# MBI BIOREFINERY:
# CORN TO BIOMASS, ETHANOL TO BIOCHEMICALS AND BIOMATERIALS

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## Final Technical Report
## for the Period
## May 19, 2004 – November 17, 2005

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## PROJECT SUMMARY

The project is a continuation of DOE-funded work (FY02 and FY03) that has focused on the development of the ammonia fiber explosion (AFEX) pretreatment technology, fermentation production of succinic acid and new processes and products to enhance dry mill profitability.

The primary objective for work beginning in April 2004 and ending in November 2005 is focus on the key issues related to the

1. design, costing and construction plan for a pilot AFEX pretreatment system, formation of a stakeholder development team to assist in the planning and design of a biorefinery pilot plant, continued evaluation of corn fractionation technologies, corn oil extraction, AFEX treatment of corn fiber/DDGs;
2. development of a process to fractionate AFEX-treated corn fiber and corn stover – cellulose and hemicellulose fractionation and sugar recovery; and
3. development of a scalable batch succinic acid production process at 500 L at or below $.42/lb, a laboratory scale fed-batch process for succinic acid production at or below $.40/lb, a recovery process for succinic acid that reduces the cost of succinic acid by $.02/lb and the development of an acid tolerant succinic acid production strain at lab scale (last objective not to be completed during this project time period).

### AFEX Pretreatment

Two processes were developed for fractionation of corn stover into cellulose and hemicellulose rich fractions: 1) Base extraction followed by ethanol precipitation of solubilized hemicellulose and 2) selective hydrolysis of hemicellulose by cellulase-free xylanase. Base extraction results of AFEX-treated and untreated corn stover were similar and about 51% of xylan was recovered as a hemicellulose pellet, while about 87% of cellulose was recovered as a cellulose-rich cake. The cellulose- rich cake was enzymatyically hydrolyzed and 100% of the cellulose was converted into glucose.

Seven cellulase-free xylanases were tested for selective hydrolysis of hemicellulose of AFEX-treated corn stover and corn fiber. For AFEX-treated corn stover, the best case gave about a 51% yield of xylose while leaving more than 94% of cellulose in the biomass. None of these enzymes were able to hydrolyze corn fiber xylan and the maximum xylose yield was only 4%, with no detectable arabinose.

A process was developed for selective hydrolysis of cellulose by cellulase hydrolysis, followed by ethanol precipitation of solubilized hemicellulose for separation of cellulose and hemicellulose of AFEX-treated corn fiber. Using this method, 57% of xylose and 54% of arabinose were recovered in a pentose-rich stream, and 83% of glucose was recovered in a glucose-rich stream.

A process was developed to produce a mixed C5 and C6 sugar stream from AFEX-treated corn fiber by enzyme hydrolysis followed by acid hydrolysis. The final yields from this process were 89% for glucose, 86% for xylose and 91% for arabinose.

Products Developed under the AFEX Pretreatment Project

Teymouri, F., Selig, M., Decker, S., Dale, B. "Hydrolysis of Ground and Unground AFEX-treated Corn Stover with Different Combinations of Cellulase and Xylanase". 27th Symposium on Biotechnology for Fuels and Chemicals, Denver, CO, May 1-4, 2005.

**Succinic Acid Production**

MBI has developed a robust succinic acid production process that includes a stable bacterial strain, a fermentation process with good productivity and a recovery method. MBI has successfully validated the process at the 250 L scale. In an attempt to further enhance the production of succinic acid, fed-batch strategies were tested that used a substrate feed (glucose), nutrient and/or glycerol feed. We demonstrated that all feeds increased the amount of succinic acid produced and the effects on yield, titer, and extended growth phase and biomass integrity were determined. Additional tuning will be needed to reap an economic benefit from these improvements.

Metabolic flux analyses identified reducing equivalents as the primary limiting factor in the fermentations. Nine recombinant strains were generated and tested for their ability to alter carbon routing and maximize the generation of reducing equivalents in *A. succinogenes*.

