Final Report
Submitted on behalf of the Midwest Consortium

By

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Summary:

Collaborative efforts of Midwest Consortium have been put forth to add value to distiller's grains by further processing them into fermentable sugars, ethanol, and a protein rich co-product consistent with a pathway to a biorenewables industry (Schell et al, 2008). These studies were recently published in the enclosed special edition (Volume 99, Issue 12) of Bioresource Technology journal. Part of them have demonstrated the utilization of distillers' grains as additional feedstock for increased ethanol production in the current dry grind process (Kim et al., 2008a, b; Dien et al.,2008, Ladisch et al., 2008a, b). Results showed that both liquid hot water (LHW) pretreatment and ammonia fiber expansion (AFEX) were effective for enhancing digestibility of distiller's grains. Enzymatic digestion of distiller's grains resulted in more than 90% glucose yield under standard assay conditions, although the yield tends to drop as the concentration of dry solids increases. Simulated process mass balances estimated that hydrolysis and fermentation of distillers' grains can increase the ethanol yield by 14% in the current dry milling process (Kim et al., 2008c). Resulting co-products from the modified process are richer in protein and oil contents than conventional distiller's grains, as determined both experimentally and computationally. Other research topics in the special edition include water solubilization of DDGS by transesterification reaction with phosphite esters (Oshel el al., 2008) to improve reactivity of the DDGS to enzymes, hydrolysis of soluble oligomers derived from

DDGS using functionalized mesoporous solid catalysts (Bootsma et al., 2008), and ABE (acetone, butanol, ethanol) production from DDGS by solventogenic *Clostridia* (Ezeji and Blaschek, 2008). Economic analysis of a modified dry milling process, where the fiber and residual starch is extracted and fermented to produce more ethanol from the distillers' grains while producing highly concentrated protein co-product, has shown that the process is economically viable resulting in an increase in net present value (Perkis et al., 2008). According to the study, the revenue is expected to increase further with improved amino acid profile of the protein rich co-products and lower cost of cellulase enzyme mixture. Also, Kim and Dale (2008) discuss using life cycle analysis to enhance the environmental performance of the corn based ethanol.

On the second phase of the research, concerted efforts were directed on assessing compositional variability of dry milling co-products collected from 4 different dry grind ethanol plants has been measured and its effect on enzymatic digestibility and fermentability. Fermentation utilized a recombinant glucose/xylose co-fermenting yeast (*Saccharomyces* cerevisiae 424A (LNH-ST)). No significant compositional variability among the samples was found. Simultaneous saccharification and glucose/xylose co-fermentation of the pretreated distillers' grains at solids and cellulase loadings of 150 g dry solids per liter and 6.4 mg protein per g dry substrate, respectively, yielded 74-801% of theoretical maximum ethanol concentration using recombinant *Saccharomyces cerevisiae* 424A (LNH-ST). The paper summarizing the results from the second phase of the Midwest Consortium is currently submitted to Bioresource Technology journal. The copy of the paper submitted is enclosed.

Detailed Results:

Below summarizes combined results and progress achieved throughout the project. Experimental methods and discussions on the results are presented in the enclosed papers published in the special edition of Bioresource Technology.

1. ***Composition of Dry Mill Co-Products*:**

Chemical compositions of DDGS, wet distillers' grains and thin stillage were determined by a series of analyses adapted from NREL's standard cellulosic biomass compositional analysis procedures. The compositional analysis of DDGS and wet distillers' grains following the established procedures produced reproducible and accurate results, with a close to 100% mass closure. DDGS and wet distillers' grains are rich in glucan, xylan and arabinan, the source of fermentable sugars for ethanol production. A summary of the average composition (dry basis) of DDGS, WDG (wet cake) and thin stillage determined by three consortium partners (Purdue, USDA NCAUR, and U. of Illinois) are given in Table 1. Total available sugars (glucan and xylan) of DDGS and wet distillers' grains for producing ethanol were measured to be approximately 30-35% based on a total dry mass basis. Crude protein comprises 25% of the total dry mass of DDGS. Crude oil measured as ether extractives is 11.6%. Table 2 summarizes composition of wet distillers' grains samples collected from four different dry mill plants in Midwest area. With the exception of sample #4, which was collected after mixing of the syrup and prior to drying step, the WDG were found to have similar compositions. Variation in feedstock composition is not expected to be a major concern for applying our processes for ethanol production.

Table 1. Composition of DDGS and WDG (wet cake) by cellulosic biomass compositional analysis (average from three research groups, Purdue, University of Illinois and USDA NCAUR).

	DDGS	WDG (Wet cake)
Dry Matter	88.8%	35.3%
Water Extractives	24.7%	8.8%
Ether Extractives	11.6%	9.6%
Crude Protein	24.9%	36.6%
Glucan (total)	21.2%	18.5%
Cellulose	*(16%)*	*(12.6%)*
Starch	*(5.2%)*	*(5.9%)*
Xylan and Arabinan	13.5%	20.9%
Xylan	*(8.2%)*	*(14.9%)*
Arabinan	*(5.3%)*	*(5.5%)*
Ash	4.5%	2.0%
Total Dry Matter Mass Closure	100.4%	96.4%

Table 2. Composition of DDGS and WDG by % dry weight. Numbers are average of Purdue, MSU and USDA-NCAUR results. Errors in 95% CI are less than 4% for all values.

Component	**DDGS**				**WDG (Wet Cake)**			
	1	2	3	4	1	2	3	4
Dry Matter (%)	90.3	90.7	90.0	90.4	36.9	36.1	36.2	51.7
Water Extractives (%)	23.1	23.0	24.6	21.7	7.8	8.4	8.7	31.9
Ether Extractives (%)	12.0	12.3	11.8	10.7	8.7	8.5	7.9	12.7
Crude Protein (%)	28.0	31.1	30.4	30.9	33.3	35.6	35.5	28.7
Glucan (total) (%)	20.1	18.2	19.0	18.3	20.2	18.8	19.6	17.6
Starch (%)	6.1	4.7	6.1	5.9	5.5	4.2	4.7	5.8
Cellulose (%)	14.0	13.5	12.9	12.4	14.7	14.5	14.9	11.9
Xylan and Arabinan (%)	18.0	16.4	15.8	16.7	23.1	21.4	21.0	14.9
Xylan (%)	11.9	10.7	10.4%	10.8	14.4	13.5	13.4	9.7
Arabinan (%)	6.2	5.7	5.5	5.8	8.8	7.8	7.7	5.2
Ash (%)	4.69	4.52	4.37	4.50	2.23	2.19	2.04	5.55
Total Dry Matter Mass Closure (%)	105.9	105.5	106.0	102.8	95.3	94.8	94.7	111.3

2. Pretreatment Enhances Enzymatic Digestibility of Distillers' Grains:

Enzymatic digestibility of #2 and #4 WDG and DDGS samples were tested at 5% dry solids loading (50 g dry solids/L or 10 g glucan/L) with commercial cellulase enzymes. The

material was pretreated by either liquid hot water (Purdue U) or AFEX (Michigan State U) at its corresponding optimal pretreatment conditions. Enzymes were added at 15 FPU cellulase (Spezyme CP) and 40 IU β-glucosidase (Novozym 188) per g glucan, which is equivalent to 32 mg protein/g glucan. A low solids concentration was selected to minimize inhibition effects by product or other substances released during the hydrolysis, as well as mass transfer resistance due to mixing difficulties, become minimal. Yields of glucose from hydrolysis of WDG and DDGS samples pretreated by either AFEX or LHW are compared in Figure 1. For all cases, hydrolysis reactions were mostly finished within the initial 6 hrs. The maximum glucose yields were 70-80% for both LHW and AFEX pretreated WDG samples and 98% for LHW pretreated DDGS. Yields for untreated WDG were much lower than for pretreated samples: 40% glucose yield for #2 WDG and 52% for #4 WDG, as measured after 24 hrs. The results clearly demonstrated that the pretreatment of the distiller's grains significantly enhanced both the rate and extent of hydrolysis. This is of significance when the large throughput of DDGS in the dry mill plants is considered.

