Project Title:
Separation of Corn Fiber and Conversion to Fuels and Chemicals
Phase II: Pilot-scale Operation

DE-FC36-03GO13147

Abstract

The purpose of the Department of Energy (DOE)-supported corn fiber conversion project, "Separation of Corn Fiber and Conversion to Fuels and Chemicals Phase II: Pilot-scale Operation" is to develop and demonstrate an integrated, economical process for the separation of corn fiber into its principal components to produce higher value-added fuel (ethanol and biodiesel), nutraceuticals (phytosterols), chemicals (polyols), and animal feed (corn fiber molasses). This project has successfully demonstrated the corn fiber conversion process on the pilot scale, and ensured that the process will integrate well into existing ADM corn wet-mills. This process involves hydrolyzing the corn fiber to solubilize 50% of the corn fiber as oligosaccharides and soluble protein. The solubilized fiber is removed and the remaining fiber residue is solvent extracted to remove the corn fiber oil, which contains valuable phytosterols. The extracted oil is refined to separate the phytosterols and the remaining oil is converted to biodiesel. The de-oiled fiber is enzymatically hydrolyzed and remixed with the soluble oligosaccharides in a fermentation vessel where it is fermented by a recombinant yeast, which is capable of fermenting the glucose and xylose to produce ethanol. The fermentation broth is distilled to remove the ethanol. The stillage is centrifuged to separate the yeast cell mass from the soluble components. The yeast cell mass is sold as a high-protein yeast cream and the remaining sugars in the stillage can be purified to produce a feedstock for catalytic conversion of the sugars to polyols (mainly ethylene glycol and propylene glycol) if desirable. The remaining materials from the purification step and any materials remaining after catalytic conversion are concentrated and sold as a corn fiber molasses. Additional high-value products are being investigated for the use of the corn fiber as a dietary fiber sources.

Executive Summary

The multidisciplinary project team from the National Corn Growers Association (NCGA), Archer Daniels Midland (ADM), and Pacific Northwest National Laboratory (PNNL) intend to economically derive high-value chemicals and oils from lower value corn fiber. In the process, starch is recovered as glucose, which is then converted to ethanol. The hemicellulose fraction is hydrolyzed to mainly yield the 5-carbon sugars, arabinose and xylose. The xylose is converted to ethanol, and the arabinose is catalytically converted to ethylene glycol, propylene glycol, and glycerol. The small amounts of six carbon sugars in the hemicellulose are metabolized in the ethanol fermentation. In addition, high-value oil components, sterols and stanols, are recovered. The residual fiber (~50% by weight of the original corn fiber) contains primarily hemicellulose, cellulose, and protein. The residual solubilized components are combined and concentrated to produce a high-protein content, liquid animal feed.

The subject of this project was pilot-scale testing to validate the process prior to full-scale commercial implementation. The pilot-scale testing phase entailed bench-scale process optimization testing, pilot-plant selection, testing, and an economic evaluation of the integrated process. Piloting of the process is necessary so that the technical (i.e., processing and operation of key equipment) and economic aspects of the process can be more thoroughly evaluated prior to commercialization of the process. The corn fiber conversion process can produce an additional 0.25 gallons of ethanol per bushel of corn processed. An economic analysis of the process gave a return on investment of 34% and additional revenue of US$86M.

Table of Contents

Table of Figures

Table of Tables

Final Progress Report

Status

Background

The United States currently produces about 280 million metric tons (11 billion bushels) of corn annually. Of this production, over 25 million metric tons (1.0 billion bushels) are processed through corn wet milling at a variety of locations throughout the United States for the production of starches and modified starches, corn syrups, corn oil, chemicals, animal feeds, ethanol and other fermentation products, and additional co-products. During the corn wet-milling process, corn fiber hulls (pericarp, approximately 5.6 million metric tons/yr nationwide) are produced as a co-product and are currently mixed with stillage from the ethanol distillation step, dried, and sold as relatively low-value animal feed (corn gluten feed), which is mainly fed to cattle due to the high fiber content. The fiber in corn fiber is mostly indigestible to monogastric animals and poultry, but relatively digestible to cattle.

Over 60% of the corn gluten feed produced is exported from the United States. A large market for this product is the European Union. As the amount of genetically engineered "biotech" corn planted increases dramatically, currently 50% of the corn grown in the United States is genetically modified, the resistance to utilizing this animal feed is increasing. This pushes the market price down.

Under a previous DOE/EE/OTT-sponsored project entitled *Corn Fiber Separation and Conversion to Fuels and Chemicals*, bench-scale testing led to the development of a technically and economically feasible integrated process for recovery of the hemicellulose, protein and oil components from corn fiber and subsequent conversion of these components to value-added products. The success of the first project in accomplishing the goals set forth led to the granting of the current DOE sponsored project *Separation of Corn Fiber and Conversion to Fuels and Chemicals Phase II: Pilot-scale Operations.* This project is concerned with scaling up the corn fiber conversion process for validation of the process on the pilot-scale. The project team for the first two projects is multidisciplinary, consisting of business managers, chemical and agricultural engineers, chemists, food scientists, biologists, and biochemists from Archer Daniels Midland (ADM), the National Corn Growers Association (NCGA), and Pacific Northwest National Laboratory (PNNL). Additional collaborations involving the National Renewable Energy Lab (NREL) and four full professors at the University of Illinois occurred during the second phase of the project. These collaborations have been extremely beneficial for the completion of the second phase of the project.

In the simplified block diagram of the corn fiber conversion process, Figure 1, the first step is a thermochemical hydrolysis pretreatment step. This step utilizes the sulfur dioxide present in the corn fiber from the corn steeping step as an acid catalyst to hydrolyze the polysaccharides, starch and hemicellulose, to soluble oligosaccharides and monosaccharides. A small amount of sulfuric acid can also be utilized during this step to balance differences seen in the sulfur dioxide concentration of the corn fiber, as the sulfur dioxide concentration in the corn fiber has been seen to be variable. The hydrolysis pretreatment step hydrolyzes the starch to soluble starch

oligosaccharides, and the arabinoxylan hemicellulose fraction to soluble fractions of the hemicellulose. Corn hemicellulose, as shown in Figure 2, is an arabinoxylan composed of a xylan backbone with short side chains consisting of arabinose, galactose, glucose, glucuronic acid, coumaric acid and ferulic acid. To hydrolyze the xylan backbone, the side chains must first be removed to reduce the steric inhibition. Therefore, during the hydrolysis, the first components solubilized are the side chain monomers. The ferulic acid on the side chains can also form diferulic bonds, which are structurally similar to lignin, and provide the same protection from degradation.

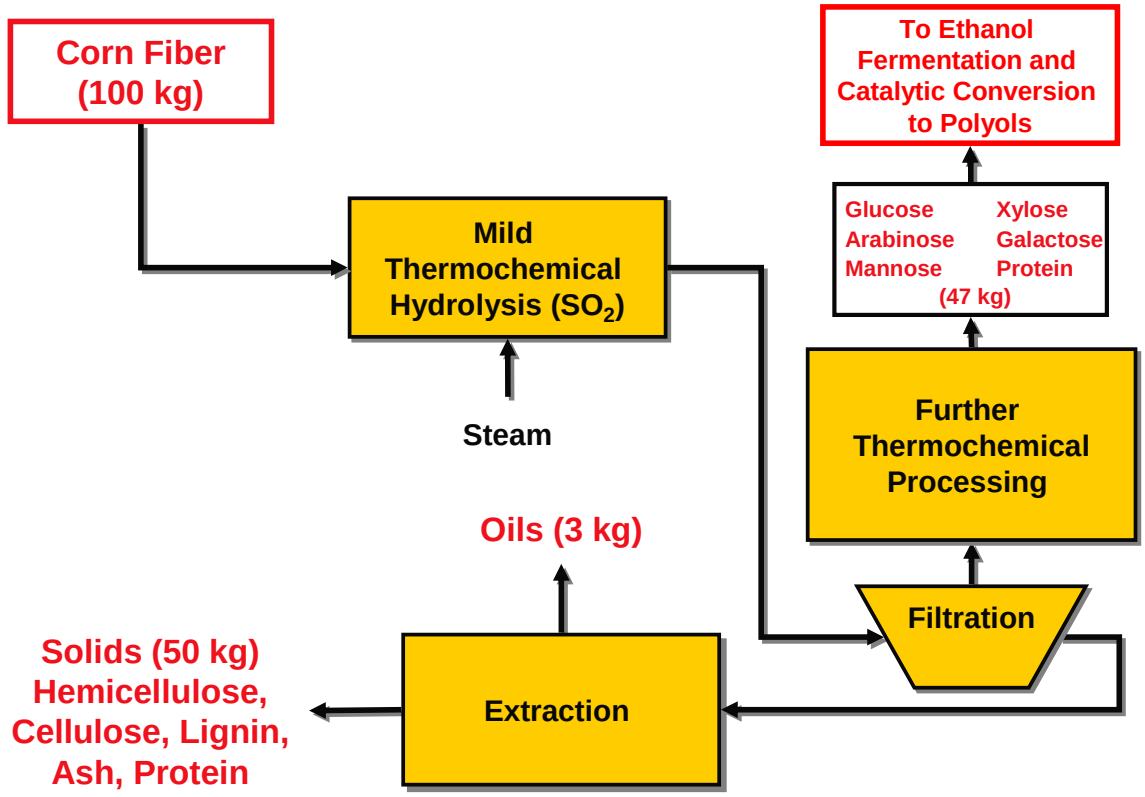

Figure 1: Corn Fiber Fractionation and Utilization Process

After the initial hydrolysis pretreatment step, the soluble portion of the fiber is removed by a step incorporating liquid/solid separation and washing. The corn fiber hydrolysate containing the soluble starch and hemicellulose oligosaccharides is then subjected to enzymatic hydrolysis utilizing a blend of enzymes tailored to convert the corn fiber starch and hemicellulose oligosaccharides to monosaccharides. This step will produce glucose from the starch, and xylose, arabinose, galactose, and mannose from the hemicellulose, as well as ferulic and coumaric acid and glucuronic acid.

The remaining hydrolyzed corn fiber insoluble solid is extracted with solvent to extract the corn fiber oil. The corn fiber oil contains valuable phytosterols, which are used by pharmaceutical, nutraceutical, and food companies. Pharmaceutical companies convert the sterols to steroids and the nutraceutical and food companies sell the phytosterols for human consumption. Phytosterols have been shown to decrease the cholesterol in the bloodstream. Under Phase I of the project, the

extracted oil was fully characterized, and a typical oil composition is shown in Table 1. The oil contains 0.2-0.6wt% sterols on a dry corn fiber basis. In Phase II, significant improvements were made related to recovery of the oils.