Products Developed under the Succinic Acid Production Project

Yi et al.: International PCT Application No. PCT/US2005/045714 (2005), *Recombinant Microorganism for Iincreased Production of Organic Acids.*

Kleff, S. "Succinic Acid Production from Biomass." 2004 Annual Meeting of the Society for Industrial Microbiology, Anaheim, CA, July, 2004.

Kleff, S. "Succinic Acid Production from Biomass." Presentation to proprietary company, September 2004.

Yi et al. "Metabolic Flux in *A. succinogenes*: The effect of different carbon sources". 2004 Engineering Conference International, Metabolic Engineering V, September 2004.

Kleff, S. "Succinic Acid Production in *A. succinogenes*". World Congress for Industrial Biotechnology, Orlando, FL, April 20-22, 2005.

Yi, J. "Metabolic Engeering of *A. succinogenes* to Enhance Succinic Acid Production." 27th Symposium on Biotechnology for Fuels and Chemicals, Denver, CO, May 1-4, 2005.

Yi, J., Guettler, M., Kleff, S., Stowers, M. "Metabolic Engineering of *A. succinogenes* for Succinic Acid Production". Gordon Conference on Applied & Environmental Microbiology, July 2005, New London, CT. *Metabolic Engineering of A. succinogenes for Succinic Acid Production.*

**Biorefinery Development**

We have developed a biorefinery plan for a dry mill corn-to-ethanol pilot plant. We have modeled this plant with Aspen Plus, reviewed it with a stakeholders committee and completed a business plan for its development. This pilot plant design incorporates a currently operating dry mill ethanol plant producing 8.9 million gallons of ethanol per year. The biorefinery model

consists of a footprint of the current plant with 2004 operating numbers and a module for corn fractionation prior to saccharification and fermentation, a module for extracting corn oil from corn germ, a module for AFEX treatment of the corn fiber and a plan for further processing the AFEX treated material. To develop this biorefinery plan we have completed the design engineering and costing estimates for a continuous AFEX treatment pilot plant operating at 3000 pounds-per-hour. Economic analysis indicates the MBI Biorefinery Pilot Plant will operate more efficiently than the current ethanol plant. With the same feedstock and ethanol price, the pilot plant projection indicates a 6.53% return on total capital whereas the current plant shows a return to its depreciated capital base of 6.29%.

Products Developed under the Biorefinery Development Project

Rajagopolan, S. "integrating Emerging Technologies with Biomass Refining: Establishing the Foundation for Replacing Petrochemicals". 2004 Annual Meeting of the Society for Industrial Microbiology, Anaheim, CA, July, 2004.

Rajagopalan, S., McCalla, D. and Stowers, M. "Enhancing the Profitability of Dry Mill Ethanol Plants – MBI's Biorefinery Model". Appl. Biochem. Biotechnol. Vol 120(1):37-50, Jan 2005.

Hoffman, A., Guettler, M., Tiedje, T., Rajagopalan, S., McCalla, D. and Stowers, M. "AFEX Pretreatment of Corn Fiber – Ethanol Fermentation and Animal Feed Analysis". 27th Symposium on Biotechnology for Fuels and Chemicals, Denver, CO, May 1-4, 2005.

Rajagopolan, S., McCalla, D., Stowers, M. "Enhancing the Profitability of Dry Mill Ethanol Plants – MBI's Biorefinery Model". 27th Symposium on Biotechnology for Fuels and Chemicals, Denver, CO, May 1-4, 2005.

Hanchar, R., Rajagopalan, S., McCalla, D. and Stowers, M. "Recovery and Value of Oil from Distillers Dried Grains and Solubles". World Congress on Industrial Biotechnology and Bioprocessing, Orlando, FL, April 20-22, 2005.

Rajagopalan, S., McCalla, D. and Stowers, M. "Enhancing Profitability of Dry Mill Ethanol Plants – MBI's Biorefinery Model". World Congress on Industrial Biotechnology and Bioprocessing, Orlando, FL, April 20-22, 2005.