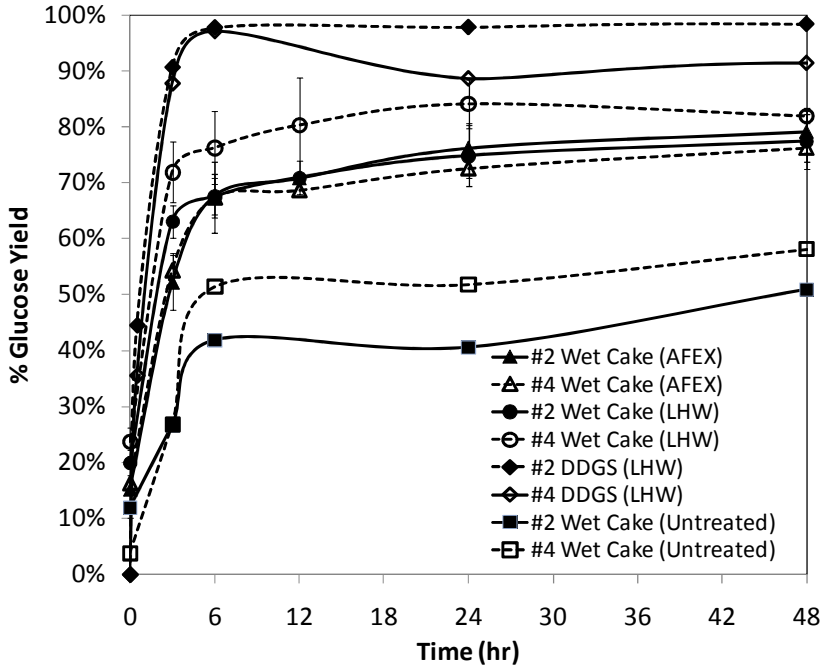


Figure 1. Digestibility of LHW pretreated and AFEX pretreated distillers' grains (wet cake, DDGS). Hydrolysis conditions: modified LAP 009, 5% (w/w) dry solids loading, 15 FPU Spezyme CP and 40 IU Novozym 188 per g glucan (or 32 mg total protein/g glucan). 50 ^{o}C, pH 4.8, 200 rpm, 48 hr hydrolysis. Error bars represent 95% CI.

3. Xylanase and Feruloyl Esterase Improves Overall Sugar Yields :

Cellulose was readily converted to glucose from both LHW and AFEX treated DDGS using a mixture of commercial cellulase and β-glucosidase; however, these enzymes were ineffective at saccharifying the xylan present in the pretreated DDGS. The heteroxylan of corn fiber (corn bran) is known to be highly branched with ferulic acid ester-linked to

arabinofuranosyl residues, requiring concerted action of various enzyme activities to facilitate the hydrolysis of heteroxylan in distillers' grains.

Several commercial enzyme preparations were evaluated in combination with cellulase to saccharify pretreated DDGS xylan and it was found that adding commercial grade (e.g. impure) pectinase and feruloyl esterase (FAE) preparations were effective at releasing arabinose and xylose. The two enzymes, Multifect Pectinase PE and Depol 740, were supplemented, in addition to Spezyme CP and Novo 188. Yields of both glucose and xylose from hydrolysis of #2 and #4 WDG, pretreated by either LHW or AFEX, were improved by addition of the supplementary enzymes (Figure 2). As expected, the yield of xylose was greatly improved (11% → 50%). Also, there was 10-15% increase in the glucose yield by the action of these supplementary enzymes.

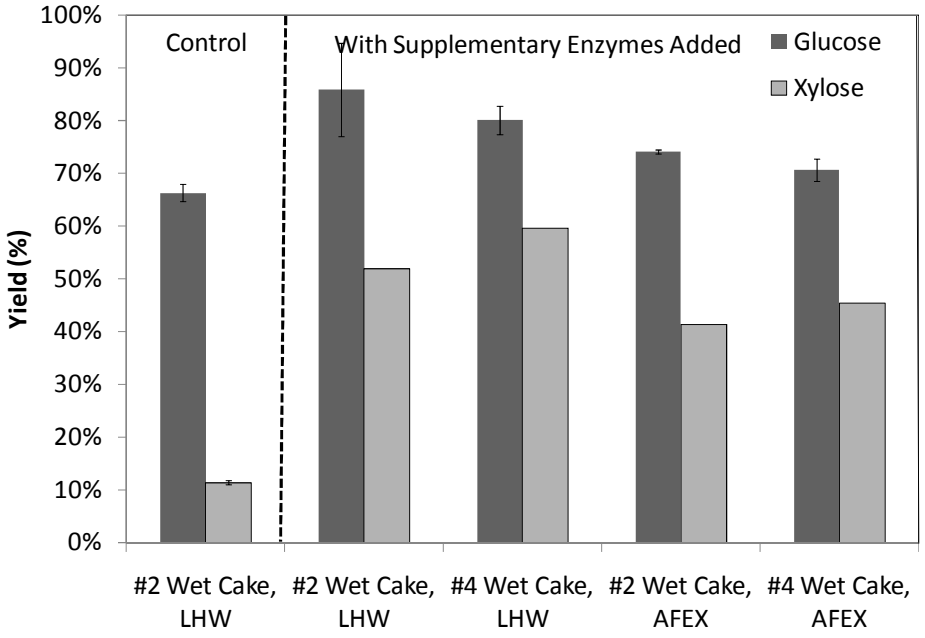


Figure 2. Effect of supplementary enzymes (Multifect Pectinase PE and Depol 740) on sugar yields from pretreated wet cake. Hydrolysis conditions: 15% (w/w) dry solids loading, 50 ^{o}C, pH 4.8, 200 rpm, 48 hr hydrolysis. Control: 15 FPU Spezyme CP and 40 IU Novozym 188 per g glucan. Supplementary enzymes were added at 50 U/g dry solids xylanase (Multifect Pectinase PE) and 2 U/g dry solids feruloyl esterase (Depol 740). Error bars represent 95% CI.

***4. Expression of supplementary enzymes in Saccharomyces cerevisiae* :**

Due to the strong effect supplementary enzymes have on enzyme hydrolysis as described above, three enzymes specific to hemicelluloses conversion to fermentable sugars were cloned into *Saccharomyces cerevisiae* to determine if these proteins could be expressed and excreted in active form by this biofuel-relevant fermentative microorganism. The three enzymes were α-glucuronidase, β-xylosidase, and β-arabinosidase. The α-glucuronidase enzyme cleaves glucuronic acid side-chains from the GAX hemicelluloses found in the corn pericarp. The gene chosen to clone this enzyme was Y15405 from *Aspergillus tubingensis* (a fungus). The β-xylosidase enzyme cleaves xylose dimers released from the hemicelluloses backbone from exo-active hemicellulases. The gene chosen to clone this enzyme as Y15405 from a closely related

fungus *Aspergillus nidulans*. The β-arabinosidase enzyme cleaves the arabinose side-chains from the GAX backbone. The gene chosen to clone this enzyme was AF040720 from *Selenomonas ruminantium*, an important bacterium in the stomachs of ruminant animals. The gene was obtained from work done at USDA-NCAUR in Peoria, IL.