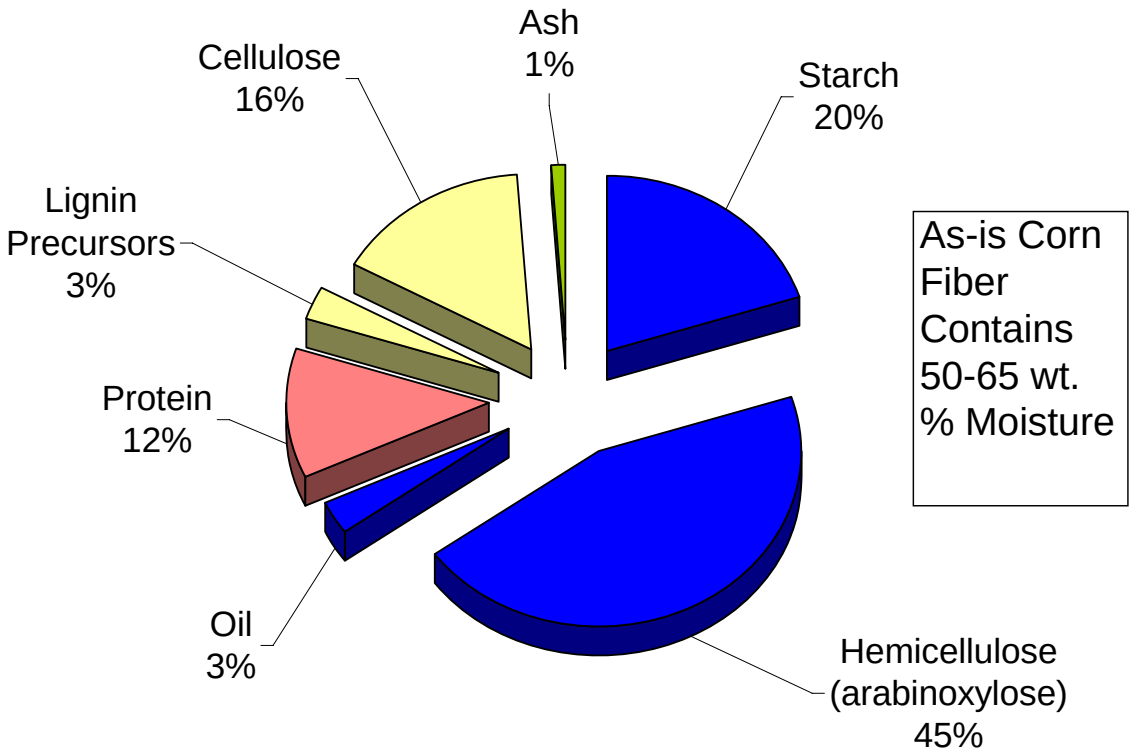

Figure 2: Corn Fiber Composition

Table 1: Extracted Corn Fiber Oil Composition

	% of Corn Fiber	% of Oil
Total Oil Yield (less sterols and stanols)	3.00%	83.37%
Total Sterols + Stanols	0.60%	16.63%
Sitosterol	0.21%	5.93%
Sitostanol	0.22%	6.21%
Campestanol	0.07%	2.02%
Campesterol	0.06%	1.75%
Stigmasterol	0.03%	0.73%

The de-oiled, solvent-extracted corn fiber is then processed to remove the solvent leading to a dry fiber stream, which is utilized as a carrier for the corn stillage in the plant. The mixture is dried, pelletized and sold as an animal feed. The corn fiber hydrolysate liquid is fermented utilizing a recombinant organism to ferment the glucose and xylose to ethanol. The ethanol is removed by distillation and the remaining slurry is purified to produce a sugar stream, which can be catalytically converted to polyols.

Project Progress:

Bench-scale and pilot-scale hydrolysis testing, fermentation testing and corn fiber extraction were conducted at ADM, and fiber extraction and pretreatment/catalyst testing were conducted at PNNL during this project. Subcontract work was completed at the University of Illinois and the National Renewable Energy Laboratory.

University of Illinois Projects

ADM subcontracted four University of Illinois Professors to work on projects directly related to the corn fiber project. The four professors are George Fahey, Rod Mackie, Yuanhui Zhang, and Hans Blaschek. The projects include testing the hydrolyzed, extracted fiber for use in animal diets; creating enzymes to hydrolyze the fiber or solubilized fiber; continuously hydrolyzing the corn fiber on a small scale; and testing the corn fiber hydrolysate with several organisms for fermentability to various products including ethanol.

Throughout the project, meetings occurred between ADM and the University of Illinois staff subcontractors regarding the DOE project. The specific projects that the Professors are working on are:

Dr. George Fahey – "*Separation of Corn Fiber and Conversion to Fuels, Chemicals, and Value-Added Feed Additives.*" Evaluating the extracted corn fiber as a pet food fiber source.

Dr. Roderick Mackie and Dr. Isaac Cann – "*Arabinoxylan Degradation & Utilization.*" Examining novel hemicellulases cloned from rumen microorganisms.

Dr. Yuanhui Zhang – "*Development and Piloting of a Continuous Thermal Hydrolysis System for the Fiber Stream.*" Continuous hydrolysis of corn fiber in a small-scale reactor.

Dr. Hans Blaschek – "*Corn Fiber Hydrolysis and Fermentation to Butanol using Clostridium beijerinckii BA101.*" Screening the fermentability of the corn fiber hydrolysate with several butanol-producing organisms, including Clostridium beijerinckii BA101.

<u>Dr. Fahey</u>

This project was concerned with the suitability of the residual corn fiber after hydrolysis and extraction as a pet food fiber source. The corn fiber was analyzed at three conditions: as-is, after hydrolysis, and after hydrolysis and extraction. The composition of the fiber on a monosaccharide basis was obtained to determine if the monosaccharides contained in the fiber would be beneficial as a prebiotic for hind-gut fermentation organisms, and thus, for animal health. The screening also included animal *in situ* trials – 2-stage digestion and 3-stage fermentation. The fiber samples were also incorporated into dog food kibbles and utilized in a feeding trial.

<u>Dr. Mackie</u>

This project was concerned with cloning fiber-degrading enzymes found in rumen organisms and determining the activity of those enzymes on the corn fiber. The goal is to produce a blend of

enzymes that can be commercially produced for use on corn fiber. Over twenty rumen enzymes with complementary fiber-degrading activities were cloned and produced and tested on the corn fiber.

Dr. Zhang:
This project approached the continuous thermochemical hydrolysis of corn fiber with a first set of optimization experiments in a batch reactor followed by operation of the continuous hydrolysis reactor. A design of experiments was completed for the thermochemical hydrolysis of corn fiber and carried out on a modified Parr Reactor. The continuous thermochemical hydrolysis system was modified and several experiments were executed on the hydrolysis of corn fiber.

Dr. Blaschek
This project was only concerned with the screening of ABE-producing (acetone, butanol, ethanol) organisms on corn fiber hydrolysate. Concentrated corn fiber hydrolysate was shipped to their lab and screening of the fermentability of the hydrolysate with several organisms, including the "hyper butanol producing organism" *Clostridium beijerinckii* BA101, was completed. Optimization of the fermentation conditions was attempted.

ADM Corn Fiber Hydrolysis

ADM Experiments
Run 4232-71
A total of 26.1 kg of fiber (as-is) were hydrolyzed in the tumbler reactor in three batches. The reactor was vented and the fiber was removed and weighed. A screw press was used to dewater the fiber. The total dry weight of the corn fiber after hydrolysis and washing was 4.6 kg, which gives a total solubilization of 58.7%. The fiber was frozen for future oil extraction experimentation. The liquid was subjected to secondary acid hydrolysis by heating the hydrolysates with 1% sulfuric acid. The hydrolysate was concentrated to a solids content of 40% for use as a fermentation feedstock.

The results are shown in Table 2, Table 3, Table 4, and Table 5. Table 2 shows the protein and degradation product concentrations in the corn fiber hydrolysate concentrate. The results are similar to what has been previous results. Table 3 shows the amino acid analyses from the concentrated corn fiber hydrolysate. The last column is the concentration in wt. % on a d.w.b. of amino acids in corn gluten feed, which is largely composed of corn fiber. The ratios of the amino acids in the experimental results from the hydrolyzed corn fiber and the levels of amino acids in the corn gluten feed are similar. Table 4 shows the elemental analysis of the corn fiber hydrolysate concentrate along with the elemental analysis of corn gluten feed in wt % on a d.w.b. The main differences seen are the elevated levels of sulfur, sodium, and iron in the hydrolysate. The sulfur level is high because of the sulfuric acid used to perform the secondary acid hydrolysis of the corn fiber hydrolysate, the sodium level is high because sodium hydroxide was used to elevate the pH, and the iron is slightly higher than in corn fiber most likely due to the steel equipment used to process the corn fiber.

Table 2: Acetic Acid, Degradation Products, and Protein Results for Thermochemical Hydrolysis of Corn Fiber 4232-71 (Values are in g/l)

	Acetic Acid	Protein	HMF	Furfural
4232-71	3.40	28.30	0.45	0.20

Table 3: Amino Acid Results for Thermochemical Hydrolysis of Corn Fiber 4232-71

	4232-71 Hydrolyzed (g/L)	Corn Gluten Feed %
Aspartic Acid	2.28	1.3
Threonine	1.14	1.0
Serine	1.58	1.1
Glutamic Acid	5.52	3.7
Proline	2.59	1.9
Glycine	1.93	1.1
Alanine	2.37	1.7
Cystine	0.22	0.55
Valine	0.96	1.1
Methionine	0.50	0.55
Isoleucine	0.57	0.7
Leucine	2.67	2.1
Tyrosine	1.01	0.7
Phenylalanine	1.22	0.9
Lysine	0.50	0.65
Ammonia	1.40	
Histidine	0.90	0.8
Arginine	0.75	1.1

Table 4: Elemental Analysis Results for Corn Fiber Hydrolysate Concentrate 4232-71

	4232-71	Corn Gluten Feed %
P	0.68	1.0
S	11.40	0.18
Na	17.70	0.13
Mg	0.36	0.46
K	1.61	1.4
Ca	0.08	0.2
Fe	0.10	0.03

g/L

The corn fiber hydrolysate was analyzed for sugars by gas chromatography and the results are shown in Table 5. As the concentration of the hydrolysate is 40% solids, over half of the solids are sugars. The remainder of the solids are composed of protein, degradation products, and organic acids. The ratios of sugars is similar to theoretical (44% xylose/29% arabinose/7% galactose in the hemicellulose) except for the high galactose concentration. The galactose and arabinose compose the side chains of the corn fiber hemicellulose, while the xylose is the

backbone, so it would be reasonable to have slightly higher levels of galactose and arabinose since the xylose chain is not completely hydrolyzed.

