes over the years. Historically, the technology of sugarcane production has been based on manpower and associated with the pre-harvesting burning of straw to decrease production cost, improve field conditions for rural workers and reduce risk of poisonous animals. Over the last decade, however, a variety of economic, social and environmental issues have pushed the sugarcane sector to mechanical-based agricultural operations mainly in Center-South region of Brazil, especially those of harvesting and planting. The consequences of such technological shift, however, are not fully comprehended when multiple perspectives are considered such as economic results, environmental regulations and social aspects. The main goal of this study is to generate comprehensive information to subsidize decision making processes not only in Brazil but also in other countries where sugarcane production is still under development. Manual and mechanical technologies for planting and harvesting were evaluated (with and without pre-harvest burning), as well as straw recovery (baling and integral harvesting systems), seeking to identify their advantages and disadvantages, considering economic, environmental and social aspects. Considering vertically integrated production systems (agricultural + industrial operations), sugarcane production scenarios were compared under the metrics from Engineering Economics, Life Cycle Assessment, and Social LCA. Manual technologies were related to the highest job creation levels, however, lower internal rates of return and higher ethanol production costs were also observed. In general, mechanized scenarios were associated with lower ethanol production costs and higher internal rates of return due to lower biomass production cost, higher ethanol yield and higher electricity surplus. Considering the restrictions for sugarcane burning and practical difficulties of manual harvesting of green cane, environmental analysis showed that mechanical harvesting of green cane with straw recovery presents, in general, the best comparative balance of environmental impacts.

Energetic potential based on the gravimetric composition and calorific power of urban solid waste from the city of São Bernardo do Campo - SP

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Today's modern western society experiences a range of environmental problems from anthropogenic actions, such as the inefficiency of municipal solid waste management (MSWM). In an attempt to mitigate this adversity, it turns to be necessary to develop and carry out joint actions, with technical and economic efficiency, applied to the processing of energy from these wastes. The estimate of the MSW variation is mainly because of the higher moisture of its components due to rainfall. However, it is important to emphasize that the periods of higher rainfall coincides with the end of the school year, holidays and festivals, which leads to a significant increase in the composition of Municipal Solid Waste (MSW) generated in the city of São Bernardo do Campo, SP. Nineteen (19) neighborhoods of the city of São Bernardo do Campo were sampled, from a research in the historical series of precipitations in the city and the collected samples, it was possible to perform a prognosis on the composition of MSW for the wettest period of the year. It should be noted, however, that this prognosis was performed without a statistical basis, which would allow more statically accuracy to the performed tests. Nevertheless, it is believed that this may be an indicative for eventually arrangements in the area of MSW

management. For the calorific value of the main components sampled in the USW, the results showed a good correlation between São Bernardo do Campo and cities with similar size and purchasing power, even when observing the sensitivity of this fuel regarding the ash and the humidity content. This work aimed to describe a prognosis for the generation of MSW in the city of São Bernardo do Campo, in the monsoon season, and the impact of the climate on the potential generation of energy in the city. With the increase of non-biogenics in the gravimetric composition of the MSW, due to the improvement of the economical buy-power of the population as well as the industrialization level, will result in higher values for energetic values obtained from such raw material. The obtained average value of 18,4 MJ/kg (Superior Calorific Value), corresponding to 4,395 kcal/kg, it could be considered for energetic input value compatible to similar cities around the world. In conclusion, the MSW from Sao Bernardo do Campo represents an interesting source of energy, still contributing to mitigate the climate change due the use of fossil based energy sources.

Pretreatments applied to sugarcane bagasse to obtain enriched-cellulosic fractions for the production of the second- generation ethanol

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The conversion of biomass into ethanol using fast, cheap and efficient pretreatments is the major challenge in the production of the second-generation ethanol. Pretreatments are carried out to disrupt linkages between lignin and cellulose structures in order to make cellulose more accessible to chemicals and enzymes that convert carbohydrate polymers into fermentable sugars. An effective pretreatment would fulfil the following requirements at industrial scale: low incubation temperature and residence time and lower cost of chemicals as much as possible. In the present work, the efficiency of pretreatments applied to sugarcane bagasse (SCB) were compared concerning the formation of cellulose-enriched fractions. Chemical pretreatment to obtain cellulosic fractions consisted in the reflux of SCB particles suspended in 1% NaOH solution in a hot plate for 1h. On the other hand, the SCB particles suspended in solutions of 1% NaOH and 2% H2SO4 were incubation for 3 min under microwave-assisted irradiation. Acid based-pretreatments can be very corrosive to incubation reactors used in pretreatments. The resulting enriched-cellulose fractions were compared by measuring their components removals, abundance of lignocellulose components (FTIR spectra), X-ray diffraction patterns (degree of crystallinity), SEM (Scanning Electron Microscopy), degree of polymerization (DP), and average molecular weights (Mv). As result of microwave-assisted irradiation pretreatment, the lowest cellulose removal was obtained with the pretreated fractions PT-6 (8.05%) and PT-7 (5.63%). Thermal analysis of the cellulosic fractions and samples from SCB showed significant differences in char and ash (char-ash) yields, indicating great differences in thermal resistance.

Electronic tongue based on modified electrodes with metal oxides for selective determination of reducing sugars and sucrose in the sugarcane industry

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With the increased consumption of sugar and ethanol, the sugarcane industry sector is in front of a major challenge: to ensure and prove their sustainability. It is the main goal increasing productivity without increasing cultivated area, for this selective quantification of sugars (sucrose, glucose and fructose) is highly important for assessing the quality of the raw material for the production of ethanol and sugar. Importantly, the alcoholic fermentation is a fundamental step in the ethanol production process, such frequent measurements of sugars are essential for the optimal process for high efficiency of the desired products, in particular, continuous monitoring of sugars is very important in bioprocess of sugars and ethanol. The method widely adopted by industry branch of sugar and ethanol production is the Somogyi-Nelson, which is used for quantification of reducing sugars. This method, although widely used, present major disadvantages such as low selectivity, relatively large analysis time and temperature controlled use. For assessing the efficiency of the various processes involved in the production of sugar and ethanol for the detection of loss of sugar in industrial effluents is essential an accurate quantification and precise sugars. Given that the objective of this work was to develop an electronic tongue that allows the quantification of sugars selectively sucrose, glucose and fructose during the manufacturing process of sugar and ethanol mills, from cane sugar input in plant and monitoring losses in the industrial process. As, such, it combines the responses from an array of voltammetric the electronic tongue developed was based on screen printed carbon electrode (SPE) modified with metal oxide-hydroxide (Copper, Cobalt, Nickel and Gold) SPE/Metals/oxy-hydroxides, plus an advanced response model obtained using artificial neural networks (ANN). Since the departure data is highly complex, providing each sensor in the array a complete voltammogram, initial pretreatment of the data is also necessary, for which different compression methods are evaluated. The electrochemical parameters for sugars studied and were observed that the electronic tongue SPE/Metals/oxy-hydroxides showed a good amperometric sensitivity ranging from 19.0 μA $mmol^{-1}$ to 43.0 μA $mmol^{-1}$ according to each sensor. The values of limit of detection were around 4.0×10^{-4} mol L^{-1} and quantification were around 1.2×10^{-3} mol L^{-1} for each sugar, the values between sugars are very close, meaning that the behavior of the different sugars are comparable, thus we may apply the electronic tongue SPE/Metals/oxy-hydroxides in the same concentration range for sugars considered. Therefore this electronic tongue SPE/Metals/oxy-hydroxides has enormous potential to be applied in sugarcane industry sector.

Production of biodiesel from waste cooking oil (WCO)

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Biodiesel is a reality in the current energetic world scenario, having as a barrier the price that is higher than the price of diesel oil. One way to reduce the final cost of this biofuel is to produce it from waste cooking oils (WCO). In this context, the objective of this work was to collect different WCO samples to produce biodiesel, through the alkaline transesterification reaction, a simple and economical chemical route, using different parameters in the process, aiming to reach higher conversions. Six samples of OGR were collected, characterized by acidity (IA) and peroxide (PI). These samples were then subjected to the alkaline transesterification reaction in a jacketed glass reactor, under mechanical stirring at 400 rpm, at a fixed temperature of 60°C, reaction times of 2.5 and 5h and ethanol / WCO molar ratios of 6 and 9. The final product was subjected to separation and purification steps and the esters conversion was determined by Nuclear Magnetic

Resonance (NMR). The acid and peroxide values of the WCO samples showed values between 0.2511 and 3.6589 mg KOH / g oil and 3.5588 and 33.6916 mEq / kg oil, respectively. These results showed that the collected WCOs suffered some type of degradation, being unfit for use in food. It was possible to produce biodiesel from waste cooking oil (WCO). The NMR results showed that the transesterification reactions showed higher conversions in esters (biodiesel) under conditions of 2h reaction and ethanol / WCO molar ratio of 9, obtaining values of up to 100%.

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Determination of a kinetic model for xylose and glucose mixture fermentation by yeast *Spathaspora passalidarum* NRRL Y-27907

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Yeast *Saccharomyces cerevisiae* is the most used microorganism for bioethanol production, either from sugarcane molasses, corn starch or lignocellulosic materials. However, hydrolysis of cellulose and hemicelluloses results in a mixture of glucose, xylose, arabinose and others monosaccharides. This becomes a problem, once *S. cerevisiae* is not capable of metabolizing pentoses (xylose), unless it's genetically modified to express the paths of xylose assimilation. Therefore, the conversion of pentoses in ethanol becomes one of the most important challenges to be solved considering the production of ethanol from lignocellulosic biomass (second-generation bioethanol). It's in this context, thus, that microorganisms that are naturally capable of fermenting pentoses are desired. In this project, we focused on *Spathaspora passalidarum*, a non-conventional yeast with xylose fermentation capacity already reported on literature. Concerning the fermentation process, temperature is an operational variable that plays a key role on the reaction, with influence on ethanol productivity, substrate consumption, cell growth and even on microorganism viability. Small deviations on temperature can dislocate the reaction from optimum conditions. Therefore, the estimation of temperature-dependent parameters is one of the key strategies for kinetic comprehension and process optimization. In this work, we propose a kinetic model to describe the fermentation process for yeast *S. passalidarum* NRRL Y-27907 with high cell concentration using a mixture of xylose and glucose as substrate. For that, batch fermentations were executed in duplicate in bioreactors BioFlo® 115 1.4 L (New Brunswick Scientific Co., Inc., Edison, NJ) with 900 mL of working volume, during 48 hours, at five different temperatures (26, 27, 28, 30 and 32°C). Initial cell concentration was 20 g L^{-1}. Substrate concentrations were 63 and 27 g L^{-1} of xylose and glucose, respectively. Aeration was kept at 0.1 vvm, with a volumetric coefficient of oxygen transfer of 4.9, which represents microaerophilic condition. In these conditions, *S. passalidarum* consumed all the glucose in the first 12 hours and started to consume xylose at a considerable rate only after glucose exhaustion, which characterizes catabolite repression effect. Xylose was completely consumed before 44 hours. During glucose consumption, there was a significant increase on cell biomass, with maximum concentration at 8 h. Right after this point, cell concentration decreased and was kept around 20 g L^{-1} till the end of fermentation. Ethanol was produced since the starting point until there was no sugar left in the media, with different specific production rates for the two carbon sources. With these results, it was possible to propose a model including catabolite repression

of glucose over xylose for substrate consumption, cell growth and ethanol production.

Optimization pretreatment of sugarcane straw with sulfuric acid to obtaining fermentable sugars for ethanol production

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The present work proposes the optimization of the process of production of second generation ethanol from sugarcane. To this end, pretreatment of the lignocellulosic material will be performed with dilute sulfuric acid solution. In the proposed experimental design - a rotational composite central delineation (RCCD), given by a factorial design 2^2 including four tests in the axial conditions and three repetitions in the central point, totaling eleven experiments. The factors evaluated in the optimization were: the concentration of the sulfuric acid solution (0,10 – 2,90% m / v) and the pretreatment time performed in vertical autoclave at 121°C and 1Kgf / cm^2 (14 – 56 minutes). The response variable defined for the optimization was the mass of total reducing sugars hydrolyzed as a function of the mass of pretreated sugarcane straw. The results showed that the optimum straw conversion condition in total reducing sugars (TRS) was in the condition that sulfuric acid concentration was 2.5% m / v with the time of 40 minutes in an autoclave at 121°C and 1Kgf/cm2, where a concentration of 0.18 g of TRS / g straw was obtained. In addition, a statistical analysis performed on the Statistica 7 software demonstrated that the concentration of sulfuric acid (linear and quadratic) and autoclave time (linear and quadratic) were statistically significant (by p-value analysis) at a confidence interval of 95%, While ANOVA (analysis of variance) analysis showed that the predicted model is statistically significant with R^2 of 96.35%. It was also observed that the calculated F was nine times larger than the F table, confirming the high significance of the predicted mathematical model. Thus, it was concluded that the concentration of sulfuric acid is the factor with the greatest impact on the conversion of sugarcane straw to fermentable sugars, which will later be hydrolyzed with cellulite enzymes and fermented with Saccharomyces cerevisiae.

Comparative profiling and stress ethanol analysis during fermentations to characterize the resistance of *Saccharomyces cerevisiae*

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During industrial scale biofuel ethanol fermentations, yeasts are subject to various stress factors that affect their growth and fermentative metabolism. Some strains of Saccharomyces cerevisiae are highly tolerant to ethanol, but when exposed to high concentrations, they may undergo growth inhibition and cell viability, which limits fermentation productivity and ethanol yield. Yeast strains which are tolerant to such stress and able to synthesize high concentrations of ethanol in their presence would be more desirable for use in the industrial scale biofuel production. In this study, two fermentation systems were used in the medium with industrial glucose concentration for the analysis of two different strains of yeast, one industrial used as control (PE-2) and the other strain selected in the solid-state resistance of ethanol 12% (Y-12632). One system was characterized by the addition of 12% ethanol at the beginning

of the process, and in another system the addition of 12% ethanol was carried out from two hours of the process. It has been shown that, contrary to analysis on a solid medium, strain Y-12632 was the most affected. The substrate consumption profile and the ethanol production were applied to evaluate the influence of the stress response on the relative productivity of the yeast. Significant differences were found in metabolic responses to stress conditions, particularly the industrial strain PE-2 showed higher fermentation performance and produced significantly higher final ethanol concentrations ($P < 0.01$) when compared to Y-12632.

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Water hyacinth 2G ethanol production as alternative to decrease the environmental problems caused by its proliferation in water bodies

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Water hyacinth, *Eichhornia crassipes*, is a native macrophyte from Brazil that has a great ability to adapt itself and at very high growth rate. For these reasons, currently, water hyacinth is considered a pest because its large amount causes environmental damage to the rivers and lakes, such as eutrophication of these habitats; and the economic sector, affecting navigation and energy production because they are arrested to engines and turbines respectively. A considered alternative to mitigate the excess water hyacinth problem is to use its biomass to second generation ethanol production, which technology use cellulose like feedstock. In the present work was studied how to optimize the production of second-generation ethanol using two different chemical process: alkaline (hydrogen peroxide) and acid (acetic acid). Both hydrolyzed raw material were used for the production the 2G ethanol by Simultaneous Saccharification and Fermentation (SSF). Through the gas chromatography analysis was possible to determine that hydrogen peroxide hydrolysis is the pretreatment more effective in this parameters. Considering the observed results, it was calculated the process yield based on the quantity of biomass dry matter that was used. The optimization was made considering the time of the enzymatic hydrolysis and alcoholic fermentation step, referent of the better yield. When the process time decreases its productivity increases. From an industrial process, the yield is measured in liter of ethanol/tons of biomass (L/t). Since the water hyacinth for natural water hyacinth has only 3% dry material its yield is low. For the calculus was utilized the result of best productivity process: a) Ethanol/dry water hyacinth yield: 0,042mL/g → 42L/t; b) Ethanol/natural water hyacinth: 0,0013mL/g → 1,3L/t. In 1 hectare grows 220 kg of water hyacinth per day, therefore in 1 year, there are 80 ton of Eichornia crassipes16,18. Although these figures can varies depending climate, water conditions, etc, with this rate is possible to produce in 1 year, 100 L of ethanol per each hectare of water hyacinth in hydric bodies. Of course cannot be compared to the sugar cane, which produces, in average in Brazil, 7,000 L/ha/yr, showing that this process is more environmental than economical. This production followed the concept "Eradication through utilization", and it is interesting for decrease the problems caused by excess of water hyacinth in rivers.

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Lignin valorization: using the CRISPR/Cas9 system to engineer the biotechnologically important bacterium *Rhodococcus jostii* RHA1.

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Lignocellulosic biomass is a cheap and abundant resource which arises as an attractive alternative to oil for conversion into fuels and chemicals. Up to a third of lignocellulosic biomass is composed of lignin, a highly recalcitrant aromatic polymer, which is disposed as waste or burned for power in biomass processing biorefineries and pulp/paper industries. These streams represent an untapped source of aromatic compounds that could be converted to high value molecules. Ferulic acid (FA) is one of the main components of lignin and present potent antioxidant capacities, which can be exploited by the pharmaceutical and cosmetic industries, and is also an important precursor for other molecules of aggregated interest, such as eugenol, vanillin and coniferyl alcohol. The lignin-degrading bacterium *Rhodococcus jostii* RHA1 is able to depolymerize lignin, using the peroxidase DypB, into lower molecular weight (LMW) aromatic compounds such as ferulic acid (FA), which are catabolized. Due to its robust and versatile metabolism, *Rhodococcus* species are of biotechnological interest; however, the paucity of genetic tools available hinders its exploitation. In this project, we designed and constructed novel vectors to apply the CRISPR/Cas9 system in *Rhodococcus*, allowing quick and efficient gene targeting procedures. As proof of concept, we have applied this system to knockout the *Cou*M gene of p-hydroxycinnamate catabolism in *R. jostii* RHA1 and RHA045 Δvdh (a vanillin-accumulating mutant), to increase yields of ferulic acid production using sugarcane bagasse as substrate. This is the first use of the CRISPR/Cas9 system in *Rhodococcus*, and the novel *E. coli-Rhodococcus* shuttle vector could be a tool of interest to other researchers in the field.

Sugarcane straw pre-treatment by softer conditions using low-cost protic ionic liquids: the impact on enzymatic hydrolysis

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Ionic liquids (IL) are raising attention as catalyst for various applications, including lignocellulosic biomass (LC) processing. Due to its relevance, sugarcane residues, such as straw (SW), have been approached as alternative feedstock for electricity production and biomolecules synthesis. However, because of LC's crystalline structure, its application as substrate requires a pre-treatment, which disrupts cellulose, hemicellulose and lignin matrix improving their conversion in other molecules, such as glucose and xylose. Although traditional methods are able to pre-treat LC, high energy input and inhibitory by-products are some drawbacks. Alternatively, use of IL as pre-treatment strategy can be highlighted as an efficient process without inhibitor production, lower energetic demand and which may increase biorefineries' target markets possibilities. Nevertheless, it is crucial to implement low cost IL regarding economic aspects. In this work, two easy synthetized and low-cost IL were assessed as low temperature pre-treatment catalysts to enhance LC's enzymatic hydrolysis. Monoethanolamine acetate ([Mea][Ac]) and hexanoate ([Mea][Hex]) were synthetized by single step acid-basic reaction and mixed with SW, 6% (w/w) loading. Reactions were conducted with both washed and non-washed straw, assessing the impact of field's

contaminants in the process. Pre-treatments were conduct in triplicate at 90°C in dry oven for 4 and 12h. Then, pre-treated SW were hydrolyzed for 24h at 50°C, 5% (w/v) loading in acetate buffer using Cellic CTec2 (Novozymes) at 20 FPU.gbiomass^{-1}. SW's chemical composition was measured according NREL's methodology. Hydrolysis yield of Cellulose (Y%Cel) and Hemicellulose (Y%Hem) were assessed by Glucose, Xylose, Cellobiose and Arabinose quantification. Non-pre-treated LC was used as control condition. [Mea][Ac] was more impacted by impurities from dirty SW, yields were 25% (cellulose) and 36% (hemicellulose) lower for dirty SW pre-treated during 4h. No statistical difference (significance 5%) was observed for dirty SW pre-treated with [Mea][Hex]. Time was significant for both IL. For cleaned SW, increasing from 4 to 12h Y%Cel and Y%Hem enhanced 81% and 100% for pre-treatment with [Mea][Ac], and 66% and 90% for [Mea][Hex], respectively. Moreover, the best values for Y% were obtained from [Mea][Ac] process, which reached YCel = 77.1% and YHem= 52.3% from theoretical, almost five and seven times superior than obtained from control, 16.2% and 7.6% respectively. Although both IL were efficient to enhance enzymatic hydrolysis, no statistical difference (significance 5%) was observed within SW's chemical composition before and after pre-treatments. Thus, suggesting that hydrolysis improvement was impacted by structural changes within LC's matrix rather than its delignification.

Bioinformatics applications in biotechnology: bioenergy production

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Actually, the world energy consumption has been mostly based on oil, coal and mineral gas, which are characterized as non-renewable sources and principal responsible for emissions of pollutants into the environment with consequences for global warming. The substitution of these sources by renewable energy is under development by researchers at universities and biotechnology companies. Among the various possibilities, one of most interesting is the development of genetically modified organism for the production of biochemical using, as carbon source, soluble sugars generated by the deconstruction of lignocellulosic biomass (second generation technology). Among the possibilities are the production of second generation ethanol using genetically modified *Saccharomyces cerevisiae* yeasts and the production of biobutanol using bacteria of the genus *Clostridium*. One of the challenges of large-scale production of biochemicals is the difficulty in making the industrial process economically viable, especially because of the high costs of soluble sugars extracted from biomass and the low fermentative yield of microorganisms. In this context bioinformatics plays a fundamental role, since through computational analysis applied to the different omics data and its integration using methodologies of systems biology is possible to (1) construct and analyze transcriptomic atlas from new interesting plants to be used as feedstock in order to prospect candidate genes for genetic manipulation or molecular markers; (2) prospect and identify new hydrolytic enzymes to be used for the biomass deconstruction stage, and (3) identify new metabolic routes or their bottlenecks in genetically modified microorganisms for industrial applications. In this way, this poster will present bioinformatics applications for bioenergy production focusing in the

transcriptomic analysis of energy cane and *erianthus sp.* from several tissues and developmental stages, metagenomic and metatranscriptomic analysis of termites and their microbial symbionts for prospecting carbohydrate-active enzymes and, finally, an integrated omics analysis of xylose-fermenting industrial yeast for second-generation bioethanol production and ABE fermentation (Acetone, n-Butanol and Ethanol) using bacteria from the genus *Clostridium*. Moreover, all of these results are being used for experimental validation producing valuable insights into the diverse biological systems studied, increasing the performance and reducing the costs of bioenergy production in Brazil.

Indigenous yeast strains: persistence through harvest seasons

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Fuel-ethanol fermentations are subject of huge selective pressures resulting in the growth and persistence of yeasts adapted to stress conditions. In alcoholic fermentation processes in Brazilian distilleries, indigenous strains from feedstock input as contaminating microorganisms and usually dominate the process along the harvest period. In this study, we examined yeast population dynamic of industrial fuel-ethanol fermentation processes during three consecutive harvest seasons in order to evaluate the permanence of indigenous strains during different harvests. The assessed industrial unit is located in the State of São Paulo/Brazil, herein designated Unit A, where a follow-up of dynamics of yeast population was performed from 2013 to 2015 seasons. Along these seasons, Unit A started its processes with different commercial strains, in different periods. Collections were made from May to December, in intervals of around 30 days. Yeast colonies were selected based on their cell morphology. Biotypes found in concentrations higher than 10^6 CUF/mL were selected. 85 colonies with different biotypes were isolated from 26 different samples stemming from industrial fermented during three successive harvests and submitted to DNA extraction. Strain differentiation was performed using karyotyping (PFGE – *Pulsed Field Gel Electrophoresis*). It allowed us to assess the composition of yeast population during the bioethanol fermentation process along the harvest period and quantify representative indigenous strains for all harvests evaluated. In 2013, we found 25 biotypes related to 8 different strains during the harvest period, followed by 33 biotypes represented by 11 different strains in 2014. However, in comparison to 2013, 2014 harvest presented 4 strains that also appeared in previous year and represent 87,9% of total yeast population. On the other hand, considering the 2015 harvest, we found 15 different strains, from which 2 appear in 2013 samples (17,4% of total 2015 yeast population). Similarly, 2 strains that turned up in 2014 appeared in 2015, representing 25,2% of total yeast in 2015. Experimental data in previous studies have demonstrated that indigenous strains of *S. cerevisiae* input as contaminating microorganism and usually dominate the process along the harvest period. Our results support this information and show the effects of composition and dynamic population of indigenous communities from previous harvest in a microbiome composition for the next harvest season. These findings show dynamic population of yeast strains in bioethanol fermentation as result of introduction, selection and maintenance of indigenous strains from feedstock in alcoholic fermentation processes in Brazilian distilleries. Thus, feedstock can be considered a determinant factor in yeast population selection of fermenters. This work has implications on the understanding of indigenous yeast population in Brazilian industry and yeast selection for the bioethanol fermentation since recently the use of selected

indigenous yeast to start fuel-ethanol fermentation processes has been a trend.

Microbial assimilation of liquor from the combined pretreatment of ozonolysis and liquid hot water on sugarcane bagasse

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A combination of ozonolysis and liquid hot water pretreatments produced a biomass enriched with more than 70% of cellulose which was addressed to second-generation ethanol through enzymatic conversion and C6-fermentation. Besides the solid cellulose, this pretreatment released a residual liquor as a byproduct. Biological assimilation of liquor was evaluated using xylose-assimilating yeasts and filamentous fungi to estimate the potential of carbohydrate consumption in the presence of inhibitors, such as furanic compounds and low molecular phenolics originated from lignin fragmentation by ozone. Crude liquor composition was (in g/L): xylose 15.3, glucose 3.6, arabinose 1.8, acetic acid 2.1, formic acid 1.2, furfural 2.1, HMF 0.5, p-hydroxybenzoic acid 0.18, 4-hydroxybenzaldehyde 0.15, vanillin 0.04, total phenolics 2.2 (such as gallic acid). Different cultivation strategies were applied to selected strains with more tolerance to these chemicals. A total of 45 isolates were evaluated, including 20 yeasts and 25 filamentous fungi. Four yeast isolates (2 *Pichia* sp., *Candida shehatae*, *Trichosporon laibachii*) and 6 filamentous fungi showed ability to grow in this composition with an inhibition rate lower than 20% in comparison to a synthetic medium with the same proportion of carbon source without inhibitors. Fungi cultivation was performed in 50 mL erlenmeyer flasks using glucose as an initial carbon source for mycelial development, followed by two sequential feds with liquor diluted by 50%. Six isolates were able to produce cellular biomass under these conditions. After 192 hours, pretreated bagasse was added as an inducer for cellulolytic activity for 120h of cultivation. FPase, xylanase and β-glucosidase activities were monitored every 24h using standard procedures for enzyme activity. Highest production was achieved by *Trichoderma harzianum* P49P11 in 96h (29 UI/mL xylanase; 3 UI/mL β-glucosidase; 0.3 FPU/mL). Another 5 fungi were also able to produce cellulolytic enzymes at a lower concentration. This work showed the potential use of the liquor obtained from ozonolysis for the production of cellulolytic enzymes by selecting microorganisms with some tolerance to the inhibitory compounds. From this finding, there is now an opportunity to optimize the cultivation of these 10 selected isolates (4 yeasts, 6 filamentous fungi) aiming to obtain high-value products (such as enzymes) from a residual byproduct of second-generation ethanol processing.

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A perspective of ethanol production at pilot scale from modified sweet potato (*Ipomoea batatas* L.) leaves as renewable agroindustrial residue

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Currently, agroindustrial residues as lignocellulosic biomass have attached more attention for biofuels production and have been considered as the most promising feedstock for biorefinery. However, bioconversion of

lignocellulosic biomass is still challenging at both technical and economic aspects. Sweet potato has several agronomic characteristics that determine its wide adaptation to marginal lands such as drought resistant, high multiplication rate and low degeneration of the propagation material, short grow cycle, low illness incidence and plagues, and covers rapidly the soil and therefore protects it from the erosive rains. In this study, the effects of acid hydrolysis for second generation ethanol production were evaluated. The modified roots of sweet potato (*Ipomoea batatas* L.) used was cultivar 'Duda' from Universidade Federal do Tocantins' germplasm bank. Acid hydrolysis of modified sweet potato (*Ipomoea batatas* L.) leaves (MSPL) was studied as strategic insight for a process development in a pilot-scale reactor. Studies were conducted under different concentrations of sulfuric acid (between 1.0 and 3.0% w/v) at different solid loading (between 10.0 and 30.0% w/v) for different times (between 15 and 90 min) at 121°C. The composition of MSPL was mainly composed of cellulose (46.48%), hemicelluloses (19.28%), lignin (19.16%), ash (6.88%) and extractives (8.22%). High glucose (70.30 g/L) and xylose (23.22 g/L) concentrations were observed after hydrolytic process. About inhibitors concentrations, it was reported that, the indices obtained of 5-hydroxymethyl-2-furfural, furfural and acetic acid were 0.25 g/L, 0.30 g/L and 0.04 g/L, respectively. The results demonstrated the MSPL had the highest content of cellulose (glucan) and can be used for second generation ethanol production.

LPMOs from *Aspergillus fumigatus* can increase the efficiency of lignocellulosic biomass breakdown.

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Sugarcane bagasse has been considered as the lignocellulosic residue for the second generation ethanol (2G) produced by breaking down biomass into fermentable sugars. We have observed that, *Aspergillus fumigatus* secrete a set of glycoside hydrolases and lytic polysaccharide monooxygenases (LPMOs) to degrade exploded sugarcane bagasse (SEB). LPMOs were recently discovered and can increase the efficiency of the lignocellulosic biomass breakdown and conversion in biofuels. These enzymes catalyze the oxidative cleavage of polysaccharides, acting synergistically with cellulases and hydrolases. Two different LPMOs were described in *A. fumigatus* secretome, AfuLPMO9A (AFUA_1G12560) and AfuLPMO9C (AFUA_4G07850), classified as AA9 in the CAZy database. Time course expression profile of LPMOs encoding genes in A. fumigatus were established in different culture media, fructose, xylan, β-glucan, xyloglucan, xylose, avicel and SEB, added to a final concentration of 1%. Our results, by qRT-PCR, showed that AA9 LPMOs are induced by different polysaccharides in a time-dependent manner. For an example, in SEB culture conditions, AfuLPMO9A and AfuLPMO9C were 7 and 988 times more expressed, respectively, after 6 and 18 hours of cultivation, when compared to fructose (control). To gain more insight into AfuLPMO9A and AfuLPMO9C, all recombinant proteins were expressed in E. coli BL21(DE3) strain for 6 hours under 30 and 25°C respectively, with 0.5 mM IPTG, but both proteins accumulates in inclusion bodies (IBs). After IBs purification, denaturation followed by refolding, soluble AfuLPMO9A (38 KDa) and AfuLPMO9C (26 KDa) were obtained. Protein purification was performed by affinity chromatography on a Nickel-NTA column (Qiagen). To identify conserved residue, we performed multiple sequence alignment of different AA9 LPMOs and compared with 3D structures modeling. We observed β-sheet cores and conserved amino acids residues (H20; H105; Y194) and (H22; H107; Y196), in AfuLPMO9A and AfuLPMO9C, respectively. Histidine residues coordinate a

copper ion in a histidine brace arrangement, which is responsible for LPMOs activities. Circular Dichroism analysis has shown changes in proteins secondary structure after incubation with Cu^{2+}, corroborating with the predicted 3D models. The synergistic effects of AfuLPMO9A and AfuLPMO9C on the enzymatic hydrolysis of Avicel® PH-101 were investigated. Avicel were hydrolyzed by commercial cellulase, Celluclast 1,5L® either with or without AA9s in the presence of Cu^{2+} and ascorbic acid. Synergistic activities were similar for both LPMOs and the reducing sugar yields was 1.4 fold higher than the control without AA9s in the hydrolysis. Our data shows that activity of the recombinant LPMO on cellulose is necessary to maximize the hydrolysis efficiency and important for industrial utilization.