Using a combination of combination of OE-PCR and PCR, each of the three genes were combined with PGI (a constitutive promoter) and MFa (protein secretion leader) to form PGI-MFa-gene which was then cloned into the pKS2μKM XK vector. Yeast were then cloned with each of the three vectors. Transformed yeast were then selected and then tested for expression and secretion of active enzyme. The enzyme tests included growing up a cell culture in 100 mL of YEP with 2% dextrose in 300 mL side-arm flasks at 28°C under aerobic conditions for 24 hours. Optical density of the cells was measured periodically during the aerobic phase. Samples of the media were also collected and then assayed for enzyme activity. After 24 hours, glucose was added to the cell culture to a concentration between 75 – 100 g/L. The 300 mL flasks were then sealed with plastic film to generated microaerobic conditions to induce fermentation. Fermentation at 28°C was continued for an additional 24 hours (48 hours total). Optical density of the cells was measured and samples were collected for enzyme activity assay. The media samples were also analyzed by HPLC as described above to monitor glucose fermentation to ethanol and glycerol.

Figures 3-5 show the results of the three transformed yeast with the three supplementary enzymes. The data from hours 0 – 24 are from the aerobic culturing of the cells. At hour 24 additional glucose was added (note dilution of enzyme and cell mass), after which the fermentation was allowed to proceed microaerobically. All cloned yeast fully fermented the glucose to ethanol within 24 hours. For all three genes, active enzyme was expressed and excreted into the media during the aerobic growth phase. Additional enzyme was synthesized and excreted for both α-glucuronidase and β-xylosidase during the fermentation phase. The activity for α-glucuronidase remained constant after the glucose was depleted (between 36 and 48 hours). However, the β-xylosidase activity decreased after the glucose was fully fermented. Unlike the other enzymes, β-arabinosidase activity did not increase during the fermentation phase. The β-arabinosidase activity decreased approximately 75% over the fermentation time course. These results indicate that supplementary enzymes can be expressed and excreted by *S. cerevisiae*, a biofuel producing microbe, during fermentation of sugars to ethanol under laboratory conditions.

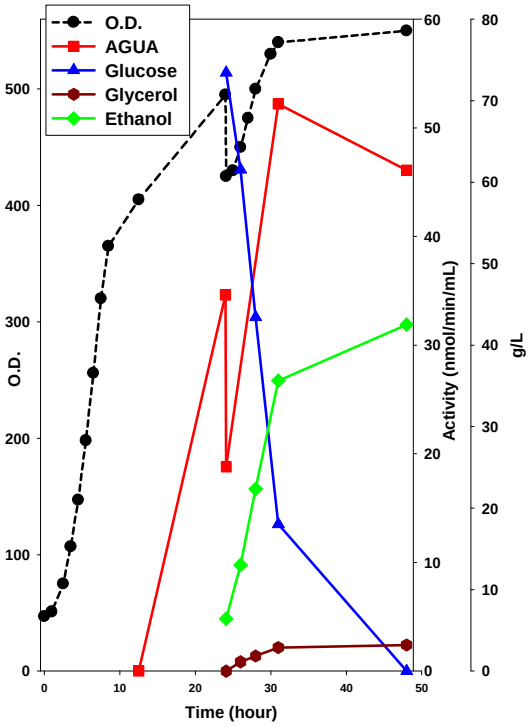


Figure 3. Production of *A. tubingensis* α- glucuronidase by *S. cerevisiae* during growth and fermentation.

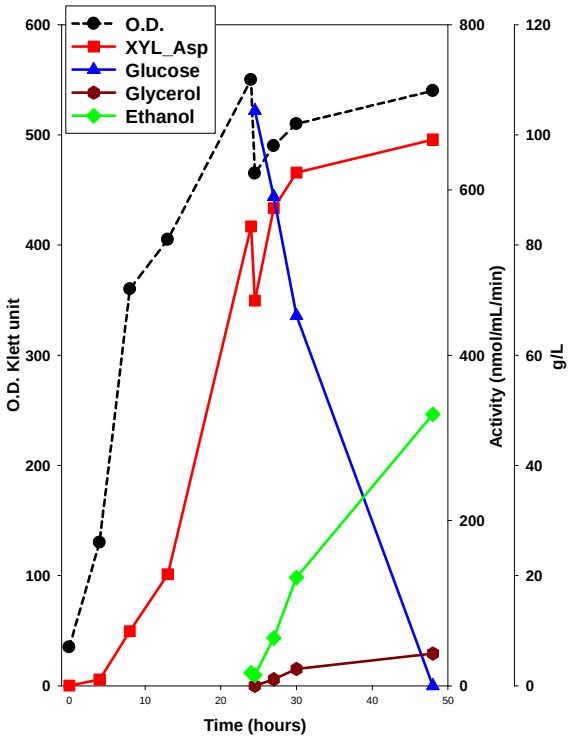


Figure 4. Production of *A. nidulans* β-xylosidase by *S. cerevisiae* during growth and fermentation.

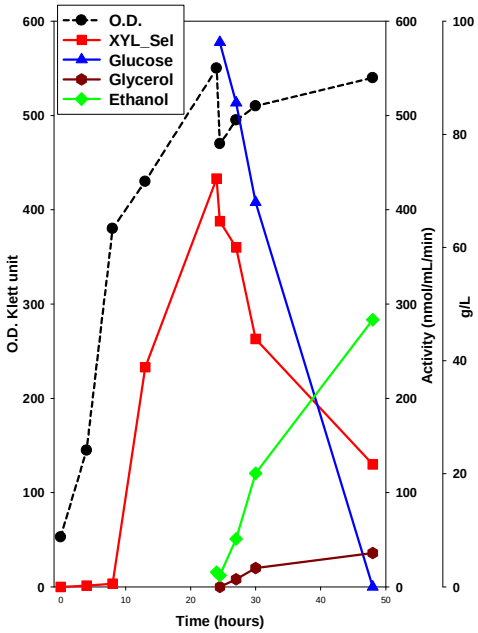


Figure 3. Production of *S. ruminantium* β-arabinosidase by *S. cerevisiae* during growth and fermentation.

5. ***Inhibition of cellulase enzymes mixture during hydrolysis of pretreated distillers' grains*** **:**

Liquid hot water pretreatment of wet cake solubilizes arabinoxylan and other soluble components during the pretreatment, some of which are identified as potential inhibitors to cellulase enzymes. It was expected that removal of the potential inhibitors would improve the overall enzymatic digestibility from the pretreated WDG. The identified inhibitory compounds include ferulic acid, sugar oligomers, starch hydrolysis products, monomeric sugars, etc. Since all these compounds are found in the pretreatment liquid of the WDG, its removal from the whole pretreated WDG slurry on the hydrolysis was assessed as following.

The liquid fraction of the pretreated WDG slurry was removed by filtration. The resulting pretreated solids were then washed with hot DI water and re-suspended in a pH 4.8 citrate buffer to give 23% (w/w) dry solids slurry. The resulting slurry was hydrolyzed by a cellulase enzyme cocktail for 48 hr. As a control, the whole pretreated WDG slurry at 23% dry solids was hydrolyzed at the same conditions. Yields of glucose are given in Figure 6. In Figure 6, the former is referred as "washed" and the latter is referred to as "unwashed." As both runs were carried out at the same dry solids level, mixing characteristics and mass transfer were assumed to be identical between the runs. As shown in Figure 6, removing the inhibitor-containing pretreatment liquid of WDG improved the overall glucose yield by as much as 25%. The increase was observed at all enzyme loadings tested. This result demonstrates the benefit of removing inhibitors on improving digestibility of cellulose.