Table 5: GC Analysis of Corn Fiber Hydrolysate Concentrate 4232-71.

Sample Id	Xylose	Arabinose	Fructose	Mannose	Galactose	Glucose	Sucrose	Maltose	Total Sugars
4232-71	54.19	45.32	0.80	0.71	36.52	57.64	0.39	16.40	212.0

Run 4232-86

An additional 43.2 kg of corn fiber were hydrolyzed in the tumbler reactor. The fiber was hydrolyzed without the addition of water. The fiber was removed, weighed, and dewatered by processing with the screw press. The fiber was rinsed twice with water and dewatered by processing with the screw press.

The liquid hydrolysates and washes weighed a total of 85 kg. The hydrolysates and washes were combined and subjected to 1% sulfuric acid hydrolysis to hydrolyze the oligosaccharides to monosaccharides. The final hydrolysate was concentrated to a weight of 19.9 kg with a solids concentration of 26.4%.

The results for the hydrolysate concentrate are shown in Table 6. The ratios of xylose:arabinose:galactose are much closer to what would be seen in the corn fiber hemicellulose (44% xylose/29% arabinose/7% galactose in the hemicellulose) than the previous corn fiber hydrolysis results.

Table 6: ADM Analytical Results for Experiment 4232-86

	Glucose	Xylose	Arabinose	Galactose	Mannose	
Concentrate	66.7	55.0	49.4	7.9	1.2	
	Maltose	Acetic Acid	HMF	Furfural	Protein	Total Concentration (g/L)
Concentrate	15.6	2.6	0.5	0.3	22.2	221.4

Run 4232-128

An additional 30.0 kg of corn fiber was hydrolyzed in the tumbler reactor. The fiber was hydrolyzed without the addition of water. After hydrolysis, the fiber was dewatered using the screw press. The three batches of hydrolyzed corn fiber were combined and washed and dewatered using the screw press. The corn fiber hydrolysate extracts and washes were combined and subjected to secondary acid hydrolysis by adding 0.8% sulfuric acid and hydrolyzing. This step hydrolyzes the oligosaccharides present in the hydrolysate to monosaccharides. The mixture was then transferred for use as a fermentation media for fermentation.

Corn Fiber Thermochemical Hydrolysis, Secondary Hydrolysis, and Concentration – A3 hydrolysate preparation

Nine batches of corn fiber at 5 kg each were thermochemically hydrolyzed to prepare an ethanol fermentation media and to prepare the fiber for ethanol extraction of the phytosterols and oil. The fiber was treated in the tumbler reactor at high temperatures for the required time. Each batch of

fiber was dewatered, washed with water, and dewatered. The liquid hydrolysate and solid were collected and stored for further processing. The average solubilization for the runs was 49.4%.

A total of 193 kg of liquid hydrolysate from the corn fiber hydrolysis experiments were subjected to secondary acid hydrolysis by addition of sulfuric acid to the hydrolysate followed by heating for the required time. After hydrolysis, the corn fiber was concentrated using a forced circulation evaporator. Approximately 44.4 kg of 31 wt/wt % hydrolysate concentrate and an additional 5.2 kg of 16 wt/wt % hydrolysate evaporator wash were obtained. Samples of the hydrolysate concentrate were sent to PNNL for analysis and the analysis gave expected results in terms of monosaccharide and inhibitor concentrations.

In addition, some hydrolyzed corn fiber was provided to PNNL by ADM for oil extraction testing. Three oil extraction tests were completed and the oil samples were recovered and are being analyzed for sterol/stanol content.

The hydrolysate concentrate was pH adjusted and then enzyme hydrolyzed to hydrolyze oligosaccharides present in the hydrolysate to monosaccharides. A sample of the hydrolysate concentrate was submitted to PNNL for analysis and also ADM Analytical. The analytical results were received and utilized in the fermentation experimentation. The hydrolysate was used as a fermentation feedstock, and the corn fiber residue will be extracted to obtain the corn fiber oil.

The corn fiber hydrolysate was processed by secondary acid hydrolysis and concentrated by evaporation. The corn fiber hydrolysate concentrate was fermented by microorganism strains in two fermentation media blends. The spent fermentation media was centrifuged and the solids were removed. The liquid portion was evaporated in a forced circulation, long-tube vertical evaporator.

Samples of this distilled, spent corn fiber hydrolysate concentrate fermentation media were sent to PNNL for catalysis testing for converting the remaining sugars to polyols.

NREL Corn Fiber Hydrolysis Experimentation

NREL was subcontracted by ADM to conduct continuous hydrolysis experiments on corn fiber in their Pilot Development Unit (PDU). The work plan included conducting small scale experiments based on a 2 factor central composite experimental design. The factors are time and temperature. After the small scale experiments were completed, a large scale experimental design was undertaken. The fiber used in the small-scale experiments was supplied by the ADM corn wet mill in Columbus, NE. An analysis of the shipped corn fiber from Columbus and a sample of corn fiber from the ADM corn wet mill in Decatur, IL, showed that the amount of starch in the Columbus sample was slightly lower than the Decatur sample, and the amount of protein was higher in the Columbus sample. The differences in composition were determined to be similar to the differences seen between same-plant samples, so the Columbus fiber was utilized in the experiments.

Small-Scale Experimentation

The small scale experiments were completed by NREL and the results showed that as the temperature and time increase, which can be used to calculate a "severity factor", the solubilization of the fiber continually increases. Or in other words, as the severity factor increases, the solubilization of the fiber increases. After a certain point, however, the production of soluble sugars begins to decrease as the sugars produced begin to react and form degradation products. Based on the results of the small-scale experiments, conditions were chosen for the pilot scale experimental design. Further work at NREL included solid/liquid separation experiments and secondary acid hydrolysis experiments on the solubilized fiber.

The small-scale experimental samples at NREL were pressed to remove the liquid from the solids and samples of the liquid and solid were sent to ADM (PNNL also received samples for analysis). The liquid samples were subjected to secondary acid hydrolysis to determine the sugar monomer yield under typical hydrolysis conditions. It was found that as the time and temperature of the initial hydrolysis increased, the amount of monomer sugars obtained from the secondary acid hydrolysis initially increased, and then decreased as the degradation product concentration increased due to the harshness of the initial hydrolysis.

Pilot-Scale Experimentation

A pilot scale trial of corn fiber hydrolysis occurred on Feb. 16th, 2005 at the NREL PDU. During the testing three conditions were evaluated. The corn fiber was used as a feedstock for the continuous reactor [300 grams (dry weight basis)] of each condition was returned to ADM and the remaining material from the three conditions was dewatered and washed in the Pneumapress to form a corn fiber cake, which was shipped back to ADM for oil extraction experimentation.

NREL analyzed the corn fiber hydrolyzed solids and solubilized hydrolysates from the three conditions in the PDU. The pretreatment conditions were designed to overlap what had previously been attempted in the small scale Zipperclave reactor experiment. The results correspond with what has been seen previously for the corn fiber hydrolysis in the small scale Zipperclave reactor. The amount of solubilized sugars in the hydrolysate nearly equaled the amount of corn fiber solubilized, which gives a good mass balance closure.

NREL Pilot-scale Test 1

Four thousand pounds of corn fiber from the ADM, Columbus, NE corn wet mill were processed through the NREL continuous hydrolysis system in their pilot development unit on July 27th and 28th, 2005. The corn fiber extract and washed fiber were shipped back to Decatur, IL for further processing.

NREL Pilot-scale Test 2

Ten thousand pounds of corn fiber were shipped to NREL from the ADM, Decatur, IL corn wet mill for use as a feedstock in their continuous reactor. Hydrolysis of the fiber utilizing previously validated time and temperature conditions followed by centrifugation, was conducted for production of a soluble oligosaccharide fraction, and a fiber residue fraction.

The liquid fraction of the hydrolyzed corn fiber processed at NREL was concentrated from 45 gallons to 2.5 gallons in preparation for use as an ethanol fermentation media. The concentrated

corn fiber hydrolysate was treated with 1% sulfuric acid at 121°C for 30 minutes to hydrolyze the oligosaccharides to monosaccharides.

The concentrated liquid fraction of the hydrolyzed corn fiber processed at NREL in February, 2005 was utilized in fermentation experiments as described in the fermentation section.

NREL Pilot-scale Test 3
Twenty thousand pounds of corn fiber were shipped to NREL for use as a feedstock in their continuous reactor. Hydrolysis of the fiber utilizing previously validated time and temperature conditions followed by centrifugation, was conducted for production of a soluble oligosaccharide fraction, and a fiber residue fraction. Two conditions were selected. The hydrolyzed fiber was been shipped to ADM for corn fiber oil extraction.

Twenty thousand pounds of corn fiber had been previously hydrolyzed at NREL. The corn fiber hydrolysate and wash from the centrifuge were concentrated in their evaporated and two drums of concentrated hydrolysate were shipped to ADM. The hydrolysate was been tested with secondary acid hydrolysis as well as enzyme hydrolysis.

The hydrolysate from this step was utilized in fermentation experiments.

NREL Further Processing
Twenty thousand pounds of corn fiber had been previously hydrolyzed at NREL. The corn fiber hydrolysate and wash from the centrifuge were concentrated in their evaporator and two drums of concentrated hydrolysate were shipped to ADM. The hydrolysate has continued to be tested with enzyme hydrolysis using experimental enzymes and for fermentability with multiple ethanologens.

NREL Results
Corn fiber hydrolysis test data reported by NREL for 16 small-scale and 3 pilot-scale corn fiber hydrolysis tests were evaluated by PNNL to develop empirical predictive correlations for the yields of glucose, xylose, arabinose, furfural, and solids solubilized. The effects of hydrolysis time and temperature, in the small-scale tests, on the total glucose and xylose yields could be predicted with a good degree of confidence, and for total arabinose, monomeric xylose and soluble solids yields with a fair degree of confidence.

Corn Fiber Fermentation-Screening of Ethanologens and Fermentations

Work on optimization of the corn fiber hydrolysate fermentation continued throughout the project. Several organisms were screened for fermentation and adapted on corn fiber hydrolysate. Multiple shake-flask and batch fermentations were carried out using corn fiber hydrolysate as the fermentation media.

The various batches of concentrated corn fiber hydrolysate (A1, A2, A3 – collectively A-series hydrolysates and N1, N2, N3 – collectively N-series hydrolysates) were tested for fermentability by shake flask testing as well as fed-batch and continuous fermentations. Figure 3 shows a

typical corn fiber hydrolysate fermentation run on the A-series hydrolysates in a 4-liter controlled fermentor.

