Brown-rot fungi biomimetic pretreatment to bioethanol production from wood

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Introduction

The conversion of lignocellulosic biomass into ethanol presents important technical and economic challenges that have limited its implementation and commercialization, among them, the necessity of a pre-treatment which represents 20% of the total cost and has an enormous significance in the further steps of the process. Pretreatment is necessary since lignin and hemicelluloses form a structural network that encapsulate cellulose microfibrils, preventing the access of cellulases to the cellulose chains. Fungal pretreatments have been proposed mainly using white-rot fungi to decay lignin and expose cellulose fibers, however, brown-rot fungi (BRF) have an even better wood deconstructing machinery where is possible to observe losses in mechanical properties before that a loss mass can be detected. The wood biodegradation system of BRF starts with non-ezymatic oxidative reactions that result in an efficient process that facilitates the further enzymatic hydrolysis. BRF developed a mechanism that reduce Fe (III) to Fe (II) and produce hydrogen peroxide, which are the Fenton reaction reagents. In Fenton reaction ·OH radical is produced by the reduction of H_2O_2 and oxidation of Fe(II), in an acidic medium (Fe(II) + H_2O_2 $\rightarrow$ Fe (III) + •OH + OH-). The oxidative reaction produces a progressive decrease in wood strength due to selective carbohydrate removal, mainly hemicelluloses. A synergy between the pretreatment of lignocellulose by brown rot fungi and saccharification was recently reported. Is it possible to biomimic the BRF process and use it as pretreatment. Its disadvantage is the modifications on the reducing end of cellulose chains, the carbonyl group of the reducing end change to carboxylic acid. This change is not recognized by the commercial enzymes, mainly cellobiohydrolases from *Trichoderma reesei*, and consequently the fermentable sugar yields decrease.

Results and Conclusions

To evaluate the effect of oxidation on saccharification, two different substrates, alpha-cellulose and holocellulose from *Pinus radiata*, were oxidized by Fenton reaction in four different levels. After oxidation, the substrates were hydrolyzed with cellulases from brown rot fungi, because their enzyme complex is adapted at oxidized substrate, also, commercial cellulase (Celluclast 1.5 L. Novozyme) from *T. reseei* and, a mixture of both, were used to compare the efficiency of hydrolysis. First, the two substrates were impregnated with 0.05, 0.1, 1.0 and 2.0 mM Fe (II). The impregnated samples were oxidized with 0.1, 0.2, 2.0 and 4.0% of H_2O_2, respectively. The oxidation was evaluated with FT-MIR, to determine the increase of carboxylic acid on the substrate, and Elemental Analysis to determine additions of oxygen on the substrate. The results showed a increase in the band at 1710 cm^{-1} in both substrate, with the increase in the concentration of Fenton reagents, this implies an increase of carboxylic groups. Intrinsic viscosity, showed a significant decrease on depolymerization degree, in both substrates. The molar mass decrease was 33, 52, 74 and 85% in holocellulose and 21, 43, 57 and 62% in alpha-cellulose, with increasing the concentration of Fenton reagents. Hydrolysis assays showed that the activity of cellulases from *Gloeophyllum trabeum* are unaffected by the oxidated substrates as opposed to commercial cellulases, however, a mixture of both enzymes, increased the saccharification efficiency. The best hydrolysis, in holocellulose and alpha-cellulose was on the substrates oxidized with 1.0 mM Fe (II) with 2.0 % H_2O_2 and 2.0 mM Fe (II) with 4.0 % H_2O_2. The samples of holocellulose were hydrolyzed with 129 UCMC from *G. trabeum* and 32.2 UCMC (0.13 FPU) from Celluclast 1.5 L and 161 UCMC from *G. trabeum* were used to alpha-cellulose. A pretreatment that combine wood oxidation followed by hydrothermolysis or organosolv pretreatment could be a promising alternative to reduce the severity of the process and cellulases consumption.

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A list of recently published articles by the main author of this proposal is presented bellow:

1. Chemical composition and wood anatomy of *Eucalyptus globulus* clones: variations and relationships with pulpability and hansheet properties. Ramírez M., Rodríguez J., Balocchi C., Peredo M., Elissetche JP., Mendonça R., Valenzuela S. Journal of Wood Chemistry and Technology 29(1): 43-58 (2009).
2. Wood anatomy and biometric parameters variation of *Eucalyptus globulus* clones. Ramírez M., Rodríguez J., Peredo M., Valenzuela S., Mendonça R. Wood Science and Technology 43(1-2): 131-141 (2009).
3. Tribromophenol Degradation by a Catechol-Drive Fenton Reaction. D. Contreras, K. Rojas, J. Freer, J. Rodríquez. J. Chil. Chem. Soc. 54(2): 141-143 (2009).
4. Evaluation of a combined brown rot decay–chemical delignification process as a pretreatment for bioethanol production from *Pinus radiata* wood chips. Antonella Fissore, Lissete Carrasco, Pablo Reyes, Jaime Rodríguez, Juanita Freer and Regis Teixeira Mendonça. J. Ind. Microbiol. Biotechnol. 37: 893-900 (2010).
5. Behavior of *Ceriporiopsis subvermispora* during *Pinus taeda* biotreatment in soybean-oil-amended cultures. Aguiar, Andre, Mendonca, Regis, Rodriguez, Jaime, Ferraz, Andre. International Biodeterioration & Biodegradation 64(7):588-593 (2010).
6. Linoleic acid peroxidation initiated by Fe3+-reducing compounds recovered from Eucalyptus grandis biotreated with *Ceriporiopsis subvermispora*. Horta, Maria Augusta; Masarin, Fernando;, Rodriguez, Jaime; Ferraz, Andre. International Biodeterioration & Biodegradation 65(1):164-171 (2011).
7. Transcript abundance of enzymes involved in lignin biosynthesis of *Eucalyptus globulus* genotypes with contrasting levels of pulp yield and wood density. Juan Pedro Elissetche & Sofía Valenzuela & Renán García

& Marcela Norambuena & Carolina Iturra & Jaime Rodríguez & Regis Teixeira Mendonça & Claudio Balocchi. Tree Genetics & Genomes. En Prensa (2011)

Book Chapter

1. Tree Endophytes and Wood Biodegradation. Jaime Rodríguez, Juan Pedro Elissetche and Sofía Valenzuela. En: Endophytes of Forest Trees: Biology and Applications. Series: Forestry Sciences, Vol. 80. Pirttilä, Anna Maria; Frank, A. Carolin (Eds.) ISBN: 978-94-007-1598-1