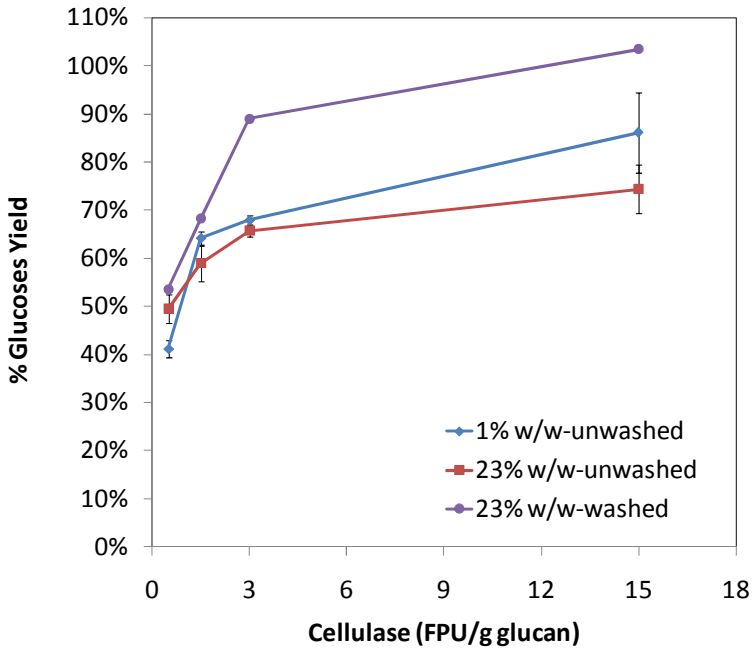
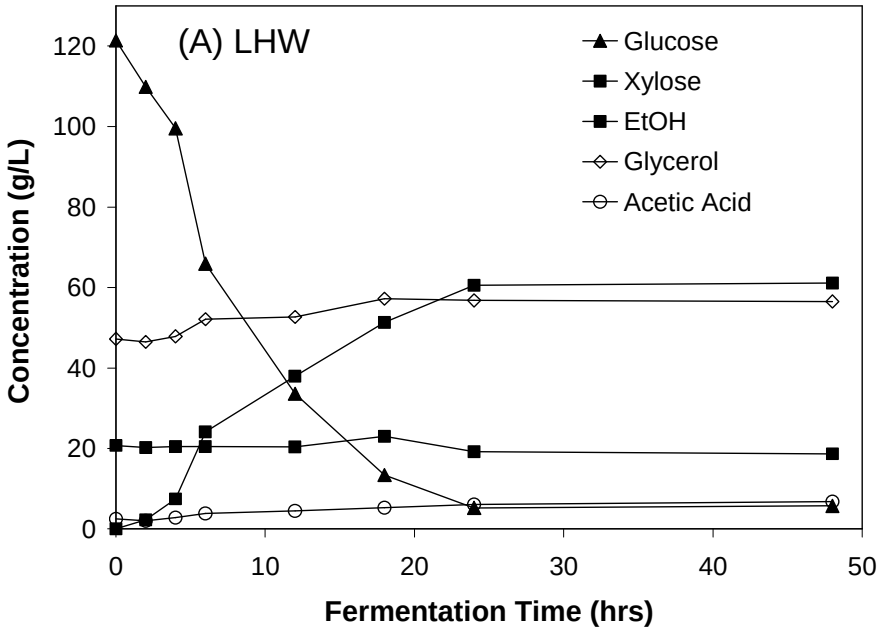


Figure 6. Effect of removing soluble cellulase inhibitors on glucose yield. Hydrolysis conditions: 1% (w/w), 23% (w/w) dry solids loading of whole slurry of liquid hot water pretreated wet cake, 23% (2/2) dry solids of washed solids of liquid hot water pretreated wet cake. 50 ^{o}C, pH 4.8, 200 rpm, 48 hr hydrolysis. Enzyme: 15 FPU Spezyme CP and 40 IU Novozym 188 per g glucan. Supplementary enzymes were added at 50 U/g dry solids xylanase (Multifect Pectinase PE) and 2 U/g dry solids feruloyl esterase (Depol 740). Error bars represent 95% CI.

6. Fermentation of Pretreated and Enzymatically Hydrolyzed Distillers' Grains:

Glucose-only fermentation: Concentrated hydrolysates of both LHW and AFEX pretreated wet distiller's grains were successfully fermented by *Saccharomyces* yeast with 100% of the theoretical (metabolic) ethanol yield being achieved (Figure 7). Over 95% of the glucose was consumed within 24 hours for the LHW treated WDG hydrolyzate. Glucose was completely consumed within 6 hrs for the hydrolyzate of AFEX treated WDG. The slower fermentation rate for the hydrolyzate of LHW treated WDG is due to the high concentrations of initially present sugars and fermentation inhibitors that may affect the yeast metabolism. The metabolic yield after 48 hrs of fermentation was 0.53 g ethanol/g consumed sugar or 104% of the theoretical yield for the LHW treated WDG. It was 0.61 g ethanol/ g consumed sugar for the AFEX treated WDG hydrolyzate, which is equivalent to 120% of the theoretical yield. The yields higher than 100% of the theoretical ethanol yields imply that co-current hydrolysis and fermentation of oligosaccharides or colloidal cellulose in the WDG hydrolysates is occurring in the fermentor. In both cases the glucose was almost totally consumed, with the difference in ethanol being proportional to the initial sugar yields. Results suggest that, although the sugar consumption rate was slower for the LHW treated WDG, the extent of the fermentation was not significantly affected at the levels of sugars and inhibitory substances found in the concentrated hydrolyzate.



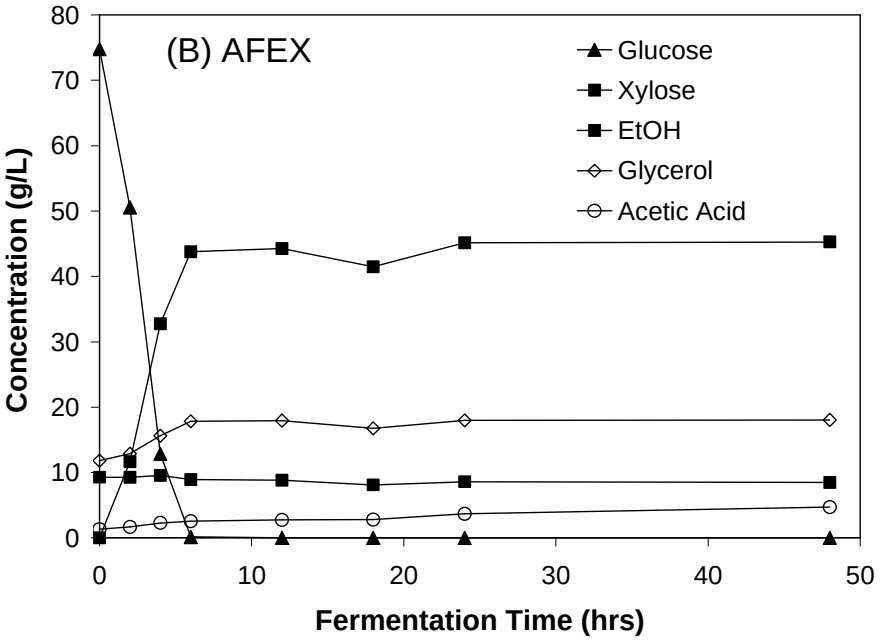


Figure 7. Fermentation of (A) hydrolyzate of LHW treated WDG at 13% solids (w/w), and (B) hydrolyzate of AFEX treated WDG at 15% solids (w/w). Enzyme loading: 15 FPU/g glucan cellulase (Spezyme CP) and 40 IU/g glucan β-glucosidase (Novozyme 188). Hydrolysis at 50^{o}C, 200 rpm for 72 hrs. Final hydrolyzates were concentrated by 5 times for LHW treated WDG and 3.4 times for AFEX treated WDG via lyophilization, prior to the fermentation.