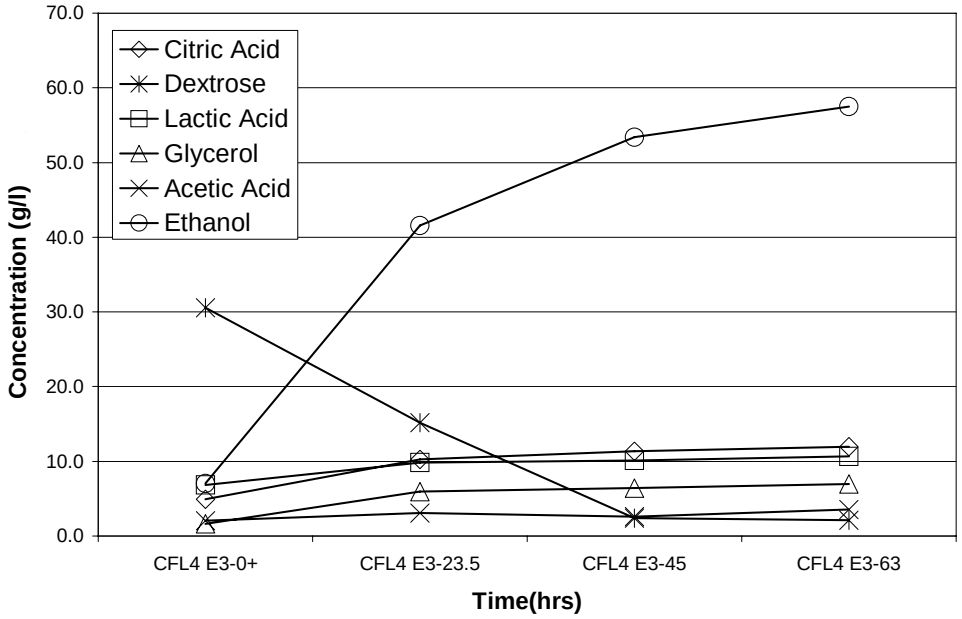

Figure 3: Typical Ethanol Fermentation Profile of A-series Corn Fiber Hydrolysate Concentrate (xylose not shown)

Shake Flask Fermentations

Several new yeast strains were obtained, which are able to ferment glucose and xylose to ethanol. An experiment was designed to screen the performance of the new organisms on corn fiber hydrolysate alongside previously tested organisms.

In one test, pentose/hexose fermenting organisms were grown on 20% corn fiber hydrolysate (CFH) enriched with glucose and xylose to the levels normally contained in 100% CFH. The levels used in the shake flasks were 70 g/L of glucose and 60 g/L of xylose. Eight inoculation shake flasks were inoculated with eight yeasts, Y1-Y8. Yeast counts were performed on the inoculation flasks, the broth was centrifuged and the pellets were washed, and the inocula volumes were adjusted in order to achieve uniform concentrations of yeast cells in the fermentation flasks. The fermentation flasks were sampled every 24 hours for five days and the samples were analyzed for yeast counts, organic acids, and carbohydrates.

A ranking of the highest ethanol concentration of each flask is shown in Table 7. Peak ethanol production was found in strains Y7 and Y6 followed by Y2 and Y4. It is interesting to note that Y2 was able to produce the peak ethanol at 24 hours, while the other strains produced the maximum ethanol at 48 hours. The amount of glycerol produced was also determined and the ratio of ethanol produced to glycerol produced was calculated for each strain. The highest ethanol:glycerol ratio was found in Y1 followed by Y5 and Y7. Yeast strain Y7 was able to utilize the highest amount of xylose at 45% utilization, and Y2 and Y8 were able to utilize the highest amounts of galactose at 54% and 52% utilized, respectively.

Table 7: Performance of Yeast Strains Grown on Corn Fiber Hydrolysate

Yeast Strain	Ranking of Peak EtOH Production @ Peak Time	Ranking by Lowest Glycerol Production	Ranking by Average Xylose Utilization	Ranking by Average Galactose Utilization	Ratio of Ethanol/ Glycerol
Y1	8@24	1	6	8	8.1
Y2	3@24	3	2	1	6.9
Y3	6@48	4	5	3	6.1
Y4	4@48	6	4	6	6.5
Y5	5@24	2	7	7	7.6
Y6	2@48	8	3	5	6.7
Y7	1@48	7	1	4	7.5
Y8	7@24	5	8	2	6

Previous studies showed that the yeast strains which fermented the largest amount of pentose from 20% CFH/1% yeast extract/2% peptone broth are Y1, Y2, and Y3. All three of these organisms fermented xylose on this broth but none exhibited the ability to ferment arabinose to any notable extent. Additionally, of the three organisms, only Y2 fermented xylose from 50% CFH broth. The other strains utilized a majority of the glucose, but were only able to ferment a small portion of the pentoses.

An additional experiment, in which broth containing 20% CFH/80% blender mix (plant ethanol fermentation broth) was utilized as the fermentation media, suggested that two bacterial strains, B1 and B2, as well as Y2 and another yeast strain, Y9, fermented xylose within 72 hours; however, as was observed previously, no organisms appeared to ferment an appreciable amount of arabinose.

The hexoses in all the tested broths seemed to be fermented preferentially to pentoses with D-glucose being completely fermented, **D**-xylose and **D**-galactose only being partially fermented, and L-arabinose not being fermented to any extent.

The CFH was supplemented with an industrial fermentation media (70% CFH/30% industrial media). A strain coded Y2 was grown in two stage CFHH/Industrial Media shake flasks and the ethanol production was measured. The pH was varied between shake flasks and it was found that higher ethanol production occurred with higher pH.

The CFH was supplemented with an industrial fermentation media (20% CFH/80% industrial media). Strains coded Y1 and Y2 were grown in two stage CFH/Industrial Media shake flasks (stage 1: 90%/10%, stage 2: 80%/20% Industrial Media/CFH), then transferred to non-aerated fermentors containing 80%/20% Industrial Media/CFH. Strain Y2 utilized nearly all the available glucose and 60% of the available xylose.

Agar Plate Growth

Eight ethanol-producing organisms were streaked on individual 20% Corn Fiber Hydrolysate (CFH) agar plates. One organism type grew well but the other strains grew very slowly, if at all. The strains which were not growing well on the CFH plates were streaked on YPD agar to check for viability and all strains grew well on YPD. Tubes of CFH broth were inoculated with the eight strains and after incubation, 20% CFH plates were streaked with this broth. All strains but one grew well on these plates.

The strains grown on the CFH agar were run with api Caux strips to determine if the strains had adapted on the CFH agar to utilize other sugars.

Retest on Agar Plates

Eight selected ethanologenic yeasts were re-screened on prepared corn fiber hydrolysates (CFH) for growth on agar plates prepared with old and new CFH. The same selected yeast were further evaluated for ethanol production using the new CFH in a liquid broth medium. The results of these experiments are provided in Table 8 and Table 9 indicate that four of these yeast, Y1, Y2, Y5, and Y8 are the most promising. All of the above yeast were further analyzed using API Caux strips to confirm sugar fermentation profiles. The results obtained showed that the selected yeast differed from each other in sugars fermented with some showing **D**-xylose C5 fermentation, good **D**-galactose fermentation, and good fermentation of the disaccharide, maltose.

Other Fermentation Organisms Test on Agar Plates

Several bacterial process organisms used at ADM BioProducts Plant were screened on CFH agar. These organisms were streaked onto tryptic soy agar (TSA) and 20% CFH agar, pH 5.5. All grew on the TSA but only one organism grew on the CFH. This suggests that the lower pH used in the selection may be responsible for the lack of growth on CFH agar.

Table 8: Screening of Selected Yeast on Corn Fiber Hydrolysate

Yeast Designation	May, 02 CFH	June, 04 CFH
Y1	+	+
Y2	+	+
Y3	+/-	+
Y4	+	+
Y5	+	+
Y6	-	+ Slow
Y7	+	+
Y8	+	+

Medium Composition 20% Corn Fiber Hydrolysate (CFH), 1% Yeast Extract, and 2% Peptone adjusted to pH 4.6-5.5 with α-amylase and glucoamylase added.
Incubation Conditions May: 30˚C, 200 rpm. June: 28˚C, 150 rpm

Table 9: Evaluation of Selected Yeast for Growth and Ethanol Production from CFHH

Yeast Designation	Peak EtOH Hour
Y1	++
Y2	++
Y3	-
Y4	-
Y5	++
Y6	-
Y7	+
Y8	++

++ Peak ethanol production at 24 hours
+ Peak ethanol production at 48 hours
- No growth or ethanol production
Medium composition: 1% Yeast extract and 20% Corn Fiber Hulls Hydrolysate
Adjusted to pH 4.7-4.8 with α-amylase and glucoamylase added.
Incubation conditions: 30˚C, 150 rpm.

One non-ethanologenic process yeast was screened on Yeast Extract Dextrose (YED) medium at dextrose levels which were similar to those found in 20% and 50% CFH, as per PNNL analysis of CFH. This yeast grew well at both 20% and 50% CFH dextrose levels, leading to product concentration measured above normal levels. Dextrose was completely consumed at the 20% CFH level but not at the 50% CFH level. (~30 g/l remained). This yeast was also screened on 20% CFH broth but unlike the concurrent study on YED, no growth was observed on CFH broth. The yeast also showed poor growth when streaked onto 20% CFH agar plates. The above confirmed that the CFH is toxic to non-ethanologenic yeast and other bacterial process organisms used at ADM.

Organism Classical Mutagenesis

An ethanol-producing strain, coded Y1, was mutagenized with 263nm UV at 20 second intervals for 60 seconds. The mutagenized organisms were diluted and incubated in the dark at 29°C. The best kill was obtained at 60 seconds of exposure to the UV light but it was still < 20%. It appears that longer exposures will be needed for this organism.

Four strains, coded Y1, Y2, Y3, and Y4, were mutagenized with the chemical NTG (N-Methyl-N'-Nitro-N-Nitrosoguanidine). Dilutions of treated and untreated cultures were spread onto YPDA (yeast, peptone, dextrose agar) and incubated at 30°C. Kill rates from 18-82% were observed with the best rates being achieved with Y1. Colonies were transferred to 20%, 30%, 40%, and 50% CFHA (corn fiber hydrolysate agar) and those colonies which grew on 50% CFHA were frozen at -70°C. The mutants that grew at 50% CFHH (corn fiber hulls hydrolysate) will be further screened in liquid hydrolysate and cells rewashed and mutagenized further to select for rapid robust growth.

