Developing a Biomaterials Industry in North Dakota
Response to Reviewer's Comments

Rating Category 3: The quality of the methodology displayed in the proposal.

The proposal lacked information on the hydrolysis, fermentation and animal feeding work since they are not included in the work package being proposed; however, we have extensive information on the work completed to date and future plans in these areas. Enzyme hydrolysis and fermentation are unit operations that are capable of being downsized to the scale appropriate to this project and MBI has completed a significant amount of work in this area at the appropriate scale. Much of this information can be found in the final report of our previously funded project by the North Dakota Industrial Commission. In summary, AFEX-treated biomass responds exceptionally well to enzyme hydrolysis and the resulting hydrolysate is easily fermented (with no inhibition noted in the process) to titers in excess of 45g/L ethanol.

Our AFEX process will result in a stable, high density Conversion Ready Biomass Commodity (CRBC) from lignocellulosic feedstocks that can be used to produce biobased fuels and other products, including animal feed. Our team does plan to perform animal feeding trials (not funded under this proposal) with CRBC as soon as scale allows the appropriate amount of material required. The CRBC product has significantly increased digestibility (as measure by rumen fluid digestion) compared to untreated material. In phase one a palatability study will be conducted using CRBC pellets. In phase two a 28 day cross over feeding trial with 32 cows will be conducted (16 test cows and 16 controls each period, 2 periods) to assess net energy content of the biomass. While prior work has shown nutritional gains from the process, the material may not produce the net energy that our digestibility studies infer. Feeding trials are the only viable way to demonstrate feasibility for this product application.

Palatability experiment

Objective: evaluate short-term effects of AFEX treated forage on feed intake compared to other fiber sources.

Substitute AFEX treated forage in diet for corn, and 2 high fiber byproduct feeds (e.g. soyhulls, beet pulp) at ~20% of diet dry matter(~10 lbs/cow/day) and evaluate dry matter intake over 7 days. Design 4x4 Latin square with 4 cows per treatment sequence and 7 day periods. 32 cow period observations total.

Production crossover experiment (not part of this proposal)

Objective: evaluate effect of production level of cows on milk production response to AFEX treated forage substituted for corn grain at ~20% of diet DM.

A crossover design will be used with a preliminary period in which baseline milk yield and feed intake will be determined. Cows (n=32) will be selected from the herd with a wide range, and uniform distribution of milk yield. Effects of treatment will be evaluated for milk yield and composition, feed intake and efficiency of nutrient utilization across level of milk production.

Rating Category 4: The scientific and/or technical contribution of the proposed work to specifically address North Dakota Industrial Commission/Renewable Energy Council goals.

We are well aware that public awareness along with education, and public policy will have a significant impact on acceptance of new bio-based technologies. In a separate program with Michigan State University, the University of Wisconsin, and the University of Minnesota we plan to address these issues in a long term effort to address public awareness/perception, changes in farming practices, public policy, sustainability and environmental issues and their impact on commercialization of these technologies. This program will leverage the Extension efforts of several land grant institutions, along with the Great Lakes Bioenergy Research Center (GLBRC), and several large bioenergy companies to address these issues.

Rating Category 5: The principal investigator's awareness of current research activity and published literature as evidenced by literature referenced and its interpretation and by the reference to unpublished research related to the proposal

As one reviewer pointed out, this is a more advanced development project rather than a typical research project. Therefore our team did not focus on an extensive literature review in this proposal due to the limitations of space. We felt it was more beneficial to focus on the status and readiness of the technology and its overall import to North Dakota. In the final report for our previous project there is an extensive review of the technology, its benefits and competitiveness.

Rating Category 7: The project management plan, including a well-defined milestone chart, schedule, financial plan, and plan for communications among the investigators and subcontractors

The milestones for a development project of this type typically follow the completion of major tasks. In this case the primary milestones are:

1. Complete final design for the 1.5kg reactor: sixth week of project
2. Complete reactor assembly: five months into project
3. Complete reactor shakedown: seven months into project
4. Complete hydrolysis studies: eight months into project
5. Complete early animal studies: nine months into project
6. Complete pilot scale reactor design: nine months into project
7. Complete techno-economic analyses: fifteen months into project

The budget for materials includes reagents for analytical testing of feedstock and hydrolysis products, ammonia, general lab supplies, biomass feedstock, and minor equipment components. The primary reactor equipment is not budgeted under this funding request but will be funded internally by MBI. Restrictions in North Dakota State University contracting policy would not allow MBI to show these costs as cost share; however primary equipment and fabrication costs for the AFEX PB reactor and hydrolysis equipment is estimated at approximately $350,000. This includes stainless steel reactor columns, valves, gauges, heat exchanger, pumps, ammonia compressors, hydrolysis tank/mixer and control systems.