Glucose/Xylose Co-fermentation: As illustrated in Figure 8, both glucose and xylose were successfully fermented by glucose/xylose co-fermenting yeast, 424A (LNH ST). Glucose was completely consumed within the initial 3 hrs of fermentation. Ethanol production yield at 3 hr, based only upon glucose was 80% for LHW treated WDG and 70% for AFEX treated WDG hydrolysate. Xylose was also quickly consumed as the fermentation proceeded. The ethanol production yields calculated suggest that at least 74-80% of the fermentable sugars (glucose and xylose combined) present in the beginning WDG and added xylose were converted to ethanol during the xylose co-fermentation. Metabolic ethanol yield (% of theoretical ethanol based on the consumed sugars) was 103-105% for all runs, suggesting that oligomeric sugars were present in the liquid hydrolysate which were further hydrolyzed and converted by the yeast to ethanol during the fermentation. A reference fermentation of reagent grade glucose (115 g/l) and xylose (50 g/l) was completed within 48 hr and the metabolic ethanol yield was 97% (data not shown). These results suggest the presence of fermentation inhibitors in the hydrolysate of WDG that impedes xylose consumption.

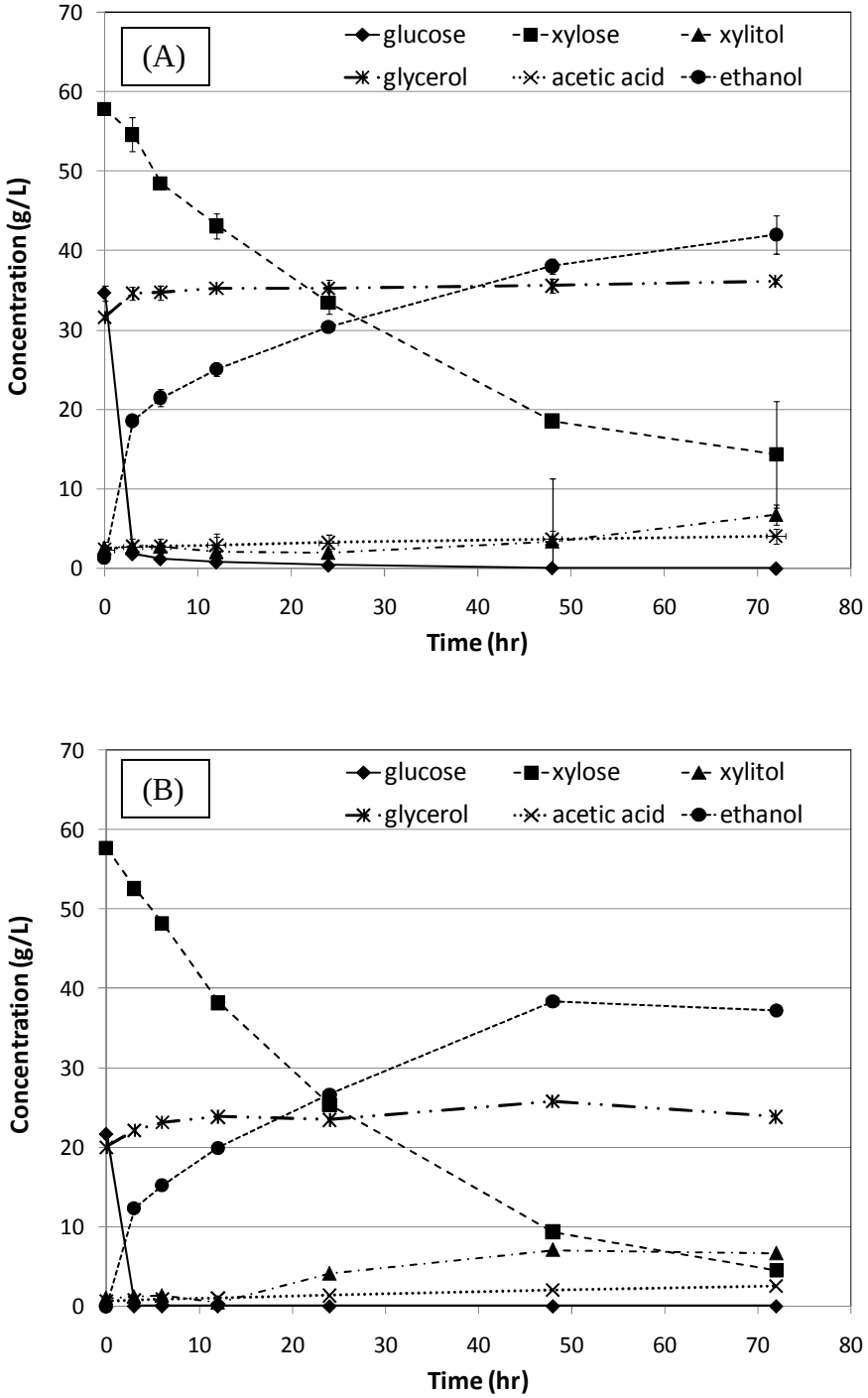


Figure 8. Fermentation time course of LHW pretreated and AFEX pretreated wet cake with added xylose. (A) LHW treated; (B) AFEX treated. Hydrolysis conditions: 15% (w/w) dry solids loading for 48 hrs using 15 FPU Spezyme CP and 40 IU Novozym 188 per g glucan, Multifect Pectinase PE at 50 U xylanase/g dry solids and Depol 740 at 2 U feruloyl esterase/g dry solids. 50 ^{o}C, pH 4.8, 200 rpm. Fermentation conditions: liquid fraction of the hydrolysate, *Saccharomyces cerevisiae* 424A (LNH-ST), 28 ^{o}C, 100 rpm 72 hrs. Error bars represent 95% CI. Data without error bars are average of duplicate runs.

7. Feed analysis of enhanced DDGS:

The solids remaining after fermentation had significantly higher protein content and are representative of a protein-enhanced wet DG that would result in enhanced DDGS. Enhanced DDGS refers to the solid product of a modified dry grind process in which the distiller's grains are recycled and processed further to extract the unutilized polymeric sugars. The laboratory prepared enhanced DDGSs were found to contain 20-50% higher proteins than conventional DDGS (see Table 3). Feed analysis and amino acid profiles of the enhanced DDGS indicated that several heat-sensitive amino acids were damaged during heat treatment of the distiller's grains. This could be caused by Maillard reaction during pretreatment of distiller's grains, which also explains the color change of the processed distiller's grains. These preliminary observations necessitate a study of utilization of the enhanced DDGS as animal feed, in much greater depth, to evaluate its value over that of DDGS accurately.