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Improving the robustness of equation-oriented simulators through multilinear interpolator-based surrogate models: 1G-2G ethanol biorefinery case

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Surrogate models based on multilinear look-up tables can be used to approximate nonlinear models and improve the robustness of equation-oriented simulators. These models have several advantages, including controllable accuracy and low computational power demands. Although they have been successfully applied to individual equipment (Furlan et al., 2016), convergence problems may arise, when several simple models are linked, e. g., for the heat and power section of a biorefinery producing 1G-2G bioethanol from sugar cane, and bioelectricity. Therefore, the extension of this methodology to whole process sectors would be worthwhile. Nevertheless, the number of input variables in a surrogate model of an entire sector a sugarcane biorefinery would probably be greater than the practical limit for look-up tables (three to five variables, Nelles, 2001). In this context, this study presents a methodology for identifying the most important input variables through global sensitivity analysis (GSA) and principal component analysis (PCA), in order to decrease the dimensions of look-up tables. PCA is used to correlate output variables in a principal components. Then, GSA is applied to determine the input variables with greater influence on the principal component. It is important to explain that the output variables are intensive ones and that both mass and energy balances are satisfied within the surrogate model. The output variables are only used to make the degrees of freedom of the mass and energy balances equal to zero. The methodology was applied to the train of evaporators responsible for sugarcane juice concentration and to the combined heat and power co-generation sector. In both cases, the methodology was able to identify the most relevant input variables.

Evaluation of the addition of cornstarch as a binder in the elemental analysis and calorific value of charcoal briquettes

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Brazil is one of the largest orange producers in the world. Due to this, there is a large generation amount of solid waste (bagasse). Currently, much of the bagasse is destined for the production of ruminant feed. However, it is known that the use of biomass for the production of fuels has been increasing, aiming at the substitution of fossil fuels. With this in mind, an alternative to the use of the orange bagasse would be carbonization of these for the production of charcoal. However, this technique produces charcoal particles, which does not

have cohesive properties to one another and are intended for the production of activated charcoal. This work proposed as the main objective the production of charcoal briquettes and, for this purpose, it is necessary to add a binder to join the coal particles together. In this study, the chemical properties of charcoal particles and briquettes produced from the mixture of these particles with the cornstarch binder were evaluated. In addition, the influence of the amount of cornstarch in these chemical properties was also studied. For this, three different proportions of the cornstarch were applied in the briquette composition (5, 10 and 15% w/w). The analyses that were performed are the proximate analysis, elemental analysis, and calorific value. The proximate analysis shows that the amount of fixed carbon in the charcoal particles is 50.864%, 9.965% for the cornstarch and 33.568, 32.275, 35.213% for the briquettes with 5, 10 and 15% of cornstarch, respectively. By elemental analysis, the charcoal particles have a carbon content of 77.304%, while the addition of starch (40.787% carbon) causes that value to drop to 74.095% when using 5% of the binder, to 74.761% when using 10% of the binder and for 71.934% when 15% of cornstarch was used. Regarding the calorific value, the value obtained for pure charcoal particles and pure cornstarch are respectively 28968.50 and 16092.00 kJ/kg. However, the briquettes produced with 5, 10 and 15% of cornstarch in their composition presented values of 26875.00, 26379.00 and 25640.50 kJ/kg, respectively. The combined analysis of the results shows, in general, that when there is an increase in the amount of binder in the final charcoal briquette mixture, there is a reduction in the calorific value of the briquette. This is due to the fact that the cornstarch itself has a lower calorific value than the pure charcoal particles, in addition to having a lower amount of fixed carbon and carbon element, as shown by the proximate and elemental analysis, respectively.

Metastillation process applied to neutral alcohol production

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Neutral alcohol is a product with high added value obtained from the extra purification of the hydrated alcohol by a system of three additional distillation columns. The first one, known as hydroselection column, is an extractive distillation equipment in which water is used as extracting agent. The main function of this column is to withdrawn, as distillate, the higher alcohols present in fuel bioethanol, through the increase of their volatility in aqueous dilute solutions. The hydroselection column has as feature a high internal liquid stream due to the water addition. As a consequence, the liquid-vapor contact inside the column can be impaired, since the ratio of internal liquid/vapor flows is high. In this way, metastillation columns may be a good alternative for the traditional hydroselection columns, since, in the case of metastillation, the total liquid flow rate is divided, at the top of column, into two descending streams contacting with one unique ascending vapor flow. This liquid division allows operations with smaller ratios of liquid to vapor streams in each tray, increasing the separation efficiencies and decreasing the column diameter. Taking this into account, this work investigated the advantages of the metastillation technique applied to neutral alcohol production, considering the feed as a hydrate bioethanol, composed by ethanol, water and others six minor compounds. For this purpose, simulations were carried out in MatLab® by adaptations of the method suggested by Naphtali-Sandholm, originally proposed to conventional distillation simulations. The results showed that the metastillation is suitable to replace the conventional process, using a column with a column diameter 10% smaller.

This reduction represents a decrease of about 15% in investment costs, indicating that metastillation can be an important alternative to conventional processes.

Cloning, expression and purification of GH10 xylanase gene from *Aspergillus fumigatus* involved in biomass hydrolysis

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Xylan is the most abundant hemicellulose polymer and it is composed by a main chain of xylose monomers linked by β-(1,4) glycosidic bonds and by branching of arabinose and glucuronic acid. Due its complex structure, xylan degradation requires a diverse range of hydrolytic enzymes that includes endo-1,4-β-xylanases, β-xylosidases, α-L-arabinofuranosidases, α-glucuronidases, acetylxylan esterases and feruloyl esterases. Xylanases catalyze the cleavage of β-1,4 linkages in homopolymeric backbone of xylan and they are classified according to the CAZy database into glycoside hydrolases families 5, 7, 8, 10, 11, 26, 30 and 43, being the majority confined into families 10 and 11. Xylanases have several industrial applications, including animal feed, paper and biofuels industries. In the second-generation ethanol production, these enzymes play an important role in the enzymatic cocktails used in the lignocellulose hydrolysis step. *A. fumigatus* is a thermophilic fungus and it is known as a great xylanases producer. Previously, our group performed the determination of xylanases activities in the supernatant from *A. fumigatus* grown in SEB (sugarcane exploded bagasse) culture, using Azo-Xylan Birchwood substrate (Megazyme). After 24 hours of cultivation, the xylanase activity resulted in approximately 7,5 U mL^{-1}. In addition, Secretome and RNA-seq analyses of *A. fumigatus* in the same condition revealed many xylanases significantly up-regulated in this biomass, including the gene Afu6g13610 (log2FC 9,831), that encodes a GH10 xylanase (*xylA*). The *xylA* gene encodes a 397 amino-acid protein with an estimated molecular mass of 42.14 kDa and a CBM1 domain at residues 361-397. SignalP analysis revealed a signal peptide at N-terminal residues 1-19. The potential N-glycosylation site N120 was predicted by NetNGlyc 1.0 Server and it is conserved among several *Aspergillus* species, such as *A. fischeri*, *A. lentulus* and *A. udagawae*. Homology modeling performed in Phyre2 software revealed 83% identity with a characterized xylanase from *Cellvibrio japonicus* (PDB_id: xylanase10c (mutant e385a) from *Cellvibrio japonicus* in2 complex with xylopentaose). Multiple sequence alignments were performed with ClustalW tool and indicated two putative catalytic residues E150 and E257. For further characterization of this enzyme, it was performed the cloning of *xylA*-encoding gene into the pET-28a(+) vector and expression in *E. coli* BL21(DE3) strain. After expression with 0.25 mM IPTG in 25°C, the *xylA* protein was obtained in the soluble fraction from cell lysates. The purification was performed by affinity chromatography and the protein was eluted at about 40-80 mM imidazole. For biochemical characterization, the determination of *xylA* activity on beechwood xylan (Megazyme) by DNS method is being performed as well as the characterization of the optimal pH and temperature for the enzyme activity. Moreover, the *xylA*-encoding gene expression levels on different carbon substrates (xyloglucan, barley beta glucan and beechwood xylan) will be investigated by qRT-PCR.

Study of non-productive adsorption of beta glucosidases

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Converting polysaccharides present on the cell wall of lignocellulosic materials is not simple due to the high recalcitrance that the presence of the lignin confers to the lignocellulosic material, reducing the accessibility of the hydrolytic enzymes to the cellulosic fibers. Non-productive adsorption of glycoside hydrolases onto lignins is an important mechanism that negatively affects the enzymatic hydrolysis of lignocellulose biomass. In this work, was used the molecular docking, molecular dynamics(MD) simulations and enzymatic assay to describe the binding process between phenolic compounds who are lignin components (tannic acid, ferulic acid, coumaric acid and synapylic acid) and two thermostable β-glucosidases of *Thermotoga petrophila* one from the GH1 family (TpBGL1) and outher from the GH3 family (TpBGL3). During MD simulations, coumaric, sinapinic and syringic acids presented a similar behavior, leaving the docking starting position but interacting with TpBGL1 or TpBGL3 proteins in an unspecific way through hydrophobic interactions from aromatic rings found in the compounds and hydrophobic spots of the proteins, formed mainly by tryptophan, valine and tyrosine. Hydrogen bonding were observed involving unspecific main chain and lateral chain of tryptophan or tyrosines with hydroxyl or carboxyl groups found in phenolic compounds. Otherwise, tannic acid MD simulations presented stable complexes in both enzymes, assuming it spatial conformation inside the active site. In TpBGL1 tannic acid made hydrogen bonds inside and around the active site through hydroxyl groups. On the other hand, TpBGL3/tannic acid complex presented a more number and stabler hydrogen bonds in both active site and in whole protein. Notably, tannic acid was the best binding molecule compared to all phenolic compounds studied in present work not only because the length of the structures but its chemical group substitutions. In despite of the aromatic rings, tannic acid presents high quantity of hydroxyl groups while the others present methoxy groups. Methoxy group is reported to form weak molecular interactions mainly with CH groups, suggesting a small polarization between oxygen and carbon atoms. On the other hand, hydroxyl group is widely known as a polar group found in many molecules that is able to both accept and donate hydrogen electrons, being part of stronger molecular contacts. In conclusion the tannic acid was the most responsible for the inhibition and deactivation of the activity of both enzymes, showing that it is the compound most responsible for the unproductive adsorption caused by lignin because of its molecular structure very well mimics that of cellobiose.

Evolutionary engineering of *Clostridium saccharoperbutylacetonicum* for enhanced tolerance toward lignocellulosic inhibitors in butanol production

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Brazil has been recognized as an attractive place to develop biomass technologies due to its vast availability of sugarcane as raw material. Moreover, the possibility of adding value to

sugars derived from lignocellulosic biomass will help the country to become more competitive in this field. Production of second-generation (2G) bio-based products includes butanol, which is a molecule with a diverse range of industrial applications, including paints, biofules, etc. Butanol has been traditionally produced by chemical synthesis or anaerobic fermentation by solventogenic clostridia species. Clostridia are able to metabolize a wide range of carbon sources such as glucose, cellobiose, arabinose, manose, including xylose, the main sugar in the hemicellulosic hydrolysates. However, the fermentation of hemicellulose hydrolysate is severely limited due to the presence of inhibitory compounds that affect growth and product synthesis. Therefore, the aim of this work is to develop strains able to tolerate the main inhibitors (acetic acid and HMF) present in hydrolysate used for 2G butanol production. In this work, a wild strain of C. saccharoperbutylacetonicum (DSMZ 14923) was submitted to serial batch cultivations with increasing concentrations of a hemicellulosic hydrolysate (HH) produced by hydrothermal pre-treatment follow by acid hydrolysis (sulfuric acid-0,4%), in order to obtain an evolved population able to withstand the main inhibitors present in hydrolysate. Preliminary results showed that after 60 generations, this evolved population (EP) was able to growth in a media containing 33% (in volume) of the hemicellulosic hydrolysate. Additionally, the EP-33 showed an increase of 23% in maximum specific growth rate (umax) and 29% in specific rate of substrate consumption (qs), compared to wild strain in a defined medium supplemented with acetic acid (1,13 g/L) and HMF (0,02 g/L). However, butanol yield was slightly lower in the EP (Ybut/s = 0,27) when compared to the wild strain (Ybut/s = 0,29). In continuation to adaptive evolution, the EP-33 was challenged in a media containing 35% and 40% of HH, in order to obtain robust mutants able to tolerate the inhibitors present in HH. The results showed that EP-40 has a better fermentative performance in media containing 40% of HH compared to wild type, achieving a higher cellular density with a faster microbial growth. The EP-40 was screened in solid media in order to obtain single clones that were challenged in media supplemented with acetic acid and HMF (same concentration of MD +40% of HH). Further studies will include the selection of best mutants able to tolerate the inhibitors (AA and HMF) and the investigation of the molecular basis for the evolved phenotype.

Pretreatment-dependent surface lithium distribution affects the performance of the enzymatic cocktail used in saccharification of sugarcane bagasse

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Sugarcane bagasse must be pretreated to be efficiently hydrolysed by enzymes. The physical-chemical pretreatments decrease the recalcitrance of the substrate and increase both surface area and binding sites for enzyme action. This work evaluated the neglected effect of current pretreatments on the composition, concentration and distribution of metallic ions on the surface of the sugarcane bagasse and their correlation with enzyme cocktail activity. Different pretreatments of sugarcane bagasse were carried out: steam explosion; Microwave: H2SO4; Ethanol: Dymethyl Sulfoxide: Ammonioum Oxalate (EtOH:DMSO:AO) and NaOH. Sugarcane bagasse was surfaced-analyzed using an ION-TOF ToF-SIMS GmbH. The most affected metal ion during pretreatment was Lithium. Furthermore, during

hydrolysis, reduced concentration of surface-bounded Lithium and the increase in the distance between Lithium clusters correlated positively with the increased efficiency of the enzymatic saccharification process. Steam explosion and microwave:H2SO4 pretreatments increased 1.74-fold the hydrolytic release of reducing sugars compared to controls after 6-hour reaction times. NaOH pretreatment produced ultrastructural changes related to Steam Explosion but released surface-bounded Li+, obtaining 2.04-fold more reducing sugars than did controls. EtOH:DMSO:AO pretreatment decreased 1.90-fold reducing sugars release. Apparently, Lithium clusters blocked binding sites on the substrate preventing both enzyme access and enzyme walking. Thus the presence and distribution pattern of lithium in the substrate acted as an inhibitor for the enzymatic saccharification of sugarcane bagasse.

Optimization of commercial enzymes mixtures for enzymatic hydrolysis of sugarcane biomass aimed fermentable sugars and xylooligosaccharides

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The sugarcane biomass is a cheap, abundant and renewable raw material that can be employed for sustainable production of biofuel, bioenergy and several value added biomolecules such as xylooligosaccharides. The biomass recalcitrance due the carbohydrates–lignin complex is the main obstacle in bioconversion. On the face of it, researchers has invested in technology to make this process economically feasible, such as the development of an efficient pretreatment step and optimization of enzymatic cocktails for cell wall deconstruction. The pretreatment with ionic liquids (IL) is a promising option that results in superior hydrolysis rates and yields when compared to conventional pretreatment methods. In this context the objective of this study was to optimize the enzymatic hydrolysis of sugarcane biomass using mixtures of commercial hemicellulases and cellulases in a biomass mixture 50% (w/w) (bagasse + straw) pretreated with ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) and dilute sulfuric acid to obtain xylooligosaccharides and fermentable sugars (glucose and xylose). A Plackett & Burman design was carried out with 12 trials and three repetitions of the central point, totalizing 15 trials. In the experimental design, the five independent variables (factors) were the commercial enzymes: α-L-arabinofuranosidase and β-xylosidase from Megazyme®, endo-1,4-xylanase, cellulase and β-glucosidase from Novozyme®. The response variables were the yields of xylose and glucose and the concentration of xylooligosaccharides released in the hydrolysates. Samples were incubated at 50°C for 48 h under agitation (1000 rpm). The enzymatic products were analyzed by chromatography (HPLC-PAD), using a Dionex DX-500 system (Sunnyvale, CA, USA). The analysis of each factor was performed using the software online Protimiza Experimental Design. The enzymatic hydrolysis of biomass pretreateated with IL provided the highest yields of glucose and xylose achieved 99.96 (%) and 86.52(%) respectively. Among the five enzymes studied, the best result was obtained by adding only celullase and endo-1,4-xylanase, which had great effect on the release of glucose and xylose for both pretreatments. The enzymatic hydrolysis of biomass pretreated with IL also favored in the release of xylooligosaccharides, reaching concentrations around 97.4 (mg/L). Except for the α-L-arabinofuranosidase and endo-1,4-xylanase enzymes, the other enzymes had no effect on the release of the xylooligosaccharides in both pretreatments.

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

Response surface methodology to improve enzymatic hydrolysis of corn cob pre-treated by diluted acid sulfuric and Tween 80

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Corn cob is a renewable biomass used to produce fermentable sugars from its cellulose and hemicellulose fractions, respectively. The sugars can be converted by fermentation to different byproducts as ethanol. However, the lignin fraction presents in the plants cell wall makes the lignocellulosic materials as corn cob highly recalcitrant to enzymatic hydrolysis. To improve the release of sugars from lignocellulosic materials is necessary to conduct its pre-treatment previously its enzymatic hydrolysis. Diluted-acid pretreatment of corn cob can break up polysaccharides fractions and release D-xylose as major sugar in hemicellulosic hydrolysate (liquid fraction) and the pre-treated corn cob (solid fraction) can be more accessible for the saccharification enzymes to release the D-glucose. New methodologies have been proposing the use of surfactants as additives during pre-treatment to improve lignin removal from solid residue. Enzymatic hydrolysis, in turn, is one of the most costly steps in the production of bioethanol and the reduction of enzymes loading is therefore very desirable. The aim of this work was to use the response surface methodology to improve enzymatic hydrolysis of the pretreated corn cob by diluted acid sulfuric and surfactant (Tween 80). In this context, this work had the challenge of efficiently hydrolyses the cell wall of corn cob in fermentable sugars by optimizing the commercial enzymatic complex Cellic CTec2 (5, 17.5 and 30 FPU/g) (E) from Novozymes and the concentration of surfactant (Tween 80) (0, 5 and 10% w/w) in both diluted acid pretreatment (SP) and saccharification medium (SS) by using a 2^3 factorial design with three replicates at the central point. According to the levels of the variables E, SP and SS the enzymatic hydrolysis were conducted using 10% of solid in Erlenmeyer flasks (125 mL) containing of 50 mL of medium prepared with sodium citrate buffer (50 mmol.L-1, pH 4.8), under 200 rpm at 50°C. The enzymatic hydrolysis showed maximum glucose yield (80.54%) and D-glucose concentration (61.98 g/L) by using a pre-treated corn cob at the highest surfactant concentration (10% w/w) in both pretreatment and saccharification medium. In this case, the enzyme dosage was reduced to 25.50 FPU/g. Also, the enzymatic hydrolysis of the hemicellulose that remained in the solid fraction of corn cob diluted acid pretreatment was converted to D-xylose (70.66%) in the saccharification medium. Corn cob pretreated with dilute acid in the presence of Tween 80 (5 and 10% (m/m) presented the morphology more disordered and decompressed form than that material processed without Tween 80, thus signaling the biomass pretreated in presence of Tween 80 had enzymes action facilitated during hydrolysis. The use of tween 80 in the diluted acid pretreatment and saccharification medium of the corn cob improved the enzymatic hydrolysis and reduced the dosage of the enzymatic complex.

Optimization of the sequential acid-alkali pretreatment of rice husk to obtain fermentable sugars for bioethanol production

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Lignocelluloses from agricultural, industrial and forest residuals are regarded as the largest known renewable carbohydrate source for ethanol production. About 140 million tonnes of rice husks are produced worldwide every year, containing pentoses and hexoses for ethanol production. The structural complexity of the

lignocellulosic materials hinders enzymatic hydrolysis for what their conversion to bioethanol requires a pretreatment step. The aim of this work was to optimize a sequential acid-alkali pretreatment applied to rice husk, improving sugar release by enzymatic hydrolysis in these samples. Samples were pretreated with diluted H2SO4 and subsequently with NaOH. Five pretreatment effects were evaluated, using a 25-1 fraction factorial design: 1. H2SO4 concentration in step 1 (no acid step to 2% v/v H2SO4 in water); 2. NaOH concentration in step 2 (from 0.5 to 4.5% w/v); 3. Temperature in step 2 (from 85 to 125°C); 4. Reaction time in step 2 (20 to 100 min) and 5. Solid/liquid ratio (from 5 to 12.5). Enzymatic hydrolysis was carried out using the enzyme cocktails Celluclast and Novozyme 188, at 50□C for 8h at pH 4.5. Untreated rice husk released 7 mg of sugar/g substrate and the acid step alone is able to increase this amount to 25 mg/g, with 1 or 2% H2SO4. Sugar release around 40 mg/g substrate or higher can be obtained in samples that had a first 2% H2SO4 step, followed by an alkali step with higher NaOH concentration (4.5%). Statistical analysis of sugar release showed that acid and alkali concentrations were important parameters for these samples. Temperature, time and solid/liquid ratio no significant effect on sugar release in pretreated materials. The model fitted to the results predicted that the maximum sugar release would be obtained in rice husks treated under the following conditions: 2% H2SO4; 4.5% NaOH; 85°C; 100min and 12.5 ratio.

Influence of several variables on xylose simultaneous isomerization and fermentation (SIF) by native *Saccharomyces cerevisiae* strain co-encapsulated with xylose isomerase for 2G-ethanol production

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Xylose is a plentiful sugar in lignocellulosic biomass and currently it is underused. This pentose is a great carbon source for 2G ethanol, however, the native yeast Sacharomyces cerevisiae cannot assimilate this sugar. If previously isomerized to xylulose, the conversion of xylose to ethanol by S. cerevisiae could be feasible. The isomerization reaction is catalyzed by the enzyme xylose isomerase (XI) and it has a chemical equilibrium of 5 xylose to 1 xylulose. A process of simultaneous isomerization and fermentation (SIF) could enable the process by leading to the displacement of the chemical equilibrium for product formation, once the yeast would be assimilating xylulose parallel to its formation, and consequently allowing full conversion of xylose to xylulose. The enzyme and cell immobilization technique has many advantages, such as easy recovery of biocatalyst and high biocatalyst density in the reactor, which could compensate the low xylulose fermentation rate by S. cerevisiae. Taking this into account, the present work evaluated the ethanol production from xylose in a SIF process using as biocatalyst XI immobilized on chitosan, co-immobilized with baker's yeast in calcium alginate gel particles. The influence of several variables on ethanol productivity and ethanol/xylitol selectivity were evaluated: biocatalyst composition (enzyme and yeast load), pH and temperature. The biocatalyst composition was varied using enzyme load from 5 to 20% (w/v) and cell concentration of 5 to 17% (w/v). Batch assays were performed under stirring, at 35°C, initial pH 5.6 and 65 g.L-1 of xylose. Productivity and ethanol yield increased with yeast concentration, while selectivity increased with enzyme concentration. The best condition was using 10% (w/v) of yeast (50 g.L-1) and 20% of enzyme (120.103 UI.L-1reator), that led to 98% of conversion within 11h, providing a yield of

0.35±0.02 gethanol.gxylulose-1, 2.07± 0.17g.L-1.h-1 of productivity and ethanol/xylitol selectivity of 2.42±0.01. The pH range to be studied is limited: isomerization requires pH above 5 but increasing pH would facilitate bacterial contamination. Thus, it was tested the pHs 5.6 and 6.5, in batches performed under stirring at 35°C and 65 g.L-1 of xylose. The ethanol yield and productivity were similar between the tested pH, however the selectivity for pH 5.6 was twice than the observed using initial pH 6.0 (2.42±0.01 and 1.23±0.12, respectively). The tested temperatures were 32, 35 and 37°C. The batches were carried out under stirring, initial pH 5.6 and xylose 65 g.L-1. In all assays, ethanol yield and productivity were around 0.35 gethanol.gxylulose-1 and 2.03 g.L-1.h-1. However, although higher temperatures being better for XI activity, the temperature of 35°C was chosen, once for this temperature the cell viability remained unchanged while for 37°C the final cell viability decreased slightly to 95%.

A new approach of large-scale production of nanocellulose isolated by mechanical method

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Feedstocks generated from processing forest wastes have traditionally been considered as a low value product. The eucalyptus is a forest residue, with a high generation (3.6 million tons of sawdust produced in Brazil in 2014). The only destination of these wastes is the incineration. The economic potential of these materials can be enhanced by emerging biomass conversion technologies, such as the isolation of cellulose nanostructures to obtain bionanocomposites. This work presents a new approach for converting lignocellulosic biomass of eucalyptus wastes into cellulose nanostructures. To minimize environmental impacts, the isolation was done via ball milling, for 6 hours, without the presence of toxic reagents, aiming an environmentally friendly isolation. For the isolation of cellulose nanostructures from the eucalyptus wastes, three pretreatment steps were performed to remove lignocellulosic components, such as oils, hemicellulose, and lignin. The effectiveness of the proposed methodology was also evaluated, and the yield of process could be obtained. The chemical composition of the eucalyptus wastes and the treated biomass was evaluated by Fourier transformed infrared spectroscopy (FTIR) and compositional analysis. Cellulose was the major component detected, and oils, hemicellulose and lignin were removed after the treatments. It was observed that the obtained cellulose nanostructures have similar structure to that of native cellulose. Scanning electron microscopy (SEM) shows the morphologies of the fibers after each treatment. The SEM images revealed that pretreatments led to fibrillation and fiber breakage. The greater exposure of the fiber results in easier isolation of cellulose nanostructures. The nanosizes were measured by dynamic light scattering technique (DLS) and the average was 192 nm. This result was confirmed by AFM analysis, which showed fibrillar morphology of the particles that were isolated. After the treatments and nanocellulose isolation, the yield was 43 wt.%. The results demonstrated that eucalyptus biomass residues can be considered as promising source of nanocellulose isolation and is a potential reinforcement to bionanocomposite applications. Also, the others fractions of the eucalyptus biomass obtained from its pre-treatment as the hemicellulosic hydrolysate and the lignin can be used to produce chemicals and biomaterials. The areas in which lignin is applicable in function of its great chemical and physical properties include: emulsifiers, dyes, synthetic floorings, sequestering, binding, thermosets, dispersal agents, paints and fuels. Diluted-acid pretreatment of can break up polysaccharides

fractions and release D-xylose as major sugar in hemicellulosic hydrolysate (liquid fraction), which can converted by fermentation to ethanol and others products of aggregated value as xylitol. This study demonstrated the technical potential for the large-scale co-production of cellulose nanostructures from eucalyptus wastes.

Detoxification of hemicellulosic hydrolysate from sugarcane bagasse using MgAl-layered double hydroxides adsorbents

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Biotechnological processes using lignocellulosic materials in most cases require preliminary steps to release the components of cellulose and hemicellulose. A common way of performing this process is through acid hydrolysis, but a detoxification process is necessary to remove inhibitory substances resulting from the decomposition of lignin and hydrolysis of the sugars during the pretreatment. The detoxification can be carried out with adsorption processes using alternative adsorbents, such as Layered Double Hydroxides (LDHs). LDHs are lamellar mixed hydroxides containing positively charged structural layers capable of anion-exchange. Hydrotalcites is the most representative LDHs of the group, whose divalent and trivalent cations and intercalated anions are Mg2+, Al3+ and CO2−3, respectively. This study describes the performance of two different hydrotalcites to adsorb inhibitory compounds (formic acid, acetic acid, hydroxymethylfurfural, furfural). The hydrolysis of the sugarcane bagasse was carried out using diluted H2SO4 (100 mg of H2SO4 per gram of dry matter) and a solid/liquid ratio of 1:10 at 121°C for 20 min. It was used two different hydrotalcites with pre-treatment (calcined at 500°C for 4 h), PURAL MG 63HT (HT63c) and PURAL MG 70 (HT70c), which have 63 and 70% MgO in the composition, respectively. Experiments were carried out isothermally (50°C) employing glass stirred reactors containing 50 mL of hemicellulosic hydrolysate and 3 g of hydrotalcite as adsorbent. Samples were periodically withdrawn from the reactors and the adsorption kinetics was observed by the inhibitors concentrations. Sugars were also determined (glucose, xylose and arabinose) to confirm that the adsorbent do not adsorb them. According the results the equilibrium time was defined as 3 h. At this time it was noticed the follow relative concentrations: C/C0 = 0,00 (formic acid), C/C0 = 0,45 (acetic acid), C/C0 = 0,00 (hydroxymethylfurfural) and C/C0 = 1,00 (furfural) for HT63c adsorbent; C/C0 = 0,91 (formic acid), C/C0 = 1,00 (acetic acid), C/C0 = 0,00 (hydroxymethylfurfural) and C/C0 = 0,90 (furfural) for HT70c adsorbent. The hydrotalcites were able to total removal of some inhibitors (C/C0 = 0,00). Under the conditions studied, it was not possible to observe the adsorption for furfural with HT63c adsorbent and acetic acid with HT70c adsorbent. The hydrotalcites used did not adsorb sugars, which is good for feasibility of the inhibitors removal by adsorption process. It can be verified that hydrotalcites could be alternative adsorbents for detoxification of hemicellulosic hydrolysate from sugarcane bagasse.

Economic potential of 2-methyltetrahydrofuran (MTHF) produced from the hemicellulosic fraction of biomass

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Furfural is easily produced via hydrolysis of biomass. When produced from cheap biomass, it can be obtained at a very low production cost and can be used as precursor for other

commodities. In processes focusing on the use of cellulose to produce fermentable sugars such as glucose, most of them have problems to deal with the sugars obtained from the hydrolysis of hemicellulose, such as xylose. One option to consume this sugar is the synthesis of furfural. However, when looking at the potential market for products from the cellulose fraction of biomass, the market for furfural seems rather small. However, 2-methyltetrahydrofuran (MTHF) can be produced from furfural through a series of hydrogenations. MTHF is a furan with potential to be used in advanced blends of gasoline because its octane number is compatible with that of gasoline. Moreover, because of its high oxygen content, MTHF can help achieve mandated oxygen content of reformulated gasoline. Therefore, this process route could open a new market for furfural and the hemicellulose fraction of biomass. In this study, the economic potential of the synthesis of MTHF from furfural was evaluated considering the operational costs associated with feedstock, energy consumption and equipment. Process conditions from the Literature were simulated using the software Aspen Plus™ 8.6. According to simulation results and using the price of gasoline in Brazil as reference for the selling price of MTHF, it was determined that furfural should be available at prices below than $ 350/t in order to achieve an IRR of 10% in a plant with an annual capacity of 50 kt of MTHF. Costs with natural gas for production of hydrogen and electricity to drive compressors represent 34% of the operational expenditure in this scenario. According to the Literature, furfural has potential to be produced at such low costs using current technology and sugarcane bagasse in a plant integrated to a sugarcane mill, an aspect that motivates further development for this process route that focuses on the use of the hemicellulose fraction of biomass.

Impacts of mechanization technologies on the different stages of sugarcane production system: use of multi-criteria decision analysis to assist the decision-making process

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Mechanization in the sugarcane agriculture has increased over the last decades, especially in harvesting and planting operations, mainly in Brazilian Center-South region. The consequences of such technological change, however, are not fully understood when multiple perspectives are considered such as economic results, environmental regulations and social aspects. In this study, a multi-criteria decision analysis (MCDA) was used to evaluate the broader impacts of mechanization technologies on the different stages of sugarcane production system. MCDA is a procedure that combines the performance of decision alternatives across several, contradicting, qualitative and/or quantitative criteria and results in a compromise solution. It consists of a group of approaches which allow to account explicitly for multiple criteria, in order to support individuals or groups to rank, select and/or compare different alternatives (e.g. products, technologies, policies). The obtained results show that mechanized scenarios presented the best sustainability performances which was later confirmed by a sensitivity analysis for three different biased perspectives: economic, environmental and social. However, it is important to mention that the main purpose of this study was to provide

quantitative subsidies for specific decision making processes, so further interpretation on the meaning of results presented may vary according to the local economic situation, environmental conditions and social context of sugarcane industry.