Section C. Overall Comments and Recommendations

The overall benchmarks or targets for this conversion technology are:

1. Achieve biomass conversion to sugars in the PB AFEX reactor and hydrolysis that is comparable to previous batch/bench scale experiments (≥ 60% yield)
2. Utilizing the biomass derived sugars from the PB AFEX reactor and hydrolysis to achieve ethanol productivity and titer comparable to previous bench scale experiments (45-50 g/L)

Achieving these productivity targets, coupled with the estimated reduced capital and operational costs of the new PB AFEX system is expected to result in an economically viable technology at commercial scale.

The animal feeding studies are meant to demonstrate the flexible nature of this technology in producing a stable intermediate biomass product that can be sold into a variety of markets. See the following justification and concept:

Commercialization of biobased products faces formidable challenges including 1) the market and supply chain dilemma for emerging technologies, and 2) logistical, contracting, transportation and storage issues connected with the current concept of large centralized biorefineries. Instead of large centralized biorefineries this project proposes regional production harvesting, preprocessing, and storage of a uniform, biomass commodity through Regional Biomass Depots (RBDs)(Carolan et al., 2007). Our primary objective is to develop an integrated system producing a stable, high density Conversion Ready Biomass Commodity (CRBC) from lignocellulosic feedstocks that can be used to produce biobased fuels and other products. The oil refining and corn processing industries are both built on commodity feedstocks that are reactive, shippable, storable and stable. We believe a large processing industry based on cellulosic biomass will be slow to emerge without a similar commodity feedstock. With the possible exception of pyrolysis bio-oil, our CRBC product is the only such commodity material currently on the horizon.

This integrated system is based on critical technological innovations that enable the production of the CRBC. The RBD concept fully integrates feedstock supply chain logistics on a local level, with 5-6 local RBDs (100 ton/day processing capacity) supplying biomass to a regional conversion facility (20 million gal/yr capacity) for production of biobased fuels and/or chemicals. This project will produce butanol, but prior work indicates that the CRBC product can be used to produce almost any fermentation based biofuel or biobased chemical. We will also establish the feasibility of using CRBC directly as an animal feed. Animal feed coproduction with biofuels largely eliminates the “food vs. fuel” conflict as well as the related issue of indirect land use change, so this is a crucial innovation.

The technological innovation that will enable and support our concept is a novel process that can be “right-sized” and practiced locally to produce a dry, high density, stable, uniform and high value intermediate feedstock (CRBC) that improves storage and transportation efficiency.

Our approach provides multiple system level benefits to promote environmental and economic sustainability through the entire value chain from field to product. Logistical benefits include: supply of feedstock using conventional harvest equipment; allows the biorefinery to contract with a few RBDs vs. hundreds of farmers; and simplifies storage and transport of feedstocks. Market and supply chain benefits include: incentivizes rural interests to participate in the biofuel value chain, including potential ownership in the RBDs; reduces risk to farmers by addressing market volatility associated with a single product; and reduces risk to biorefineries due to increased robustness of the supply chain.

The planned approach has the potential to achieve >65% reduction in GHG emissions. Distributed regional biomass processing as proposed will greatly increase the net value of feedstock and have a significant positive impact on the rural community. We estimate each RBD will generate $3 million per year in sales and will provide about 30 direct jobs. The ability of a regional depot to have multiple markets for treated biomass will improve business stability in volatile fuel markets.

The following is an excerpt from the final report of our previous project with the North Dakota Renewable Energy Council/Industrial Commission, and provides a brief literature review on pretreatment technologies:

AFEX is a biomass treatment process in which biomass, water and concentrated ammonia are mixed in a specified ratio, and maintained at a specified temperature and pressure for a specified time period. AFEX treatment causes significant physical and chemical changes in the biomass, resulting in reduced recalcitrance to enzymatic hydrolysis, and more effective conversion of sugars to fermentation products. The applicability of AFEX to a broad range of agricultural feedstocks has been described in the literature (Alizadeh et al., 2005, Teymouri et al., 2004, Teymouri et al., 2005a, Murnen et al., 2007).