Table 3. Feed analysis results of DDGS and enhanced DDGS. Results are expressed on a dry matter basis (wt/wt %).

% Compositions	DDGS Average Value[+]	DDGS, (this work)	Enhanced DDGS (from LHW WDG)	Enhanced DDGS (from AFEX WDG)
Moisture	11.1	10.4	6.6	11.5
Crude Protein*	30.2	28.3	41.2	50.8
Crude Fat	10.9	14.5	14.7	7.2
Crude Fiber	8.8	6.5	2.9	0.5
Ash	5.8	4.8	5.3	6.0
Pepsin Digestibility**	----	86.7	86.7	92.2
Carbohydrates***	----	52.5	38.8	36.0

* Crude protein by Kjeldahl.
** Percentage of crude protein digested by pepsin.
*** Carbohydrates calculated by difference from proximate data.
[+]Spiehs et al., 2002.

8. Process simulation of modified dry grind ethanol plant:

A simulated material balance model was established for evaluation of the impact of incorporating pretreatment and fermentation of distillers' grains in conventional dry grind process. The simulated material balance model presented provides an initial frame work for an economic study and for estimating impacts of improvements.

Our simulated material balances for the three different proposed dry grind processes with recycle and saccharification of the distillers' grains showed that the modified dry grind processes yield 14% higher ethanol yield than the conventional process. The theoretical mass balance showed that the modified processes yield 134 gal of ethanol based on input of 1000 kg of dry corn. However, the results (see Figure 9) also predicted at least 2-5 times higher concentrations of by-products and inhibitory components in the fermentation step of the modified processes as compared to the conventional dry grind process. The impact of toxic substances at anticipated

levels on kinetics of enzymatic hydrolysis and fermentation of the distillers' grains requires further investigation to validate the process modifications. The water balance for the modified dry grind processes showed that the proposed processes require less fresh water input for the liquefaction than a conventional dry grind process, and hence compensates for the increased overall water requirement for the modified processes. The final co-product, eDDGS, contains a higher amount of protein per total mass. The total solids sent to the drier, as well as the total water, are decreased, thus decreasing the drier load. A complete analysis on protein quality, digestibility, amino acid and mineral profiles of the enhanced DDGS is still needed to verify its value as an animal feed.

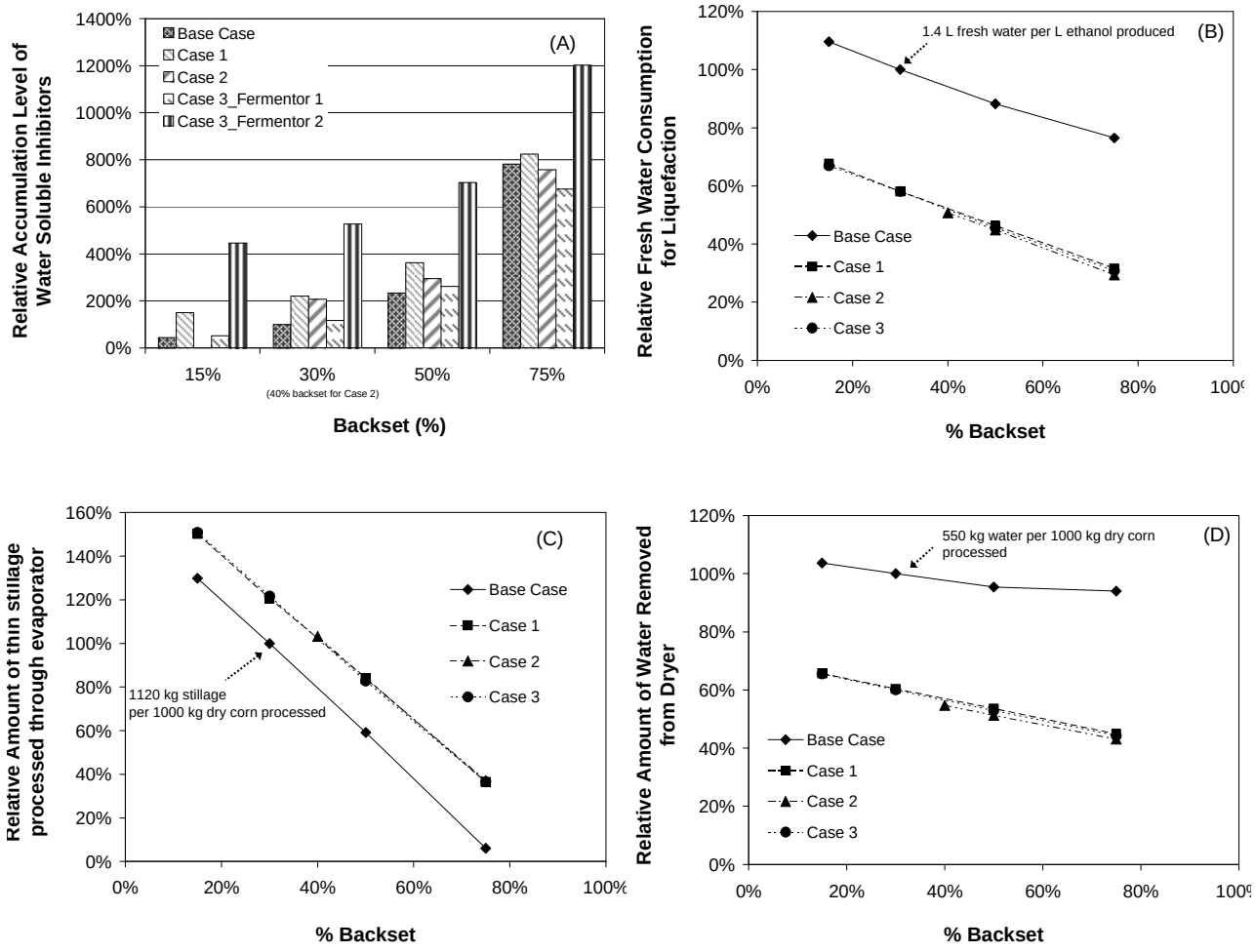


Figure 9. Effect of levels of backset in the process on relative (A) water soluble inhibitors in fermentation mash; (B) amount of fresh water input to liquefaction; (C) amount of thin stillage processed through evaporator; (D) amount of water removed from dryer. Conventional dry grind process (base case) with 30% backset represents the base case (i.e. 100% on y-axis).

9. Economic Analysis of modified dry grind ethanol plant:

A dry grind financial model, which has been validated against other financial models in the industry, was utilized to determine the financial impact of the process changes in a modified dry grind process. A 32% increase in net present value (NPV) for the overall operation is expected when applying the process modifications to a 100 million gallon ethanol plant, and an enzyme cost of $0.20 for each additional gallon of ethanol produced (Table 4). However, there may be no value added to the enhanced dried distillers' grains (eDDGS), even in light of its higher protein levels, as current pricing is expected to be more sensitive to the amino acid profile than the total protein level, and the eDDGS has lower lysine levels, a key amino acid. The financial improvements are a result of the increased revenue from higher ethanol yields outpacing the sum of all added costs, which include higher capital costs, larger loan payments, increased operating costs, and decreased revenues from dried distillers' grains. At current ethanol prices, the economics of the pretreatment technology are encouraging, and finding a way to maintain lysine levels in the dried distillers' grains would likely make the technology even more economically attractive. Any changes which either increase the value of the eDDGS or decrease the cost of the enzyme mixture would help to lower this breakeven price even more, thus making ethanol production by the dry grind process feasible over a larger range of ethanol prices.