Three NTG (N-Methyl-N'-Nitro-N-Nitrosoguanidine) mutagenized strains were plated on corn fiber hydrolysate agar (10-50% CFH). The strains only grew at the lowest level of corn fiber

hydrolysate. Five NTG mutagenized strains were inoculated into a broth before being plated on corn fiber hydrolysate agar. Three of the strains were able to grow on 30% CFH agar after growing in the broth, but two would not grow in the broth.

Two NTG (N-Methyl-N'-Nitro-N-Nitrosoguanidine) mutagenized strains were plated on corn fiber hydrolysate agar (10-50% CFH). The strains that had been mutagenized once and frozen did not grow. Strains that had been mutagenized twice grew on the plates within one day. It appears that freezing may have caused die-off of the first mutagens. The twice mutagenized cultures will be tested next in shake flasks.

Four Liter Controlled Fermentation Optimization Experiments

Several fermentation optimization experiments were conducted on the corn fiber hydrolysate. Several organisms were utilized for fermentation and adapted on corn fiber hydrolysate. Several batch fermentations were carried out utilizing corn fiber hydrolysate.

Over the course of the project, over 100 batch fermentation experiments were completed using all of the A- and N-series corn fiber hydrolysates.

Testing of new A-series and N-series Corn Fiber Hydrolysates

Several strains of organisms were adapted on the corn fiber hydrolysates from the NREL and ADM runs. Shake flask fermentations were also run to validate the fermentability of the hydrolysates. Batch fermentations were carried out utilizing the NREL and ADM-produced hydrolysates.

Hydrolyzed Corn Fiber Extraction

The oil in corn fiber contains approximately 10-20% phytosterols and phytostanols. These sterols and stanols are high-value feedstocks for pharmaceutical and nutraceutical applications. The extraction of the corn fiber oil is accomplished after hydrolysis of the corn fiber and liquid/solid separation. The oil remains in the fiber portion during these steps, and is therefore at a higher concentration for extraction. Several small-scale and pilot-scale extractions were completed on the hydrolyzed corn fiber

Bench Scale Extractions

Run 4232-88

Hydrolyzed corn fiber was dried in a convection oven at 220°C overnight and extracted. After the solvent extraction of the dried, hydrolyzed corn fiber, the solvent-oil mixtures were concentrated by evaporating. The oil analysis was performed to determine the sterols and stanols concentrations. The components included in the analysis were free fatty acids, monoglycerides, diglycerides, triglycerides, free sterols and free stanols.

The results are shown in Table 10 and Table 11. The results show that the % free sterols and stanols extracted from the corn fiber is 0.23% in the saponified oil. The composition of the sterols and stanols in the saponified oil is similar to previous results with the highest levels of sitosterol and sitostanol followed by campesterol, campestanol, and stigmasterol. The largest

amount of oil component before saponification is the triglyceride component followed by free fatty acids (FFAs). After saponification, the largest component of the oil is the free fatty acids since all the ester bonds were cleaved in the glyercides.

Table 10: Corn Fiber Extraction Sterol and Stanol Results 4232-88.

	Oil Concentrate
1. Campesterol (wt %)	1.1
2. Campestanol (wt %)	1.5
3. Stigmasterol (wt %)	1.12
4. Sitosterol (wt %)	4.8
5. Sitostanol (Stigmastanol) (wt %)	4.9
Total (wt %)	13.5
Saponification Yield	26.5%
% sterols and stanols extracted from starting corn fiber	0.23%

Table 11: Corn Fiber Extraction Oil Results 4232-88.

Sample Id	Total FFAs	Total Monoglycerides	Total Diglycerides	Total Triglycerides
Steady State Oil	29.13	1.27	7.12	91.50
Saponified Steady State Oil	135.00	0.00	0.00	0.00

(g/kg sample)

Run 4232-125

An additional batch of hydrolyzed corn fiber was dried and extracted for oil. The extracts were concentrated by vacuum evaporation and submitted to analytical for determination of the concentrations of mono-, di-, and tri-glycerides, and cholesterol, brassicasterol, campesterol, campestanol, stigmasterol, sitosterol, and sitostanol. The sterol and stanol results were obtained and are shown in

Table 12. Again, the ratio of sterols and stanols is consistent with what has been seen previously. The yield of 0.29% total free phytosterols extracted from the starting corn fiber is in the expected range also.

Table 12: Sterol and Stanol Results from Batch 4232-123.

	Wt. % of product based on Saponified oil			
	Replicate 1	Replicate 2	Replicate 3	Average
1. Campesterol	0.95	1.01	1.05	1.00
2.Campestanol	1.75	1.83	1.80	1.79
3. Stigmasterol	1.09	1.20	1.05	1.12
4. Sitosterol	5.04	5.05	5.25	5.11
5. Sitostanol (Stigmastanol)	6.26	6.27	6.31	6.28
Total Sterol and Stanols	15.09	15.35	15.46	15.30
Saponified oil yield based on crude oil	34%	34%	34%	34%
% sterols and stanols extracted from hydrolyzed corn fiber	0.56%	0.57%	0.58%	0.57%
% sterols and stanols extracted from starting corn fiber	0.28%	0.29%	0.29%	0.29%

Rapid Corn Fiber Extraction

A Gerhardt Soxtherm rapid extraction system was tested at ADM. Twenty four grams of hydrolyzed corn fiber at 42% dry solids were extracted with 150 mL of solvent for the required time using the steps of 1) rapid boiling extraction; 2) reduction; 3) washing/extraction. The samples were sent to PNNL for sterol and stanol analysis.

Pilot-Scale Extraction

44.8 kg of thermochemically treated corn fiber was extracted in the pilot extractor. After the fiber was extracted, it immediately entered a desolventizer (DT) where it was heated and agitated with two ribbon mixers. The DT was kept at 140°F (60°C) with steam heating and was under vacuum. It should be noted that the fiber was heated and agitated in the DT to a point where it was ground into a fine powder. This is not perceived as a problem; however, since the DT was operated in batch mode, and in an industrial process, the DT would be continuous.

The oil-containing mixture from the extraction was concentrated in a forced-circulation, long vertical-tube evaporator with pumps on the inlet and outlet for continuous operation. The oil extract was pumped into the evaporator and evaporated to a total of 3 gallons (11.4 L). The concentrated oil extract was fed into a smaller natural circulation evaporator. The 3 gallons (11.4 L) of concentrated oil extract were concentrated to 2.84 kg of oil extract. The oil was submitted to PNNL and ADM Analytical for oil and sterol and stanol determination. Analysis showed that the composition of the oil was equal to theoretical levels, showing that all of the oil had been extracted.

The solids fraction of the corn fiber hydrolysis run completed in July, 2005 at NREL were extracted at ADM in the continuous extractor. The oil-containing extract was evaporated. A sample of this oil was submitted by ADM for analysis.

Analytical results (provided by PNNL) for four of the oil extraction runs conducted at ADM are shown in the Table 13. As can be seen the sterols and stanols content for these samples ranged from 5.81% to 18.82% (saponified oil basis).

Experiments were also conducted at PNNL using hydrolyzed corn fiber samples from a pilot plant test to examine the effect of combining two key steps involved in the extraction process into one step. The experiments showed that under certain conditions the one-step process resulted in the recovery of significantly more oil and total sterols as compared to the two step process.

Table 13: Composition of ADM Oil Extraction Samples.

Sample Label	ADM-1-KEB	ADM-2-KEB	ADM-3-KEB	ADM-4-KEB
Sample weight, g	**0.710**	**0.699**	**0.699**	**0.720**
Saponified Oil weight, g	**0.501**	**0.105**	**0.202**	**0.520**
Campesterol Concentration, Wt % of Saponified Oil	1.75	1.41	0.67	1.57
Campestanol Concentration, Wt % of Saponified Oil	2.02	1.07	0.60	2.02
Stigmasterol Concentration, Wt % of Saponified Oil	0.73	1.18	0.39	0.84
Sitosterol Concentration, Wt % of Saponified Oil	5.93	5.70	2.18	7.15
Sitostanol (Stigmastanol) Concentration, Wt % of Saponified Oil	6.21	3.93	1.97	7.23
Total Sterol Concentration, Wt % of Saponified Oil	**16.63**	**13.30**	**5.81**	**18.82**

Extraction of NREL Hydrolyzed Fiber

ADM Extractions

The solids fraction of some hydrolyzed corn fiber processed at NREL was extracted at ADM. The total mass of fiber extracted was 11.6 kg. The oil-containing extract was evaporated. A sample of this oil was submitted by ADM for analysis.

The analytical data were used to prepare empirical models to investigate the separate effects of hydrolysis time and temperature on oil and total sterol/stanol yields for the NREL tests that were conducted. These models, which showed very good correlation with the data, indicated that the oil and sterol yields, with respect to the dry corn fiber weight prior to hydrolysis, increased in a regular manner, with respect to both hydrolysis time and temperature (for a given reactor system).

100 g d.w.b. samples of the hydrolyzed corn fiber from the NREL pilot scale experiment were extracted at ADM. Each sample was agitated with solvent, and then the solvent was removed by

filtering the slurry through cheesecloth over a vacuum filter. The process was repeated a total of three times to ensure thorough or quantitative extraction of oil.

Two separate pilot-scale counter current extractions were conducted at ADM on the pretreated corn fiber from some of the NREL pilot-scale hydrolysis tests. Over 50 kg of corn fiber was extracted in the two experiments.

PNNL Extractions

In addition to conducting oil analyses in support of ADM and NREL hydrolysis/extraction testing, PNNL also conducted extraction testing. In particular, PNNL was involved in testing a one-step extraction process (with added reactants) as compared to a two step extraction process.

The one-step process was optimized with respect to solvent concentration, temperature and time. Through this testing, conditions were identified in which about 95% of the extractable oil and sterols can be achieved in the one-step process. This one-step process leads to the extraction of a significantly higher percentage of the oils and sterols that are present in the fiber as compared to the two-step process. An example comparison is given below in Table 14for two tests that were conducted.