Woody biomass with CCS can provide negative emissions from sustainable forests in Brazil?

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The bioenergy process with Carbon Capture Storage (BECCS) allows CO2 to be collected and stored directly from the atmosphere, not from a fossil source as in the case of CCS, this implies that eventual storage emissions can be collected and restored by reiterating the BECCS process. Therefore, as suggested by the (IPCC, 2011), it could be a key technology to reverse the emission trend and create a global system of net negative emissions, which is not possible only with CCS, because it is from fossil sources. Currently, most of the schematic BECCS systems are not economical compared to normal CCS, mainly because of the costs of the technology for post-combustion capture, as well as sustainability restrictions on large-scale biomass production. In this context, the objective of this paper is to develop a CCS technology with the ability to link it to bioenergy systems through forest plantations directed to solid biofuels. This will also look to initiate a carbon pre-capture program with the use of wood as an alternative to CCS with bioproducts, for example replacing concrete and structural steel, with wood involving the projected bioenergy demands and potentially linked to CCS technology to promote negative BECCS emissions in the power systems. The study presents an analysis of forest biomass energy with CO2 capture and storage (BECS) including a new method of pre-combustion capture carbon with woody biomass from sustainable forests produced in Brazil.

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Effects of ultrasound treatment on the activity of a commercial cellulase enzyme

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Cellulases are important enzymes employed in second-generation bioethanol production by acting on lignocellulosic biomass and promoting its hydrolysis. Although the enzymatic step represents a high cost to the process, it has a great potential of cost reduction. Cellulase related strategies that can make bioethanol production more economical include producing enzyme preparations with higher activity and greater stability. Ultrasonic technology has received great attention recently in the biotechnology field, since appropriate intensity and application time can increase the enzyme activity by improving mass transfer. The aim of this study was to evaluate the effects of ultrasound application on the cellulase complex activity and to investigate the optimal conditions to enhance the enzymatic activity. Commercial cellulase Celluclast 1,5L (Novozymes®) was diluted in 0.2 M acetate buffer (pH 4.8) and then placed in a jacketed reaction beaker and sonicated. An ultrasonic probe was used (maximum ultrasonic power of 800 W and 19 kHz of frequency). A Central Composite Rotatable Design (CCRD) was performed to assess the effects of exposure time, intensity and temperature on the enzyme activity subjected to the ultrasound treatment. The range of evaluated temperatures was 40°C to 60°C, exposure time from 60 s to 600 s, and intensity from 160 to 600 W. After the ultrasound application the enzymatic activities (endoglucanase, total cellulase and β-

glucosidase) were determined. The control sample was the unsonicated enzyme and the results were expressed in terms of relative activity of the control. The results were analyzed using the software Protimiza Experimental Design®. The highest relative activities for endoglucanase (101%) and β-glucosidase (107%) were achieved with 330 s of exposure time, 160 W intensity and temperature of 50°C. However, the total cellulase activity (FPase) was reduced at all the evaluated conditions. For endoglucanase and total cellulase activity, time of exposure, intensity and temperature were significant ($p < 0.05$), but for β-glucosidase activity none of the parameters were significant. From the CCRD results, we could conclude that shorter times of exposure and smaller intensities can lead to higher enzymatic activities. Temperature resulted in a positive effect on endoglucanase activity, but negative effect on FPase activity. These results are relevant to select appropriate conditions to increase the cellulase enzymatic activity through ultrasound application, and subsequently apply them combined to solid substrates (as sugarcane bagasse) to evaluate its efficiency in terms of fermentable sugars release.

Corncob pretreated with the combination of electron beam irradiation and enzymes to enhance fermentable sugars for biofuel production

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Lignocellulosic biomass pretreatment technologies have been taken up as a global challenge as it comprises to increase enzyme accessibility to biomass and yields of fermentable sugars. The reducing sugars released from pretreatment of the corn cob can be converted into biofuels. Corncob is a lignocellulosic material composed of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are polysaccharides constituted of simple sugars (hexoses and pentoses). However, these sugars are difficult to access, due to the presence of lignin, which is a polyphenolic molecule that provides a high recalcitrance to plant tissue. An appropriate biomass pretreatment disrupts the hydrogen bonds in crystalline cellulose, breaks down cross-linked matrix of hemicelluloses and lignin, and raises the porosity and surface area of cellulose for subsequent enzymatic hydrolysis. There are several pretreatment methods including, physical pretreatment (electron beam irradiation, grinding and milling, microwave, and extrusion), chemical pretreatment (alkali, acid, organosolv, ozonolysis, and ionic liquid), physico-chemical pretreatment (steam explosion, liquid hot water, ammonia fiber explosion, wet oxidation, and CO2, explosion), and biological pretreatment. This study evaluated electron beam irradiation (EB) in combination with enzymatic hydrolysis on corncob at different grain size to produce fermentable sugars. Dry biomass samples after characterization were exposed to EB radiation doses of 0, 30, 50, 70, 100, and 200 kGy. Enzymatic hydrolysis of the pretreated biomass samples were conducted using 10% of solid in Erlenmeyer flasks (125 mL) containing of 50 mL of medium prepared with sodium citrate buffer (50 mmol.L-1, pH 4.8), CellicCTec 2 25.50 FPU/g dry lignocellulosic material) and Tween 80 (9.8% w/w) under 200 rpm at 50°C. using the Cellic® CTec2 from Novozymes. The structural changes and degree of crystallinity of the pretreated biomass were studied by FTIR, DRX, DSC, TG and SEM analyses. Corncob in natura showed 6.3% extractives, 40.3% cellulose, 31.8% hemicellulose, 17.3% lignin, and 0.7% ash. The highest conversion of cellulose to glucose (44.2%) was by using EB radiation doses of 200 kGy and reduced corn cob grain size. Significant

improvement in the enzymatic saccharification (80.4%) of the EBI exposed biomass was observed compared to control. The sugars released can be converted to biofuel or another bioproduct. The EB in combination with enzymatic hydrolysis of corncob is an environmentally sound biomass pretreatment.

Montmorillonite: an efficient support for immobilization of *Candida rugosa* lipase aimed at biodiesel production by hydroesterification

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The biodiesel production from waste cooking oil (WCO), crude oils and other raw materials with high acidity and water has become a challenge for the supply chain of biodiesel. Therefore, hydroesterification is an alternative to this scenario, a process that involves two consecutive steps:, hydrolysis followed by esterification. In this study was investigate the immobilization of *Candida rugosa* lipase in KSF Montmorillonite clay for application in the first step: hydrolysis, a chemical reaction between vegetable oil (mono-, di- and triglycerides) with water, generating glycerin and fatty acids. This process can be used enzymatic catalysts, as well as lipases, enzymes capable of catalyzing reactions of hydrolysis for the transformation of triacylglycerides (TAG) into free fatty acids (FFAs). KSF Montmorillonite is a cationic clay with a 2:1 structure. Initially, the clay was reacted with 3-aminopropyletriethoxysilane (APTS) and glutaraldehyde was used as a coupling agent to covalently immobilize lipase. Two reactions medium (aqueous solution and the organic solvent) were studied to provide the greatest immobilization potential. In order to evaluate the reaction medium, an experiment design (2^{4-1}) was applied to estimate the most significant effects of the immobilization of lipase from Candida rugosa in KSF Montmorillonite. For the aqueous solution, the variables analyzed were temperature, acid treatment of clay, lipase loading and pH. For immobilization in the organic solvent the variables were temperature, acid treatment of clay, lipase loading and solvent. The results showed that the significative variable for two reaction medium was temperature. The highest values obtained in immobilization yield in aqueous medium was 62% (m/m) and in the organic medium, 97%(m/m). These results indicate that the substrates immobilized in the organic medium had a potential for the immobilization of *Candida rugosa* lipase bigger than immobilized in the aqueous medium since they provided biocatalysts with high values of hydrolytic activity and yield. The best biocatalyst from the design experiment in the organic medium showed a hydrolytic activity of 1607.0 U/g. The biocatalyst was applied in a hydrolysis reaction with OGR in a comparative study using free Candida rugosa lipase. In 10 hours of reaction, the biocatalyst produced 49.0%(m/m). FFAs and the free *Candida rugosa* 52.5%(m/m) FFAs. Immobilization of lipase in KSF Montmorillonite clay in organic solvent was successful, making it a suitable candidate for biocatalyst biodiesel processes by hydroesterification.

Biochemical and biophysical influence of an accessory domain on the enzymatic activity of the endo-beta-1,4-glucanase CelE2

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Cellulose is the most abundant natural polymer on Earth, presenting a great potential for conversion into fermentable sugars through the action of cellulases, which represent a key enzymatic activity for the depolymerization of this polysaccharide. In addition to the catalytic domain, some cellulases exhibit accessory domains, which can provide direct influence on its enzymatic activity. The metagenomic-derived endoglucanase CelE2, has in its modular architecture an N-terminal domain typical of the GH5 family and a C-terminal domain with identity for the Calx-β domain, called Dom2, previously identified in other glycoside hydrolases, but without clearly defined function. In this context, in order to investigate the influence of the C-terminal accessory domain on the enzymatic activity of cellulase CelE2, truncated derivatives of the catalytic and the accessory domain were constructed. The constructs of interest were amplified and cloned into the expression vector pET28a, submitted to heterologous expression in *Escherichia coli*, and the proteins fractions recovered were purified in two steps to obtain high purity. The enzymatic activity analysis showed that one of the construction representing the catalytic domain was able to retain the enzymatic activity in the absence of the accessory domain Dom2. Moreover, the removal of Dom2 did not interfere in the enzymatic activity regarding the preferential substrates (β-glucan, carboxymethylcellulose and lichenan), optimum values of pH (5.3) and temperature (45°C), thermal stability at 40°C and 50°C and cleavage pattern, which is typical of endoglucanase. A positive effect on enzymatic activity was observed in the presence of calcium chloride, as well as it was observed for the full-length enzyme. Regarding structural properties of the catalytic domain, circular dichroism (CD) analysis indicated an α-helix predominance with thermal denaturation at 49.5°C and the small-angle X-ray scattering (SAXS) revealed that calcium has an influence in the conformational stability. Together, these results indicate that the acessory domain is not essential for the CelE2 enzymatic activity. In order to investigate the possible biological function of this domain, further studies will be performed in other to evaluate the ability of the accessory domain to bind to insoluble and soluble substrates.

Enzymatic hydrolysis of sugarcane bagasse with high solid concentration for bioethanol production

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One of the great challenges to make the second generation bioethanol production process viable is related to the cost of ethanol distillation in the fermentation broth, which requires ethanol concentrations greater than 4% in fermentative media. Thus, high concentrations of fermentable sugars in the enzymatic hydrolysis step are necessary, which are only achieved if the hydrolysis is performed at high solid concentration. However, the hydrolysis with high solids leads to problems related to mass transfer limitations. Several promising studies on hydrolysis at high substrate concentrations have been performed, such as the work of Xue et al. (2012), which proposes a hydrolysis technique in two separate steps: a stage of cellulase adsorption on the pretreated biomass at a solid concentration of 5% (w/v) for 10 min and a subsequent step of solid/liquid separation to achieve solid concentrations around 20% (w/v). However, that study has some limitations. The first step of

enzyme adsorption occurs at 50°C (optimum hydrolysis temperature) and therefore should have a reduced operating time. Recently, our study has shown that the adsorption of cellulase on pretreated sugarcane bagasse reaches equilibrium in about 2 hours. Thus, the filtration step performed by Xue et al. (2012) may lead to significant loss of non-adsorbed enzyme in the liquid. The objective of this work is to propose changes in the process of Xue et al. (2012) to obtain higher glucose concentration and cellulose conversion for hydrolysis of sugarcane bagasse pretreated with the hydrothermal method, with minimum enzyme loss. The results showed that the strategy allowed to maintain the conversion of a process performed at ~ 20% (w/v) in the same range of the obtained in a conventional hydrolysis at 5% (w/v) solids. Modifying the process proposed by Xue et al. (2012) it was possible to design a two-stage hydrolysis scheme from a low solids concentration in which the conversion remains the same at high solids concentrations. As a result it is possible to obtain a higher concentration of glucose in solution after the hydrolysis step.

Structure change in the corncob pretreated by diluted acid with the combination of surfactant

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Corncob, lignocelullosic material, is a potential feedstock for the production of biofuels as bioethanol, biogas and biodiesel. Corncob is composed of cellulose, hemicellulose, and lignin. Cellulose is built of C6 sugars; hemicellulose mainly of the C5 sugars xylose and arabinose. Lignin consists of phenolic macromolecules. In order to efficiently convert biomass into sugars, it should maximally remove lignin and minimally modify polysaccharide by pretreatment. The pretreatments may be physical, chemical, biological, or a combination of them. New methodologies propose the use of surfactants as additive in both pre-pretreatment and in the enzymatic hydrolysis of lignocellulosic materials to obtain bioethanol. Surfactants as Tween 80 due to its hydrophilic and a hydrophobic chain have the function of increasing the exposure of the cellulose and forming emulsions with the lignin removing toxic compounds of low solubility that adhere to the surface of the active site of biocatalysts. This study evaluated structure change of the corncob pretreated by diluted acid with the combination of different Tween80 concentrations (0, 5 and 10% m/m). The results showed that Tween 80 (10%m/m) had a greater effect on the removal of lignin and hemicelluloses concomitantly with reduction of 21.1% of cellulose. The SEM analysis showed higher porosity and fragmentation in the morphology of the pretreated corncobs by increasing surfactant concentration. The FTIR showed 2% change in lignin by using 10% (m/m) surfactant. The corncob pretreated in this work was practically free of hemicellulose which may facilitate the enzymatic saccharification of the cellulose to bioethanol production.

Identification and evaluation of major phenolic compoundsderived from sugarcane biomasson yeast physiology

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Yeasts will play an important role as microbial platforms in the conversion processes of sugars from lignocellusic (LC) biomass into fuels and chemicals. However, pre-treatment processes for LC-biomass utilization generate various yeast inhibitors, including phenolic compounds, that reduce the overall efficiency of the fermentation process. In this context, the isolation and identification of the major phenolic compounds derived from sugarcane biomass, and their subsequent evaluation on yeast physiology are of paramount importance to this emerging industry. In the present work, we aimed to identify and to quantify major phenolic compounds in various sugarcane bagasse hydrolysates. We used high-performance liquid chromatography in reverse phase (C18) coupled to mass spectrometry for that purpose. To improve resolution, an extraction method was also employed. The main phenolic compounds present in high concentrations were p-coumaric acid, ferulic acid and vanillin. We also aimed to investigate their effects on the physiologyof industrial yeast strains. For that, strains of *Saccharomyces cerevisiae*, including one laboratory (CEN.PK113-7D) and two industrial strains (PE-2 and SA-1), were grown in the presence of these compounds, both in defined medium and in industrial hydrolysates. The presence of 5mM of p-coumaric acid (p-CA), inhibited in more than half the growth rate of strain CEN.PK 113-7. Under these conditions, biomass and ethanol yields were higher in PE-2 when compared to the lab strain. The growth of the laboratory strain and industrial PE-2 strain werevirtually abolished at 7 mM p-CA, whereas SA-1 was still able to grow under this condition, with a maximum specific growth rate of 0.20 1/h. Similar results were obtained in the presence of ferulic acid. In sugarcane bagasse hydrolysate fermentations, although fermentation kinetics were quite similar for all strains, industrial strains showed a higher ethanol yield in relation to the lab strain. Overall, these results highlight the improved robustness of the fuel industrial strain SA-1 toward LC-phenolic inhibitors.

Improvement of Isopropanol-Butanol-Ethanol production by flash fermentation technology

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Butanol is an advanced biofuel conventionally produced through the acetone-butanol-ethanol (ABE) fermentation. However, in this process approximately one-third of the sugars are converted to acetone, which cannot be used as a fuel. Moreover, there is a risk of oversupply of acetone in case butanol penetrates the fuel market. Alternatively, butanol can be obtained from the IBE fermentation, whose three products (isopropanol-butanol-ethanol) are suitable for fuel purposes. On the other hand, superior process performance, especially butanol titer and productivity, still makes the ABE process more attractive. In the IBE fermentation, microorganism tolerance to butanol is low (~5-7 g/L) and productivity in batch operation is lower than 0.50 g butanol/L.h. In this regard, the IBE fermentation can certainly benefit from in-situ product recovery technologies originally developed for the ABE process. Nevertheless, the suitability of these technologies still needs to be investigated due to the presence of isopropanol and different kinetics of the microorganism. In previous works, the continuous flash fermentation considerably improved butanol concentration and productivity in the ABE process. As such, we hypothesized that similar gains could be achieved for the IBE fermentation. In this technology, concentrated sugar solutions are continuously fed to a tank fermentor connected to an external flash tank. The fermentation broth circulates through the flash tank, where the low pressure allows for partial vaporization of products. To investigate the performance of IBE production using the flash fermentation, we used computational modeling (Microsoft Visual Studio Community® and Fortran language) considering (i) mass

balance equations based on the kinetics of *Clostridium beijerinckii DSM 6423*; and (ii) the UNIQUAC model for the multicomponent flash calculation. For a feed stream (30 m^3/h; 100 g/L sugars) processed in a 500 m^3 fermentor, the technology achieved 96% sugar conversion, butanol productivity of 0.97 g/L.h, and final butanol titer of 16.2 g/L. Isopropanol was also recovered from the fermentation broth, and the outlet stream contained 9.6 g/L isopropanol. Whereas the process significantly improved in relation to the batch control (60 g/L sugars; 59% sugar conversion; 0.13 g/L.h butanol; 6.4 g/L butanol; 3.8 g/L isopropanol), performance is still below the gains reported for the ABE fermentation (100 m^3/h; 140 g/L sugars; 95% sugar conversion; 9.0 g/L.h butanol; 27 g/L butanol). Nonetheless, since the flash technology efficiently recovered IBE from the fermentation broth, further gains are expected from the combination of this technology with enhanced IBE-producing microorganisms.

Multi-omic analysis of the fungus *Laetiporus sulphureus* on the deconstruction of sugarcane bagasse

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Multi-omic approaches such as genomic, transcriptomic and proteomic data can allow the identification of genes, transcripts and proteins involved in the degradation of lignocellulosic biomass. Such studies may provide advances on development of enzyme cocktails since it is possible to determine which genes are induced and which proteins are produced during the deconstruction of the plant cell wall. In the present study, the genome of the brown rot fungus Laetiporus sulphureus was analyzed, as well as its transcriptome in response to sugarcane bagasse induction. Additionally, studies of the microorganism secretory were carried out to identify proteins involved on plant biomass deconstruction. Analysis of the L. sulphureus genome identified 363 carbohydrate active enzymes ("Cazymes") of which 231 were expressed and 71 were overexpressed in response to growth on bagasse. The secretomic identified enzymes interesting for the breakdown of the vegetal cell wall such as xilanase, cellobiohidrolase.and auxiliary (redox) activities. The present study supported the initial understanding of the functional role of genes and proteins involved in the degradation of lignocellulosic material by the fungus L. sulphureus, which may contribute to commercial enzymatic cocktails improvement.

Evolutionary approach for proposing sites for rational modification in enzymes of xylose metabolism

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First-generation (1G) ethanol is accepted as a biofuel with environmental and economic benefits over oil-based fuels, and second-generation (2G) ethanol became a desired commodity to many countries. In 2G processes, redox balance in yeast cells is one of the major features accounting for yield and productivity of ethanol, especially when metabolizing xylose. Despite most yeasts being poor xylose-fermenters, some can naturally produce higher amounts of ethanol from this pentose, such as *Spathaspora passalidarum* and *Scheffersomyces stipitis*, members of a xylose-fermenters phylogenetic clade. In these yeasts, many unknown aspects of its metabolic pathways

changed along evolution allowing the current observed performance. But to surpass the bottlenecks of redox unbalance the most used the strategy is comparing enzymes of these yeasts to Saccharomyces cerevisiae, ignoring the underling evolutionary history that had shaped the species metabolisms. This work presents, to our knowledge, the first description of positive selection acting in specific sites of important enzymes from yeasts pentose metabolism, like xylose reductases and alcohol dehydrogenases, in xylose-fermenters' clade. Through comparative genomics of 18 yeast species we assigned proteins in homolog gene families and used *Saccharomyces cerevisiae* annotated proteins to identify gene families involved in xylose, glucose, ethanol, glycerol and acetate metabolisms. We reconstructed the phylogeny of each gene family and found dN/dS evidences of selection fingerprints in sequences of xylose-fermenters' clade, suggesting putative important sites for rational modification possibly related to cofactor preference. Opposed to the former methodology of comparing sequences by alignments and ignoring that they had diverged a long time ago in evolutionary history, we used an approach that account on possible multiple substitutions per site, trying to minimize time effects when looking for these patterns. Also, we have found positive selection marks in enzyme sequences of glycolysis, previously supposed to remain unchanged along time due possible loss of function when under higher mutational rates, such as fructose-1,6-bisphosphate aldolase and triose-phosphate isomerase. Lining up both strategies for searching important sites along sequences may render new insights to rational modification of important proteins. We are now engaged in testing the suggested sites in vitro and simultaneously exploring other important genes in the yeasts genomes using the presented evolutionary approach.

Study of the anticorrosive action of natural products used as biodiesel additive in zinc plates

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The impacts caused by the use of petroleum-derived fuels have led to greater awareness regarding the use of renewable energy. Therefore biodiesel presents itself as a solution for the use of diesel. The low stability of biodiesel alters its quality standard which in turn increases its corrosive action on equipment and metal structures. The present work deals with the study of the improvement of the thermo-oxidative property of biodiesel of soybean oil with the use of natural antioxidants in order to verify its action in the improvement of the corrosiveness of biodiesel in metallic plates. The ASTM G1-03 and G31 standards were used, in which mass measurements were performed before immersion of the specimens and during the experiment as control for the study. The specimens were immersed in biodiesel suspended by a nylon wire in a closed glass container. Four analyzes were carried out, at controlled temperature at 60°C, supplemented with extracts the of sea almond tree, blackberry and guava solubilized in alcohol, named: BP (pure biodiesel), B0.12A (sea almond tree extract), B0.12B (blackberry extract) and B0.12C (guava extract). At 1080 h an excellent result was verified for biodiesel added with extract sea almond tree, with mass loss of only 0.33%, therefore BP (12.05%), B0.12B (9.82%) and B0.12C (9.06%). For 1440 h a mass loss of more than 14% was verified for all the analyzes, and the acidity index was higher than that stipulated by the National Agency of Petroleum (ANP) standards. At the zero time, the acid index was 0.24 mg KOH/g for pure and after 1440 h was 10.80 mg KOH/g. For the biodiesel additives, the best results were obtained in relation to the pure biodiesel at the

end, for guava (reduction of 14.17%) > almond tree (reduction of 13.33%) > blackberry (reduction of 10.92%). The loss of thickness for all samples and the infrared bands corresponding to the formation of compounds such as hydroperoxides and carboxylic acids (range of 3600 cm-1 - 3250 cm-1) were also verified at the end of the test, justifying the increase of the acidity index of the biodiesel.

Immobilization of xylanase Novo NS22036 in chitosan-glutaraldehyde support modified by *S. cerevisiae* addition

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Xylanases are enzymes that catalyze the hydrolysis of beta-1,4-xylan, one of the major components of biomass and the second most abundant renewable polysaccharide in nature. These enzymes can be applied in the production of ethanol, xylitol and xylooligosaccharides (XOs). XOs are high value added oligomers constituted by 2−10 units of xylan monomers, with important prebiotic properties. Considering the advantages of enzyme immobilization, such as easy recovery of biocatalyst and enzyme stabilization, the application of immobilized xylanases in XOs production may decrease the industrial process costs. Multipoint enzyme immobilization, in particular, can lead to a high degree of enzyme stabilization through a covalent link of enzyme molecule to several activated groups of support, such as glyoxyl groups. The Xylanase Novo NS22036 (XynNovo), have a great potential on XOs production. However, this enzyme have a low external lysine concentration, which makes unfeasible its multipoint immobilization once enzyme amination cause loss of enzyme activity. The addition of yeast cells during support gel formation, on the other hand, could change gel structure and increase internal surface for enzyme immobilization, leading to better immobilization parameters. Among available supports for enzyme immobilization, chitosan is a cheap and abundant support obtained by the deacetylation of the chitin from fungi cell wall and from the shell of shellfish. Taking this into account, this study evaluated immobilization and stabilization of XynNovo in chitosan activated with glutaraldehyde supports prepared with (chit-SC-glut) and without (chit-glut) addition of *Saccharomyces cerevisiae* cells. For this, chitosan gel (2.5% v/v) was prepared with 5% v/v of *S. cerevisiae* cells and after gel formation in KOH 0.5M solution, protein and membrane cell debris were removed by treatment with commercial powder soap (24h, 40°C and pH 9.0). Immobilization experiments were conducted at 25°C and pH 7.0 (100 mM phosphate buffet) with 1:10 vsupport/v ratio. High immobilization yields were obtained, 98% for chit-SC-glut and 91.6% for chit-glut and an increase in recovered activity (51%) was observed when *S. cerevisiae* cells were incorporated in gel formation, with recovered activities of 47% and 31%, respectively. However, no significant improvement on enzyme thermal stability was observed (70°C, pH 5.5), immobilized xylanases were 1.35-fold more stable. Glutaraldehyde activated supports leads to unipontual immobilization and this technique does not promote significant improvements on thermal stability. However, *S. cerevisiae* addition does improve immobilizations parameters and its application is a good strategy in xylanase immobilization.

In silico analysis of product formation during syngas fermentation

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Acetogenic bacteria such as *Clostridium ljungdahlii* are facultative autotrophs that are capable of metabolizing CO/H2/CO2 (syngas) through the Wood-Ljungdahl pathway for synthesis of cell mass, acetate and ethanol. Syngas, which is obtained via industrial gasification of a wide range of lignocellulosic feedstocks, is also a traditional building block for the metal-catalyzed synthesis of chemicals and fuels. Alternatively, the biological conversion of syngas using acetogens, a fairly young technology, has the potential to offer advantages such as gas composition flexibility and lower energy use due to operation under mild conditions of temperature and pressure. It does, however, pose design challenges due to the scarce solubility of the gaseous substrates and the formation of undesired acetate, besides possible inhibition of the cells due to trace contaminants in syngas. While syngas composition can be tuned in upstream stages of the process, it is also decisive for the proton and carbon balance of the cell metabolism, therefore the integrated process is greatly affected by this variable. In the present study, syngas fermentation was investigated using mathematical modeling tools with the goal of generating a robust model and evaluating the influence of main process parameters on the outcomes of the fermentation. For this purpose, Flux Balance Analysis (FBA) and dynamic FBA techniques were employed. The genome-scale reconstruction of *C. ljungdahlii* available in the literature was used as source for the stoichiometric matrix containing 785 reactions and 698 metabolites. The flux distribution was computed using the constraint-based approach, where the space of feasible solutions is constrained with the stoichiometry of the reactions and the defined bounds for the various fluxes, with the solution giving the optimal value of the specified objective function. The modeling method was divided in two distinct phases, with distinct objective functions, namely: (i) the acidogenic phase, in which the problem was optimized for biomass growth; and (ii) the solventogenic phase, in which the hypothesis of minimization of metabolic adjustment was considered. The FBA model developed was then used to evaluate the influence of varying the maximum uptakes of CO, H2 and CO2, which, in real process, would correspond to different availabilities of these molecules in the liquid medium. While the model predicted mostly acetate formation during the first phase, it also indicated that ethanol could be formed under certain gas compositions. In the second phase, the model correctly predicted that ethanol would be favored, with little to no biomass formation, and acetate being re-assimilated by the cell. Furthermore, the FBA model was integrated with the dynamics of the extracellular environment (uptake kinetics and gas-liquid mass transfer) in order to simulate the dynamic behavior of product formation over time.

Biomass sorghum as new substrate in solid state fermentation for the production of hemicellulases and cellulases by *Aspergillus niger* SCBM1 and *A. fumigatus* SCBM6

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Biomass sorghum (BS) is a promising lignocellulosic biomass for the production of enzymes and cellulosic ethanol (second generation ethanol) due to its rapid development, high productivity and potential to produce more than 50 t ha-1 of dry matter per half-yearly average cycle. Furthermore, its

composition resembles to other agroindustrial materials conventionally studied for reuse in the bioethanol production, such as sugarcane bagasse (SCB). Nevertheless, BS comprises relatively lower lignin content and higher amounts of hemicelluloses and cellulose than SCB. Among the enzymes generally used in biomass saccharification, commercial microbial cellulases and hemicellulases have been commonly employed. However, their high cost represents a great limitation for the advance of 2G ethanol production in large scale. In this context, the in home biosynthesis of cellulases and hemicellulases employing cheap raw materials has been widely studied. Thus, in this work, the production of such enzymes was performed by solid state fermentation (SSF), using two strains of Aspergillus (A. fumigatus SCBM6 and A. niger SCBM1) as inocula and biomass sorghum (BS) and wheat bran (WB) as carbon sources. Additionally, different nitrogen sources were evaluated (peptone, yeast extract, ammonium sulfate and urea). Variable SSF conditions were investigated in order to select the best association between microbial inoculum, carbon and nitrogen sources for the production of hemi- and cellulolytic enzymes. In the first stage, A. niger SCBM1, A. fumigatus SCBM6 and the culture in consortium of both strains were analyzed for enzymatic production in BS and WB (1:1) as substrates and ammonium sulfate as nitrogen source. After the selection of the best inocullum, SSF were conducted on BS and the best nitrogen source was evaluated. At last, the ideal combination of carbon sources (BS and WB) was studied, in ratios of 1:1, 1:2 and 2:1 (w/w). The production of five essential enzymes for the hydrolysis of lignocellulosic biomass was evaluated (β-glucosidase, β-xylosidase, xylanase, exoglucanase and endoclucanase. The SSF carried out with BS associated to peptone and A. niger SCBM1 as inoculum was chosen as the best condition for the induction of the most of the enzymes. In this condition, the highest xylanase and exoglucanase productions were obtained in 72 hours of fermentation (300.07 and 30.64 U g-1, respectively), β-glucosidase and endoglucanase in 120 hours (54.90 and 41.47 U g-1, respectively) and β-xylosidase in 144 hours (64.88 U g-1). The chemical characterization indicated the predominance of cellulose (39.84%) in BS, which could be associated to the significant production of cellulases, especially exoglucanase. The present work represents the first study in which BS is employed as carbon source for the production of cellulases and hemicellulases by SSF, presenting it as a new and promising source of biomass for several biotechnological applications, such 2G ethanol production.

Saccharification of sugarcane bagasse using cellulases and oxidoreductases enzymes

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During the enzymatic hydrolysis of lignocellulosic biomass mixtures of enzymes are used: cellobiohydrolases, endoglucanases, oxidoreductases and other accessory enzymes. One of the methods for assembling enzymatic cocktails is to use a number of microorganisms producing the enzymes of interest, especially fungi. The fungus *Trichoderma reesei* is widely known to produce large amounts of cellulases (essentially cellobiohydrolases), but is relatively poor in other enzymes necessary for the conversion of biomass. Thus, other fungi are being investigated for their ability to produce more diverse and efficient cocktails or complement the enzymes of *T. reesei*. The aim of this study is evaluate the enzyme cocktails for enzymatic hydrolysis of sugarcane bagasse. In this study, two fungal species, *Cochliobolus sp*. and *Trichoderma reesei*, were selected for their ability to produce oxidoreductases and cellulases enzymes, respectively. The fungi were grown in kirk medium with sugarcane bagasse in natura (0,5%) (*Cochliobolus sp*.) or liquid medium containing avicel 1% (*T. reesei*). Crude

cellulase of *T. reesei* and oxidoreductases of *Cochliobolus sp*. was further tested for the release of reducing sugars during the saccharification of sugarcane bagasse in natura. The saccharification of 4% sugarcane bagasse in natura with crude enzyme was found to be 3.2 g.L-1 of reducing sugars in 0.05 M citrate buffer, pH 4.5, 50 °C after 48 h. The combined use of *T. reesei* and *Cochliobolus sp*. enzymes resulted in a significant synergistic enhancement in enzymatic activity. Our data suggest that fungal enzyme from *T. reesei* and *Cochliobolus sp*. could be used to saccharification process of sugarcane bagasse.