Table 4. Annual Revenue and Operating Cost Details of the Base and Alternative Processes

Revenues	Base Process	With Pretreatment
Ethanol	$223,000,000	$251,254,754
DDGS or eDDGS	$37,694,685	$28,735,669
CO2	$2,004,506	$2,271,200
Total Revenue	**$262,699,191**	**$282,261,623**

Costs	Base Process	With Pretreatment
Materials	**$166,843,443**	**$171,035,283**
Energy and Water	**$33,707,243**	**$39,552,795**
Indirect	**$27,917,734**	**$30,813,358**
Total Operating Costs	**$229,165,569**	**$241,401,436**

Net Benefits	Base Process	With Pretreatment
without loan	**$33,533,622**	**$40,860,187**

10. Pretreatment of DDGS via derivatization with phosphite esters:

In addition to liquid hot water and AFEX pretreatment, derivatization of DDGS by phosphite esters was studied as a mean to increase reactivity of cellulose in DDGS by disrupting hydrogen bonding between cellulose polymers. Water solubilization of cellulose and hemicellulose in DDGS facilitate access to them by cellulases and other hydrolytic enzymes. DDGS was pretreated with commercially available trimethylolpropane phosphite

[P(OCH2)3CEt] in the presence of a slight molar excess of water. Greater than 90% DDGS solubility was achieved (Table 5). FTIR spectral analysis indicates that the mode of action of this pretreatment method is to cleave glycosidic bonds of carbohydrates, thus leading to solubilization of DDGS.

Table 5. LHW-DDGS Solubility with Varying Amounts of Water in the Presence of phosphite ester **3**.[a]

Entry	Phosphite (g)	H_2O or MeOH (mL)	% Soluble[h]
1	7.14	0.00	19%
2	7.14	1.00	99%
3	0.50	0.07	86%
4	7.14	0.08	37%
5	0.93	1.00	89%[c]
6	4.50	1.00	100%[d]
7	7.14	1.76 MeOH	36%

[a]All treatments were performed with 250 mg LHW-DDGS heated at 150 ^{0}C for 48 h. [b]Determined from (weight of insoluble material after methanol extraction)/(initial weight of lignocellulosic material)x100%. [c]10 mol% **3** based on water; reaction mixture turned black. [d]50 mol% **3** based on water.

11. Acid-functionalized mesoporous silica as a catalyst for hydrolysis of oligosaccharides:

As an alternative to enzymes, propylsulfonic acid functionalized mesoporous silica was examined for the hydrolysis of oligosaccharides released from the hydrothermal pretreatment of distiller's dry grains. The use of solid acid catalysts would obviate the need for the neutralization and separation step unlike a liquid acid. Acid-functionalized mesoporous silica was shown to be an effective catalyst for the hydrolysis of the oligosaccharides at temperatures above 175 ^{o}C particularly if an activated silica material was used to remove impurities. A simple activated silica treatment was appeared to be effective in removing deactivating impurities such as proteins.

Yields of released monosaccharides by propylsulfonic acid functionalized catalyst are compared to yields obtained from hydrothermolysis as shown in Figure 10. Glucose degradation over 90 min of the solid acid catalyzed reaction was negligible. Solid acid catalysts were effective in this hydrolysis, but the more desirable high temperature precludes the use of standard acidic resin materials. The optimal hydrolysis temperature for high recovery of glucose and xylose was different from that for arabinose. Glucose and xylose recovery favored high temperature at which arabinose started to degrade. Results suggest that, while these organic-inorganic hybrid materials appear to be promising, the rate of monosaccharide degradation particularly for the C5 sugars may limit the utility of solid acid catalysts in the hydrolysis of oligosaccharides released from hydrothermal pretreatment of distiller's dry grains. A multi-step, continuous fed plug flow-type reactor would improve selectivity to the monosaccharides, minimizing sugar degradation.

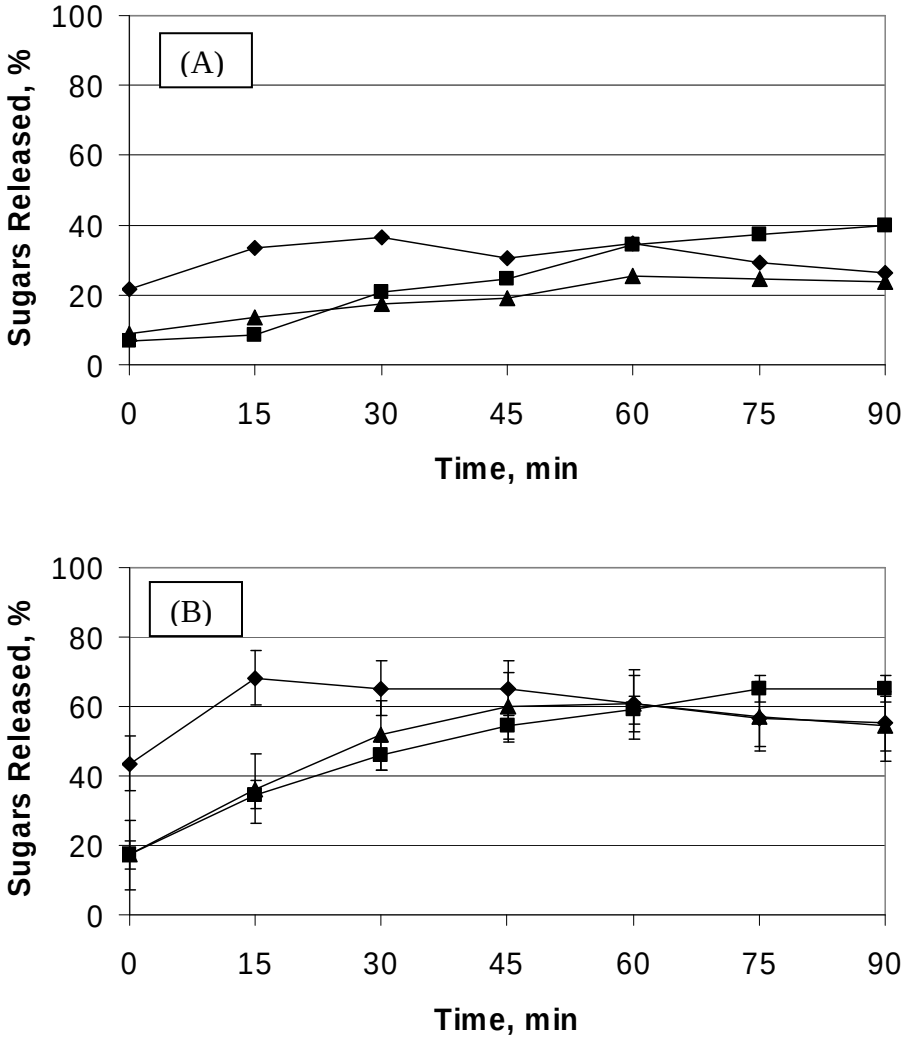


Figure 10. (A) Monosaccharides released at 175 °C due to hydrothermolysis (■ glucose, ▲xylose, ♦ arabinose); (B) Monosaccharides released using the propylsulfonic acid-functionalized catalyst at 175°C (■ glucose, ▲xylose, ♦ arabinose).

12. ABE (Acetone-Butanol-Ethanol) production from DDGS by solventogenic clostridia:

Another potential use of DDGS includes production of ABE by solvent producing clostridia. Ability of solventogenic clostridia to ferment the hydrolysates of pretreated DDGS was examined. The effect of sugars and inhibitors present in DDGS hydrolysates on the cell growth and ABE production was also investigated.