Table 14: One Step vs. Two Step Extraction Process

Test No.	Component	Weight % Dry Hydrolyzed Fiber Basis	Weight % Wet Hydrolyzed Fiber Basis	Weight % Dry (prehydrolyzed) Corn Fiber Basis*
One-Step Process (w/reactant addition)	**Total Oil (saponified oil)**	7.0667	2.3398	4.4075
	Campesterol	0.0659	0.0238	0.0411
	Campestanol	0.0914	0.0303	0.0570
	Stigmasterol	0.0500	0.0165	0.0312
	Sitosterol	0.2775	0.0919	0.1731
	Sitostanol	0.2593	0.0859	0.1617
	Total Sterols/Stanols	0.7441	0.2484	0.4641
Two-Step Process	**Total Oil (saponified oil)**	4.1668	1.3796	2.5988
	Campesterol	0.0304	0.0101	0.0190
	Campestanol	0.0295	0.0098	0.0184
	Stigmasterol	0.0255	0.0085	0.0159
	Sitosterol	0.1009	0.0334	0.0629
	Sitostanol	0.0573	0.0190	0.0357
	Total Sterols/Stanols	0.2435	0.0806	0.1519

*Yields corrected for sample loss during extractions

Pretreatment/Catalyst Testing

This task took place at PNNL and focused on the catalytic conversion of the unfermented sugars to polyols. Efforts focused on pretreatment of the feedstock to minimize the detrimental affects on downstream catalysis.

Conversion of sugar components (e.g., from fermentation broth) to value-added products is accomplished by a two-step catalytic processing, involving 1) conversion to the sugars to sugar-alcohols (hydrogenation), followed by 2) conversion of the sugar alcohols to the final products (hydrogenolysis) – propylene glycol (PG), ethylene glycol (EG) and glycerol.

Several pretreatment scenarios were investigated during this testing.

Ultrafiltration

Prior testing of hydrolysate indicated that a 10 kDa (kiloDalton) membrane was sufficient in demonstrating activity of the hydrogenation catalyst to convert sugars in hydrolysate feedstocks to sugar alcohols. Initial hydrogenation testing with fermentation derived feedstock filtered through the 10kDa UF membrane resulted in negligible catalyst activity (see Figure 4), similar to that seen with the raw unfiltered feed. Hydrogenation conditions were 150g of liquid feedstock, 2.5g of catalyst, processing temperature of 150°C, and operating pressure of 1800psi hydrogen.

Fresh fermentation derived feedstock was subjected to further ultrafiltration using successively tighter membranes. The following ultra- and nano-filtration membranes shown in

Table 15 were used to generate feedstock for testing.

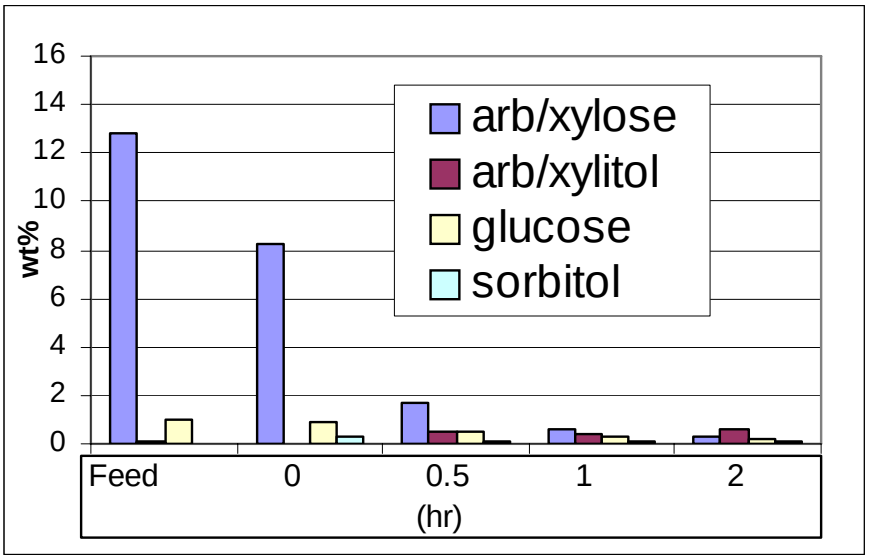

Figure 4: Hydrogenation of filtered 10kDa UF fermentation derived feedstock.

Table 15: Ultrafiltered Feeds Generated from Fermentation Derived Feed

Membrane	Type	Cut
GN	Ultrafiltration	10,000 Da
GM	Ultrafiltration	8,000 Da
GH	Ultrafiltration	2,500 Da
DL	Nanofiltration	150 to 300 Da

Subsequent hydrogenation testing of filtrate from a 2.5kDa UF membrane at 170°C resulted in similar results to those obtained for the feedstock resulting from 10kDa UF membrane, demonstrating arabinose and xylose conversion with minimal observable production of arabitol and xylitol. This is shown in the 0.5 hr sample below in Figure 5.

This testing demonstrated a few primary observations. The fermentation derived feedstock appears to have some small molecular weight materials (perhaps in addition to longer chain proteins) that interfere with the hydrogenation of the sugars present. This is in contrast to the hydrolysate derived feedstocks, in which the interfering compounds were most likely only longer chain protein materials that were more easily removed by ultrafiltration. Thus it was decided that the next step should be to continue towards screening adsorbents to remove interfering contaminants while leaving the sugars in solution. This screening was conducted using the combinatorial processing unit that exists at PNNL.

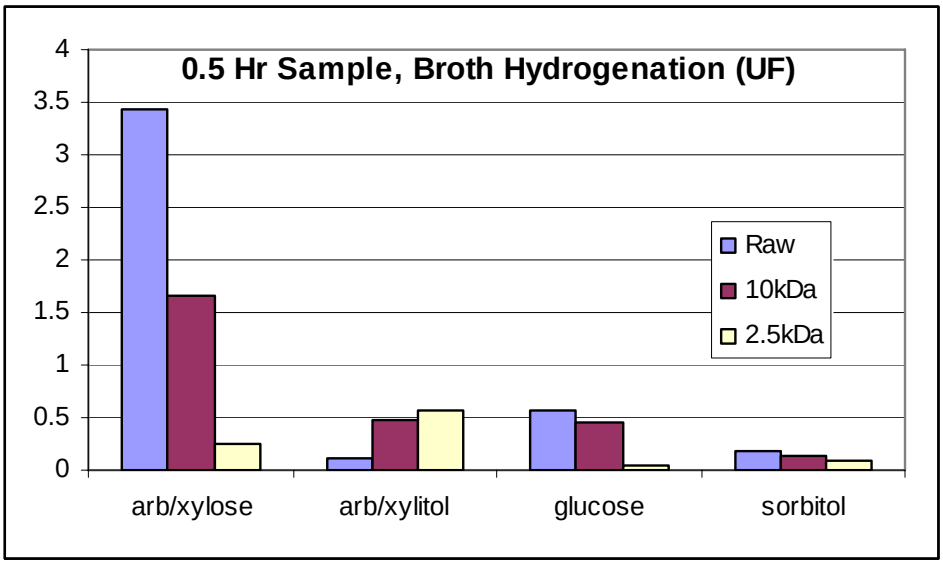

Figure 5: Comparison of 0.5 Hr Sample of Hydrogenation of multiple UF Cuts.

Combinatorial Feed Pretreatment Testing

Adsorbent testing was performed on a micro-scale parallel processing unit based on a 96 well titer plate. Each plate was divided into separate zones. Each zone was loaded with the same set of selected adsorbents, loaded with a fermentation derived feedstock, and shaken for a minimum

of 4 hours. Following agitation, each cell was filtered and the supernatant was transferred to a second plate containing hydrogenation catalyst in every cell. This plate was transferred to a reactor and processed at 150°C under hydrogen. Following hydrogenation, the supernatant from each well was analyzed via fast HPLC method for xylose, arabinose, xylitol, and arabitol. In each plate, a number of cells were designated as control and loaded with model compound in DI water to benchmark the results from the fermentation feedstock.

The first plate was divided into 3 zones. In the three zones, the following fermentation derived feedstocks were loaded: Xylose Feed model compound, DL filtrate (150-300 Da), and GH filtrate (2.5 kDa).

In each of the zones the following adsorbents were loaded: Gamma alumina, Dowex Marathon (H) Ion-exchange resin, Davicat ZL 5100 Synthetic Zeolite, Saint Gobain NORPRO XZ 5100 16052 1/8" Pellets, CS-1030E 1/16" Silica Extrudate, Titania Support P25 Extrudates, Degussa 7702 Titania, Engelhard Coconut Carbon CTC=125, Norit R1 Extra Activated Carbon, Norit RB 1 Activated Carbon, Aldrich Silica-Alumina Catalyst Support grade 135, Norit KBB, Ceca L2S Carbon, CaCO3, Amberlyst 15 Ion Exchange Resin, and Amberlyst 36 Ion Exchange Resin.

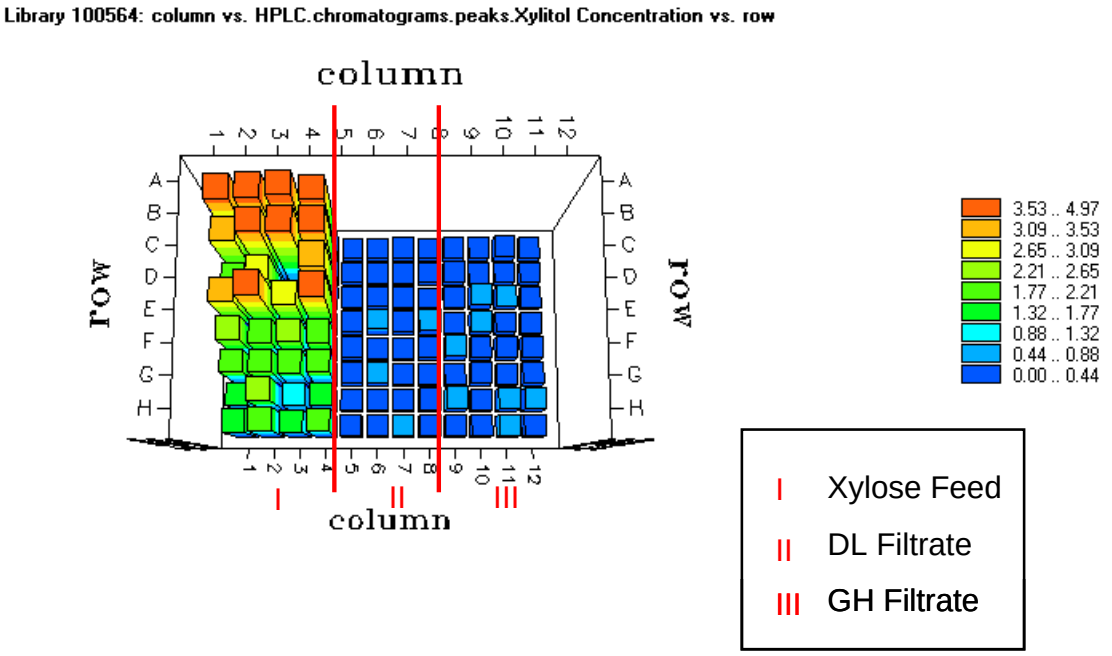

Figure 6: Plate 1 Xylitol Profile.