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Excess volumes and partial molar volumes of binary mixtures of 2–methylfuran with alcohols at 298.15 K

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Ethanol and biodiesel have been thought of as the market−leading gasoline alternative due to the strong environmental appeal and to its mass production methods. However, some limitations of these biofuels as a direct substitute for petrol have inspired the development of chemical catalytic transformation for producing 2−methylfuran (MF), a promising furan−type biofuel, from carbohydrates or cellulosic biomass. MF is considered to be a better biofuel than ethanol in terms of production efficiency, energy density, handling and storage. It is particularly very attractive due to its physical and chemical properties, such as the research octane number (RON≈101) and its motor octane number (MON≈86), that are similar to those of gasoline. Additionally, MF is not soluble in water and can be used directly or blended with gasoline in motor vehicles. The MF production process involves its separation from the synthesis (hydrogenolysis) of HMF to MF over a CuRu catalyst and its separation from the extracting solvent and unreacted intermediates. Several solvents have been tested in the MF reaction and purification, among them 1−butanol, 2−butanol, 1−hexanol, methylisobutylketone and toluene. In order to explore the MF production process and its applications as a fuel or as a gasoline additive, it is necessary to characterize some thermodynamic properties. A survey of the literature shows that no densities and volumetric properties data are available for the MF and alcohols. The densities and excess molar volumes of the MF and their mixtures with alcohols are required, for instance, for relating excess enthalpy and excess Gibbs free energy values. From a practical point of view, the data are useful for the design of mixing, reaction, separation and storage equipment. In this work, we present the densities, excess molar volumes and partial molar volumes for binary mixtures of MF with 1−propanol/2−propanol/1−butanol/2−butanol at 298.15 K and atmospheric pressure over the entire range of composition. The densities were determined experimentally using a digital densimeter (Anton Paar model DMA−5000, Austria) with oscillatory U−tube, precise to ± 0.00001 $g.cm^{-3}$, previously calibrated with air and water (distilled and deionized) at known temperature. The calculated excess molar volumes were negative over the entire range of composition considered, suggesting influence of the chain carbon on the molecular interactions between MF and the alcohols in the mixtures. The excess molar volumes deviations were determined from the experimental data and fitted to a Redlich−Kister type equation satisfactorily to correlate their dependences on composition.

Hemicellulosic hydrolysate: the use of adapted *Scheffersomyces stipitis* as an alternative to prevent the action of inhibitors

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The increase in ethanol demand at national and global levels encourages the search for alternatives to maximize the production of this biofuel. The use of raw materials for ethanol production, such as the reuse of the biomass generated (bagasse and sugarcane straw) in the process itself can result in a raise of up to 40% in production. Among the main challenges related to the development of the cellulosic ethanol production technology, the presence of inhibitory compounds in lignocellulosic hydrolysates (non-desired generation due to sugars degradation) still represents a highlighted issue, since it negatively affects the microbial metabolism and, consequently, ethanol formation. Among these constituents, organic acids, such as acetic acid, are generated when the hemicelluloses structure is degraded. Its inhibition is related to the accumulation of anions intracellularly, promoting cytoplasm acidification. The use of adapted yeasts during fermentation processes is a widely-used strategy for first generation ethanol, and may be a suitable action to collaborate for E2G production viability, regarding the tolerance improvement against inhibitors from hemicelullosic hydrolysates. In this work, the performance of an adapted *Scheffersomyces stipitis* (accomplished through cellular recycles batch fermentations containing non-detoxified hemicellulosic hydrolysate as carbon source) was compared to the wild-type strain using increasing acetic acid concentrations (from 0.2 to 3.5 $g.L^{-1}$) added to xylose-rich synthetic media. The results revealed that the adapted strain showed better performance than the wild-type, considering growth, ethanol production and sugars consumption. In the presence of 3.5 $g.L^{-1}$ of acetic acid, the use of the adapted strain led to to three times higher C5 sugar consumption and 25 $g.L^{-1}$ of EtOH production, while the fermentation with the wild-type strain showed 64 $g.L^{-1}$ of residual xylose and a decrease of 80% on ethanol formation. In this way, the adaptation of *S. stipitis,* along cellular recycle batch fermentations using the hemicellulosic hydrolysate as an adaptive factor, demonstrated to be a promising strategy.

Biodiesel production by subcritical hydroesterification of macauba crude oil (*Acrocomia aculeata*)

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Macauba (Acrocomia aculeata) is a palm tree native to South America, usually cultivated in regions of the Brazilian cerrado. The oil production is around 4000 to 6000 Kg of oil ha-1, and the oil content in pulp is 40-70% composed mainly of oleic acid (C18:1) and palmitic acid (C16:0). However, this oil has high acidity and can not be used as raw material for biodiesel production by conventional method (basic transesterification), whereas it can cause secondary reactions decreasing the reactional yield. Hydroesterification is an alternative process to conventional biodiesel production methods, as it can be carried out using low quality raw materials such as crude or residual oils that have a high acidity (above 3 mg NaOH/g) and water content (0,1% w/w). The raw material represents 75% of the biodiesel production cost. Therefore, the use of low quality raw materials is essential to ensure the lowest cost of production of biofuel. In addition,

the use of this oil allows a diversification of the oil crops used for biodiesel production in Brazil and provides the Social Seal to biodiesel producers that buy this oil from family farmers. The aim of this study was to produce biodiesel by hydroesterification from macauba oil. This reaction occurs in two steps, hydrolysis of the triglyceride with water that produces FFAs and glycerol followed by esterification of FFAs with an alcohol to obtain biodiesel. The hydrolysis reactions were performed under pressurized conditions based in factorial design 23 with central point. The influence of the molar ratio (oil:water), temperature and reaction time were evaluated watching the FFAs yield. In the hydrolysis reaction, the best result (94.5% w/w of FFAs) was attained with 250°C and 2 hours. Molar ratio showed no significant influence. After evaluation of the model, the equation of the regression with R^2 of 0.9914 was obtained. In order to optimize the results, a new reaction was carried out at 263°C for one hour, obtained 96.3% (w/w) of FFAs, without increasing the energy costs of the reaction. The FFAs produced in the optimum condition were esterified with methanol and ethanol using 1.0% acid catalyst (H2SO4) at 65 ± 5°C for 1 h. The ester conversion using the methyl route was 70.0% (w/w) and for ethyl route 48.4% (w/w). The water content was 346.4 mg/Kg and 317.5 mg/kg for the methyl and ethyl ester respectively.

Production of biodiesel from soybean oil using heterogeneous and enzymatic catalysts

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The rapid depletion of non-renewable fossil fuels has accelerated the development of new environment friend energy sources. Biodiesel, known as fatty acid alkyl esters, is seen as an alternative, ecofriendly, biodegradable and renewable non-fossil fuel. Currently, the industrial process for fatty acids alkyl esters production by transesterification reaction with methanol via homogeneous catalysis in alkaline medium still predominates. This process has the disadvantages of using a non-renewable source of alcohol (methanol) and the formation of soaps, which goes into the glycerin phase, increasing the production costs and the environmental impacts. On the other hand, heterogeneous and enzymatic catalysts have shown great potential to facilitate the production of alkyl esters fatty acids, since the final products do not need undergo complex purification processes to remove catalyst residues. Therefore, this work aims to improve the process of production of fatty acids ethyl esters via heterogeneous catalysis using Novozyme 435 (immobilized Candida antarctica lipase B) and Amberlyst A26OH (anionic exchange resin). The catalytic activities between those heterogeneous catalysts will be compared under different conditions: (ethanol:oil molar ratio and% catalyst) at 60°C. Commercial soybean oil from Cargill, absolute ethanol from Merck (purity 99.9%), Amberlyst A26OH purchased from Dow Chemical and the enzyme Novozyme 435 donated by Novozymes S/A were used to perform the experiments. All the other reagents were of HPLC or analytical grade. The samples were analyzed by GC method according to the norm ASTM D6854. The results revealed that the fatty acid ethyl esters (FAEE) yield increased by increasing the amount of anionic resin and lipase. The highest FAEE yield was 89% at 5h with 6% of resin content (based on oil weight). The effect of Novozyme 435 dosage on FAEE yield increased by increasing lipase dosage, reaching 94% at 8h by using 7% of enzyme. Regarding the optimal oil:ethanol molar ratio, it was observed that the resin Amberlyst A26OH requires a higher amount of ethanol:oil (9:1) when compared to the enzyme Novozyme 435 (4:1), to reach FAEE yields of around 90%.

Improvement on enzymatic cassava bagasse treatment to obtain glucose for ethanol biosynthesis

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The search for economical bioprocesses able to hydrolyze complex carbohydrates, such as the combination of starch and lignocellulosic compounds, has been a current concern. This fact is due to the need for better use of waste as a renewable platform for the production of chemicals, food and biofuels, considering that oil and its derivatives are non-renewable, and expansion of agricultural land is limited. Furthermore, the environmental crisis has turned public attention to the use of sources of energy that are environmentally correct and renewable, such as bioethanol. Currently, the majority of the cassava bagasse is discarded as waste, however, this residue can be considered as an excellent source of fermentation for higher-value biochemicals. A fast and cost-effective bioprocess able to hydrolyzing cassava bagasse (CB) in fermentable sugars to be used for ethanol production was developed. The enzymes were provided from both commercial source (Cellulase complex - NS-22086) and microorganism cultivation (fungal amylase produced by solid state fermentation). Three-level full factorial design (3^3) was performed to optimize the enzymatic proportion varying from 0 to 30 enzyme units per gram of substrate. The response was validated through hydrolysis assays in order to get high yields of CB hydrolysis using the minimum proportion of enzymes. The optimized enzyme mixture, comprised of fungal amylase and cellulase, converted 61.67% of cassava bagasse into reducing sugars. When the proportion of amylase and cellulase were reduced to a half and 3 times, respectively, compared to the original amount, the yield of conversion was 51.79%, showing that it is possible to significantly reduce the proportion of enzyme used, maintaining yield levels higher than those reported in the literature under similar conditions. Modifications on the surface of CB samples caused by the enzymatic treatment were observed using scanning electron microscopy. In order to scale up the process CB was hydrolysate in a bioreactor producing more than 60 g/L reducing sugar, corresponding to 47% yield. The concentrated hydrolysate up to 88.07 g/L glucose, determined by HPLC, was subsequently applied in fed batch process producing 35.25 ± 0.75 g/L ethanol with 78.33% efficiency and 1.47 g/L.h productivity. Therefore, the proposed bioprocess would have the potential to produce 238.4 liters of additional ethanol per ton of dry cassava bagasse in a total period of 48 h for a single step hydrolysis and alcoholic fermentation. Thus, the approach of using different enzymes for biomass hydrolysis using simplified and efficient strategies can contribute to glucose and ethanol production and other various applications.

Saccharification of sugarcane bagasse using the whole material obtained from fungal cultivation by solid state fermentation without enzymes extraction

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The endophytic fungus *Phomopsis stipata* SC 04 was cultivated by solid-state fermentation and the whole fermented material was used as enzymes source for saccharification of sugarcane bagasse submitted to alkaline hydrothermal pretreatment, aiming to obtain glucose for the production of second generation in a future work. The initial saccharification experiments were performed using the whole fermented material, pretreated bagasse (10% dry weight/volume), sodium citrate buffer, in 500 mL Erlenmeyer flasks, under 200 rpm, at 50ºC, for 24 hours. Glucose and xylose concentrations (4.72 ± 0.1947 and 1.08 ± 0.1260 mg/mL, respectively) were determined by High Performance Liquid Chromatography (HPLC). Afterwards, a complete factorial design 2^2 was carried out, with 4 replicates at the central point, evaluating the influence of bagasse load and Tween 80 and glucose concentration was the response evaluated. The variables time, shaking and temperature of saccharification were maintained at the level used in the initial experiments. The highest glucose concentration (5.53 mg/mL) was obtained when using bagasse at 10% and Tween 80 at 0.17%. A R^2 of 0.999 was obtained from variance analysis, and the curvature test confirmed a linearity of the model. As a next step, for curvature confirmation, 4 experiments as axial points will be performed.

Simulation of integrated first and second generation bioethanol production from sugarcane hybrids: comparison of different configurations

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In the integrated first and second generation bioethanol production, sugarcane bagasse and straw are used as fuels in the cogeneration system, and biomass surplus can be used as feedstock in the second generation ethanol production process. The first step in the second generation process is to produce fermentable sugars from lignocellulosic feedstock. This route is comprised of pretreatment, required to decrease biomass recalcitrance and improve accessibility of enzymes for the subsequent step, enzymatic hydrolysis, in which cellulose is converted to glucose. Genetic engineering and plant breeding approaches are strategies to develop more readily fermentable energy crops. To reduce biomass pretreatment costs and increase cell wall digestion efficiency, modulating lignin content could be an alternative. Pretreatment for these plants (hybrids) may be less severe and therefore, less costly than for conventional plants. Some studies have reported that alkaline-sulfite pretreatment significantly improves cellulose enzymatic hydrolysis rate and glucose yields because they remove part of the original lignin and hemicellulose from the lignocellulosic biomass. The degree of removal of each individual lignocellulose components depends on the reaction severity, but optimized processes can provide glucose yields as high as 90% after enzymatic digestion of the pretreated material. In the present work, different configurations of the second generation ethanol production process (e.g. pretreatment with different chemical loads, 24h or 72h enzymatic hydrolysis, with or without pentose fermentation) were evaluated in an integrated first and second generation ethanol production process through computer-aided simulation within the Virtual Sugarcane Biorefinery framework. Simulation data for 1G-2G-ethanol production suggest advantages of plants with characteristics found in hybrid H89 (low lignin content, high fiber and sucrose content and high field productivity). The results showed that the integrated first and second generation

process using high chemical load of alkaline-sulfite, 72h enzymatic hydrolysis and pentoses fermentation presented the highest ethanol production. 2G ethanol production using alkaline-sulfite pretreatment was responsible for an increase in ethanol production of up to 30%. Evaluation of processes parameters along with economic assessment are crucial aspects for selection of sugarcane characteristics and of 1G-2G configurations to develop a feasible biorefinery configuration.

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Production of glucose by enzymatic hydrolysis of type II wheat flour

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Currently, the interests in obtaining biomolecules from processes of low cost and biased sustainable are growing more and more in international scopes. The use of byproducts from agroindustries is an alternative to produce glucose by reusing products with low added value. The objective of this work was to obtain an expressive amount of glucose by enzymatic hydrolysis of type II wheat flour in different proportions. Type II wheat flour solution was prepared to obtain the concentrations: 15, 20 and 25% (m/v), in acetate buffer pH 4.5, 0.05 M. The substrate for enzymatic hydrolysis was gelatinized 80°C for 30 minutes. Then, 15 U/g of glycoamylase produced by the filamentous fungus Rhizopus oligosporus in solid state fermentation were added and the substrate was saccharified for 36 hours at 50°C. After this period, the hydrolysate was centrifuged at 4000 rpm for 15 min at 6°C and the released glucose was estimated by the 3,5-dinitrosalicylic acid (DNS) method for determination of yield conversion. The glucose concentrations statistically higher ($p < 0.05$) in the hydrolysate with 25% type II wheat flour. In agreement, the yield of conversion was also higher at that concentration, around 46%. Thus, 25% type II wheat flour was the best concentration tested to obtain glucose.

Extraction and application of *Eucalyptus urograndis* hemicellulose fraction as substrate for the single cell oil production by *Rhodosporidium toruloides* CCT7815

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Single cell oil (SCO) is a promising substitute for the vegetable oils as raw material for biodiesel production since it does not compete with food supplies. Also, oleaginous microorganisms can grow in a variety of substrates including agricultural residues. However, studies are still required to reduce the production costs, for example, by using low cost carbon sources for the fermentation step such as hemicellulosic hydrolysates. The objective of this work was to study the extraction of the hemicellulose of Eucalyptus urograndis (extensively cultivated in Brazil for papermaking) and to use its undetoxified hydrolyzate as carbon source for SCO production by the yeast Rhodosporidium toruloides CCT7815. The hemicellulose extraction studies were conducted in a laboratory shaking oven under different temperatures (145-160°C), solid to liquid ratios (S:L, 1:4-1:8), and reaction times (60-240 min). The extracts obtained were hydrolyzed and used for lipid production according to a 22 factorial design experiments to evaluate the effects of C/N and C/P ratios. Virtually 100% of the hemicellulose was extracted at 160°C, S:L ratio of 1:8 at 195 min but the xylose content of this extract was relatively low, 12.2 g/L. SCO production by the yeast using the undetoxified hydrolyzate was only affected by the C/N ratio. The best results–lipid concentration of 1.45 g/L

and lipid yield of 26.4%–were obtained at C/N molar ratio of 100. We expect to increase lipid production by using hydrolysate concentration or by supplementing it with raw glycerol and salts.

Heterologous expression of *Acremonium strictum* in *Saccharomyces cerevisiae* aiming to second generation bioethanol production

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The yeast *Saccharomyces cerevisiae* is the most used microorganism to produce ethanol, because it presents an excellent fermentative capacity and is tolerant to the stress generated in industrial fermentation processes. However, this yeast can't metabolize complex carbohydrates into ethanol, such as cellulose, the major component of sugarcane bagasse, making it necessary to hydrolyse the cellulose into glucose for subsequent conversion of glucose into ethanol. In this sense, an industrial strain of *S. cerevisiae* (Pedra-2) was transformed with cellulases from *Acremonim strictum*, a wild microorganism isolated from the Brazilian Biome, aiming to the production of second generation ethanol through simultaneous saccharification and fermentation process (SSF). For the heterologous expression, the vector pRS426 with uracil selection marker (URA +) was used and one vector models were assembled with simultaneous expression of the two enzymes. The enzyme activities in anaerobiose were 0.3 U/mL for endoglucanase and 0.054 U/mL for β-glucosidase, after 72 hours of fermentation. The endoglucanase activity expressed by the genetically modification *S. cerevisiae* FGY050 presented similar output at the expression of the same enzyme by the *A. strictum* fungi, however the fermentation time for expression of the enzyme decreases significantly. In the case of the β-glucosidase, the expression was 138% higher than the activity expressed by the fungus from which the cellulase genes were extracted. Regarding the fermentations, they were performed using a combination of different substrates: AVICEL® (commercial cellulose) + glucose, cellobiose + glucose andjust glucose, in anaerobic conditions at 37°C for 120 hours. The highest ethanol content (14,930 g/L and 12,945 g/L) occurred at 120 h of fermentation when glucose + cellobiose and commercial cellulose (AVICEL®) + glucose was used as carbon sources respectively. This results were 53% and 38% higher than fermentation conducted with justglucose (control).

Flash point of methyl ester binary mixtures

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The global increase necessity of energy associated with the decrease in petroleum supplies, high energy prices, the awareness about the greenhouse effect and the release of gases to atmosphere lead the researches to develop a cleaner and sustainable source of energy which is not based on fossil fuels. In this scenario, biodiesel seems to be an appropriate renewable alternative fuel to the conventional fossil fuel (diesel, gasoline etc). Biodiesel has been presenting many advantages over fossil fuel like lesser carbon monoxide emission, biodegradability, non-toxicity and it can be used in engines without significant modifications. Animal or vegetable fats in presence of a catalyst and an alcohol (methanol/ethanol usually) can produce biodiesel, a mixture of fatty acid methyl or ethyl esters (FAME or FAEE) and glycerol. Besides the potential of biodiesel their physicochemical properties are still scarce in the literature. Some of these properties are important since they influence directly in regards to the aspects as combustion, safety

and performance of the biofuel. Among these properties, the Flash Point (FP) is one that is important to be defined as the minimum temperature at which vapor pressure of the hydrocarbon is sufficient to produce the vapor needed to spontaneous ignition of the fuel with air in the presence of an external source, i.e., spark or flame. The FP can be used to determine the fire hazard of fuels that are stored or transported. Besides, some researches have been demonstrated that the FP associated with other properties can indicate the quality of the refining process and the initial material from the biodiesel was originated. The main objective of this work was the determination of FP of different mixtures formed by FAME. To perform the experiments a Miniflash FLPH (Grabner Instruments, Austria) was used with pure chemicals and their binary mixtures (methyl caprylate, methyl caprate, methyl laurate and methyl myristate). The equipment was operated according to the standard test method ASTM D6450, recommended to biofuel. The results show that the ideal model of liquid phase is adequate to describe the system and the carbon chain of methyl ester influences the FP of the mixture. It was also observed that larger carbon chain presents a higher flash point as expected.

An integrated omics approach to the characterization of genetically modified yeast for second generation ethanol production

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Brazil is one of the biggest producers of ethanol in the world, a pioneer in the ethanol industry. However, the country is already facing a major limitation imposed by the first-generation ethanol production technology, in which the sugarcane juice is converted by ethanol using industrial yeast Saccharomyces cerevisiae. Therefore, a new alternative approach has been proposed, called second generation, which is based on lignocellulosic residues of sugarcane (bagasse and straw) for ethanol production using recent methodologies for biomass deconstruction that generates soluble sugars, majority represented by glucose and xylose. One of the biggest challenges of this technology is the development of genetically modified industrial yeast that can not only produce ethanol from glucose as usual, but also from xylose that represents 15% to 45% of the lignocellulosic material. Several works have developed xylose-fermenting yeast using different exogenous genes and genetic engineering approaches, but always resulting in very low yield and productivity mainly caused by unbalanced redox potential and metabolic bottleneck. Nowadays two metabolic pathways for the consumption of pentoses are known: oxido-reductase (OXR) pathway, identified in fungi, and xylose isomerase (XI) pathway frequently found in bacteria. Using genetic engineering tools is possible to insert these two metabolic pathways into the industrial yeast in order to enable it to consume pentoses with different fermentative performances. The combination of omics data (transcriptomic, proteomic and metabolomic) and bioinformatics analysis is an essential step for a better understanding of this system. In this work we studied four genetically modified strains for OXR and XI pathways with different fermentative performance in xylose. For OXR, metabolomic data in different conditions in two carbon source (YPD: glucose medium and YPX: xylose medium) were generated by our group in association with public transcriptomic data. For XI, the transcriptomic and proteomic data were generated by our group in similar conditions to

OXR. For OXR analysis, the statistically significant metabolites and gene expression profile were integrated by the web platform IIS (Integrated Interactome System) generating metabolite-genes interaction networks and their enriched KEGGs. These enriched pathways were used to perform Petri Net simulations in YPD and YPX medium using transcriptomic data as input. In addition, the FBA (Flux Balance Analysis) simulations were carried out of the OXR strains. Both simulations were used to understand how these genetic modifications affected the ethanol production and metabolite concentrations profile. These results gave us new insights about the flux optimization on pentose phosphate pathway and unbalanced redox correction. In parallel, the data from XI pathway are being analyzed by integrating proteomic and transcriptomic data using IIS and Petri Net/FBA simulations.

Biogas from microalga: a novel computational approach for estimating biochemical methane potential and energy efficiency

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Biogas from microalgae is often claimed as a carbon-null energy route. Most invariably, downstream and upstream operations are ignored, and anaerobic digestion efficiency is the sole parameter considered, focusing on effects of the species being digested, biomass pre-treatment and operational conditions which significantly impact biochemical methane potential (BMP). Estimation of the net energy production requires mass and energy balances for the operations down and upstream to the anaerobic digester. Most simulation approaches focus exclusively on the complex metabolic description of the biodigester, subtracting the global analysis, necessary for screening species with best potential for enhanced performance and process optimization. A systems approach embodying heat integration, pre-treatment, CO2 separation to produce biomethane and its compression to dispatch through existing distribution grid benefits from the framework available in commercial process simulators. The present approach simulates the microalgae suspension as a process stream composed of model molecules (lipid, protein and carbohydrate) and water, used to investigate performance of anaerobic digesters in commercial process simulator (Aspen Hysys). A perfectly mixed adiabatic reactor is assumed, at constant temperature and pressure, with the system behaving as in equilibrium, with constant mass hold up. The set of species (the model molecules, water and digestion products) is subject to simultaneous phase and chemical equilibrium. An equation of state describes the state of matter under the given physical conditions (e.g., Peng-Robinson). The calculation consists in varying the quantity of product species in each phase to find a solution that minimizes the total Gibbs energy of the reacting system, using a general minimization algorithm available in Gibbs reactor model (Hysys library). Although kinetics effects by biocatalysis are not embodied in the modelling approach, a biodigester will eventually reach equilibrium if sized for sufficient hydraulic retention time. Hence, predictions from equilibrium conditions are the thermodynamic upper limit of BMP. The approach is amenable for screening microalgae for biodigestion applications and for preliminary evaluation of capital and operational expenditure of a biogas plant, involving upstream and downstream operations.

Alcoholchemistry, Sugarchemistry, Oil Chemistry and Biorefineries

Use of natural eutectic solvents in sugar cane straw pretreatment

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In order to make lignocellulosic biomass available for the production of a wide range of bioproducts it is necessary that the lignocellulosic material goes under pretreatment in order to break the lignocellulosic matrix and expose its carbohydrates, improving the performance of the cellulolytic enzymes responsible for such saccharification. Aligned with this fact, the conversion of current biomass cannot be considered totally environmental friendly due to the use of acids or bases during its pretreatment. The concept of green solvents then emerges as an alternative to the use of these harmful substances for the pretreatment of lignocellulosic materials. In this context, this study aims to evaluate sugarcane straw pretreatment under deep eutectic solvents. For this, seven different organic acid and amino acid based deep eutectic solvents were considered. Initially the raw material was submitted to pretreatment under the same circumstances of 110°C, 1:10 biomass: solvent ratio during ninety minutes for every solvent. Then, the total polysaccharides and lignin dissolution of the liquid phases of each sample after pretreatment were measured using the spectrogram obtained for each test individually. Among the solvents, both propionic acid/proline and lactic acid/alanine presented the best lignin solubilization results, 9.76 g/L and 8.95 g/L of lignin, respectively. It was also pointed out that the other three lactic acid based solvents lignin dissolution did not differ much with the one prepared with alanine. The leading total polysaccharides dissolution was observed for oxalic acid/proline and propionic acid/proline, which presented 49.97 g/L and 33.95 g/L of total polysaccharides, respectively. For the lactic based solvents the best dissolution was achieved by lactic acid/proline solvent with 32.62 g/L. The eutectic solvents whose pretreatment allowed high dissolution of polysaccharides and lignin can be said to have caused more disruption in the lignocellulosic complex, removing both lignin and hemicellulose, thus presenting better performance. Taken together, the solvent that better interact with the straw fibers and were able to extract lignin and turn the fibers into an amorphous structure, facilitating a subsequent hydrolysis reaction of the sugarcane straw was the propionic acid/ proline deep eutectic solvent.

Sucrose hydrolysis has been completely abolished in *Saccharomyces cerevisiae* using a single CRISPR/Cas9 transformation step

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Sucrose is an inexpensive substrate for industrial fermentation since it is abundant and readily available. Additionally, use of sucrose for fuel and chemicals' production does not represent a threat to food prices as demonstrated by the Brazilian ethanol industry. Although studies on sucrose fermentation by *Saccharomyces cerevisiae* are not a new topic, a platform strain unable to consume sucrose has never been constructed. Such strain would find diverse applications both in academia (e.g. characterization of plant sucrose transporters)

and in industry (e.g. to increase product yields). In this study, deletion of the transporter genes (*MAL11, MAL21, MAL31, MPH2, MPH3*) were sufficient to abolish sucrose uptake. However, deletion of genes coding for sucrose-hydrolysing enzymes (*SUC2, MAL12, MAL22, MAL32*) was not enough to eliminate sucrose hydrolysis and the resulting strain could still grow on sucrose-based medium with a specific growth rate of 0.08 h^{-1}. After 70 sequential batch cultivations, three evolved strains were isolated and characterized. The growth rate after evolution increased to 0.25 h^{-1} accompanied by a 3-fold increase of sucrose and isomaltose hydrolysis and 3- to 5-fold upregulation of two isomaltase-coding genes (*IMA1 and IMA5*). An innovative single step CRISPR/Cas9 transformation (where one targeting sequence was chosen for the simultaneous deletion of 5 *IMA* genes) was carried out to eliminate the entire *IMA* gene family. The resulting strain could no longer grow on sucrose medium and no sucrose hydrolysis was detected. This strain is a new platform for studying and engineering sucrose metabolism in *S. cerevisiae*.

Mathematical modeling of a packed bed column for a vegetable oil extraction with biosolvent

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The vegetable oil extraction processes from the Brazilian biodiversity are exclusively done by mechanical press method, which produce marcs with significant lipid contents. The extraction process can also include the use of solvents, with hexane, a petrochemical product, being the most commonly used. Their substitution by biosolvents, such as ethanol, is an opportunity for the industrial sector. The design of this new process can be developed with the help of mathematical modeling. This study presents a new mathematical model for vegetable oil solvent extraction in a packed bed column with porous and nonporous particles. The mathematical model is based on three differential equations that describe the oil concentration, in a position and time function, in three regions of the column: I) solid, ii) particle pore (stagnant liquid) and iii) bulk (convective liquid). These equations were numerically solved using the finite difference method with a mobile control volume for the bulk region. This form establishes a direct relation between the discretizations in space and time, proportional to the velocity of miscela flow, which results in a good mathematical stability and allows the numerical validation in the global mass balance. Moreover, the proposed mathematical model was evaluated with experimental data of soybean (porous) oil extraction with hexane and Brazil nut (nonpourous) oil extraction with ethanol in a packed bed. The average deviation between calculated and experimental data was 8% and an error in the global mass balance less than 0.3%. These results demonstrate the coherence and the consistency in the extraction phenomenon for different materials and solvents.

Influence of the support polarity for immobilizing *Rhizopus oryzae* lipase to be used in a packed bed reactor to yield isoamyl laurate by esterification of fusel oil with lauric acid under continuous runs

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Fusel oil, the less volatile fraction obtained during the distillation process of ethanol fuel, is composed of a mixture of primarily and

secondary alcohols and can be considered a potential low cost raw material for the synthesis of esters. Thus, the present work aimed to synthetize emoliente esters by direct enzymatic esterification of fusel oil with medium-chain carboxylic acid (C12:0) using lipase from *Rhizopus oryzae,* previously selected as suitable to mediate synthesis of secondary esters. The experiments were carried out in packed bed reactor (D = 15 mm, L = 55 mm and V = 10 mL) running on continuous basis using feeding medium made up of fusel oil and lauric acid molar ratio of 1:1.5 (0.40 M/0.60 M) in the presence of isooctane. The water content in the substrate was maintained at initial values less than 500 ppm by applying dehydration techniques using molecular sieves. The lipase was immobilized on different matrixes having different polarities: homemade hybrid silica matrix hydroxyethyl cellulose (silica-HEC) and HP Diaion resin-20 (Sigma-Aldrich) aiming at determining the behavior of water formed as by-product in the catalytic bed. The immobilized derivatives showed similar hydrolytic activities of 3500 ± 120 U/g and water content lower than 4%. The loading of the lipase immobilized was set at 80% of the catalytic bed and runs were carried out at 45°C using in a suitable flow rate to give a fixed space-time of 3.5 h. The progress of the esterification was monitored by withdrawal samples for determining the levels of both starting materials consumed and product formed (mainly isoamyl laurate). For both experiments, the state steady was attained with less than 2 space times (6 h) and the best reactor performance was found in the system operated with the lipase immobilized on resin Diaion HP-20, which allowed to achieve an average productivity of 690 ± 47 µmol/g/mim and biocatalyst half-life at around 285 h. On the other hand, the system operated with the immobilized derivative on silica-HEC the half-life time was estimated in 173 h and the average productivity was 277.8 ± 19.3 µmol/g.min. These results can be credited to the higher affinity of the hybrid matrix for the water formed as by-product, modifying the substrate interaction with the essential water around the enzyme limiting the substrate migration to the solid phase and thereby reducing the enzyme activity. Regardless of what mechanism involved, our results demonstrated the continuous bioreactor model developed in this study was more effective than other systems previously reported and highlighted the importance of taking into account a rigid control of water in the whole process, including the support polarity used as a matrix for immobilizing the selected lipase.