ABE production by solventogenic clostridia using pretreated and enzymatically hydrolyzed DDGS is summarized in Figure 11. Both hexose and pentose sugar were concurrently utilized for growth and ABE fermentation by the solventogenic clostridia, although the rate of sugar consumption was sugar specific. Metabolic yield of ABE from fermentation of DDGS hydrolysate ranged from 0.3 to 0.35, while it was 0.36-0.39 for a reference fermentation of a mixture of reagent grade monosaccharides. Ferulic and ρ-coumaric acids were found to be

potent inhibitors of growth and ABE production from DDGS. Presence of furfural and HMF (2.0 g/L) in the DDGS hydrolysates was not inhibitory to the solventogenic clostridia; rather they are stimulatory to some of the clostridia.

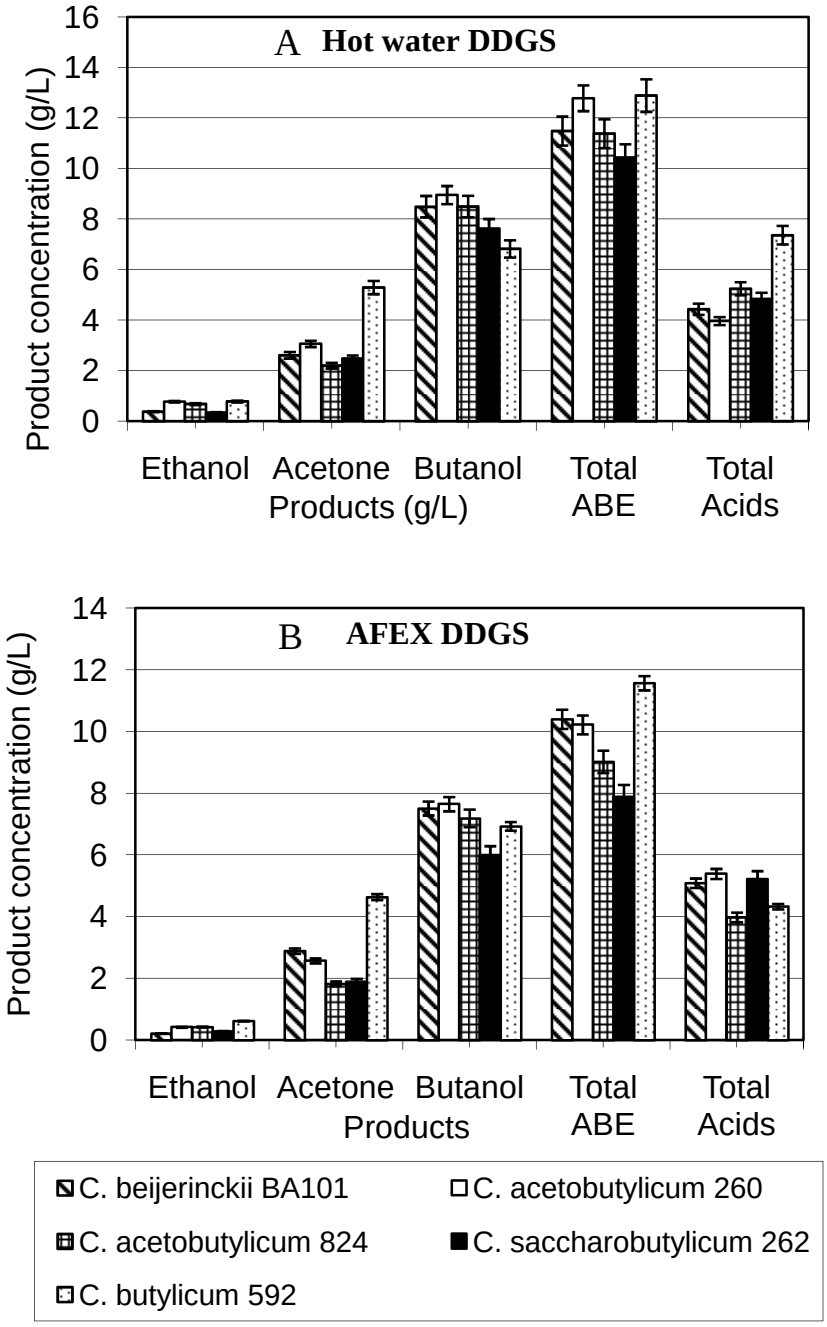


Figure 11. ABE production by solventogenic clostridia using pretreated and enzymatic hydrolyzed DDGS as carbon. **A.** Hot water pretreated DDGS; **B.** AFEX pretreated DDGS.

13. Life cycle assessment of fuel ethanol from corn:

Life cycle analysis serves as a useful tool for assessment of environmental and economic impact of enhancing corn ethanol yields using cellulose conversion technology in a conventional dry milling process. Based on data from eight counties in seven Corn Belt states as corn farming sites, the study has shown that using ethanol derived from corn grain dry milling as liquid fuel (E10 fuel) would reduce nonrenewable energy and greenhouse gas emissions but would increase acidification, eutrophication and photochemical smog, compared to using gasoline as liquid fuel. The environmental performance of corn ethanol depends heavily on the farming site due to different crop management practices, soil properties, and climatic conditions. Nitrogen losses from soil are the dominant factor determining green house gas emissions, acidification, eutrophication and photochemical smog formation. Planting winter cover crops is suggested as a mean to reduce the negative environmental impacts of corn ethanol by reducing nitrogen losses from soil (See Table 6).

Table 6. Environmental impacts associated with the scenario analysis

	HIA	FIL	TMI	MMN	FMN	MMO	HNE	CSD	AVG
Nonrenewable energy [MJ kg^{-1}]									
Base case	-15.6	-14.4	-13.6	-14	-15.4	-13.6	-14.3	-15.6	-14.6
Winter Cover Crop	-16.0	-15.1	-14.5	-14.0	-15.7	-14.3	-15.0	-15.6	-15.0
Greenhouse gas emissions [kg CO_2 eq. kg^{-1}]									
Base case	-1.06	-0.94	-0.83	-0.76	-0.78	-0.22	-0.86	-1.00	-0.80
Winter Cover Crop	-1.43	-1.53	-1.28	-1.07	-1.29	-0.80	-1.39	-1.30	-1.26
Acidification [moles H^+ eq. kg^{-1}]									
Base case	0.28	0.53	0.57	0.56	0.18	0.65	0.33	0.34	0.43
Winter Cover Crop	0.09	0.19	0.23	0.31	0.07	0.48	0.09	0.18	0.21
Eutrophication [g N eq. kg^{-1}]									
Base case	0.51	1.82	2.44	2.00	0.33	3.60	0.84	0.56	1.51
Winter Cover Crop	0.09	-0.14	-0.24	0.41	0.04	0.12	-0.23	0.24	0.04
Photochemical smog formation [mg NO_x m^{-1} eq. kg^{-1}]									
Base case	10.75	17.67	17.75	18.83	7.60	21.02	12.13	12.68	14.80
Winter Cover Crop	5.14	7.53	7.76	11.25	4.42	16.39	5.04	7.85	8.17

Publications:

The special edition of Bioresource Technology on Cellulose Ethanol in Dry Grind Plants has been published in August 2008. The manuscript entitled “Effect of Distillers’ Grains Product Variability on its Conversion to Fuel Ethanol” summarizes the works done in the second phase of Midwest Consortium project and has been recently submitted to Bioresource Technology.

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