AFEX is fundamentally different from "pretreatment" as defined by the U.S. Department of Energy (DOE). The DOE definition of pretreatment is intimately tied to the concept of "prehydrolysis" of sugars from the biomass, as described in the March 2008 DOE Biomass Multi-year Program Plan document (page 3-31): "*In this [pretreatment/pre-hydrolysis] step, the biomass feedstock undergoes a thermochemical process to break down the hemicellulose fraction of the feedstock into a mixture of soluble five-carbon sugars…and soluble six-carbon sugars. This partial solubilization makes the remaining solid cellulose fraction more accessible for enzyme saccharification later in the process."*

Unlike most pretreatments, AFEX retains the hemicellulose and cellulose sugars in their polymeric form in the treated biomass. AFEX causes physical and chemical changes in the biomass, but there is no pre-hydrolysis of the hemicellulose fraction nor conversion to monomeric sugars during the process. No sugar-bearing liquid stream is produced during the AFEX process, as is seen in steam or dilute-acid pretreatment processes. Consequently, unlike pretreated biomass, AFEX-treated biomass may be considered a stable intermediate, one that can be stored, transported and integrated with subsequent processing steps.

The DOE-supported Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) studies conclude that AFEX is effective and economical. Following extensive comparative studies at laboratory bench-scale, the CAFI group concluded that: 1) AFEX is an effective biomass treatment method to enhance enzymatic hydrolysis of corn stover to produce clean fermentable sugars, and 2) the estimated cost of commercial production of ethanol using AFEX is competitive with other technologies (Wyman et al., 2005, Eggeman and Elander, 2005).

AFEX batch reaction conditions that are effective for pretreatment of a variety of biomass feedstocks are known. Batch AFEX treatment of corn stover at scales from a few grams to several hundred grams per batch, followed by enzymatic hydrolysis under standard conditions, have consistently demonstrated that hydrolysis yields improve significantly, as compared to untreated stover. In one published study (Wyman et al., 2005b), combined yields of glucose and xylose from corn stover were shown to exceed 94% of theoretical yield after AFEX treatment followed by enzyme hydrolysis. In another batch study of AFEX treatment (Teymouri et al., 2005a), the key independent variables were found to be ammonia loading, feedstock moisture level, temperature, and reaction time. Effective conditions for AFEX batch treatment were found to be ammonia mass loading of 1 kg/kg of dry stover, 60 to 80 wt% feedstock moisture (dry weight basis), 90 to 110^{o}C temperature, and 5 minutes reaction time. A more recent study (Sendich et al., 2008) has shown that lower ammonia loadings can be effective for AFEX-treatment of stover. The results in Figure 1 show that ammonia loading could be reduced to about 0.3 kg of ammonia per kg of dry biomass before AFEX treatment effectiveness declined.

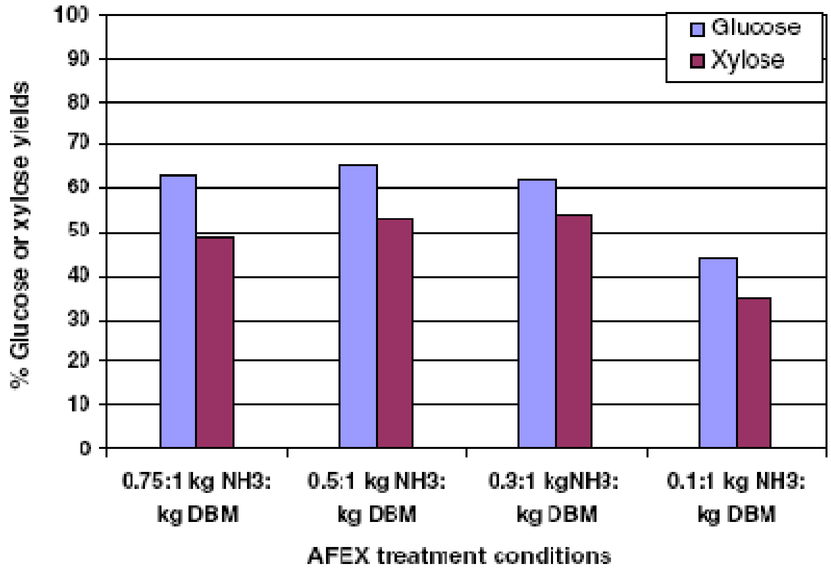

Figure 1. Glucose and xylose yields after enzyme hydrolysis of AFEX-treated corn stover at different ammonia loadings, 60% moisture content at 90 ºC. (Adapted from Sendich et al., 2008).