Microbial fuel cell applied for vinasse treatment

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Vinasse is a by-product of the sugar-ethanol industry produced in large quantities. This by-product possesses high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), and other nutrients in its composition. Since vinasse increases the temperature of the receiving water body and reduces dissolved oxygen, its direct discharge in rivers and lakes causes serious pollution problems. Due to these characteristics, by-product has been considered an environmental challenge to treat. Microbial fuel cells (MFCs) are a promising approach to apply for this type of residue. The advantages of this technology include non-pollution, high energy efficiency, mild operating conditions, strong biocompatibility and a great application potential in various areas, which have received a great deal of attention from scientists. In this context, our study evaluated the performance of different two-chambered MFC prototypes operated with variable distance between

electrodes-Nafion membrane and inoculum concentration applied for vinasse treatment. The performance of a developed MFC resulted in a maximum current density of 1200 mA m−2 and power density of 800 mW m−2 in a period of 61 days. MFC performed a chemical oxygen demand (COD) removal in a rate ranging from 51 to 60%. Taking our preliminary results into consideration, we concluded that the MFC technology presents itself as highly promising for the treatment of vinasse. However, further studies on the configuration and operation of the MFC are required for a higher number of cycles. In addition, microbial community that form the biofilm in the anodic chamber should be identified.

Economic competitiveness of lignocellulosic sugars production in biorefineries annexed to kraft pulp mills

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Following the global trend of transformation of the forest sector, pulp producers in Brazil are interested in expanding their business to the bio-economy. Entry points are varied and among several process-product options that can be integrated to Kraft pulp mills, those based on the sugars platform are promising. In this platform, physicochemical and enzymatic treatments convert biomass to sugars and lignin. The sugars are used to produce chemicals and fuels and the lignin can be used for energy purposes or converted into products. However, direct competition against the sugarcane industry worries investors. In fact, although the integration to a pulp mill may offer economic gains, other factors can limit the economic attractiveness of producing lignocellulosic sugars, such as biomass type and price, costs of the pretreatment technology, and sugar yields. As such, the main question addressed in this research is whether biorefineries annexed to pulp mills can offer affordable lignocellulosic sugars for fuels and chemical production while keeping the investment attractive. Also important is whether competitiveness can be improved by sourcing biomasses other than the conventional eucalyptus wood such as the short rotation eucalyptus (energy forest) or even energy crops. To answer these questions, we developed conceptual designs of lignocellulosic sugars plants annexed to a eucalyptus kraft pulp mill considering three types of biomass (conventional eucalyptus wood, energy forest, and energy cane) and pretreatment technology (steam explosion and organosolv). Processing capacity of the biorefinery is 1000 dry ton biomass per day and the economic criterion is the minimum selling price (MSP) of glucose that ensures an Internal Rate of Return (IRR) of 10%. Xylose price, sold separately, was assumed to be 80% of the MSP of glucose. Energy and mass balances of a kraft pulp mill were modeled in Excel spreadsheets based on process data from an existing modern mill located in Brazil. Balances of the biorefinery options were calculated using conversion and consumption factors available in the literature and lignin is used for energy generation. The option that combines energy cane with steam explosion offered the best MSP of glucose (200 USD/ton against 250-380 USD/ton for the other options), which is similar to the 2014-2016 average price of table sugar in Brazil (216 USD/ton). Although extraneous to pulp producers, energy cane price is very competitive (39 USD/dry ton against 52-56 USD/dry ton eucalyptus or forest energy) and this highly productive crop also contains sugars in its juice. Equipment-wise, steam explosion demands lower capital investment (25 MMUSD against 64 MMUSD of the organosolv technology) and reduced power consumption (0.3 MW against 8.1 MW). The organosolv technology can only be competitive if organosolv lignin is marketed at a price above 350-480 USD/ton, depending on the type of biomass.

Synthesis of bio-lubricant by transesterification of palm kernel oil with isoamyl alcohol: batch and continuous processes

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Conventional petroleum-derived mineral lubricants are poorly biodegradable and contain toxic aromatic hydrocarbons and sulfur. Annually, millions of tons of engine, hydraulic and industrial oil are released into the environment all over the world. Substitutes for lubricants of fossil origin are known as bio-lubricants and are obtained from vegetable oils. This study proposes the production of lubricant esters using palm kernel oil and larger chain alcohol, like isoamyl alcohol under batch and continuous processes. Microbial lipase from Burkholderia cepacia immobilized on epoxy matrix silica-hidroxyethylcellullose having an average hydrolytic activity of 1900 ± 165 $U.g^{-1}$ was used to mediate the transesterification reactions. Batch process was carried in a jacketed cylindrical glass reactor (volume=70 mL) containing 40 g of reaction medium at molar ratio of 1:4 and 1:6 and biocatalyst (500 units of activity per gram of oil), temperature of 45°C to 72 h of reaction with magnetic stirring of 150 rpm. For continuous reactions, a jacketed glass column (14 mm internal diameter, 210 mm length, and 32.3 cm^3 volume) was running on substrate containing palm kernel oil-to-isoamyl alcohol at molar ratio of 1:4 and 1:6. The column was packed with 20 g of immobilized lipase (40.000 $U.g^{-1}$) and the feeding medium pumped through the fixed bed at flow rates from 2.1, 2.6 and 3.5 $mL.h^{-1}$ at 45°C. Flow rates were maintained until a steady state was observed as indicated by at least five consecutive samples in the reactor outlet, and multiple steady states were obtained by adjusting the space time (10, 8, 6 and 4h). Concentrations of isoamyl esters were monitored by gas chromatography and biodiesel samples were purified by successive washes with water and subsequent rotary-evaporation, further analyzed to determine viscosity, density and residual monoacylglycerols (MG) and diacylglycerols (DG) by high performance liquid chromatography. For both process the best oil to alcohol molar ratio was found to be 1:4. High formation of isoamyl esters (99 wt%) was attained in 48 h reaction and this result was corroborated with the low values of viscosity (4.03 $mm^2.s^{-1}$) of the purified products which were between six and eight times lower than its lipid precursor (palm kernel oil viscosity = 30 $mm^2.s^{-1}$). In addition, low levels of monoacylglycerols (MG=0.4 wt%) and absence of diacylglycerols (DG) were observed. Under continuous runs at space-time of 8 h, similar biodiesel properties (ester= 98 wt. %, viscosity=4.93 $mm^2.s^{-1}$ and MG=1.07wt %) were found in relation to batch runs though attained much higher productivity ($4.22e^{-6}$ mol $ester.min^{-1}.gcat^{-1}$) as expected when comparison is made between batch ($1.04\ e^{-7}$ mol $ester.min^{-1}.gcat^{-1}$) and continuous runs. In addition, the biocatalyst was found to be active during the whole process revealed half-life time (t 1/2) of about 43 days.

Techno-economic analysis of industrial enzyme production using *E. coli*: the recombinant β-glucosidase case

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The enzymatic conversion of lignocellulosic biomass into fermentable sugars is a promising approach for producing renewable fuels and chemicals. However, the cost and efficiency of the fungal enzyme cocktails that are normally

employed in these processes remain a significant bottleneck for manufacturing low value-added products from biomass. A potential route to increase hydrolysis yields and thereby reduce the hydrolysis costs would be to supplement the fungal enzymes with their lacking enzymatic activities, such as β-glucosidase. In this context, we present a techno-economic analysis of the production of a model industrial low-cost enzyme, based on a fed-batch process using recombinant E. coli. Simulating a base scenario for a β-glucosidase demand in a hypothetical 2G ethanol plant in Brazil, the production cost of the enzyme was calculated at 316 US$/kg of protein, owing primarily to the facility-dependent cost (45%), the cost of consumables (23%) and the cost of raw materials (25%). For the last, the carbon source (12%) and inducer molecule (10%) were the main culprits. In terms of process sections, it was found that the seed train, the main fermentation and the downstream section all contributed significantly to the enzyme cost, accounting for 11%, 43% and 46% of the cost, respectively. The cost was also shown to decrease steeply with the production scale, going from 457 US$/kg for a 25 m3 fermenter to 316 $/kg for a 100 m3 fermenter. The results finally indicated that low volumetric productivity and downstream processing costs are the main concerns regarding the development of promising strategies for producing recombinant enzymes at a low cost using recombinant *E. coli*.

Engineering xylose metabolism for production of polyhydroxybutyrate in the non-model bacterium *Burkholderia sacchari*

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Burkholderia sacchari has been proposed as a microbial platform to generate biopolymers from pentoses, obtained from hemicellulosic sugarcane bagasse hydrolysates, thus allowing introducing new added-value products into biorefineries. Despite its natural ability to grow and accumulate high-value molecules using xylose, arabinose and other renewable carbon sources, two main factors must be improved to establish *Burkholderia sacchari* as a chassis for bioproducts production at industrial scale: the lack of molecular tools available to engineer this organism, and the inherently slow growth rate and accumulation of the biodegradable polyester poly-3-hydroxybutyrate [P(3HB)] using xylose. In this work, we addressed both. First, seeking to develop basic tools for synthetic biology and metabolic engineering in *B. sacchari*, we adapted a set of BglBrick plasmids using pBAD and placUV5 promoters and showed tunable protein expression by measuring red fluorescent protein (RFP) fluorescence under different inducer concentrations. Both promoters were able to efficiently drive RFP expression, showing a maximum induction of 65 and 2.5 fold respectively compared to uninduced control plasmids. We then used these validated plasmids to overexpress xylose transport, metabolism and regulation proteins in *B. sacchari*. Individual effects on growth rate and production of P(3HB) were evaluated. Xylose transporters overexpression did not improve growth rate on xylose, whereas increased expression of xylose activated regulator *xylR* did enhanced growth rate, achieving a growth rate of 0.25 h^{-1}, polymer yield of 0.39 $g \cdot g^{-1}$ and a percentage of P(3HB) accumulated up to 71% of cell dry weight. These values are the highest P(3HB) yield reported from xylose in *B. sacchari*. The data presented in this work highlight the importance of tuning gene expression levels to achieve efficient xylose utilization in *B. sacchari*.

Identification and expression of sugarcane culm-specific expansin genes

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Sugarcane (Saccharum spp.) is one of the main crops grown worldwide. The increasing energy demand requires an abundant and efficient source of raw materials to be supplied. Lignocellulosic biomass is one of the most promising raw material for ethanol industry. The production of ethanol from lignocellulosic biomass or second generation ethanol (2G) has been considered as part of the solution for the transition from fossil fuels to renewable energy sources in the medium and long-term. However, the efficient use of lignocellulosic biomass requires the optimization of several steps. Pretreatment is a pivotal step involved in the production of bioethanol from lignocellulosic biomass, reducing cellulose crystallinity, increasing the porosity of the biomass, thus allowing a better action of the enzymes in the conversion to fermentable sugars. Currently, it has been noted that several methods of delignification of lignocellulosic material and amorphogenesis increase the rate of enzymatic saccharification. Some genes coding for accessory proteins are required to improve the activity of fungal enzymes complementing the enzymatic cocktail to break down the carbohydrates present in the plant cell wall. Expansins refer to a family of closely related nonenzymatic proteins found in the plant cell wall are involved in the cell wall loosening. Such accessory proteins are present in all plants and in some microbial organisms. The addition of expansins in the enzymatic cocktail could drastically improve the saccharification process by weakening the existing hydrogen bonds between polysaccharides under acidic pH. In this study, seven different expansins genes were selected with expression restricted to culm tissue. The expression level of the selected expansins genes was quantified in different parts of asses and leaves in sheets +1. Six segments of stem were collected and the leaf +1 of plants of sugarcane of 8 months. Total RNA was isolated from each part and RT-qPCR analysis was performed. The RT-qPCR analysis of the seven selected expansins, three (one alpha and two beta-expansins) showed a significant high-level expression in the third upper segment of internode 1 (TSI1) and no expression was observed in leaves. The three expansins were cloned into the expression vector Komagataella (Pichia) pastoris and the proteins produced will be used in enzymatic cocktails to evaluate the resulting saccharification efficiency.

Hydrogenation of fumaric acid to 1,4-butanodiol in supercritical CO_2

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Hydrogenation is one of the most important processes in industry namely on energy and pharmaceutical industries. The use of supercritical carbon dioxide (scCO2) as reaction medium for hydrogenation can improve not only the reaction rates but also the selectivity of the process. Moreover, the use of scCO2 also improves the safety of the process and large-scale hydrogenation processes can be carried either in mono or biphasic approaches. Here we report a highly selective process for the hydrogenation of fumaric acid towards 1,4-butanodiol in supercritical carbon dioxide (scCO2) using a ruthenium catalyst (MNP@SiO2NH2@Ru). The reaction has taken place in a variable-volume high pressure cell. In this reactor, a biphasic system was being established between water and gas (CO2+H2O). The reaction was followed through sampling

from the gas phase. In the present work, it has been studied the impact of temperature pressure and hydrogen concentration on conversion of fumaric acid to 1,4-butanodiol. The results show that that the conversion of fumaric to succinic acid was complete and the conversion towards 1,4-butanodiol was 92%. These results show a high selectivity of the process for 1,4-butanodiol. In fact, as far as our knowledge goes this is the first time that this reaction has been described in scCO2. The higher conversion was obtained at 260 bar of CO2 and 18 bar of H2 and 40°C.

Production of N-methylated amino acids with *Corynebacterium glutamicum*

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N-methylated amino acids are present in diverse biological molecules in bacteria, archaea and eukaryotes. They have diverse cellular functions, e.g. as parts of complex molecular structures of microbial peptides or of nucleic acid binding proteins involved in gene regulation and gene expression. There is an increasing pharmacological interest in N-methylated compounds due to their altered affinity and selectivity, increased membrane permeability or stability of peptide drugs. Cyclosporine A for treating Alzheimer disease, the antibiotic actinomycin D and the food flavoring theanine which naturally occurs in tea leaves are prominent examples of bioactive molecules containing N-methylated amino acids. N-methylated amino acids can be synthesized chemically e.g. by nucleophilic methylation of α-bromo-amino acids or N-methylation by alkylation. Based on the established million-ton-scale sustainable production of amino acids by fermentation primarily using the GRAS organism *Corynebacterium glutamicum*, we describe here metabolic engineering for the fermentative production of N-methylalanine by *C. glutamicum*. A *C. glutamicum* strain shown to produce up to 18 g L-1 pyruvate in flask cultures was engineered for conversion of pyruvate to N-methylalanine using heterologous reductase when monomethylamine was added to the medium. After optimization of the fermentation parameters in flask cultures, about 9 g L-1 N-methylalanine accumulated within 48h, which is the highest titer for fermentative production of an N-methylated amino acid. Moreover, production of N-methylalanine using alternative carbon sources such as pentose sugars present in lignocellulosics was shown. Moreover, the strategy was applied to the production of additional N-methylated amino acids.

In silico analysis of derivatives of xylonic acid in *Escherichia coli*: development of a renewable biotechnological platform

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Renewable feedstocks are not as widely used for the production of chemicals in industrial processes as petrochemical feedstocks mainly due to a higher production cost. Xylose, the most abundant sugar at the fraction known as hemicellulose, has attracted attention among the renewable feedstocks since it is a residue of the agroindustrial business. In this context, studies that seek for adding value into xylose are gaining importance and are supporting the development of renewable technologies. An effective way to perform this would be generating diverse value-added chemicals from a single building block produced from xylose by a fermenting microorganism, resulting in a

renewable biotechnological platform. Actually, the concept of microbial renewable platform arises as an answer to the development of versatile production and competitive processes, where Metabolic Engineering combine the efficient use of renewable industrial residues and the prediction of cells behavior. In the present study, the goal was to design a biotechnological renewable platform from xylose and to perform the *in silico* analysis of a selected building block and one of its derivatives productions. *Escherichia coli* was the bacteria selected as the *in silico* host and the model *E. coli* core was used for modelling. After performing yield comparison and technological prospection, xylonic acid was shown as the most interesting building block among the options analyzed, since it presented the greatest yield and the fewer publications, which shows potential and need for technological development. Analyzing altogether compounds metabolic pathways, market and applications, the main derivatives for the platform were chosen as: 1,4-butanediol, 1,2,4-butanetriol, glycolic acid, lactic acid, malic acid and succinic acid. Then, the production of xylonic acid and 1,2,4-butanetriol were simulated at COBRApy (Constraint-Based Reconstruction Analysis for Python) with FBA (Flux Balance Analysis) method, resulting in the identification of some key points for deeper investigation: i) xylose transport across cell membrane, ii) redox balance and behavior of the NAD transhydrogenase enzyme in *E. coli*, iii) carbon flux from the substrate to metabolism products and iv) cellular respiration. In order to maximize production yields, it was also proposed the combination of some pathways that would lead to the coproduction of derivatives of the platform using redox-balanced routes. Every option included 1,4-butanediol as one of the coproducts since the pathway for its production is the only one in our platform that consumes cofactors. Therefore, this study identified xylonic acid as an interesting building block for the improved use of xylose, and it was suggested a production platform for some commercially interesting products. It was possible to map some key points to be analyzed in this metabolic pathway in order to develop an optimized platform, demonstrating the viability of FBA and the relevance of Metabolic Engineering.

Production of 5’-GMP by autolysis of residual yeast

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Currently, yeast is an underutilized waste product of brewing and ethanol industry. Moreover, the use of this biomass can be an economical source for the production of 5'-ribonucleotides such as guanosine monophosphate (GMP), which is high added value by product and have wide applications in food industry. 5’-GMP can be formed spontaneously during autolysis and in the form of its salts, as disodium guanylate are food additives used as flavor enhancers to provide the umami taste. Faced with these issues, this work aims to evaluate the influence of stirring on the natural formation of 5’-GMP during autolysis of residual yeast biomass from brewery industry. For this, evaluation of the autolysis with and without stirring was made aiming to observe the influence on 5’GMP yields. A cell suspension constituted by 15% (w/v) of *Saccharomyces cerevisiae* as by-product from Cervejaria Malta (Assis–SP) was submitted to autolysis with addition of NaCl 9.8% (w/w) under controlled conditions of temperature (55.2°C), pH (5.1) and stirring (300rpm) for 24 hours. The control test was done under the same conditions, however without agitation. After cell lysis, the 5'-GMP content (ppm) on yeast extract was determined by high performance liquid chromatography (HPLC) at 260 nm, flow rate of 1 mL/min, 45°C, using as the mobile phase a buffer solution of KH2PO4 - Na2HPO4 20 mM and pH 7. The total

cell RNA was determined according to Herbert (1971) and GMP content in RNA of yeast cells was calculated supposed that the four kinds of bases (guanine, adenine, uracil and cytosine) are contained equally. The yield of 5'-GMP forming ratio (GMP contained in yeast extract/GMP contained in the original yeast cells ratio in percentages) were 45.56% for autolysis with agitation and 42.57% for autolysis without agitation. No significant difference was observed ($p>0.05$), which means that agitation does not influence the formation of 5'-GMP. According with Zhao (2005), the rate of RNA degradation and the composition of the breakdown products varies with temperature and pH. The results presented here contribute to a better understanding of the factors that influence the spontaneous formation of flavor during yeast cell degradation. Finally, these findings can leads to improvement in the technology of fractionation and purification of *S. cerevisiae* in more valuable products according to the concept of biorefinery, i.e., co-production of biofuels, bioenergy and marketable products from renewable biomass.

Sustainable production of the non-proteinogenic amino acids pipecolate and 5-aminovalerate

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As alternative to current fossil-based production processes bio-based production of value-added chemicals from renewable resources is desirable. *Corynebacterium glutamicum* is used for the million-ton scale production of amino acids such as L-lysine. L-Lysine is used as an additive for animal feed, but can also be considered as precursor for functional ingredients in the pharmaceutical and chemical industries. Therefore, we have metabolically engineered C. glutamicum for the production of the non-proteinogenic amino acids L-pipecolic acid (L-PA) and 5-aminovalerate (5AVA). L-PA is a precursor of immunosuppressants, peptide antibiotics and piperidine alkaloids. Metabolic engineering to convert L-lysine to L-PA involved L-lysine dehydrogenase from Silicibacter pomeroyi and endogenous pyrroline 5-carboxylate reductase and use of a L-lysine producing base strain. After abolishing L-lysine export, L-PA was produced from glucose in fed-batch cultivation to a titer of 14.4 g/L with a volumetric productivity of 0.21 g/L/h and an overall yield of 0.20 g/g. 5AVA is a C5 platform chemical for the production of dicarboxylic acids (glutarate), diols (1,5-pentanediol), and lactam monomers (δ-valerolactam) for polymer synthesis. A new metabolic pathway was designed to convert L-lysine to 5AVA in three steps. Inspired by GABA formation from putrescine, cadaverine generated from L-lysine by L-lysine decarboxylase (ldcC) from E. coli is converted to 5AVA by putrescine transaminase (patA) and γ-aminobutyraldehyde dehydrogenase (patD) from E. coli. Eliminating formation of the by-products cadaverine, N-acetylcadaverine and glutarate in a genome-streamlined L-lysine producing strain expressing ldcC, patA and patD enabled glucose-based 5AVA production to a titer of 5.1 g/L at a yield of 0.13 g/g and a volumetric productivity of 0.12 g/L/h. To enable production of L-PA and 5AVA from alternative feedstocks like glycerol, starch, glucosamine, xylose and arabinose the respective pathways for their catabolic conversion to central metabolites were embedded into L-PA or 5AVA producing *C. glutamicum* strains. Tunable synthetic metabolic switches were designed and their use for on-demand gene expression to improve L-PA and 5AVA producing strains characterized.

Protein and amino acids recovery from brewer's spent grains by different pretreatment technologies

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Protein has extensive use as ingredient in the food and feed industries. With the growing population, the global demand for protein in 2030 is expected to exceed the current production capacities. Therefore, finding alternative sources of protein is a topic of great interest nowadays to meet the future demand of this component. Residual biomass and side streams are potentially interesting sources of this ingredient. However, exploitation of these sources for obtaining proteins is still at an early stage. Brewer´s spent grains (BSG) is an agro-industrial residue rich in proteins, which correspond to approximately 18-20% of its composition in a dry weight basis. In the present study, different pretreatment strategies were evaluated with the aim of recovering protein and amino acids from BSG. Protein fractions, including amino acid, were fully recovered using a pretreatment in three sequential steps (aqueous, alkaline and dilute acid). When comparing the three stages, the greatest part of protein (80.7%) was recovered during the alkaline step, followed by the dilute acid step (10.25%). HPLC analysis revealed that the total amount of amino acid recovered by this sequential pretreatment corresponded to 0.5 g/100 g BSG. The amino acids present in BSG were also identified and quantified. Extraction using only one step dilute acid pretreatment (at different acid concentrations) was also assayed but resulted in much lower protein recovery (between 13.0% and 28.6%) and higher solubilization of the hemicellulose fraction, which is not convenient in a biorefinery perspective. The results also revealed that defatting BSG previous the extraction is not necessary to improve the protein recovery efficiency.

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Ethanol tolerance investigated using data integration from 'omics', systems biology and cell biology

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Bioethanol has been shown as an excellent alternative energy source of fossil fuels. The yeast *Saccharomyces cerevisiae* is the most used microorganism for bioethanol production. However, the ethanol concentration is one of the limiting factors for ethanol production because, at high concentrations, this compound disturbs cells and reduces the productivity. Despite many studies on this topic, the integrated view of 'omics', systems biology and cell biology approaches are far from to be explored. Thus, the selection of target genes to improve the ethanol tolerance for genetic engineering is not trivial. The upper limit to tolerate ethanol stress along 1h was defined for six different haploid strains and unsupervised learning was used to cluster each one as highest tolerant (HT) and lowest tolerant (LT). Differential expression analysis using RNA-Seq for those treatments and controls were performed. The results showed that genes involved in mitochondrial metabolism (matrix, membranes and oxidative stress), peroxisome, nucleoplasm, endoplasmic reticulum lumen, nucleoplasm, protein digestion and homeostasis are deregulated in HT strains; while DNA repair, vesicle-mediated transport, transcription regulation, replication and apoptosis are deregulated in LTs. Proteomics by mass-spectrometry reported that alcohol

dehydrogenases, aldolases, phosphor-glycerol kinase and some proteins related to translation mechanisms are affected after treatment. Protein-protein interaction networks (PPIs) were obtained for five strains and enrichment analysis reported that RNA biosynthesis, vesicle transport, translation mechanisms, energetic metabolism and protein degradation are overrepresented. Since most of the analysis converge to process that affect the cell division and viability, it was performed cell growth curves concluding that most of HT grows faster and more than LTs. Flow cytometry analysis showed that most of the HTs have lower ability to survive after treatment. Taken together, genes/proteins/systemic properties directly involved with energy and cell viability are affected by treatments. In fact, HTs tolerate more ethanol not due to have lesser apoptosis rate but due to having higher cell division rate compared to the LTs. Ethanol tolerance is a multifactorial phenotype, and deeper systems analysis has to be performed for better clarify the phenomena.

Enzymatic production of nanocellulose in a stirred-tank bioreactor

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The biorefinery concept has been identified as one of the most promising routes to build the new industries of the future, as it allows the use of renewable biomass such as lignocellulosic residues for the production of biofuels including cellulosic ethanol, together with other chemicals and bioproducts. Among these bioproducts, high added-value materials such as nanocellulose can significantly contribute to the economic viability of the overall process. However, the development of a bioprocess for large-scale production of this material in a green and sustainable way still remains a challenge. Here, the estimation of scale-up parameters for the production of nanocellulose via the enzymatic hydrolysis was carried out using eucalyptus cellulose pulp as feedstock. For the enzymatic hydrolysis step, experimental central composite design (CCD) methodology was used as a tool to evaluate the effects of solids loading (SL) and enzymatic loading (EL) on glucose release and cellulose conversion. Validation of the statistical model was performed at SL of 20% and EL of 10 mg protein/g, which was defined by the desirability function as the optimum condition. For all the CCD conditions, the residual solids presented cellulose nanofiber (CNF) characteristics. Enzymatic hydrolysis experiments were made in a stirred tank reactor (5L) using a SL of 10 and 15% and EL of 5 and 10 mg/g cellulose, in order to obtain the parameters required to scale-up. The impeller used was the up-pumping and down-pumping Elephants Ears. The rotation of 470 rpm, defined by performing mixing time test, was used to evaluate the power consumption and apparent viscosity during of hydrolysis reaction. The residual solids of the hydrolysis at 5L scale presented nanocelulose with similar characteristics to the smaller scale (100 mL). In conclusion, our findings showed that the processes for nanocellulose production using exclusively the biochemical route is very promising and could contribute to implementation of future biorefineries.

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Impact of acid-tolerant strains utilization on lactic acid production process

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Lactic acid is a biotechnological product of growing interest and demand due to its versatility as a renewable chemical platform. It

can be used in the production of other useful chemicals, such as the biodegradable polymer PLA (polylactic acid). However, large scale production is hampered due to process bottlenecks such as low productivity and pH drop during fermentation. Today, in order to circumvent product inhibition and maintain pH during fermentation at the optimal value, neutralizing agents are applied, keeping fermentation pH well above the pKa of lactic acid. This technique requires a regeneration step in order to recover the acid from the final fermentation broth, which in turns generates a large amount of gypsum as process residue. This large generation of gypsum may not have place in the market, and therefore could represent a challenge for this process. Moreover, besides the negative economic impact, the large amount of waste represents an environmental problem. Research has been dedicated to developing acid-tolerant strains to reduce this environmental impact and simplify lactic acid downstream processing, therefore reducing production costs. Herein we evaluated different lactic acid producing microorganisms to assess the impact of fermentation pH on the downstream processing and production costs. Fermentation parameters reported on the literature were employed to simulate four scenarios using Aspen Plus® v8.6, and economical assessment was carried out based on these results. In each scenario, the downstream processing was adjusted according to the resulting fermentation broth in order to yield polymer grade lactic acid. According to results, production at low pH is possible even with higher costs associated with technology licensing, due to the requirements of less process inputs such as neutralizing agents. Low pH fermentation can decrease gypsum production in kg gypsum/kg lactic acid produced in about 45% for the same strain. Therefore, these results show that fermentation at low pH has potential to improve the economics of lactic acid production.

Vinasse purification procedures for application in food production

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The sugar and alcohol industry is directly related to the Brazilian development process. The study and development of biofuels in Brazil are dated to the beginning of the 20th century, being responsible for the Brazilian pioneer in the area of biofuels with ethanol production from sugarcane. Because of increased concern about the production of renewable and cleaner energy sources, the world's interest in the full exploitation of the production chain has been prominent in contemporary society. In the ethanol production is generated a residue which has high concentration of organic matter and, in turn, is considerable pollution potential, known as vinasse. Vinasse is generated in the distillation of ethanol after fermentation from sugarcane juice. In this process, for each liter of ethanol, approximately 10 to 15 L of vinasse is produced. This sub product is an effluent of extreme relevance both in terms of volume generated and in relation to its toxicity, characterizing a potential risk to surface water, groundwater, human health, animal and the environment as a whole. The demand for generation of this effluent increases each year resulting in severe environmental impacts and also invaluable economic losses. The present paper aimed to develop a technology of treatment of vinasse for its use in the biotechnological production of forage yeast, since its process has been carried out using cane molasses, resulting in the loss of this raw material, which can other uses of greater potential. The vinasse was concentrated,

purified by pH adjustment and adsorption on activated charcoal. The fermentative assays were developed according to an experimental statistical design to determine the effect of substrate concentration (undiluted and 4 fold diluted), pH (4.0 - 6.0) and concentration of the microorganism (0.5 - 1.5 g.L-1) In the production of forage yeast in vinasse. After treatment of the vinasse by changing pH up to 8.0 and with activated charcoal adsorption (2.5%) were obtained a cell growth of 46.85 g.L-1, biomass volumetric productivity of 0.58 g.L-1 h-1 and cell substrate yield factor 1.78 g.L-1 under conditions of higher substrate concentration, pH and cell concentration.

Engineering of *Corynebacterium glutamicum* for the coproduction of astaxanthin and lysine

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The GRAS organism *Corynebacterium glutamicum* is a natural L-glutamate producer and used for the fermentation of a wide range of products. Especially the amino acid L-lysine is produced in a million-ton scale and sold as a spray-dried fermenter product as feed additive. *C. glutamicum* is naturally pigmented due to the C50 carotenoid decaprenoxanthin. Over the last years carotenogenesis has been characterized in more detail, which enabled metabolic engineering of strains for the production of non-native industrial relevant carotenoids such as astaxanthin. This carotenoid can be used as colorant for food and beverages, as high-value additive in aquaculture for the coloration of salmon, or as feed additive for poultry breeding. In order to combine these positive traits as L-lysine and carotenoid producer with industrial relevance, coproduction *by C. glutamicum* was established. In a first attempt a genome-reduced strain with a deletion of the *crtR* gene encoding the repressor of the carotenoid biosynthesis was capable to coproduce decaprenoxanthin and L-glutamate. Similarly, coproduction of astaxanthin and glutamate was achieved using an engineered astaxanthin producing strain. To enable coproduction of lysine and carotenoids, the L-lysine overproducing strain GRLys1Δ*sugR*Δ*ldhA* with optimized glucose uptake and utilization was used as base strain and was further engineered for carotenoid overproduction. A collection of strains for coproduction of lysine with either decaprenoxanthin, lycopene, β-carotene (by expressing *crtY* from *Pantoea ananatis*), zeaxanthin, canthaxanthin or astaxanthin (by expressing *crtW* and/or *crtZ* from *Fulvimarina pelagi*) was generated. It was possible to produce L-lysine to titers of 2.0 – 2.8 g/L as secreted product in addition to carotenoids that were found in the cell fractions. Moreover, coproduction of L-lysine and astaxanthin was characterized in fed-batch fermentation. Strain ASTALYS produced 50 g/L L-lysine and 10 mg/L astaxanthin with volumetric productivities of 0.44 g/L/h and 0.01 mg/L/h, respectively. Since two compounds can be produced simultaneously by a single bacterial strain, coproduction of amino acids and astaxanthin offers a new strategy in the feed additive industry.