This plate generated low activity in all cases (see Figure 6). Only the model compound (Xylose Feed) demonstrated any activity on this catalyst. This plate successfully confirmed our technique for analysis, as the model compound demonstrated that this processing method would correctly generate a positive result when suitable conditions were met.

Due to low activity detected in the first plate, it was decided to divide the 2nd plate into five zones to test feedstock with modifiers. The zones were loaded with the following feeds: GH

filtrate (2.5 kDa), GH filtrate acid adjusted, DL filtrate (150-300 Da), DL filtrate acid adjusted, DL filtrate ion exchanged.

The ion exchanged DL feedstock was prepared by taking the DL filtrate and passing it across two ion exchange columns. One was packed with a cation exchange resin Amberlite 200 sodium form and one with an anion exchange resin Amberlite IRA-400 hydroxide form. The material was passed over the cation exchange resin first and the anion exchange resin second. The first and last fraction of each column was discarded to minimize dilution.

In each of the zones the following adsorbents were loaded: Engelhard Silica Gel Mod A, Engelhard Silica Gel Mod B, Engelhard Silica Gel Mod C, Engelhard Silica Gel Mod D, Engelhard Silica Gel Mod E, Engelhard Silica Gel Mod F, Engelhard Envisorb B+, Duolite C467 chelating ion exchange resin, Engelhard Clay F-115FF, Engelhard Clay F-160, Engelhard Clay F-20X, Engelhard Clay F-2, Amberlite IRA-400 acid form, Zinc Oxide, and MELCat XZO645-01.

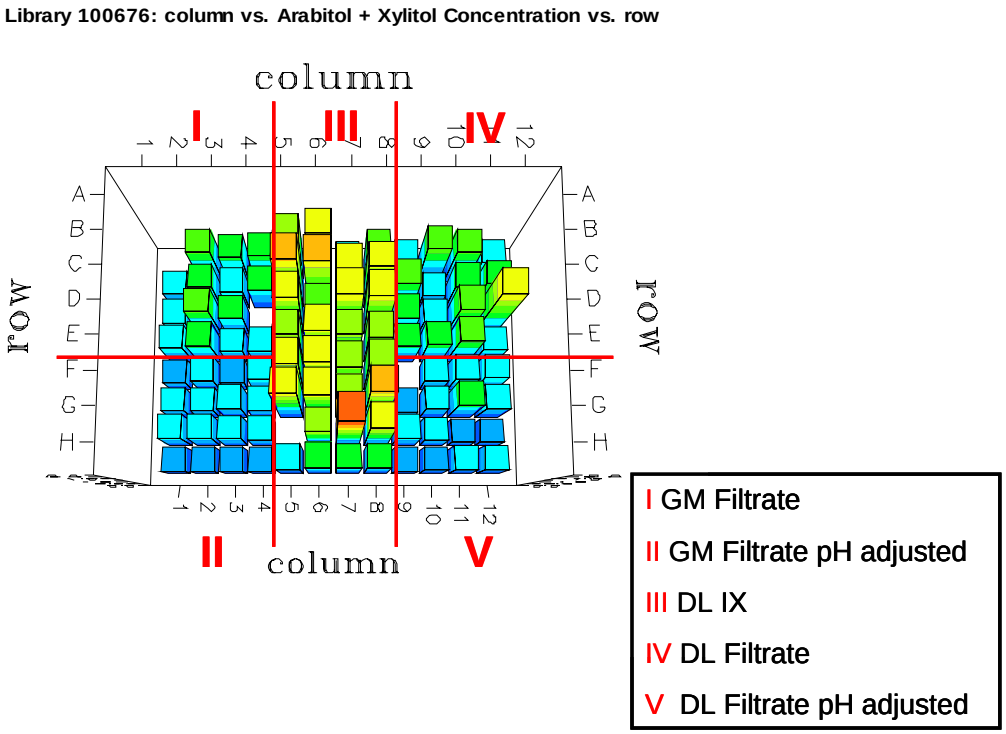

Figure 7: Plate 2 Xylitol/Arabitol Profile.

From this plate a number of conclusions were reached. It was apparent that the ion exchange treatment of the DL filtrate resulted in noticeable improvement in xylitol and arabitol yields (see Figure 7 and Table 16). The total concentration of xylose plus arabinose in this feed was 5.3%, yielding average conversions of 95% and average molar selectivities of 31%. In addition, the acid adjustment appeared to have little effect and possibly a detrimental effect on the catalyst performance. There was some marginal improvement applying the Engelhard silica gels over the blank, but it was very little improvement. In addition, it should be noted that there were organic

acids still present in the ion exchanged feed, which indicated that the feed was not completely anion exchanged (see Figure 8).

Table 16: Cells from Plate 2 with the best results

Cell	Feed	Adsorbent	Wt% C5-ose	Wt% C5-itol	Conv/Selectivity
B5	DLIX	Engelhard Silica Gel, Mod D	0.165	1.833	97 / 35
F8			0.185	1.826	97 / 35
B6	DLIX	Engelhard Silica Gel, Mod A	0.142	1.761	97 / 34
F7			0.261	1.323	95 / 26
B7	DLIX	Engelhard Silica Gel, Mod C	0.243	1.525	95 / 30
F6			0.237	1.699	96 / 33
B8	DLIX	Engelhard Silica Gel, Mod E	0.231	1.582	96 / 31
F5			0.230	1.751	96 / 34
C5	DLIX	Engelhard Silica Gel, Mod B	0.197	1.611	96 / 31
G8			0.226	1.672	96 / 33
C6	DLIX	Engelhard Silica Gel, Mod F	0.270	1.217	95 / 24
G7			0.109	2.620	98 / 50
C7	DLIX	Envisorb B+	0.197	1.721	96 / 33
G6			0.265	1.325	95 / 26
C8	DLIX	None	0.194	1.679	96 / 32
D5	DLIX	Duolite C-467 Resin	0.232	1.393	96 / 27
H8			0.286	0.797	95 / 16

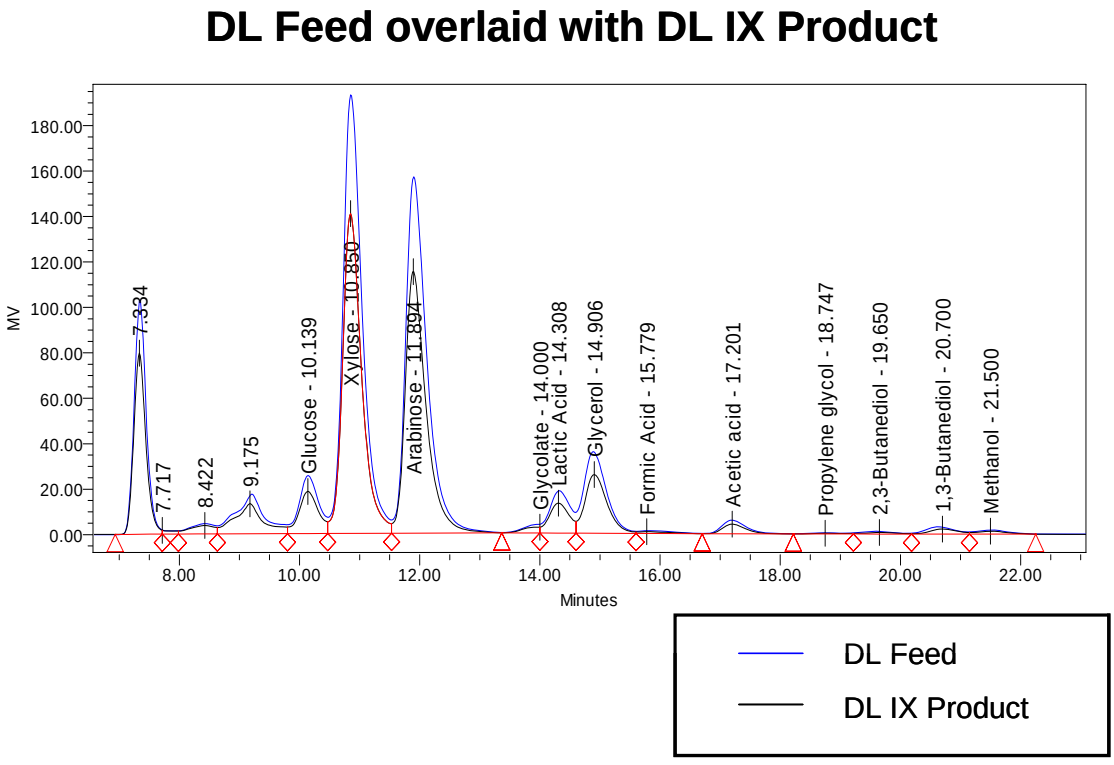

Figure 8: HPLC Trace of unmodified DL filtrate and Ion Exchanged DL Filtrate

The most promising pretreatment conditions were further evaluated, using the combinatorial catalysis system. The feedstocks for this testing were:

- DL: Broth filtered with 250 Dalton cutoff membrane
- DL IX: DL filtrate that has been cation and anion exchanged
- GH AX: GH (2500 Dalton cutoff) filtered broth after anion exchange.
- Xylose: Reference cells with model compound
- Xylose w/ GH AX Backflush: Reference with anion exchange regeneration effluent.

The plate was split in half for processing. One half of the plate (rows A-D) was processed at 100°C during the catalytic hydrogenation and the other (rows E-H) was subjected to 120°C. This is in contrast with combinatorial testing conducted previously at 150°C. 150°C was selected from model compound testing earlier in the program as being a good condition for hydrogenation.

The conversion, selectivity, and molar balance were calculated for each cell. The top ten results using the fermentation derived feedstocks are shown in

Table 17. The results from this testing were highly encouraging. Particularly at 100°C, the conversion is approximately 90%, and the selectivities range between 80% to 90%. This indicates that the sugar is being converted to sugar alcohols under these conditions instead of to unwanted byproducts. The model compound under similar conditions demonstrated up to 100% conversion and up to 90% selectivity. The ranges achieved for each feedstock processed in this plate are shown in Table 18.

The primary observation from this test is that the hydrogenation can be performed successfully at 100°C. This is a distinct improvement over previous results at 150°C where most of the sugars (~70%) were converted to undesirable byproducts.

It was observed that the backflush effluent from the anion exchange of the GH filtrate did not have a noticeable effect on the performance of the model compound. Additionally, the adsorbents used in these tests showed marginal improvement on the performance of the hydrogenation reaction. The cells that were tested without adsorbent were midrange in selectivity performance amongst the cells where adsorbents were used.