AFEX treatment gives yields of fermentable sugars equivalent to or greater than yields from leading pretreatments. Table 3 summarizes the hydrolysis yields of each treatment method evaluated under the recent Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) study (Wyman et al., 2005b). The consideration of sugar yields must account for the fact that the overall system consists of two stages for each treatment system evaluated, and that not all of the sugar is released as monomers. In Stage 1, biomass is treated to open up the structure of the residual solids and facilitate access by enzymes to obtain high yields. Enzymes are then added to the treated solids in Stage 2. For many operations, some amounts of glucose and xylose are released in Stage 1 and recovered in the liquid stream. Furthermore, although cellulase enzyme is added in Stage 2, it has enough xylanase activity to hydrolyze a substantial portion of the xylan, and both xylose and glucose are typically found in the liquid streams from Stage 2. Thus, yields of each sugar are reported for each stage as appropriate. Because some treatments produce sugar oligomers as well as monomers in Stage 1, the yields of each sugar from each stage are further differentiated to reflect this information. Comparison of the amount of each sugar monomer or oligomer produced to the maximum potential amount for that sugar would give the percent yield of each. However, it is important to recognize that corn stover, along with most other forms of cellulosic biomass, are richer in glucose than xylose, and as a result, yields of glucose have a greater impact than those of xylose. Thus, sugar yields were defined by dividing the amount of xylose or glucose, or the sum of the two, by the maximum potential amount of both sugars to better reflect the relative contribution to overall sugar production. Hydrolysis of AFEX-treated corn stover gave the higher sugar yield than corn stover pretreated with dilute acid or steam explosion.

AFEX reactors do not require acid-resistant materials of construction, so reactor capital cost is significantly lower than for dilute acid and SO_2-catalyzed steam explosion (Eggeman and Elander, 2005). The AFEX process is operated at milder conditions compared to dilute acid and SO_2 catalyzed steam explosion.

Enzyme hydrolysis of AFEX-treated corn stover yields sugars that are readily fermented to ethanol. MSU researchers have recently performed a side by side test to compare fermentabilities of sugars generated from hydrolysis after AFEX treatment and dilute acid pretreatment of corn stover (Lau et al., 2007). Fermentations were performed at low initial density and at about 20% solid loading of treated biomass. *E. coli* KO11 and *Saccharomyces cerevisiae* 424A (LNH-ST) were used in this evaluation. It is clear from Figure 2 that both organisms were able to ferment hydrolyzates generated from AFEX-treated corn stover without any conditioning or detoxification. However in contrast, hydrolyzates generated from dilute acid treated corn stover were hardly fermentable without detoxification (not fermentable at all in *E. coli* KO11 case and exhibited a very long lag phase (almost 72 hr) in *S. cerevisiae* 424A (LNH-ST) case.

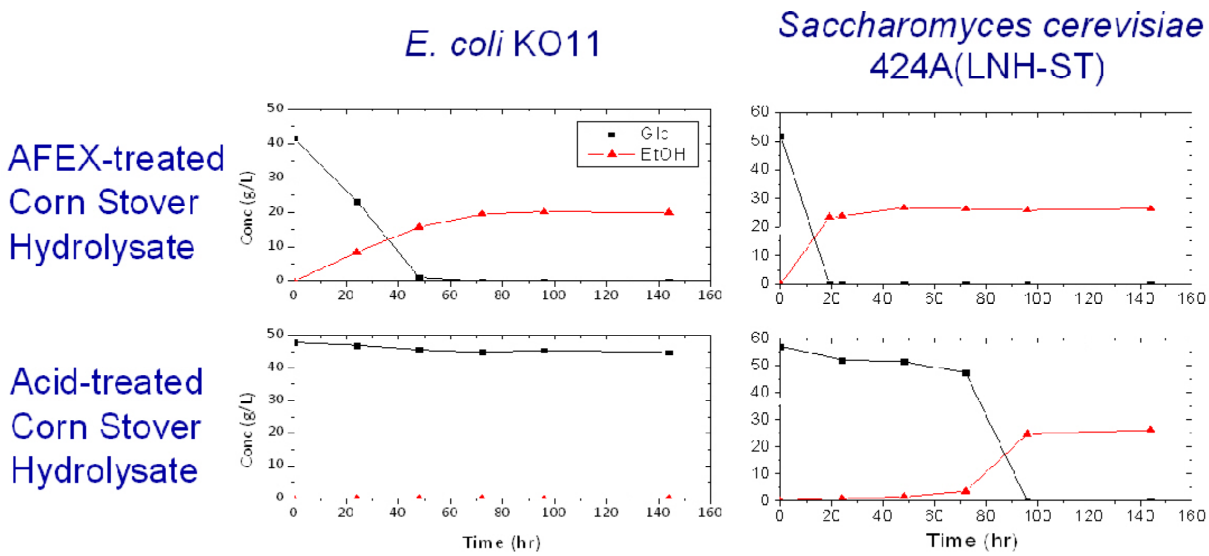

Figure 2. Comparing fermentability of AFEX and dilute acid pretreated corn stover