Conversion of lignocellulosic biomass to high value-added products: sustainable production of biosurfactants from sugarcane bagasse

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Biosurfactants (BS) are versatile molecules with tensoactive, emulsifying, antimicrobial and antitumoral properties which have many industrial applications. In a near future, these compounds may substitute the currently used synthetic surfactants, mainly considering BS are

environmentally friendly and have high biodegradability, low toxicity, superior physicochemical properties and possibility of production from renewable sources. In this context, BS can be considered as potential sustainable products for future lignocellulosic biorefineries. The present study showed different ways of producing them by yeasts using sugarcane bagasse as raw material. Fermentations were carried out to evaluate BS production using hemicellulosic hydrolysate and sugarcane bagasse as carbon sources in submerged fermentation (SF) and solid state fermentation (SSF). The yeast used in this work was Cutaneotrichosporon mucoides UFMG-CM-Y6148 that, in preliminary tests, was proved as able to assimilate xylose and to produce cellulases and xylanases. Fermentations were performed in Erlenmeyer flasks using about 1.0 x 107 total cells/ml as inoculum. For SF assays, hemicellulosic hydrolysate (40 g/L xylose) was used supplemented with mineral nutrient solution and the flasks were incubated in shaker at 200 rpm and 30°C for 68 h. For SSF assays, 2 g/L of sugarcane bagasse was put in flasks, supplemented with mineral nutrient solution and inoculated with the yeasts, resulting in a mixture with 85% relative humidity. In this case, flasks were incubated in a static microbiological oven at 30°C for 240 h, followed by extraction with 10 mL of distilled water. SF broth and SSF extract were centrifuged for removal of the cells and bagasse and the tensoactive and emulsifying properties of the produced biosurfactant were analyzed. Also, in the supernatants, acid precipitation (addition of 0.1 mol/L HCl to pH 2.0) was performed and the mixture was kept overnight at 4°C, using the obtained precipitate for analysis of the BS chemical composition. The BS produced in SF and SSF resulted in surface tension ranging from 45 to 55 mN/m and emulsifying index in kerosene between 52 and 70%, with stable emulsions by 168 h. Microscopy analysis of emulsions showed presence of small globules that promoted high stability. Chemical analysis of the produced BS showed that they are glycolipids. The results obtained in this study indicate a great potential for a sustainable production of BS using lignocellulosic biomass as raw material in a context of biorefineries. Moreover, due to interesting properties the produced BS, it can be applied in a large variety of products.

Multi-response optimization of the bio-refining of rice husk in the production of levulinic acid

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The production of levulinic acid (LA) from rice husk (RH) via the cellulose/furan pathway was studied and optimized using a desirability function approach. The RH was bio-refined using three steps of a consecutive fractionation technique: (1) acid pretreatment, (2) alkaline pretreatment and (3) catalytic depolymerization of cellulose to LA. For the third step, the influences of the H2SO4-acid concentration (*Ad*), reaction temperature (*Td*) and reaction time (*td*) on the LA concentration (*CLA*), selectivity (*SLA*) and yield percentage based on the theoretical value (*Yt,LA*), were investigated. Higher *Ad*, *Td* and *td* values did not enhance the *CLA*, *SLA* and *Yt,LA* values, which means they exhibited a simultaneous concave function. Optimal values for *CLA* of 27 g/L, *SLA* of 62.2% and *Yt,LA* of 60.6 mol % were obtained with process conditions of *Ad* of 5.0% w/v, *Td* of 175°C and *td* of 75 min. Under these conditions, a scenario based on 100 kg of dry RH produced 11.8 kg of LA with a yield of 30.7% and 42.9 mol %, as well as 12.5 kg of xylose, 12 kg of lignin and 2.9 kg of formic acid. In addition, the potential route to LA production with the

requirement to remove the ash was highlighted in this study.

Utilization of agroindustrial sugarcane by-products for coproduction of ethanol and xylitol by *Candida guilliermondii* FTI 20037

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Several by-products are generated during different phases of the sugar-and-alcohol productive chain, such as sugarcane straw in the mechanical harvest, bagasse during the milling, and molasses during sugar crystallization. These by-products can be used on bioprocesses since are sources of several carbohydrates, such as xylose and glucose, which are the main constituents of the hemicellulosic and cellulosic fractions, respectively, of straw and bagasse; as well as sucrose, which is the main component of the molasses. Among the products that can be produced from these carbohydrates, ethanol and xylitol can be highlighted. Ethanol production, which is mainly by fermentation of sucrose or glucose, has economical and environmental drivers as a low-value biofuel used currently for transportation. Xylitol is a high-added value product with important applications in pharmaceutical and food industries, since it is a special sweetener non-cariogenic and without dependence on insulin to be metabolized, which can be produced by fermentation of xylose. Coproduction of ethanol and xylitol by ⟨i⟩Candida guilliermondii⟨/i⟩ FTI 20037, native yeast recognized by its capacity of xylose-to-xylitol bioconversion, was evaluated using mixtures of sugarcane molasses and hemicellulosic hydrolysate from bagasse and straw as fermentation media. Two proportions of molasses and hemicellulosic hydrolysate were prepared based on the concentrations of sucrose and xylose, respectively: 1. Xylose and sucrose 50 g/L; 2. Xylose 50 g/L and sucrose 10 g/L. Fermentation media prepared only with sugarcane molasses (sucrose 50 g/L) or hemicellulosic hydrolysate (xylose 50 g/L) were used as control. All the media were supplemented with solution of rice bran extract (20.0 g/L), (NH4)2SO4 (2.0 g/L) and CaCl2·2H2O (0.1 g/L). Fermentations were carried out in 125mL Erlenmeyer flasks with 50 mL of fermentation medium, initial pH 5.5, at 30 °C, 200 rpm for 48h. Results showed that xylose consumption (higher than 88% in all the media) was not affected by the presence of sucrose, even in the same concentration. In fact, both carbohydrates were consumed simultaneously during the first 24h of fermentation, in which ethanol and xylitol were also obtained concomitantly. The highest productions of ethanol (approximately 47 g/L) were obtained in the media with the highest concentration of sucrose evaluated in this study (50 g/L), independently of the presence of xylose. Xylitol volumetric productivity was improved by the addition of sugarcane molasses to the hemicellulosic hydrolysate, since in the medium with the same concentration of xylose and sucrose (50 g/L) the value (0.64 ± 0.03 g/L/h) was 14,30% higher than in the medium composed only by hemicellulosic hydrolysate (0.56 ± 0.01 g/L/h). Thus, the by-products of the sugarcane agroindustry named molasses, straw and bagasse can be used for coproduction of ethanol and xylitol, which can be desirable in the context of a sugarcane biorefinery.

Amino acids determination in sugarcane vinasse by anion-exchange chromatography with electrochemical detection

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Sugarcane vinasse is a co-product generated from ethanol production through yeast fermentation of carbohydrates in sugar and ethanol plants, with high pollutant power (about a hundred times that of domestic sewage) and requires high chemical oxygen demand (COD). It is estimated that the sugarcane vinasse production in Brazil will be between 265.1 and 370.3 billion liters in 2017/2018 harvest. The development of methods for determining the chemical composition of vinasse is extremely important to add greater value to this residue and bioenergy applications. In this work, reduced graphene oxide (RGO) containing copper nanoparticles (CuNPs) on glassy carbon electrode (CuNPs-RGO/GCE) was investigated as an amperometric detector combined with high performance anion-exchange chromatography (HPAEC-PAD) for amino acids determination in sugarcane vinasse sample. Electrode modification was performed with graphene oxide and copper sulphate in sodium sulphate solution. Chromatographic separations were performed by using a CarboPac™ PA1 (Dionex®™) anion-exchange column (250×4 mm I.D., 5 µm) coupled with a guard CarboPac™ PA1 column (50×4 mm I.D.) in isocratic elution, flow rate of 0.40 mL min^{-1} and detection potential of 0.50 V. The effects of hydroxide and water as mobile phase on the retention time, retention factor, separation factor and peak resolution was evaluated. Electrochemical response and sensitivity improved dramatically owing to the application of CuNPs and RGO as the electrode modifier. The analytical parameters of the method developed were evaluated. Solutions were prepared with concentrations in range of 1.0×10^{-6} mol L^{-1} to 1.0×10^{-3} mol L^{-1}. Under the optimized HPAEC-PAD conditions, the developed method using the modified electrode demonstrated a linear relationship toward the amino acids concentration range over two orders of magnitude with the detection limits less than 1.0×10^{-5} mol L^{-1} and excellent correlation coefficient of at least 0.995. The proposed analytical method using the CuNPs-RGO/GCE was applied to detection of amino acids in the sugarcane vinasse sample with good recoveries ranging from 96 to 105%. In addition, the stability and reproducibility of the prepared modified electrode were investigated. The excellent long-term stability and reproducibility of the prepared CuNPs-RGO/GCE make them attractive in electrochemical detector. Furthermore, the modification approach with high performance could be further extended to quantify amino acids in a wide variety of sugarcane vinasse samples.

Structural biology and medicinal chemistry strategies toward the discovery of bioactive compounds as agrochemical candidates for sugarcane leaf scald disease

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Sugarcane derivatives stand out as major contributors to renewable energy sources in bioenergy production. However, the occurrence of severe diseases affects the productivity of sugarcane crops. One such condition is leaf scald disease, which is caused by the gram-negative bacteria Xanthomonas albilineans. Leaf scald disease has a dramatic impact on crop productivity, including reduced yields and reducing the quality of the juice. The absence of chemical or biological agents to treat the disease justifies research and the development of new bioactive molecules as effective and selective agrochemicals. An attractive metabolic pathway explored for this purpose is the

biosynthesis of folates. Enzymes involved in synthesis and modification of folates are validated molecular targets for inhibitor design. In this context, the enzyme N5, N10 - methylenetetrahydrofolate dehydrogenase-cyclohydrolase (XaFolD) and dihydropteroate synthase (XaDHPS) are essential for biosynthesis of folates and have been selected for structural and functional characterization. Previous kinetic and structural characterization of XaFolD and XaDHPS carried out in our lab provides the basis for exploring the enzyme as a target for inhibitor screening and discovery. The genes encoding both targets were cloned successfully. Next, an efficient recombinant protein production system was prepared, the products expressed then successfully purified (>95%). Crystallization screening and optimization assays were performed and X-ray diffraction dataset for both enzymes were collected, allowing the structural determination of the 3D structures at high resolution in the presence and absence of ligands. In addition to the structural elucidation, the functional characterization of the enzymes has been performed. For this purpose, functional assays were standardized. The assessment of the substrate affinities revealed KM values of 50 ± 10 µM for N5, N10-methylene-tetrahydrofolate and KM = 668 ± 81 µM for NADP+ for XaFolD enzyme. In the case of XaDHPS a Kd value of 1.8 µM was determined for the substrate dihydropteroic acid (DHP). In sum, high resolution 3D structures are crucial for the application of structure-based drug design strategies aiming at the discovery of new agrochemicals. The selected enzymes are promising targets for medicinal chemistry efforts aimed at developing new chemical alternatives for leaf scald control.

Immobilization by cross-linking of *Aspergillus oryzae* IPT-301 mycelium for fructooligosaccharides synthesis

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Fructooligosaccharides (FOS) are functional sugars available commercially by synthetic production, using microbial enzymes such as fructosyltransferases (FTases, E.C.2.4.1.9). *Aspergillus* spp. stand out as potentially producer sources of these enzymes. They synthesize it, by submerged fermentation, both extracelular and mycelial FTases (adhered to biomass) with transfructosylation activity (AT)(the amount of enzyme necessary to produce 1 µmol of FOS per minute under the experimental conditions) and hydrolytic activity (AH)(the amount of enzyme necessary to release 1 µmol of fructose per minute under the experimental conditions). The ratio between activities (AT/AH) is an important parameter that indicates the predominance of the transfructosylation activity over hidrolytic activity. High ratios are desired for the efficient synthesis of FOS. The biomass can be immobilized by aggregation and cross-linking with glutaraldehyde aiming to positively change the enzymatic activity of the mycelium and improve its application. Therefore, the present work had the objective to study immobilization, with glutaraldehyde, of the *Aspergillus oryzae* IPT-301 mycelium and its effect on hydrolytic and transfructosylation activities aiming at the synthesis of FOS. The microorganism was cultivated by aerobic submerged fermentation for 64 hours, in synthetic culture medium at a temperature of 30°C and 200 rpm agitation. The biomass obtained was immobilized and the mycelial activities were quantified. By means of the central rotatable composite design 2^2, the effects of glutaraldehyde concentration (0.17, 0.50, 1.30, 2.10 and 2.43%, v/v) and pH (5.75, 6.11, 7.00, 7.90 and 8.25) on the mycelial immobilization were evaluated. The immobilization reaction was performed for 60 minutes at 25°C on a stirring of 200 rpm, being

folowed by a sodium borohydride reduction in order to increase the stability of the obtained biocatalyst. By using this biocatalyst in the production of FOS from sucrose, it was possible to obtain the activities AT, AH and the ratio (AT/AH) as response variables. It was observed that the activity (AT) remained constant (120 ± 50 $U.g^{-1}$) for all studied effects. On the other hand, activity (AH) decreased in regions with higher pH (pH 7.90) and high concentration of glutaraldehyde (2.10%, v/v), getting 8.5 ± 0.8 $U.g^{-1}$, 3.5 times lower than that obtained with the use of the free mycelium (29.4 ± 11.7 $U.g^{-1}$). Consequently, there was an increase in the ratio (AT/AH), whose values of the ratios obtained for the free and immobilized mycelium were 5.4 ± 2.1 and 12.8 ± 6.7, respectively. The results indicated an increase in the prevalence of (AT) over (AH), showing that mycelial immobilization potentiates the synthesis of FOS.

Electrochemical sensors for monitoring of myo-inositol in sugarcane vinasse based on molecularly imprinted polymer and nanostructures

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The large volume of vinasse produced by the sugarcane industry has generated great interest in developing a concept of biorefinery for the production of fuels and chemical products that offer economic, environmental and strategic advantages, as is the case of the molecule of Myo-inositol (MI). This molecule can be reused by the pharmaceutical industry (synthesis of vitamins and drugs) and food industry (sweetener). In this work, the aim was to construct a highly sensitive and selective sensor for the monitoring of MI in vinasse based on molecularly imprinted polymers (MIP) modified with reduced graphene oxide (RGO) and nickel nanoparticles (NiNPs), on glassy carbon electrode (GCE), due to the fact that these nanostructures provide increased surface/volume ratio and more locations for imprinting. For this, a conventional three-electrode system was employed with a modified GCE as working electrode, a Pt electrode as the counter electrode and Ag/AgCl (KCl 3.0 M) as referenced electrode. The sensor was constructed through the electrodeposition on the GCE of a suspension of 0.5 mg ml^{-1} of graphene oxide in 0.2 M Na 2SO4 solution under a constant potential of -1.5 V for 500s. After this, the NiNPs were electrodeposited in the potential of -1.3V until the charge of 5.0 mC, using a solution of 5.0 mM NiSO4 in 0.1 M Na 2SO4. On this surface MIP was prepared by electropolymerization of 25.0 mM pyrrole (functional monomer) and 7.0 mM of MI (template) in 0.1 M LiClO4 by cyclic voltammetry (CV) at a scan rate of 50 mV s^{-1} for 7 cycles. In the electropolymerization, myo-inositol molecules are trapped in the polymer matrix through hydrogen forming the MIP sensor. After electropolymerization, the electrode was overoxidized in alkaline solution for remove the template of the polymeric matrix, creating in this way the recognition sites. After template removal the MIP can be used for recognition of MI, with incubation time of 10 min. The differential pulse voltammogram (DPV) was obtained through 5.0 mM of the electrochemical probe $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl, to monitor the variations in peak currents before and after analyte binding cavities. On optimal conditions, the sensor showed recognition 6 times higher than for interfering. The linear range was 1.0×10^{-10} to 1.5×10^{-8} M, with detection limit of 7.0×10^{-11} M and quantification of 2.3×10^{-10} M. Besides that the electrode showed considerable repeatability with 1.5% RSD (n=5) and a high stability with 2.6% RSD (n=22 days). The sensor was applied in samples of vinasse and the found concentration was 1.2±0.07mg for MI per liter of vinasse. The high recovery rate (97.1%-

100.9%) indicates that the proposed method has excellent degree of accuracy, being suitable for practical utility.

Potential of biobutanol production by means of fermentation of sugarcane vinasse

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Butanol is an interesting biofuel due to its combustion properties similar those of gasoline, which allows the mixture of such fuels without the need of engines modification while lowering their emissions. Between 1912 and 1914 Weizmann isolated a culture of butanol producer bacteria named BY, later called *Clostridium acetobutylicum*, which is currently used in many studies about butanol production due to ease of isolation from microbial consortia growing in natural environments. Other butanol producer microorganisms include *C. beijerinckii*, *C. saccharobutylicum*, and *C. saccharoperbutylacetonicum*. Such microorganisms convert carbohydrates to butanol in a solventogenic process named ABE fermentation. The biologic process occurs in two phases: volatile organic acids are produced in a first step, mainly acetate and butyrate, following an exponential growth phase; then, the solventogenic phase takes place where the organic acids are reassimilated. The big challenge has been directing the route of carbohydrate degradation involving mixed cultures towards the production of butanol. In these cases, a common procedure is the treatment of the inoculum in order to inactivate undesirable microorganisms, especially Gram-negative bacteria, allowing the predominance of endospores bacteria producers, e.g., Clostridium genus. In this study, we evaluated the feasibility of butanol production from fermentation of sugarcane vinasse diluted in a synthetic culture medium. Anaerobic sludge treating cattle manure and milking room wastewater was originally used for obtaining the tree different inoculum utilized in this work. For this, three different treatments were applied to the anaerobic sludge: thermal treatment (TT), acid-thermal treatment (AT) and autoclave sterilization (AU). The experiments were carried out in batch reactors kept at 37° C and 60 rpm. The initial pH and sucrose concentration were adjusted in 6.9 and 20 g L-1 for the fermentation with TT and AT and 6.5 and 25 g L-1 for the fermentation with AU, using 1M NaOH solution. The fermentation with TT and AT inoculum was held by 7 days and with AU was conducted in 12 days. Carbohydrates, volatile acids, and alcohols were determined by high performance liquid chromatography (HPLC). The microbial community was analyzed by massive an amplicon sequencing using Miseq-Illumina, 2x250 (Illumina Inc., San Diego, CA, USA). Although butanol was not detected, the fermentation of vinasse resulted in high concentrations of iso-butyric and butyric acids, totaling 20.82 g L-1 and 22.76 g L-1 after fermentation with TT and AT inoculum, respectively. In the fermentation with AU inoculum, the sum of concentrations of iso-butyric and butyric acids was 13.65 g L-1. The inoculum also influenced the microbial diversity and Clostridium sp. was detected in all treatments, with the highest abundance in AU. In the fermentation using TT inoculum, there was a predominance of Lactobacillus sp., a little more than in AU.

Modification on products selectivity from ethanol conversion with nickel- and copper- cationic substitution in a hydroxyapatite catalyst

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Biorefineries are the promissory proposal of traditional oil-refinery substitution in terms of

reduction of carbon emissions, due to the balanced production with the renewable sources. Over the years in Brazil, the principal biorefinery is the sugarcane industry. Ethanol is a versatile refine product from biomass which acts as a building block for organic reactions, producing a large number of products for diverse applications, such as solvents, fuels and additives, polymers, and resins. Nowadays, most part of ethanol conversion is obtained with the heterogeneous catalysis. One type of catalyst in this category is the hydroxyapatite (HAP), which results in interesting yield values. Other advantages of the use of HAP is the impact in process optimization, accomplishing fewer reaction steps than that are required by other techniques; and also its bifunctional behavior in terms of acid and base sites balance which shows more possibilities of products. Here we evaluate the influence of minor cationic substitution with two metallic elements in the catalyst structure and the consequences in the reaction experiments. Both elements were added during the synthesis by co-precipitation with pH control by the addition of an ammonia solution (NH4OH, 25% V/V). The samples were prepared from metallic nitrate precursors and diammonium phosphate at 50°C. Afterward, the solids were dried at 80 °C for 12 h and calcined at 700°C for 2 h. The solids were submitted to X-Ray Diffraction Analysis (XRD), which confirmed a pure crystalline phase of HAP. Other characterization techniques were also used, such as X-Ray Fluorescence (XRF), Temperature Programmed Desorption (CO2-, and NH3-TPD), and N2-physisorption (BET). XRF resulted in a real atomic substitution of less than 2%. Although the substitution was low, TPD showed the interference of the substitution in the parameters of each catalyst, which the pure sample reveals that acid sites were predominant, whereas the substituted catalysts presented a distribution profile of base sites mostly. The difference between the metallic elements substitutions was the distribution of sites, accordingly to the intensities of their forces, which might be explained by the different anionic radii. BET assays presented similar surface areas for all the solids (30 m^2/g catalyst). Catalytic experiments were lead in a continuous gas system with an online chromatograph and were obtained small hydrocarbons. The utilization of nickel and copper in the substitution resulted in changes in selectivity distribution of the products.

Engineering *Burkholderia sacchari* to improve PHA production from biomass

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Burkholderia sacchari LMG 19450 is a Brazilian bacterial strain isolated from sugar-cane crop soil. It is able to produce high-value bioproducts such as polyhydroxyalkanoates (PHA) from different carbon sources in sugarcane bagasse such as xylose, arabinose, sucrose and glucose among others. PHA, a family of biodegradable and biocompatible biopolymers, is a candidate product to be introduced in sugarcane mill biorefineries. Using xylose as sole carbon source, *B. sacchari* reached a maximum specific growth rate (μmax) of 0.12 h^{-1}. However, preliminary economic analysis indicates that a μmax value of 0.25 h^{-1} must be achieved so as industrial PHA production from xylose would be feasible. PHA production using xylose as the sole carbon source was evaluated in wild-type *B. sacchari* and a recombinant strain overexpressing *xylR*. Culture media were formulated containing xylose in excess and limiting amounts of nitrogen or phosphorus to reach increased polymer accumulation. Experiments were conducted in 2.5L-working volume bioreactor, pH controlled at 7.0, temperature maintained at 30°C, dissolved oxygen concentration above 30% of medium saturation. Xylose was fed in pulses to keep sugar concentration above 5 $g.l^{-1}$. Carbon

metabolic fluxes were analyzed to identify xylose consumption bottlenecks. Preliminary analysis indicated that phosphorus limitation yielded better PHA accumulation results. Recombinant strain overexpressing *xylR* achieved higher µmax rates and higher PHA productivity in comparison to the wild-type strain using xylose as the sole source of carbon.

Biomass and aromatic models for methanolic catalytic conversion in one-pot supercritical reaction using Nb/Cu-metal oxide from hydrotalcite as catalyst for methanol dehydrogenation

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The purpose of the work is to apply a new Nb/Cu metal oxide catalyst derivate from 3Mg/Al hydrotalcite for hydrogenolysis and hydrogenation of biomass and aromatic models at auto-generated H2 supercritical condition from methanol dehydrogenation in an one-pot reaction to produce bio-based chemicals. Doing so would replace petroleum with a renewable resource that in principle is carbon dioxide neutral. Porous metal oxides (PMO) were obtained by calcining 3Mg/Al hydrotalcite containing Nb(1-5%) Cu(10%) synthesized in a co-precipitation process of methanolic ionic solution into CO32- alkaline aqueous solution (pH 9~10) under strong acoustic cavitation (20kHz) to avoid Nb2O5 clusters formation. Catalystis were characterized by DRX, FTIR, BET, TGA and Zeta potential. Methanol (3mL), catalyst (50mg) and sawdust (200mg) or aromatic models (50mmol) (benzene, ethylbenzene, phenol, benzyl alcohol, catechol, o,m,p-cresol, guayacol) were submitted to 300oC for 4h in a 10mL reactor. The potential for methanol reform was evaluated by gas development and the products formed were analyzed by GC-MS. A major breakthrough in the catalytic reduction of biomass would be the ability to direct the reaction selectivity toward aromatic products and other higher valuable products using catalysts based on earth-abundant materials. This is the first time that Nb and Cu were incorporated in the same hydrotalcite. The catalyst is key to selectivity, so we addressed this challenge by developing new catalysts based on Earth-abundant elements and elucidating the mechanisms of the catalyzed biomass disassembly reactions and those pathways that can lead to selective hydrogenation/deoxygenation of intermediates formed as lignin and carbohydrates depolymerize. In general many short chain and cyclic alcohols were obtained from the biomass hydrogenolysis/hydrogenation and the ratio of them was changed by Nb presence in the Cu-Mg/Al hydrotalcite. Nb decreases the hydrogen produced from the reform process but it promotes methylation and methoxylation in the phenolic compounds. Non-oxygenated rings do not suffer any reaction of hydrogenation or methylation in this process. The presence of Nb in the Cu-PMO has a good potential to give selectivity in the biomass catalytic methanolic supercritical process.

Sustainability and Impacts

Ionic liquids and their potential applications aiming sustainability

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Due to the concern for sustainable development and, consequently, with the green solvents, the research about ionic liquids (ILs) has been raised as they are potential substitutes for volatile organic solvents in industrial processes because of their selective properties. ILs are organic salts with melting point of less than 100°C and liquids at room temperature. Also, they are formed by an organic cation and an organic or inorganic anion with physicochemical properties defined from their chemical interactions, that allows the selectivity from ions used. In addition, ILs are characterized by high thermal and chemical stability, negligible vapor pressures, non-flammable or toxic and may dissolve many organic and inorganic compounds, besides their reuse in industrial processes, that meets the green chemistry principles. Thus, ILs have been used as solvents, catalysts or reagents aiming sustainability, safety and health with a very wide application. They may be used in analytical chemistry, mainly in chromatography; electrochemistry as electrolytic batteries; separation and liquid-liquid extraction for pollutants removal and purification of fossil fuels, respectively; synthesis and catalysis, either in polymerization or enzymatic reactions; coating processes of materials to adjust the thermal conductivity. To achieve higher efficiency in the processes above and minimize environmental impacts it's necessary to know the related physicochemical properties, such as density, viscosity and heat capacity (Cp), for example, that are fundamental projects under development. Regarding thermal properties, the heat capacity is the most studied by researchers because it is indispensable for important applications in engineering and is related to energy consumption, as distillation, evaporation and heating processes, for example, utilized in petrochemical and pharmaceutical industries. So, this property may be determined by experimental procedures and computational modeling. Since there are many possibilities of combining cationic and anionic groups to provide selective properties, the first alternative becomes unfeasible due to its high cost and time for its characterization. As a result, this is the technological challenge of the work, which consists of using predictive methods for Cp determination. Among these, the group contribution (GC) models are adequate because of their simplicity and precision. First, it was obtained a database containing 2753 experimental data of 14 ILs with imidazolium cationic group for Cp, and then the parameters A, B and D were determined for each IL proposed in the equation $Cp = R[A+B(T/100)+D(T/100)2]$. The results were obtained under pressure variation (100-6000 kPa) and temperature (183-463 K), with mean relative deviations (σ) of 0.8% and $R^2 = 0.96$. Therefore, there is no need to waste reagents and time to achieve proprieties of ILs since the GC results were precise and will be a sustainable process.

Antibiotics thermal stability and environmental risk from its use in fuel ethanol production

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Brazil is the biggest sugarcane fuel ethanol producer in the world. In the last harvest/year the production reached 27.8 billio n of liters of the biofuel. A key step of this production is the fermentation, in which carbohydrates are converted in ethanol by yeasts. One problem related to fermentation is the bacterial contamination, what lead to the use of antibiotics for control it. Therewith, the antibiotics can reach the environment, due to a widespread practice in sugarcane crops, the fertigation with vinasse. In this context, the antibiotic thermal stability in distillation conditions plays a key role to the possible impacts from this practice (concerning to antibiotic release in environment). In this work, the thermal stability of antibiotics penicillin G, monensin, virginiamycin M1 and erythromycin was investigated simulating the distillation conditions. To that, 100 mL of individual antibiotic standard solution with 1.0 to 5.0 µg mL^{-1} was subjected to boiling by 3,0 h under reflux. In this period, aliquots were collected in t=0, 30, 60, 90, 120 and 180 min and then they were analyzed by LC-MS/MS to verify the behavior of each antibiotic under elevated temperature in time. The chromatography peak area from each antibiotic in various times was used to evaluate its thermal stability. Chromatogram comparison, between t=0 and other times, was used to verify the formation of new compounds. As result, it was found that the antibiotic stability under distillation conditions was more than 1.0 h for penicillin G, 1.5 h for virginiamycin M1 and 3.0h for monensin and erythromycin. These results show that all antibiotics studied are stable enough to resist to the distillation process. Furthermore, for all studied antibiotics, new chromatographic peaks were observed in t≥30min indicating new products formation as result of degradation/transformation caused by elevated temperature. Therefore, even if the antibiotics do not resist the distillation, the transformation products formation still is a risk to the environment. The effect of that new products to the biodiversity must be known for ensure a safe use of antibiotics in fermentation for fuel ethanol production.

Comparison of GHG emissions models for biofuels: the case study of ethanol produced from sugarcane in Brazil

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The use of alternative fuels in the transportation sector, particularly bio-based fuels, has been an important strategy to achieve greenhouse gas (GHG) emissions reduction compared to petroleum-based fuels. Discrepant results obtained using different life cycle assessment (LCA) tools over the years, decreased the credibility of results in measuring progress towards compliance with established targets. Four models used for GHG emissions analyses, i.e. GREET from the U.S., GHGenius from Canada, the regulatory BioGrace from the EU, and the Virtual Sugarcane Biorefinery platform developed by the Brazilian Bioethanol Science and Technology Laboratory (CTBE) were compared in this study. The objective was to identify the main differences and commonalities in methodological structures, calculation procedures, and assumptions. The estimated life cycle GHG emissions ranged from 16 to 45g CO2eq per MJ of sugarcane ethanol. The agricultural stage (N2O emissions from fertilizers, energy and fuel use, straw burning, and limestone applications) and ethanol shipping were the major sources of differences observed across models. Upon harmonization of data and assumptions, the models generated close results for sugarcane ethanol, within a range of eight percent. The elaboration and application of the emissions assessment tools ultimately align with biofuels regulations as defined by governmental agencies, which may have different and/or conflicting requirements.

Nitrogen losses by leaching and nitrous oxide emission in a sugarcane area

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Nitrogen (N) fertilizer application is fundamental for yield gain of agricultural crops. Meanwhile, major negative impacts of agriculture on the environment results from the leaching of nitrate (NO3) from the root zone to groundwater and from the nitrous oxide (N2O) emission to the atmosphere. These aspects are a priority especially when the N fertilizer is used to produce ethanol and bioelectricity derived from sugarcane biofuels, comprising the largest share of renewable sources of energy. Therefore, the aim of this study was to evaluate the losses of N fertilization (NO3 leaching and N2O emission) on a sandy-clay Oxisol under sugarcane ratoon. The experiment was carried out at APTA experimental station, in Jaú, State of São Paulo, Brazil, during 2015/2016 crop season. The treatments consisted of three N doses: 0 kg N ha-1 (TC-Control), 140 kg N ha-1 (T140) and 210 kg N ha-1 (T210), with four replications per treatment. The N fertilization was split in two doses, 50% on November 31st 2015 and 50% on January 19th 2016, applied on soil surface. Soil solution extractors were installed in the crop rows at 0.90 m depth and were kept under pressure with 0.6 bar for vacuum formation and water samples were analyzed by flow injection analysis. N2O emissions samples were taken using rectangular chambers (75 cm x 30 cm x 20 cm; 0.045 m3) sampling rows and inter-row positions and analyzed in a gas chromatograph. Regards to nitrate leaching, the average concentration detected in water samples of all treatments were lower (0.35, 0.46 and 0.97 mg L-1 for TC, T140 and T210, respectively) when compared to the standard (10 mg L-1) according to CONAMA Resolution nº 420. Therefore, no significant impact on the water quality is expected. The cumulative emissions of N2O were 170, 572 and 833 g ha-1 in TC, T140 and T210, respectively, and resulted in N-N2O emission factors of 0.29% for T140 and 0.32% for T210 treatment. Those emission factors are significant lower when compared from other scientific results (Crutzen, 2008; IPCC, 2006). Moreover, it was detected that N2O emission factor from split fertilization management is close for non-parceled fertilization values. This creates a scenario of environmental sustainability for the Brazilian agroenergy sector.

