Fundamental insights to the AFEX process that catalyzes the rapid deconstruction of lignocellulose to fuels and chemicals

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Introduction

The world is in the early stages of transitioning from a fossil fuel driven economy to one that is supplied by a portfolio of more renewable and sustainable options. The annual solar energy captured by non-food plant biomass ("cellulosic biomass"), such as grasses, woody materials, etc., is nearly ten times that of the total energy used by humans. Thus cellulosic biomass will undoubtedly play an important and increasing role in our future energy portfolio. Valorization of cellulosic biomass is expensive and inefficient due to the native recalcitrance of plant cell walls to enzymatic and microbial degradation. Three primary barriers to biomass degradation are; (1) cellulose crystallinity, (2) lignin-carbohydrate cross-linking, and (3) poor accessibility to enzymes and microbial systems. Thermochemical pretreatment by acid, base, organic solvents and ionic liquids helps overcome these barriers with varying degrees of success. We highlight a promising strategy for pretreating lignocellulosic biomass using ammonia (NH_3) as a pretreatment reagent to activate its deconstruction to fuels and chemicals.

Results and Conclusion

We use anhydrous liquid ammonia to alter the cellulose nanofibril hydrogen bonding network from its native crystalline state (cellulose I_{β}) to produce an alloform (cellulose III_{I}) that enhances its hydrolysis rate by up to 5 fold. Endocellulases, with deep and open–cleft substrate–binding sites, in combination with processive exocellulases had the highest synergistic activity on this alloform. Restructuring the hydrogen bonding network within crystalline cellulose with ammonia overcame the rate–limiting step for enzymatic depolymerization. Biomass pretreatments employing ammonia (e.g., AFEX or Ammonia Fiber Expansion) allow subtle morphological and physicochemical changes that enhance enzyme accessibility without extracting lignin and hemicellulose into separate liquid streams.

Multi-modal characterization of ultrastructural changes within ammonia treated plant cell walls was performed by various techniques to gain insight into the mechanism of AFEX pretreatment (e.g., LSCM, Raman spectroscopy, AFM, SEM, TEM, NMR, ESCA). AFEX cleaves lignin-carbohydrate ester linkages via ammonolytic reactions and thereby facilitates the extraction and redeposition of cell wall decomposition products (e.g., amides, arabinoxylan oligomers, lignin-based phenolics) on outer cell wall surfaces. Nanoporous tunnel-like networks, as visualized by 3D-electron tomography, are formed within the cell walls as a result of the extraction/redeposition process. This highly porous structure enhanced cellulase accessibility to embedded cellulosic microfibrils. AFEX pretreated biomass was also readily fermentable without washing or nutrient supplementation, unlike other pretreated feedstocks (e.g., dilute acid treated) that needed both extensive detoxification and external nutrient supplementation to support microbial catalysis to produce high titer end-product yields (e.g., ethanol).

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Author Publications

1. Chundawat SPS, Beckham GT, Himmel M, & Dale BE (2011) Deconstruction of Lignocellulosic Biomass to Fuels and Chemicals. *Annu. Rev. Chem. Biomol. Eng.* 2 (Review ahead of print; DOI: 10.1146/annurev-chembioeng-061010-114205).
2. Chundawat SPS*, et al.* (2011) Multi-scale visualization and characterization of plant cell wall deconstruction during thermochemical pretreatment. *Energy & Environmental Science* 4(3):973 - 984.
3. Chundawat SPS, et al. (2011) Restructuring crystalline cellulose hydrogen bond network enhances its depolymerization rate (*Manuscript under review*).
4. Lau MW, et al. (2011) An integrated paradigm for cellulosic biorefineries: Utilization of lignocellulosic biomass as self-sufficient feedstocks for fuel, food precursors and saccharolytic enzyme production (*Manuscript under review*).
5. Eranki P, et al. (2011) Advanced Regional Biomass Processing Depots - A key to the logistical challenges of the cellulosic biofuel industry. *Biofuels, Bioproducts, and Biorefining* (*submitted*).
6. Chundawat SPS, et al. (2010) Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and dilute-acid based pretreatments. *Bioresource Technology* 101:8429-8438.
7. Dale BE, et al. (2010) Biofuels Done Right: Land Efficient Animal Feeds Enable Large Environmental and Energy Benefits. *Environ. Sci. Technol.* 44:8385-8389.
8. Lau MW & Dale BE (2009) Cellulosic ethanol production from AFEX-treated corn stover using Saccharomyces cerevisiae 424A(LNH-ST). *Proceedings of the National Academy of Sciences of the United States of America* 106(5):1368-1373.