There is little or no (depending on biomass type) formation of degradation products during AFEX treatment. Consequently, AFEX-treated biomass is compatible with diverse organisms. In a recent study by MSU researchers (Balan et al., 2006) concentrations of inhibitory compounds in AFEX treatment are trace level and are much lower than those generated from leading pretreatments such as the dilute acid process. Furfural and hydroxymethylfurfural, inhibitory products from the degradation of pentose and hexose sugars, were not detected in hydrolyzate after AFEX treatment, but were present at concentrations of 1,797 mg/L and 88 mg/L, respectively, in acid hydrolyzate. The basis for this comparison was valid on a solid-to-liquid basis. For acid treatment, 1 gm of biomass was treated with 10 times liquid, while for AFEX-treated material, 1 gm of AFEX biomass was washed in 10 times the amount of water. Thus the amount of compounds produced in both liquid streams is on a comparable basis.

AFEX treatment offers significant advantages for densification, storage, and transport of treated biomass. Unlike leading pretreatments which result in wet treated biomass, AFEX-treated biomass is relatively dry and inert, and hence it is more easily storable and transportable. AFEX-treated biomass can also be densified to further improve bulk handling properties. Because of these unique features, biomass can be preprocessed and treated at a site close to the biomass source, then the treated materials can be shipped to a centralized biorefinery location. Initial trials at MSU have shown that the density and other properties of pellets of AFEX treated biomass are better than pellets of untreated biomass. AFEX treatment transfers some lignin and hemicellulose oligomers to the surface of biomass fibers, where it can act as a binding agent. Hard, durable pellets have been made from AFEX-treated corn stover with a specific gravity of up to 1.16 without using any binders, whereas untreated corn stover pellets are much less dense and are not durable at all.

References

Alizadeh, H., F. Teymouri, T.I. Gilbert, and B.E. Dale. 2005. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). Appl. Biochem. Biotechnol. **121-124**: 1133-1141.

Balan, V., Sh. Chundawat Sh., B. Bals, and B. Dale. 2006. The Case of Ammonia Fiber Expansion (AFEX). Presented at AIChE Annual Meeting. San Francisco, California.

Carolan, J., S. Joshi, and B. Dale. 2007. Technical and financial feasibility analysis of distributed bioprocessing using regional biomass pre processing centers. Journal of Agricultural and Food Industrial Organization, Volume 5, Article 10 (2007) SPECIAL ISSUE: Explorations in Biofuels Economics, Policy, and History.

DOE (U.S. Department of Energy) 2008. Department of Energy Biomass Multi-year Program Plan. March 2008.

Eggeman, T. and R.T. Elander. 2005. Process and economic analysis of pretreatment technologies. Bioresource Technology. **96**: 2019-2025.

Lau, M., B. Dale, and V. Balan. 2007. Comparing fermentability of corn stover hydrolysate from AFEX and dilute acid pretreatment. Presented at 29^{th} Symposium on Biotechnology for Fuels and Chemicals. Denver, Colorado.

Murnen, K.H., B. Balan, Sh. Chundawat, B. Bals, L. Costa Sousa, and B.E. Dale. 2007. Optimization of Ammonia Fiber Expansion (AFEX) Pretreatment and Enzymatic Hydrolysis of *Miscanthus x giganteus* to Fermentable Sugars. Biotechnol. Prog. **23**: 846-850.

Sendich, E., M. Laser, S. Kim, H. Alizadeh, L. Laureano-Perez, B. Dale, and L. Lynd. 2008. Recent process improvements for the ammonia fiber expansion (AFEX) process and resulting reductions in minimum ethanol selling price. Biores. Technol. **99**(17): 8429-35.

Teymouri, F., L. Laureano-Perez, H. Alizadeh, and B.E. Dale. 2004. Ammonia fiber explosion treatment of corn stover. Appl. Biochem. Biotech. **113-116**: 951-963.

Teymouri, F., L. Laureano-Perez, H. Alizadeh, and B.E. Dale. 2005a. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Biores. Technol. **96**: 2014 – 2018.

Wyman, C.E., B.E. Dale, R.T. Elander, M. Holtzapple, M. Ladisch, and Y.Y. Lee. 2005a. Coordinated development of leading biomass pretreatment technologies. Bioresource Technology. **96**: 1959-1966.

Wyman, C.E., B.E. Dale, R.T. Elander, M. Holtzapple, M. Ladisch, and Y.Y. Lee. 2005b. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. Bioresource Technology. **96**: 2026-32.