Development of passive samplers for measurements of NH_3 and NO_2 concentrations emitted from fertilized soils of sugarcane crops

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Sugarcane plays a prominent role in Brazil's current economy and has growth projections for the coming years mainly due to the use of biofuel ethanol as an alternative energy source. However, a relevant problem regarding the production of ethanol refers to the possible massive increase in the emissions of reactive nitrogen compounds, such as NH3 and NO2 to the atmosphere, due to the use of nitrogen fertilizers in the cultivation process. These compounds may undergo transformations for other gaseous species, such as nitric acid (HNO3), photocatalyses the formation of tropospheric ozone, nucleate particles such as ammonium nitrate (NH4NO3) forming fine particulate matter, associated with respiratory health problems in humans, and increase the nitrogen deposition over aquatic and terrestrial ecosystems. Considering the scarcity of updated

and precise information regarding these emissions, the development of simple and portable methodologies which allow to perform samplings in field and the determination of concentrations of such species are required. In this way, passive samplers were built from commercial polystyrene monitors where were added cellulose filters impregnated with absorbent solution specific to each analyte. To the samplers also was added a Teflon membrane to act as barrier to turbulence process of the air. After sampling, the determination of NH3 was made from the Berthelot Reaction modified by the monitoring of colored product at 660 nm formed with NH3 in the presence of phenolic compound in an oxidant media. In the case of NO2, its determination was made from Griess-Saltzman Reaction, in which there is the formation of an azo-dye monitored at 550 nm in the presence of nitrite ions. Linear behavior was obtained between NH3 and NO2 concentrations and the measured absorbance in the range of 1 to 30 µmol L^{-1} (n=3, r^2=0,9991) with quantification limit of 1 µmol L^{-1} for NH3 and in the range of 0,3 to 30 µmol L^{-1} (n=3, r^2=0,9997) with quantification limit of 0,3 µmol L^{-1} for NO2. The passive samplers were evaluated regarding repeatability of the results obtained in the measurements for samplings performed in the environment and stability of signal measured after different time of storage after sampling. The passive samplers for NH3 and NO2 presented satisfactory performance in the repeatability study with relative standard deviation of 10,8% and 3,0%, respectively, by the measurements of 15 replicates and variations of only 7,3% of concentration measured over 60 days of storage for NO2. The passive sampler for NH3 is still being evaluated in this criterion. Based on the results, the developed passive samplers were suitable for determination of NH3 and NO2 concentrations in the environment and present the necessary simplicity to enable their installation in sugarcane crops.

Production and productivity of the Brazilian sugar and ethanol industry from 2002 to 2012: some stylized facts

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Since the early 2000s, success of Flex Fuel Brazilian vehicles consuming a growing amount of the anhydrous alcohol and gasoline mixture, beside the continual escalation in international sugar demand, have projected positive expectations on sugarcane productivity. Regardless the optimistic scenario, some factors have hampered sugar and ethanol investments affecting output efficiency since 2009. Even more, there are stylized facts showing a steady reduction of output which has motivated this paper. Starting from the identification of the mentioned problem, the paper initially proposes to evaluate the performance of the industrial physical productivity of sugarcane mills between 2002 and 2012. Previous studies showed that, between 2008 and 2012, the rate of accumulated growth of productivity was negative, except in 2010. Secondly, based on date obtained from a group of 23 refineries, from 2009/2010, 2010/2011, 2011/2012 harvest, total factor productivity will be estimated. This way, using the Malmquist Index, it could be verified if productivity variations at mills were caused by technical efficiency or by technological change. Preliminarily results indicate that poor economic performance of mills was caused by negative variations of technological change, indicating that sugar and ethanol companies were not able to positively shift their production boundaries, probably because they did not invest sufficiently.

Biorefinery value-chain analysis: technology, commercialization and sustainability

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Transition from petroleum-based to biomass-based products is considered as a paradigm shift for the holistic development of a sustainable society, energy independence, and to make environmental safe and clean. With the recent changes in geopolitics, and trends over the last decade, many bio-based industries are now focusing on the core of the Bio-economy comprising bioenergy, and commodity bio-chemicals. Given that it takes more time, more efforts and financial inclusions to develop the pitch for markets for biomass-derived based energy and chemicals, renewed interest in real process engineering, engineering economics and in synthetic biology are requisite to provide enough pace to commercial market niche in a sustainable fashion. Green chemicals and biofuels are potential alternative of gasoline and petrochemicals and has befitting source for transitioning petro-economy into bio-economy. Production of second generation (2G) sugars from lignocellulosic biomass has a key role in the holistic development of bioeconomy. Given the theme 'sugar is the new oil' pretreatment is quintessential in successful deployment of biorefineries. These sugars are considered as renewable building blocks for the sustainable production of biofuels and renewable chemical commodities in biorefineries. Pretreatment is an inevitable step for the production of 2G sugars for their eventual conversion into green chemicals and fuels in bio-refineries. While the 2G sugars production at laboratory scale is quite successful, the same yield and production titers have not been achieved at the demonstration scale and commercial operations. Beside the bulk chemicals such as 1G ethanol, lactic acid, ethylene, succinic acid, among others are being scale-up with moderate success, products for example- Adipic Acid, Epichlorohydrin, 1,4 Butanediol, Dodecanedioic acid have the potential to make a ground in biorefinery value chain. This presentation will describe the obstacles and key challenges in 2G sugars recovery at large scale operations and a global view about the technology, commercialization of cellulosic ethanol and principle bulk chemicals under biorefinery concept.

Assessment of the microbial diversity associated with CH_4 production from vinasse

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Sugarcane-based ethanol has a significantly favorable energy and greenhouse gas (GHG) balance. However, its production generates several residues, where vinasse deserves attention, both in terms of quantity (ranging from 10 to 15 L for each liter of ethanol) and because of the significant CH4 emission potential during the step of storage and transportation. In Brazil, the most widespread systems of vinasse storage and transportation are uncoated and coated open channels and coated tanks. Considering that CH4 emissions are dependent on the structure and abundance of microbial communities, we hypothesized that different systems of vinasse storage and transportation harbor a distinct microbial community composition. To test this hypothesis, we used high-throughput 16S rRNA sequencing to assess the microbial community from sediments of the two main systems of vinasse storage and transportation. In addition, we focused on specific archaeal groups and functional genes related to methanogenesis and correlated to CH4 production. Our results

showed significant differences in microbial community structures, diversity and abundance between the vinasse storage and transportation systems, indicating a clear selection at both taxonomic and functional levels in relation of CH4 production. Shannon estimator revealed higher diversity of methanogens associated with open channel systems, especially in uncoated section. This result is in agreement with the methanogens abundance, 1.5 x 10^7 and 4.3 x 10^{10}, respectively to *mcr*A and *mba* copy number, and subsequent positive correlation with CH4 emissions (R^2 = 0.8). Methanogens responded to nutrient availability and anaerobic conditions provided by vinasse and the higher production of CH4 observed in the storage of vinasse comprised by uncoated channel (2.6 kg CH4 m^{-2}) are related to the direct contact between vinasse, sediment and soil. In the tank system, vinasse is stored temporally and transported under pressure through closed pipes, where it is not in direct contact with soil and short period of sedimentation, which consequently reduced the potential for CH4 formation, ~0.01 kg CH4/ m^{-2}. Overall, our data indicated that methanogen microbial communities are influenced by the agricultural residue storage as well as the residue composition. The adoption of new technologies and subsequent improvements to vinasse storage and transportation systems should significantly change the microbial community dynamic, reducing GHG emissions and thereby making sugarcane-based ethanol a cleaner biofuel.

Relating the production of biodiesel between Brazil and Argentina: An exit for the preservation or persistence of negative impacts to the environment?

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Due to questions about negative externalities to the environment, biomass-derived fuels are an alternative to minimize the pollutant emissions of fossil fuels, since a significant part of the carbon emission is absorbed by the photosynthesis of the plants used to produce this fuel. On the other hand, this alternative can only be considered sustainable in relation to fossil fuels by analyzing the production chain to confirm how it can be considered sustainable, since most of this fuel for sale to the consumer is composed mainly of petrodiesel. In Latin America, Brazil and Argentina emerge as biodiesel producers leaders and the objective of this work is to compare how sustainable is the productive chain of each country. The tool used to investigate this issue is the Life Cycle Analysis (LCA) methodology, which analyzes and catalogs the inputs and outputs of matter and energy, from the production process to the final consumption of the product. Through the LCA, is possible to discover in which of the two countries biodiesel production has the highest degree of optimization and productive sustainability by providing information to observe the actions taken at each stage of production, minimizing the negative consequences of critical stages. The world's largest soybean producers are United States, Brazil and Argentina, totaling 80.34% of the world total. Brazil is the first to export soybeans (39,481 thousand tons), followed by the USA (36,992 thousand tons) and Argentina (10,181 thousand tons). Among these countries, Argentina is the largest exporter of biodiesel. On the other hand, Brazil produces to meet domestic demand. Regional differences and specificities are important for the LCA methodology, because the countries that are the object of this research have scenarios that contribute to the differentiation of productive chains, such as transport distance, land use and even the transesterification route. These regional specificities are essential factors for understanding different results of the same process.

Modification of Hansen methodology using hot-stage microscopy

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Hansen methodology was developed to obtain quickly with good precision the partial solubility parameters of dispersion (δD), polar (δP) and hydrogen bonding (δH), and solubility radius of a compound. These parameters are useful to carry out a preliminary selection of solvents, which can be used in industrial processes. In this way, it is possible to minimize impacts, obtaining alternatives that aim at achieving better results, economy and sustainable options. The parameters are determined by qualitatively observing the solubility of the compound analyzed in solvents whose solubility parameters are previously known. Based on the results obtained (soluble or insoluble) and using a numerical algorithm, the solubility parameters and radius of solubility of the compound studied are determined. To obtain more representative results, the ideal is that the data of good and bad solvents (soluble and insoluble) obtained are in close proportion. Generally, this methodology performs the experiments at room temperature and the present work aims to improve it using the thermomicroscopy (hot-stage microscopy) to determine melting temperature of the last crystal observed in a heating ramp of the system. In this way, a temperature can be selected to obtain balanced amounts of good and bad solvents, providing more precise calculation of the parameters. Stearic and palmitic acids had been tested with 20 different pure solvents. Solubility tests were performed according to the methodology and the parameters of each acid were determined. The same binary mixtures had been analyzed by temperature-controlled microscopy to determine the dissolution temperature of the solute. Subsequently, the parameters were again calculated, taking into account the temperature data, and a comparison was made with the literature values. The results (in $MPa^{1/2}$) found by the standard methodology were δD=15,9; δP=5,8; δH=16,2 with 2 good solvents, and δD=16,6; δP=8,1; δH=10,3 with 10 good solvents, for stearic and palmitic acids, respectively. In microscopy, it was found that the ideal temperature for the experiments was 33 and 25°C, for stearic and palmitic acid, respectively, and the following parameters were obtained: δD=16,1; δP=5,8; δH=7,0 for stearic acid and δD=15,5; δP=6,6; δH=8,9 for palmitic acid. The use of microscopy has improved the results when compared with the values of the literature, which has been obtained by other techniques, thus evidencing the potential of the proposed technique.

Evaluating soil carbon and nitrous oxide emissions in South Central Brazilian sugarcane production systems using the DAYCENT model

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The rapid expansion of sugarcane production in Brazil is prompting concerns for the effects of land use conversion into sugarcane as well as the sustainability and greenhouse gas (GHG) emission impact of sugarcane management practices. Simulation models such as DAYCENT may be useful in evaluating the direct impacts of sugarcane production on soil carbon and GHG emission dynamics. However, to date the DAYCENT model has had limited application in Brazilian sugarcane systems. The objective of this study is to evaluate soil carbon and nitrous oxide emissions under land use change into sugarcane production as well as under different sugarcane management practices using DAYCENT. We assembled soil C data from 58 sites where land had been converted from pasture and active agriculture into sugarcane production, as well as sugarcane production and N2O emission data from 43 experimental treatments, drawn from 8 studies, that represent a range of sugarcane management practices (burned versus unburned harvest, application of vinasse and filtercake as well as synthetic fertilizers) in the South Central region in Brazil. We used these data to parameterize and validate the DAYCENT model, as well as compare its estimation of N2O emissions using multiple types of climate datasets to drive model simulations. Our preliminary results show DAYCENT simulated trends in measured soil C change with land use conversion from pasture into sugarcane in several regions of South Central Brazil, but with high variability. DAYCENT estimation of N2O emissions were highly sensitive to the precipitation data used to drive model simulations, with better performance simulating cumulative annual N2O emissions using climate data from the nearest weather station (adjusted r^2=0.38, slope=0.98, p=0.011). Precipitation preceding the experimental period was as much if not more important than precipitation during the experimental period when estimating N2O flux in the short-term (e.g. over 15 and 50 days). Our results suggest the need for targeted improvement of DAYCENT simulation of high N2O emission conditions, perhaps by adding simulation of DOC movement across the soil profile. Our analyses also suggest a strong need for developing a daily climate datasets to support regional evaluation of GHG emissions from sugarcane in South Central Brazil.

Maize in a biofuel crop and brachiaria intercropping: an alternative to increase nitrogen use efficiency and mitigate N_2O emissions

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Information from maize production is necessary to compare the environmental impacts of Brazilian ethanol from sugarcane and maize. This study aims to evaluate nitrogen use efficiency (NUE) and N2O emissions from maize fields intercropped with brachiaria compared with sole maize production. A field trial was set up on December 2016 in Botucatu – SP. The plots in randomized blocks with 5 replicates have 6 rows (15 m long) spaced by 0.75 cm. Treatments (6) consisted of plant combination and N rates (0 or 150 kg N ha-1) top dressed at the 4-leaf stage in maize intercropped with two Brachiaria species (B. brizantha and B. humidicola) plus a control without Brachiaria. All plots were fertilized at sowing with a common rate of N (30 kg N ha-1). N2O fluxes were measured using static chamber method and N2O concentration determined by GC. The grain yield and NUE were determined. Grain yields in the fertilizer control treatments varied from 5.1 to 5.5 t ha-1; in the fertilized plots, yields ranged from 8.7 to 9.0 t ha-1 and were not affected by the intercropping treatments. N2O emissions was monitored from sowing to harvest; the N2O emissions were higher in treatments with 150 kg N ha-1, however no effect of Brachiaria was observed in the first year. Emission factors of N fertilizer varied from 0.25% to 0.26% in treatments fertilized with 150 kg N ha-1, lower than 1% default value used by

the IPCC. The NUE varied from 48.0% to 52.6% and did not differ between treatments. The intercropping showed no negative impact on maize yields and N2O emission and did not affect NUE.

Valorization of sugar cane residues as construction materials

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Sugar cane production increased in Brazil in the last years. Consequently, residues from this production increased in the same way, as the sugar cane bagasse and the sugar cane straw. Due their interesting calorific value, they can be used in energy generation through a burning process. However, after this procedure, other residues are obtained: the sugar cane bagasse ash (SCBA) and the sugar cane straw ash (SCSA). A convenient form to valorize these residues is in the civil engineering as construction materials. In this way, a new trend of research in building construction is the alkali-activated binders. These kinds of binders present the potential to replace the use of Portland cement due technical and environmental advantages. Therefore, this study proposes the use of SCBA and SCSA as construction materials in alkali-activated binders. They were studied in binary systems with blast-furnace slag (BFS) in mortars with replacement percentage of BFS by the residues of 0-50%. These mortars were assessed by their compressive strength after 3, 7, 28 and 90 days of curing. Results showed that the SCBA maintained the mechanical properties from the control after 90 days of curing. In the other hand, SCSA showed better potential to be used in alkali-activated binders: the improvement in the compressive strength respect to the control mortars was more than 50%. These results can allow concluding that both SCBA and SCSA can be utilized as construction material, which give them a suitable valorization.

Treatment of manipueira by biotechnological process using microalga *Chlorella minutissima*

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Most agroindustries generate waste on a daily basis, which due to the toxicity or volume generated deserves special attention. Current environmental policies require waste to be treated prior to disposal in the wild, thus increasing production costs. The environmental sector looks for ways to make rational use of waste generated. The starch industry extracted from cassava produces a significant amount of waste that can be rationally used, reducing waste and contributing to the production process. The present study aims to value the effluent as a culture medium for micro chlorella minutíssima that would act in the bioremediation which will make this effluent discardable in the receiving body (rivers and seas) thus minimizing the environmental impacts, being able also to comply with the current legislation for Discard. For this, operational tests were performed in a semi-closed plastic photobioreactor to evaluate the cell growth tolerance in the effluent at different concentrations under natural conditions of temperature and luminosity. A chemical physical characterization of the "in natura" and post-treatment effluent was also performed. The main objective of this study was to study the microalgae Chlorella minutissima in different concentrations of manipueira, evaluating the growth of the microalgal population (qualitative) and the bioremediation of this residue. For this, it was aimed: to evaluate the influence of different concentrations of manipueira on the growth of

microalgae Chlorella minutíssima in closed photorobioractors under controlled conditions; to evaluate the reduction of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Organic Carbon (TOC), resulting from the bioremediation of this residue; to evaluate the detoxification of the residue in terms of cyanide reduction; and to evaluate the biodegradability of the effluent. In relation to physical-chemical characterization of the effluent in natura, the parameters BOD5, COD, Total Solids, Phosphorus and Nitrogen, presented higher concentrations than the legislation, demonstrating that the effluent should be treated and suitable for disposal. It was verified that the degree of recalcitrance of the organic matter present in the effluent, with BOD/COD, justifies the biological treatment. After the biological treatment, a percentage reduction of COD of the order of 30%, Total Solids around 75% was observed. The parameters that presented reduction, adapting to the standards of release, were phosphorus and nitrogen, with reduction percentage 98% and 65% respectively. The parameters phosphorus and nitrogen were altered due to the consumption of microalgae in the form of nutrients. The parameter that, in natura, did not surpass the standards of launches obtained a significant reduction of its levels: cyanide (99%). Analysis by X-ray spectroscopy by dispersive energy showed that the chemical structure of the effluent was modified by the biological treatment and the analysis of the micrographs showed that the structure of the solid was also modified by the treatment.

Increase in sugarcane areas and profile change in the agricultural production in Northeast of São Paulo state

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We evaluated the changes in the agricultural production profile of an area at the watersheds of the Mogi-Guaçu and Pardo rivers along a 27-year period using satellite images. The areas used for sugarcane production in 2015 were classified by updating the maps produced by the Canasat project, which were transformed into vectors and added as layers in a Geographic Information System (GIS) . We then reclassified the sugarcane areas using on-screen visual interpretation. The polygons of areas which still featured sugarcane in 2015 were maintained. New areas, identified by means of the Google Earth TM's high-resolution images used for the 2015 mapping were added. We mapped the areas of coffee, citrus, eucalyptus and rubber-tree, pastures, native forests, and urban areas using the Google Earth TM image file in a GIS environment and on-screen visual interpretation of high-resolution images, and compared the results to data of 1988. The sugarcane areas, which encompassed a little over 1.0 million hectares (21% of the region's area), increased to about 2.3 million hectares (44% of the region's area). Over this period, the return on investment produced by sugarcane crops was beyond market average in comparison to other agricultural activities, thus leveraging the prices of leaseholds and increasing the competition with other crops for space. The mapping shows a decrease in areas used for husbandry, annual crops – especially grains/cereals –, and citrus. Areas used for annual crops such as soybean and maize, decreased from 936 thousand to 352 thousand hectares. There was an intensification in crops, especially with irrigated areas. The area used for citrus decreased from 486.2 thousand hectares (9.4% of the study area) to 301.5 thousand hectares (5.8%). Low profit and phytosanitary problems contributed to this decrease in citrus area. Despite sugarcane's leading role, over the same period there was an increase in areas occupied with coffee, eucalyptus and rubber-tree, native forests and urban areas. The area used for coffee increased over 80%, from 67 thousand hectares (1.3% of

the region's area) to 123 thousand hectares (2.4% of the area). This increase took place mainly at the Mogiana region, near the border of Minas Gerais state. In 2017, the mapping using satellite images showed that about 150 thousand hectares which are currently used for sugarcane crops (7.1% of the area of both watersheds) may be used for a new crop or may be occupied with native forests in order to increase the legal reserve areas within the farms. These are areas with steepness degrees above 12% which cannot be mechanically harvested for sugarcane crop, and which may not be burned for manual harvesting due to the complete prohibition of flaming in the state of São Paulo.

Biobleaching of Kraft pulp using fungal xylanases produced from sugarcane straw and the subsequent decrease of chlorine consumption

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Xylanases was produced by *T. reesei* QM9414 using sugarcane straw as substrate in an economical method. This crude enzyme was studied in biobleach process of Kraft pulp associated with chemical bleaching, using chlorine dioxide, to investigate the bleaching effect on the final chlorine consumption. An appropriate xylanase dose and time process were evaluated on Kraft pulp, with 3% of consistency, showing the bleach boosting in optimal pH and temperature. A significant decrease of Kappa number (~12%) and an increase in the brightness (~3 points %ISO) were observed, as well as a release of sugars, proving the hydrolysis of lignin-xylan complex. The presence of chromophores compounds (237 nm) was observed in the liquid phase of the pulp hydrolysis, and also prove the discoloration attributed to lignin depolymerization. Scanning Electron Micrograph (SEM) showed the enzymatic effect on the pulp surface. Subsequently, after acid and chlorine dioxide sequence (A/Do), the pulp treated with enzymes showed 4.5 (%ISO) more points of brightness as compared with the control without enzyme treatment. The pretreatment of pulp with xylanase resulted in a remarkable reduction in chlorine consumption maintaining the same brightness as in control. These results clearly demonstrated the *T. reesei* QM9414 crude xylanase produced by sugarcane straw was effective as a pulp "bio-agent". The decrease in chlorine consumption due to previous biobleaching using fungal xylanases produced by residues can be an integrated and eco-friendly bioprocess option to be used in paper mills.

Macroeconomic and environmental impacts of a biobased economy in Brazil in 2030

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Fossil fuels dominate the current energy and chemicals' supply and this leads to a rapid growth in global greenhouse gas (GHG) emissions. One mitigation option is using renewable feedstock for materials, chemicals and mostly energy. This study develops and demonstrates a modelling framework interlinking Computable General Equilibrium and Input-Output models, which allow the analysis of the impacts on macroeconomic aspects and GHG emissions for different future scenarios for deployment of biobased economy. The analysis was done for chemicals (ethylene, propylene, butanol, polylactic acid and acrylic acid) and for energy carriers (ethanol, biodiesel, bio-jetfuel and electricity). Three scenarios for

2030 were to compare different levels of production (amount of chemicals and energy production through fossils versus biomass) for Brazil, based on sugarcane, soy and forest crops (eucalyptus and pine). Different technological levels are also considered in this study. Two important methodological aspects of this research are the financial support provided by the government to enable the bioeconomy to develop, through subsidies, and the limitations in land use established by the need to reduce emissions. Results show that the choice of a bioeconomy in Brazil generates a slight reduction in GDP due to changes in the components of final demand that integrate it (Household, government, exports and imports). Government consumption falls due to increase in subsidies, translating directly into reduction of public services provision to the Brazilian society. Following government consumption comes the investments, which drops following a drop in foreign savings. With decrease of imports, focused on fossil feedstock and fossil-based products, Brazil would reduce its dependency in foreign feedstocks and inputs in a bioeconomy scenario. Increases in the unemployment rate are projected. The increase in land demand imposes an increase in land prices, which impact the prices of products that depend on land (agriculture), which affects household food consumption negatively, mostly focused on meet products (cattle, pork and poultry). It is important to mention that impacts could improve if livestock stocking rate was improved. When it comes to Greenhouse gases emissions, a bioeconomy would allow further emissions reductions, but more attention should be paid to the land use change and forestry sector. Renewable energy has an important role in emissions reductions, in any scenario.

The Renewable Fuel Standard: formulation of U.S. Energy Policy and decision-making process in the Administration

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The objective of this paper is to analyze the Executive branch decision-making process in the United States and its relationship with Congress and interest groups in the case of the formulation, approval and implementation of the Renewable Fuel Standard (RFS). It is a consumption mandate program of biofuels in which Environmental Protection Agency (EPA) is the regulatory agency. The EPA has been the target of interest groups and advocacy coalitions linked to the ethanol industry given that the RFS directly impacted the entire supply chain of biofuels: the farmers, the ethanol and biodiesel plants, and the oil, automobile and aviation industry. The need for this study is justified to prove the hypothesis of lobbying and advocacy activities can be more effective within the agencies of the Executive. Understanding the EPA formulation mechanisms carries notable inputs for this analysis. To better evaluate the decision-making process, our methodological approach aims to combine the perspective of Agroindustrial Complex to model the Advocacy Coalition Framework (ACF) along with the methodology of Social Network Analysis (SNA), setting an innovative analytical tool. Other aspect is related to the fact that mandates have effects on biofuels international trade. On the one hand, they encourage consumption, on the other, present a series of requirements so that other countries have to provide them. Thus, as a secondary objective, we seek to assess this impact and how affect Brazilian interests. The most profound and detailed understanding of these relationships can increase the effectiveness of the work of negotiators and Brazilian policy makers in their role in relation to the United States.

Development of social impact indicator for differentiating Brazilian ethanol production technological scenarios: a sectorial approach of the human development index (HDI)

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Compared to economic and environmental assessment methodologies, social impact evaluation is on an early stage. The Social Life Cycle Assessment (S-LCA) is one of the most promising methodology with increasing systematization, propositions and case studies. Nevertheless, few of these initiatives aimed to evaluate the social effects of biofuels. In a previous study, S-LCA was combined with Input-Output Analysis (IOA) with the purpose of assessing social aspects of different technological configurations of ethanol production on the number of jobs, occupational accidents, wage profile, education profile and women participation on the workforce. However, comparing scenarios analyzing such metrics cannot be an easy task when dealing with decision-making processes. In some cases, additional information can be necessary to provide magnitude and significance to some metrics. Therefore, the current study presents a social indicator to assess the social impact on workers of different biorefinery technological configurations. The applied aggregating characterization model was inspired in the Human Development Index (HDI), whose significance and "easy to communicate" properties make it a good model to express human prosperity. The indicator is the sectorial HDI (HDIsector), which was thought to evaluate both ethanol production scenarios as well as the effects on its supply chain. Similarly, to HDI, the HDIsector is the geometric mean of the three dimension indices. The education and income indices were calculated by using the mean years of schooling and the average annual wage respectively. The challenge that this study proposes to overcome is how to estimate the life expectancy for economic sector and scenarios in order to calculate the health and long life index. The assessed scenarios included 1G-basic (1st generation ethanol - average current technology), 1G-optimized (1st generation ethanol - best current technology) and 1G2G (1st and 2nd generation ethanol - future technologies). The results indicate the 1G-basic with the lowest HDIsector value, followed by the 1G-optmized, while the scenario 1G2G ethanol production showed the highest value. A comparative analysis, considering different process alternatives, was performed. For one of the estimation alternatives the sectorial HDIsector values were 0.595 for 1G-basic, 0.664 for 1G-optimized and 0.682 for 1G2G.

Identifying potential areas for sugarcane expansion aiming to reduce negative impacts on landscape connectivity: a case study in a Dracena, state of São Paulo - Brazil

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The maintenance of landscape connectivity is fundamental to reduce potential impacts of land use changes due to the expansion of crops – e.g., for biofuels production – on biodiversity and ecosystem services. The aim of this study is to identify available areas for the expansion of sugarcane in order to mitigate effects on functional connectivity of a mammals group which require a specific forest habitat. For this purpose, the landscape was modeled according

to the Graph Theory and the Circuit Theory was used to predict animals' movement pattern according to the land cover permeability. The study area occupies around 11,000 hectares and is located in Dracena, west of São Paulo State, in southeastern Brazil, in a transition region between the Atlantic Forest and Cerrado biomes. Connectivity for medium and large mammals was estimated considering dispersal distances of 500, 1000 and 3000 m. The index of Probability of Connectivity (PC) was applied to identify the most important habitat and non-habitat areas that can contribute to maintain landscape connectivity, considering the land use and land cover in 2015. The results allow the identification of non-habitat areas suitable for sugarcane expansion, in which land use changes would not impact the dispersal routes between the remaining forest fragments for the group of mammals evaluated. Therefore, from a biodiversity point of view, these areas are more indicated for future sugarcane expansion. Moreover, the methodology allows the identification of the more critical areas where sugarcane expansion would negatively impact landscape connectivity. The methodology adopted is based on simple but rather robust theories that have been applied in ecology and conservation. The procedure can also be combined with other geospatial information (e.g., physical and environmental data) in order to minimize threatens to biodiversity caused by land use change.

Sustainability of intensified pastureland: global feedstock availability

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Global demand for biofuels will have a high increase in the next decades, especially due activities in transportation by plane, ocean ship and heavy trucks. The land to produce bioenergy crops bring up questionable aspects, as food production conflicts and sustainability index. Pasturelands are much more promising to make room for bioenergy production than forestland and cropland. The global consumption of protein and calories from cropland is 97 and 99%, and from pastureland is only 2.7 and 1.3%, respectively. Moreover the potential to intensify pasture production can be much higher than for crops, which are typically looking to maximize annual production. This project focuses on pastureland as a promising large-scale source of bioenergy feedstocks, by combining pasture intensification with sustainable expansion of bioenergy feedstock production. The potential of pasture intensification, also referred as yield gap will be performed considering the current animal production, in which the pasturelands with low productivity can reach the highest places in a similar climate conditions. However is necessary to confirm if the pasturelands with high animal production are sustainable, or if not, as an overgrazing scenario or low efficiency production of meat and milk. In the present study, global feed availability (FA) for grazed animals was estimated. The aims were: i) to identify if the grass production in the land supports stock density worldwide; ii) to evaluate the improvement due N and P fertilization in FA. The FA for animals was calculated due the estimation of net primary production (NPP) of grass according Miami model, considering ruminant stock density and pastureland data from the year 2000. The Miami model links NPP to temperature and rainfall data; another scenario was also estimated, which consider addition of N and P fertilizers. FA was expressed in% of feed needed for animals, assuming grass intake - dry matter - of 2% of the body weight (BW) per day. The livestock unit for cattle was defined as an animal of 500 kg BW, and for sheep and goats as a 50 kg BW. Around 95% of the pasture land

occupied by ruminants showed 100% of feed availability, supporting the high potential of pasture intensification. Few places worldwide had less than 100% of FA, as found in west Latin America, north Africa and south Asia. However some of these places fit with high number of animals, which can be not sustainable. Considering the scenario with N and P fertilization, 43% of pasturelands with lower than 100% FA was improved to 100% status, e.g. in Africa and south Asia. The FA data is important to set the maximum attainable production in intensified pasture, linking grass productivity with animal product, which can reflect a better sustainability view of the pastureland.

Simulation of energy and carbon balances for two sugarcane plantations in southeast Brazil

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Brazil is currently the world's leading sugarcane producer. Although the culture presents excellent adaptation to the Brazilian's climate and relative high yield levels, the knowledge of the relations between the climate and sugarcane growth and yield are necessary to predict the sugarcane potential yield under current climate and climate change scenarios. We validated a process-based sugarcane model (included as a module within the Agro-IBIS dynamic agro-ecosystem model) against eddy covariance flux tower measurements, for the energy and carbon balance components, considering two growth cycles at each of two different sites. The USR flux tower measurements site (21°38'S, 47°47'W at 552 m altitude) covered the second and third re-growth (ratoon) in a sandy soil area from April 2005 to May 2007, and the attained yields were 83 and 62 t ha-1, respectively. The FAYS site (21o 57'S, 47º 20'W at 657 m altitude) was established in a clay soil area and represented the cane plant and the first ratoon, from October 2015 to July 2017 (yields: 169 and 112 t ha-1, respectively). For the USR site the model underestimated the net radiation and sensible heat fluxes by -0.64 and -1.88 MJ m-2 day-1, respectively (observed mean net radiation was equal to 9.42 MJ m2 day-1). Latent heat flux was overestimated by 1.24 MJ m2 day-1, equivalent to 0.51 mm day-1 of evapotranspiration (observed mean latent heat flux was equal to 5.0 MJ m2 day-1, or 2.04 mm day-1). For the FAYS site the model underestimated the net radiation and sensible heat fluxes by -0.41 and -1.15 MJ m-2 day-1, respectively (observed mean net radiation was equal to 10.5 MJ m2 day-1). Latent heat flux was overestimated by 0.5 MJ m-2 day-1, equivalent to 0.2 mm day-1 of evapotranspiration (observed latent heat flux was equal to 7.43 MJ m-2 day-1, equivalent to 3.03 mm day-1). Model simulates very close the observed differences in the net ecosystem exchange (NEE) of CO2 fluxes between the two sites. At USR carbon assimilation was water limited, mainly during the third ratoon cycle (2006 to 2007 growing season), and the predictions were in phase with the observed cycle exhibiting similar accumulated NEE fluxes. At the FAYS site sugarcane assimilation increased very fast in the months after planting/harvest, and relatively high values were observed during most of the cycle. For this site although the model simulated similar patterns, the predicted ecosystem respiration was very poor (underestimation) mainly for the periods before planting and after harvest, resulting in greater accumulated CO2 absorption along the cycles.

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

Socio-environmental idiosyncrasies associated with the discourse of the development and sustainability in the agribusiness - The case of ethanol expansion and food security in the State of São Paulo, Brazil

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The recognition of the environmental benefits from sugarcane ethanol, in recent decade has grown in the context of worldwide interest in renewable fuels to reduce pollution and climate-altering greenhouse gas (GHG) emissions. Considering all countries connected to the United Nations, Brazil is the country where renewable energy contributes to the largest part of the primary energy matrix. In this country, 42% of the percentage sum of all kinds of energies (nonrenewable and renewable) is composed by renewable energy; and bioenergy of sugarcane, alone, accounts for 18% of all energy sources. The Brazilian government forecasts that 45% of the internal energy supply, in 2024, will come from renewable sources, and it define biofuels as a necessary component of this transformation. The sugarcane ethanol is presented as a "Brazilian solution" to the problems of fossil fuel dependence and mitigation of climate change. Nevertheless, the virtues of ethanol as renewable fuel, low-carbon technology, and as positive solution to oil dependence and as environmentally beneficial products has been matter of controversy regarding the possibility of inducing food security. In such context, in the present paper, we examine the relation of the ethanol expansion with other food cultures in the state of São Paulo, the richest state of Brazil in terms of GDP, and responsible for the production of around 60% of all Brazilian sugarcane ethanol.

Vinasse on soil: influences on greenhouse gases emission and soil communities of denitrifying and methanogenic

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Vinasse is an important coproduct of the ethanol production from sugarcane. Due to this coproduct be rich in organic load and nutrients, such as nitrogen and potassium, vinasse is applied via fertigation to the soils cultivated with sugarcane. However, there is a lack of information about the effects of soil vinasse application in the microbial community of soil, as well as the greenhouse gases (GHG) emission. This work aimed to evaluate the impacts of the vinasse application on N2O and CH4 emissions and in the soil community of denitrifying and methanogenic bacteria. The field area was located in Piracicaba - SP (22º35'31 ''S, 47º38'23''O). The Sampling of GHG and soil were performed in a sugarcane area without burning with the application of different vinasse doses (0, 150, 300 and 450 m^3 ha^{-1}). Soil samples were collected in four periods after vinasse application (0, 7, 15 and 30 days) during the summer, in two consecutive years. GHG analyzed were N2O and CH4 by chromatography and the abundance of the genes *16S*, *mcrA*, *nirK* and *nosZ* by qPCR technique. Soil application of vinasse increased N2O emissions, especially, in the first couple of days after the application. On the other hand, CH4 flux was variable indicating the ability of the soil to serve either as source or sink. N2O emission factor obtained by vinasse application (dose of 300 m^3 ha^{-1}) was 0.07% in the first yr and 0.04% in the second yr. These emission factors were considered low, indicating that a little part of the nitrogen applied via vinasse was lost as GHG emission. The abundance of the genes indicated that vinasse application can significantly increase the amount of bacteria and *nosZ* gene activity in the soil. However, no

differences were observed in the gene expression of either *nirK* (potential for biological denitrification) or *mcrA* (responsible for CH4 reduction). Therefore, vinasse application impacted soil microbial community, stimulating the mitigate function genes, as well as, negatively to the soil GHG emissions, and increasing the emission of N2O. We highlight the need for new studies for a better understanding of the complex dynamics of carbon and nitrogen in the environment (soil and atmosphere) during the decomposition process of agricultural coproducts, such as vinasse.

Expansion dynamics of sugarcane production

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Brazil is the second largest producer of biofuel in the world and the largest producer of biofuel from sugarcane (ethanol). The potential of biofuels to reduce GHG emissions in Brazil is highly dependent on how the land use change caused by the sugarcane expansion occurs. The planning of this crop has a direct impact on the change in the GHG emissions amount, since sugarcane is the third largest crop planted in Brazil, occupying an area of 10.1 million hectares in 2015. Due to this crop expansion potential, the objective of this work is to analyze the driving forces of the expansion dynamics of sugarcane produced in Brazil. The methodology of this work was based on questionnaires applied to specialists in the sugarcane industry and subject to an extensive literature review. Of the 121 questionnaires sent, 33 responses were obtained (27.3%), of which 25 were from researchers in the area and 8 from mills directors and managers. For the researchers, the driving forces that most influenced the expansion dynamics were: slope (72%), harvest distance to the mill (68%), domestic ethanol market (64%), land price (56%), and external sugar market (56%). Among the mills directors and managers that answered the questionnaire, driving forces that stood out were: distance from harvest to plant (87.5%), slope (87.5%), soil quality (75%), road infrastructure (75%), and land price (50%). This difference is due not only to the amount of answers obtained from each group, but also because of differences in priorities between each group when analyzing the sugarcane expansion. To complement the results of the questionnaires, different driving forces that influence the spatial dynamics of sugarcane cultivation were identified and analyzed through literature review. They are: ethanol and sugar market, agroecological zoning for sugarcane (ZAE), agroenvironmental zoning for the sugar and ethanol industry sector of the São Paulo state (ZAA), environmental code and its constrains, land use, land price, slope, distance between harvest and mill, and road infrastructure to the flow of the final product. Finally, to analyze the change in land use caused by the expansion of sugarcane cultivation, one should bear in mind that all the driving forces mentioned above influence this dynamic, being through the location or quantity of expanded area. Some driving forces, as observed in the questionnaires, are considered more than others in planning, and therefore have a greater weight in decision making, usually only related to the cost of production, leaving aside social and environmental issues.

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

An environmental performance assessment of collective urban transport in the state of São Paulo: a diesel and battery electric bus life cycle approach

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In order to reduce the potential environmental impacts caused by the collective urban transportation sector, technologies have been developed and improved to replace or complement the use of fossil fuels in urban buses. Biodiesel, an alternative to fossil diesel, can be used in internal combustion engines to entirely or partially replace diesel oil. The complete replacement of diesel oil with biodiesel, especially in future technologies, would require engines that are designed exclusively for this purpose, given the susceptibility of premature wear on engine components. Electric buses have been considered to be viable alternatives to conventional powertrains in light of several factors. For example, electric engines offer greater efficiency and produce less noise than internal combustion engines; provide higher torque at low speeds, resulting in a better acceleration out of repose; and increase energy efficiency in the regenerative brake. Therefore, this paper used a Life Cycle Assessment (LCA) to carry out a comparative analysis of the potential environmental impacts of bus-based passenger transport in the state of São Paulo, by evaluating (i) conventional internal combustion buses; and (ii) plug-in electric buses. The aim of this analysis was to consider not only energy sources (the "well-to-tank" step), but also their uses (the "tank-to-wheel" step) and productive processes. The LCA was conducted using SimaPro software, widely used in these types of studies; with regionalized data from the Ecoinvent database, a reliable and traceable information system; and CML-IA as life cycle impact assessment method, with global application and an approach directed to midpoint level impacts. The systems used in the analysis were: collective urban passenger transport by internal combustion buses, with SCR+ARLA32, using diesel S-10 (B7) and biodiesel (B100); and collective urban passenger transport by Li-ion battery electric buses, with plug-in recharge measured during times of average and high electricity demand. The results showed that, when the two electricity generation profiles were considered for electric collective transport systems, similar conclusions were reached, since relatively small variation was present in both generation profiles. However, for transport using internal combustion, the biodiesel life cycle of soybean diesel or biodiesel (B100), when used in urban buses in the state of São Paulo, presented worse environmental performance than the diesel blend (B7) in five categories: Abiotic Depletion; Freshwater Ecotoxicity; Terrestrial Ecotoxicity; Acidification; and Eutrophication. In the other categories, diesel (B7) had higher potential environmental impacts than biodiesel (B100), and demonstrated worse performance than electrical motorization (both recharge profiles) in Abiotic Depletion (fossil fuels), Global Warming, and Ozone Layer Depletion categories. Electric buses had their environmental performance results influenced with buses production, especially to the battery one.

Interventions of sugar-based energy sector prices on sugarcane plantation area

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Historically, the sugarcane industry has been an important play in the Brazilian agribusiness that has gained notoriety with the increasing global demand for renewable energy sources. The

significant expansion of sugarcane cultivation shows the extent that theis sector has in the current scenario. Brazil's production capacity is indisputable, however, there are still differences regarding the sugarcane products prices behavior. It is noteworthy that the decisions within the sector are done in the context of free pricing, but the market ambient has been heavily influenced by exogenous influences, such as deregulation of the market and launch of flex-fuel vehicles. However, it is possible that the producers decisions are been guided, predominantly, by the market prices. Within this context, this study seeks to identify whether there is an influence of sugar, ethanol, and sugarcane prices on the sugarcane planted area in Sao Paulo state, from 1995 to 2015. The methodology used was the one proposed by Box-Jenkins, Transfer Function, which is a multivariate time series method and has advantages over traditional methods of estimation. The results showed that all prices have influenced the increase in sugarcane area, in particular the sugar's price. There was verified the existence of two-way effects, in which, the area affected the prices and these influenced the area. To conclude the study, analyzes of interventions were carried out for the main occurred events in the sugar-energy market in the years under study. From this analysis, only the sugarcane area was significant, being influenced by the interventions.

Implications of N-fertilizer application method on N_2O emissions and biomass production in sugarcane fields

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The use of N-fertilizer in sugarcane fields has raised environmental issues regarding the sustainability of sugarcane-based ethanol, because it is a major source of N2O emissions. Currently, 85% of sugarcane areas in south central Brazil are mechanically harvested without burning and thereby result in large amount of straw retained on the soil surface. Certain challenges still remain regarding sugarcane management, such as the best method to apply N fertilizer once straw layer may hamper its contact with soil. This study aimed at evaluating the effects of different N fertilizer application methods on N2O emissions and sugarcane production. Additionally, N2O emission factor and emission intensity were determined for each method of N fertilizer application. The experiment was conducted during two crop years in an Oxisol under sugarcane cultivation in Iracemápolis, São Paulo, Brazil. The treatments were comprised of three most common N-fertilizer application methods (above straw, below straw and incorporated into the soil) in sugarcane fields. For all treatments, it was maintained 12 Mg ha-1 of dry straw on the soil surface and N-fertilizers were applied at a rate of 120 kg ha-1. N2O emissions were measured along the 2014/2015 and 2015/2016 crop seasons using static chambers methodology. Chambers were installed in fertilized band and in the mid row to better represent emissions from sugarcane fields. The N2O measurements were taken three times per week during the first 90 days after fertilization. After that period, they were spaced according to stabilization of the gas flux until to the harvest date. The gas samples were stored in pre-evacuated vials and analyzed in a Shimadzu gas chromatograph (GC-2014). At the end of each crop cycle, the plots were mechanically harvested and the stalk biomass of each plot was automatically transferred to a wagon fitted with a balance designed specifically for weighing biomass. N2O emission were significantly higher in the 2015/2016, corroborating with the year of higher rainfall. On average, N2O emissions from the treatments incorporated and below straw were

reduced by 56% and 41% in relation to above straw application (baseline scenario). N2O emission factors were estimated at 0.62, 0.36 and 0.24% for 2014/2015 crop season, and 1.41, 0.34 and 0.27% for 2015/2016 associated with the treatments above straw, below straw and incorporated into the soil, respectively. Sugarcane yield showed the same tendency across the assessed treatments in both years, indicating average increases of 18 and 13% when N-fertilizer was applied incorporated into the soil and below straw in comparison with above straw application, respectively. The placement of N-fertilizer in direct contact with the soil increase biomass production, reduce N2O emissions and consequently attenuate the emission intensity of sugarcane biomass, which are key opportunities to be considered towards cleaner production of sugarcane ethanol

Spatial assessment of the techno-economic potential and cost-supply curves of sugarcane straw

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Improvements in on-farm sugarcane operations are relatively recent in Brazil and its co-benefits are not entirely known. Since the phase out of sugarcane burn harvest, sugarcane straw has been targeted as a potential biomass source to supply the growing national bioenergy demand. Differently from sugarcane bagasse, straw recovery requires spatial-based information that are usually neglected in techno-economic assessments of straw to bioenergy. Hence, our study aims to assess the techno-economic potential and the cost-supply curve of sugarcane straw for all the 174 operating sugarcane mills from state of São Paulo in 2012 crop-year, taking into account the spatial variation of yield levels and the location of the mills. The geographically driven assessment was carried out through a combination of spatial datasets, sizing a straw collection radius around the mills containing fine-scale information on straw yield and recovering distance at pixel level. Based on literature surveys, we sketched a moderate scenario of straw technical potential assigning straw-to-sugarcane ratio at 140kg.t^{-1} and that 5.4t.ha^{-1} of straw should be necessarily left on the field for no-till purposes. To calculate the straw recovery costs, we used baling system route as reference accounting both straw farm-gate and transportation costs. In addition, a labeled "affordable" straw recovery cut-off cost at 30US$.t^{-1} was established to quantify the economic potential. As a result, we primarily found that the sugarcane mills altogether have 24.4Mtstraw technical potential in São Paulo that could be used for energy purposes. Among the mills, straw technical potential ranges between 5 to 545ktstraw, while the average straw recovery costs varied from 23.1 to 43.3US$.t^{-1}. Based on average cost-supply analysis at regional level, the results have shown that mills with significant straw supply (> 100ktstraw) have presented average straw recovery costs between 27 - 33US$.t^{-1}. Most of these Mills are installed in the east part of São Paulo, being strongly influenced by straw yield and the ensuing farm-gate cost. Conversely, low supplier mills (< 50ktstraw) have presented either extreme high (>35US$.t^{-1}) or low (<25US$.t^{-1}) average straw recovery costs. For mills with poor cost-supply performance, recovering distance greater than 50km were accounted as the key factor incurring to high recovery costs. In general, part of these mills were identified in the west of São Paulo where the sugarcane fields are rather sparse due to competition with other land use functions. Moreover, we estimated that 16.9 out of 24.4Mtstraw of technical potential were classified as economic potential. These outcomes are available at pixel level aggregated in each sugarcane mill of São Paulo, which is

decisive information for decision-makers at mill level as for regional bioenergy planners.

Scientific and technological activities in biofuel R&D: countries and research areas

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The aim of this study was to provide a general overview of how the development of knowledge in biofuelis related to international research networks, whose role is to diffuse this knowledge while stimulating the emergence of a new paradigm. The present paper aims to answer two questions: a) Is there an emergent pattern in scientific collaboration towards a convergence in investigations related to "enabling technologies" and simultaneously to a specialization in themes of specific raw materials (generating sub-networks whose dimension is regionally constrained)? b) What role do second and third generation ethanol play in redesigning the patterns of scientific international collaboration? The methodology is based on Bueno et al. (2017) which provides an analytical treatment to the matrix of co-occurrence of scientific collaboration of countries/organizations using the software Pajek. Two procedures were carried out: a) block modeling; b) comparisons between general and sub-networks. The identification of key actors, papers and patents enabled the analysis of changing patterns and the correspondence between scientific activity and patenting in biofuels. The results show a scientific collaboration is growing fast and new areas of research have been incorporated, generating variety. The following considerations are highlighted: i) The block models of knowledge analyzed over different time periods showed that the paradigm is in development. A more robust appearance of the networks in terms of collaboration structure and production of articles, together with the insertion of hundreds of countries, took place after the 2000s, in which 4 decades were necessary to reach the current stage of development. ii) The technological areas of the patents are interdependent, as can also be observed in the knowledge networks of the articles. This is an extremely important result, given that fundamentally, in this case, it leads to technological variety. Interdependence between the areas generated technological variety for third generation ethanol (from algae) on the sugarcane network, in the same way in which biotechnology generated technological variety for second generation ethanol. iii) This more robust structure of co-authorship of articles and interdependence between areas of knowledge could indicate a pattern related to the paradigm's development, in which international research collaboration networks appear as a structure to regulate the development of these products of knowledge. Thus, these observations lead us to believe that the adoption of international networks conditions the parameters of technological progress and variety, and not the contrary. Countries can increase technological opportunities as a result of the diffusion of knowledge in the network, given that it transmits and absorbs knowledge. This effect, of action and reaction, since all of the agents compete with each other, generates technological variety and boosts the progress of the paradigm in development, therefore establishing routes and technological trajectories.

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The Brazilian sugarcane agroindustry labor market: analysis from the recent expansion to the crisis

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The growth in demand and production of sugar and ethanol since around 2000 has transformed

Brazilian agricultural landscapes, with the growth of sugarcane cultivation. The optimistic and expansionary scenario to the sector was interrupted in 2008 and, since then, there has been a crisis, related to the low remuneration of the final products, with the occurrence of many producing units closing. In addition to these movements of economic growth and decline, the sector has undergone a technological revolution – the mechanization of the harvest, which occurred with the restriction on burning in the sugarcane process. In this scenario, Brazilian sugarcane agroindustry labor market was expressively affected and also experienced changes, and this study aims to evaluate this from 2000 to 2014. As sugarcane agroindustry, we mean the agricultural, industrial and administrative sub segments related to the production of sugarcane, sugar and ethanol. The analysis is based on microdata from the Annual Social Information (RAIS-MTE) and the National Sample Survey of Households (PNAD-IBGE) and we seek to bring the literature to a greater understanding of the sector's labor market changes in terms of numbers of workers, income and other socio-economic indicators. This paper also brings as contribution to literature a new methodology of classification of workers within the sector. Briefly, we use the activities developed by workers rather than the National Classification of Economic Activities (CNAEs) to classify, as is usually done - which has important implications mainly regarding jobs in the cane in the field. As results, we find that between 2000 and 2008 the number of formal jobs in the sector doubled and from this year to 2014 there was a 27% reduction; for the same comparison periods, average real wages increased by about 3% a year for the first and by almost 5% per year for the second. Between 2000 and 2008, the formal jobs have grown in all analyzed sub segments; from 2008 to 2014, otherwise, industrial and administrative jobs increased a little while for agricultural jobs there has been a 35% decrease. Despite this decline in quantity, average real wages have increased about 40% from 2008 to 2014 for the agriculture workers, indicating an evolution in the quality of jobs. This last result is related with the change in the profile of cane workers, mainly due to the mechanization process (which led to the replacement of sugar cane cutters by more skilled workers). As for the year of schooling, the reduction in the number of workers was 44% for those with up to 9 years of study (2008-2014). For those with 10 to 12 years of schooling, there was an increase of 35% and, for those with 13 years or more of study, a 72% increase.

Straw removal and nitrification inhibitor as mitigation strategies to N_2O emission in sugarcane fields

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Nitrogen (N) fertilizers are responsible for 40% of N2O emissions in sugarcane production and the maintenance the straw after harvest can increased such emissions. However, some management practices, such as straw removal and the use of nitrification inhibitors (NI) could result in significant N2O mitigation strategies. The present study aimed to quantify N2O emissions in the presence of different amounts of sugarcane straw, N fertilizer and NI in a commercial sugarcane area in Campinas - Sao Paulo - Brazil. Two field experiments were set up (results of only one is reported here) in a randomized block design with three replicates and a factorial 4x3, composed by four rates of straw (without straw, 5; 10, 15 Mg ha^{-1} in dry basis) and three N fertilization treatments as ammonium sulfate (without nitrogen; 120 kg N ha^{-1}; 120 kg N ha^{-1} + NI). The NI used was dicyandiamide in a concentration of 10% of the N-fertilizer applied. Nitrous oxide emissions

were measured along the 2016/2017 crop season using static chambers methodology three times a week or after rainfall exceeded 5 mm during the first 90 days after fertilization. After that, samplings were spaced according to stabilization to the gas flux. Chambers were installed in the fertilizer band application and in the mid row in order to represent the sugarcane fields emissions. The results showed that N2O-N emissions increased linearly with the amount of straw left on the field (from 0.8 to 2 kg N2O-N ha^{-1} in the treatments without straw and 15 Mg straw ha^{-1}) in all N fertilization scenarios (without N, with N and with N + NI) indicating that the straw removal can be an effective emission-relieving agent. Our results showed that regardless the amount maintained on the field, the addition NI reduced the N2O emission in 90%. For all the straw layer and N fertilizer treatments the N2O emission factors (from 0.12 to 0.82%) were smaller than that proposed by IPCC. The maintenance of straw increased sugarcane yields up to 10 Mg ha^{-1} of straw; the use of NI did not influence sugarcane yield. The N2O emission intensity, showed that the association of large amount of straw (15 Mg ha^{-1}) and N fertilizer resulted in higher emission (7.0 as opposed 4.5 kg of CO2 eq Mg of sugarcane $stalk^{-1}$ for the treatment without straw). Conversely, the use of NI reduced the emission intensity in 7%. We concluded that removal of sugarcane reduces N2O emission and sugarcane yield; the intensity emission is a useful indicator of such changes. The use of NI is a good strategy to mitigate N2O emission regardless of the straw amount without affecting sugarcane yields.

Organic carbon stock in the soil at a sugarcane cultivation area under different management systems

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The intensive cultivation of sugarcane characterized by monoculture with strong soil plowing and machine traffic has resulted in a fast process of soil degradation. In contrast, the organic matter of the soil is an essential component to maintain the production capacity of the soils. However, it is necessary for sugarcane areas to have wider studies on the most satisfactory combination of cover plants to be used during the off-season and the soil tillage system for sugarcane cultivation in order to make full use of the benefits of the system as well as improve productivity, profitability and sustainability. Therefore, the objective of this paper is to assess the effect of different cover plants and soil tillage systems on the organic carbon stocks in an area cultivated with sugarcane. The study was carried out in field conditions, at Santa Fé Mill at an experimental area in the municipality of Ibitinga, São Paulo. Because of practical matters, the experiment was conducted through a design of continuous ranges, with four treatments (cover plants) on the horizontal ranges (Crotalária juncea IAC KR1, millet BRS 1501, peanut Runner IAC 886, and sorghum BD 7607) as well as four treatments (soil tillage) on the vertical ranges (conventional tillage, minimum tillage, no-tillage, and deep tillage). Each block had three repetitions and each portion was constituted of six lines of cultivation with spacing of 1.50 m and 30 m long, encompassing an area of 300 m2. The experiment started in December 2014 with the collection of soil for characterization of the area. The cover plants were seeded in December 2014 and managed until April 2015. The sugarcane cultivation occurred in April 2015 with the variety of sugarcane CTC 4. In the end of the first sugarcane cycle, a new soil sampling was carried out to assess the effect of the treatments on the organic carbon stocks of the soil. The results of this study indicate that each cover plant required a specific soil tillage system along the sugarcane cultivation in order to maintain or even increase the carbon stocks of the soil. For sorghum, the deep tillage system is more adequate, while for Crotalaria, peanut and millet, conservationist tillage systems, such

as no-tillage and minimum tillage had better performance.

Evaluation of water footprint of sugarcane and other crops using basin level water balance components

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Recent sugarcane expansion has been raising concerns about its impacts in water availability. However, there is no consensus regarding impacts of sugarcane in water availability as hydrological processes were complex and depend on several economic and edaphoclimatic conditions. Water Footprint (WF) is one of the most applied indicators to assess water use along the supply chain of products (e.g., of energy carriers, crops and biofuels), as well as for the evaluation of water use efficiency and sustainability within river basins. However, several criticisms have been raised when employed for the assessment of water use sustainability as the indicator itself brings no measure of the impacts on water availability. Unlike other environmental indicators, such as the carbon footprint, this assessment must consider regional conditions of the river basin. This work proposes a new approach for the estimation of water footprint of crops, based on river basin water balance and its components in attempt to embed the basin water availability in the WF values. The new approach accounts not only for the crop evapotranspiration but the difference between this component and the water yield values. This work presents case studies for two Brazilian basins, one in Goiás (FMA) and another in São Paulo (MM) state, for which the water balance components were estimated using a calibrated and validated SWAT (Soil and Water Assessment Tool) model. With this new approach, the WFs of sugarcane and annual crops were the smallest ones in both basins, indicating that they contribute less than pasture and perennial crops to basin water scarcity. The new WF values for FMA were lower than those for the MM basin, suggesting that crop cultivation is less impacting in the former than in the latter. The incorporation of the water balance components in the WF estimation is meaningful since it considers basin physical issues related to the land use/land cover, soil characteristics, topography, slope and geology through the inclusion of evapotranspiration, surface and subsurface runoff and return flow. This enables the direct comparison of the impacts of different crops, both at the same basin and across different basins. However, the evaluation of other basins and cultivation practices, such as irrigation, are necessary to further elaborate and validate the new proposed approach.

São Paulo state potential to sugarcane ethanol and cattle integration

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Biofuel production is increasing mainly due to Climate Change concern, to replace fossil fuel energy and decrease greenhouse gases emission. United States is the largest ethanol (15,330 million gallons in 2016) and beef producer (11.8 million ton in Carcass Weight Equivalent - CWE) in the world. The country produces beef and ethanol in integrated system using corn ethanol by-products as animal feed. Brazil, second ethanol producer (7,295 million gallons in 2016) and second beef producer (9.5 million ton in CWE) also has potential to integrate mainly in São Paulo state. This state is the largest sugarcane (56.3%), sugar (62.2%) and ethanol (49.3%) producer in Brazil. In São Paulo state sugarcane occupies 4.8 million hectares corresponding to 52% of Brazilian total sugarcane area. On the cattle side, the state has about 11 million heads, the largest amount of

cattle finished in feedlots (8.7% of Brazilian total) and the fourth largest slaughter rate in the country (around 3 million head which corresponds to 10% of total slaughtered heads). The integration can be done with exclusive feed in feedlots using ethanol by-products, with a 50 km average distance radius between cattle feedlots and sugarcane plant, according to real cases of integration. The objective of this work is to assess the life cycle and economic feasibility of sugarcane ethanol and beef cattle integration system in São Paulo state. For that, an integrated scenario was defined using Virtual Sugarcane Biorefinery from Brazilian Bioethanol Science and Technology Laboratory (CTBE/CNPEM). The studied scenario has an sugar-ethanol plant with 6.8 million tonnes of sugarcane milling capacity per year and 100,000 cattle heads fed in feedlot with ration composed by ethanol by-products, corn, soybean bran and others. The integration was considered techno-economic and environmental feasible. Brazil has a huge potential to integrate beef and ethanol production using an adaptation of Unites States model, and São Paulo state is probably the best location to consider this new strategy.

Biofuel solutions in countries at different levels of development: case studies of Brazil, Colombia and Mozambique

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Production of biofuels has large economic and environmental importance in the global market and for regional development strategies of many countries. Major efforts are dedicated to biofuel production studies, ranging from the feedstock choice and its management options to the definition of different production technology routes, marketing strategies and uses of the products. Countries at different development levels such as Brazil, Colombia and Mozambique are looking for solutions to start or expand sustainable biofuel production and, therefore, guarantee economic, environmental and social benefits to its population. This study uses an example of the ethanol and electricity production from sugarcane to show that biofuels production in the three countries should be associated with the level and strategy of development for each country. Main differences considered in the evaluation were the agricultural production system (including sugarcane yields, management practices, harvesting, among others); industrial technology and production scale (including micro-distilleries, vinasse destination, second generation ethanol production, gas and solid biofuels as by-products, among others); and products demands and uses (including fuel matrix, fuel and electricity prices, use of ethanol for cooking, exports potential, among others). Using an innovative framework, the so called Virtual Sugarcane Biorefinery (VSB) developed by the Brazilian Bioethanol Science and Technology Laboratory (CTBE), to perform economic, environmental and social analysis of the biofuel production scenarios, it was possible to compare different realities of these countries showing that there is no “one size fits all” solution. Different levels of development require different solutions regarding biofuels production systems.

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PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE 2017 (BBEST2017)

- DESIGNING A SUSTAINABLE BIOECONOMY -

Metals adsorption by crosslinked chitosan beads in sugarcane contaminated stream sediments

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Brazil is the biggest sugarcane producer in the world, followed by India, China, Pakistan, Mexico, Colombia, the Phillipines, Australia, Indonesia and the United States. The projection for 2016/2017 is that Brazil will reach 680 million tons of sugarcane production. In the southeast region of Brazil, specifically in the state of São Paulo, sugarcane culture has an annual production of about 370 million tons covering an area of 5 million of hectare. Considering that fertilizers applied to sugarcane cultivation contain metal ions, the superficial runoff can carry them to the aquatic sediments. Chitosan beads, an alternative biopolymer that exhibits a high affinity for metal ions, are easy to prepare in laboratory conditions and low overall cost, are indicated for metals removal from aquatic sediments. This work studied contaminated stream sediments located in adjacent areas to sugarcane cultivation, without riparian vegetation. The sediments were collected from four streams, historically impacted by sugarcane activity; one of them located in a preserved area (control site). The sediments were evaluated for adsorption of Cr, Cu, Zn, Mn and Mg. The results showed that the maximum adsorption in chitosan beads (containing only 5.5% of chitosan) for metals were obtained in Sao Joao Stream, such as: 0.65 mg.kg-1 for Cr6+, 2.85 mg.kg-1 for Cu2+, 2.5 mg.kg-1 for Mg2+ and 0.85 mg.kg-1 for Zn2+. For manganese, the maximum adsorption was 0.84 mg.kg-1, for Agua Sumida Stream. The adsorption was related to initial metal concentration present in sediment and high affinity for specific metals. Chitosan presented potential and feasibility for use in the remediation of aquatic sediments systems, with good adsorption capacity and ability to be applied “in loco”.

Hydrological regime analysis of the Capivari river basin utilizing SWAT

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In Brazil the water is an abundant natural resource. However, due to spatially different distribution, disorderly growth of cities and environmental degradation by industry and agriculture, there are problems about water scarcity. These changes in soil land use have, as a consequence, a decrease in river flow, altering the hydrological behavior of the hydrographic basins. The study of the impacts of land use change on river flow is expensive and time consuming. In this context, hydrological models are an important tool for the development of better water conservation techniques. The Soil and Water Assessment Tool (SWAT) is a hydrological model with potential to be used in studies of water quantitative analysis in a river basin. This study aims to simulate a scenario for a region of the Capivari River basin (State of Sao Paulo - Brazil) and verify the reliability of the model. For the use of the model several data are required: topography, land use soil, pedology, hydrography and climatic data such as precipitation, temperature, wind, humidity and solar radiation. The meteorological database was obtained from six stations. It is important that these stations contemplate the historical series for the study. For the statistical analysis of the model were used the fluviometric data of the station operated by the National Water Agency with the coordinates 22°57'32.026 "S and 47° 17'47.047" W. The period used for the data collection was from 1995 to 2007, with two years for the heating of the model, six years for the simulation and five years for validation. The application of the

model requires the input data in spatial format, and this can be made with an interface between the model SWAT and the Geographic Information System (GIS). After the data entered in the SWAT the scenario can be carried out and the results of the simulations are presented in tables, graphs and maps. The data obtained, monthly from the flow after the simulation, are compared with the data observed in the studied basin. For the model statistical analysis, Nash coefficient and Sutcliffe and the deviation of the simulated data in relation to the observed data were used. The results indicate that the model has a good representation for studies related to the water regime of an area of the Rio Capivari/SP. This study shows the importance of the use of hydrological models as allies in the research to monitor the behavior of the water regime in watersheds. The results show a good simulation result of the SWAT model.

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