There was marginal difference between the DL raw and ion exchanged feedstocks. This was encouraging as it indicates that at some levels of ultrafiltration, ion exchange may not necessary.

Table 17. Top Performers (i.e. Top 10 Adsorbent/Feedstock/Temperature Compositions).

Adsorbent	Feedstock	Temp (°C)	Analyses		
			Selectivity to Xylitol/Arabitol	Xylose/Arabinose conversion † (wt%)	Molar Balance
Silicagel Mod C, Engelhard	GH AX Filtrate	100	87.0	88.6	93.6
Silicagel Mod C, Engelhard	DL IX Filtrate	100	85.1	92.2	91.7
Silicagel Mod D, Engelhard	DL IX Filtrate	100	85.0	88.6	91.7
Silicagel Mod A, Engelhard	GH AX Filtrate	100	84.8	89.2	91.7
Silicagel Mod E, Engelhard	GH AX Filtrate	100	84.3	88.7	91.2
Silicagel Mod D, Engelhard	GH AX Filtrate	100	83.9	91.6	90.8
Silicagel Mod B, Engelhard	GH AX Filtrate	100	83.0	88.2	89.9
Silicagel Mod C, Engelhard	GH AX Filtrate	100	82.8	91.4	89.8
Silicagel Mod C, Engelhard	DL IX Filtrate	100	82.8	90.7	89.8
Silicagel Mod D, Engelhard	DL Filtrate	120	82.6	97.6	88.4
Silicagel Mod C, Engelhard	DL Filtrate	100	82.4	97.7	88.7
Silicagel Mod E, Engelhard	DL Filtrate	120	82.4	97.9	88.0

Table 18. Summarized Selectivity, Conversion, and Molar Balance Results For Each Feedstock on Plate 100811.

Feedstock	Temperature (°C)	Selectivity to Xylitol/Arabitol	Xylose/Arabinose conversion † (wt%)	Molar Balance
Xylose	100	84.2 - 90.5	96.5 - 100	84.2 - 90.8
	120	84.5 - 90.5	99.5 - 100	84.2 - 90.8
Xylose + GH AX Backflush	100	88.7 - 89.7	97.0 - 97.4	90.1 - 91.1
	120	83.6 - 87.8	96.7 - 97.4	85.1 - 88.9
DL Filtrate	100	74.6 - 82.4	97.3 - 100	81.0 - 88.7
	120	71.6 - 82.6	97.2 - 100	77.3 - 88.4
DL IX Filtrate	100	65.4 - 85.1	85.7 - 92.6	74.4 - 91.7
	120	63.8 - 77.5	93.7 - 96.6	70.0 - 84.4
GH AX Filtrate	100	69.6 - 87.0	82.7 - 91.6	77.8 - 93.6
	120	60.0 - 76.5	95.6 - 100	66.9 - 83.1

This process was demonstrated during microscale testing resulting in conversions between 88 to 97% along with selectivities between 82 and 87% using actual feedstock with pretreatment. A combination of pretreatment and substantial reduction of the hydrogenation temperatures has resulted in a successful demonstration of the first step in the two step catalytic upgrading of the sugars.

Additional micro-scale combinatorial testing generated good hydrogenation and subsequent hydrogenolysis of fermentation derived feedstock. It was found that ultrafiltered material using 300Da and 1kDa membranes were required to allow for the hydrogenolysis step to be successful.

Scale up of Combinatorial Results

Subsequent scaled-up Parr reactor (300ml) testing of the 300Da membrane and 1kDa membrane filtrates were conducted. The conversions in these tests were significantly lower than those obtained in the micro-scale combinatorial testing.

Some successful catalyst results were obtained by extensive processing of the fermentation derived feed, requiring ultrafiltration, thermal processing with catalyst, and adsorbent filtration,

all of which lose some of the sugars prior to catalytic processing. This is in contrast to prior microscale testing which indicated that much less pretreatment was necessary.

The Parr reactor testing indicated that for fermentation broth, both catalysis steps were enabled by the following pretreatments: ultrafiltration to 150-300Da followed by a Norit RX 1.5 Extra carbon adsorbent. While enabling catalysis, pretreatment also resulted in removal of desirable substrates (e.g., sugars) along with the inhibiting compounds. For acid hydrolysate, both catalysis steps were enabled by the following pretreatments: ultrafiltration to 2.5kDa followed by a Norit RX 1.5 Extra carbon adsorbent. Again, this pretreatment resulted in removal of desirable substrate.

Following pretreatments, fermentation broth or acid hydrolysate was processed at 100^{o}C in the presence of catalyst to allow for hydrogenation of sugars to sugar alcohols, and then subsequently processed at 180^{o}C over in the presence of catalyst to allow for hydrogenolysis of the sugar alcohols to polyols (e.g., propylene glycol, ethylene glycol and glycerol).

Table 19 shows some representative results from the hydrogenolysis batch testing (2nd catalysis step) for the fermentation broth and acid hydrolysate as compared to xylitol and sorbitol model compound work. All batch reactor tests shown were performed at the 100ml size except for fermentation broth which was at 10ml.

Table 19: Summary of Hydrogenolysis Results.

Conv / Carbon Molar Select.	C5 (xylitol)			C6 (sorbitol)			Actual	
	Theoretical	Best Flow Model Cpd	Best Batch Model Cpd	Theoretical	Best Flow Model Cpd	Best Batch Model Cpd	Best Batch Acid Hydrol	Best Batch Ferm. Broth
C5 Conv	100	99	---	0	0	0	84.4	72.2
C6 Conv	0	0	0	100	100	93.2	94.8	80.9
EG	40	27.8	35.3	0	15.7	17.5	21.3	11.9
PG	60	39.8	30.4	100	46.8	32.2	26.7	9.1
Glycerol	0	7.7	13.7	0	8.1	8.1	20.9	0
Lactate	0	5.2	8.9	0	4.1	2.9	6.0	7.2
Total	100	80.5	88.3	100	74.7	60.7	74.9	28.2

One continuous test was subsequently conducted, using fermentation derived feedstock. The feed material was bulk vacuum filtered across Whatman #1 filter paper. This material was prepped and placed in a ultrafiltration system using the “GE” membrane of approximately 1kDa molecular weight cut off. This material was collected and designated as the feed for the first

hydrogenation step. While the carbon treatment resulted in significant losses of sugars in the feedstock, this was deemed necessary based on the Parr batch testing.

The pretreated material was fed to a trickle bed reactor containing hydrogenation catalyst. The hydrogenation reactor was operated at 100C, hydrogen at 84 sccm at 1800psi, and 60 ml/hr of feedstock. Material was collected and sampled over the course of the test. Sugar alcohol production started out at 100% conversion and 92% selectivity on the first day, but dropped to 93% conversion and 53% selectivity by day two. By day three, the catalyst was inactive, indicating poisoning of the catalyst had taken place over time. Materials from the first two days of the hydrogenation were collected for the hydrogenolysis test.

A second reactor bed containing 30cc hydrogenolysis catalyst was used. The hydrogenolysis reactor was operated at 180C, hydrogen at 84 sccm at 1800psi, and 60 ml/hr of feedstock. Material was collected and sampled over the course of the test. Sugar alcohol conversion of both C5 and C6 materials remained high over the course of the test. The system started out at 74% conversion of arabitol/xylitol and 97% conversion of residual sorbitol (very small amount present). The test continued at between 90% to 100% conversion of C5's and 100% conversion of residual C6's. However, overall selectivity to useable EG and PG were low. Glycerol was difficult to analyze for due to a suspected co-eluting compound. Consumption of this compound in concert with production of glycerol yielded a negative change in "apparent" glycerol. If this compound is assumed to be completely consumed and only glycerol accounts for the compound analyzed by HPLC in the product, selectivity is speculated to be a maximum of 30%.

The continuous flow test indicated that there is significant deactivation of the catalyst during the hydrogenation step over time, resulting in short catalyst lifetime. The reason for this deactivation is not understood. However, in the short hydrogenolysis test, the lifetime of the catalyst did not drop off dramatically as during the first catalysis, indicating that the poisons may have been retained in the first reactor through sacrificial deactivation.

Animal Nutrition Testing

The hydrolyzed, solvent extracted corn fiber was sent for testing to ADM Alliance Nutrition to determine the value of the corn fiber as an animal feed. The sample of extracted fiber was sent for analysis of CNCPS chemical profile. In addition, analysis for crude fiber, crude fat, NFE, TDN, and RFV were obtained. All analyses were done via standard wet chemistry method due to the novel nature of the sample.

Also the sample was subjected to *in situ* analysis. The ruminal fiber and protein digestion of these samples was evaluated for both dairy and beef application. Samples of the corn fiber were incubated *in situ* for 0, 4, 8, 16, 24, 48, 72, and 96 hours. Samples were placed in the rumens of dairy cattle receiving a lactation ration and dairy steers receiving a maintenance hay-based diet (minimum of 3 animals each). Samples were fermented in duplicate.

Finally, the sample was subjected to *in vitro* analysis. Isolated neutral detergent fiber was obtained from the extracted corn fiber. Samples of extracted corn fiber, isolated NDF from corn fiber, medium-quality grass hay, soyhulls, and wheat straw were fermented *in vitro* *In vitro* gas

production was monitored for 48 hours and residual NDF digestion was determined. Samples were fermented in duplicate using rumen fluid from animals fed both dairy and beef rations. Kinetic analysis of fiber digestion was evaluated using the table-curve program.

The thermochemically treated, solvent extracted corn fiber was analyzed for chemical analysis and feed value and compared to Corn Starch, Soyhulls, Corn Fiber, and Corn Gluten Feed. The *in vitro* gas production, *in vitro* and *in situ* digestion percentage, and chemical analysis of the samples were determined.

Process Flowsheet Development and Economics

An engineering firm, Lurgi, PSI joined the development team and a preliminary flow diagram was presented by them during the quarterly meeting held at PNNL on April 16, 2004. Based on the bench-scale optimization testing results, ADM and PNNL researchers provided updated technical information to NCGA and Lurgi, PSI for incorporation and updating the flowsheet and process economics. Lurgi PSI completed the preliminary financial model and material balance. This model was built around information from NCGA, PNNL, and ADM as well as LPSI experience with equipment and costs.

Presentations, Articles, and Patents

Presentations:

"From Cornstarch to Corn Fiber-Converting Corn Fiber to Ethanol." CIFAR Conference XXIII: Overcoming Hurdles to Implementing Lignocellulosic Biofuels. Davis, CA September 28, 2006. K. Beery and C.A. Abbas.

"Corn Fiber Hulls Conversion To Higher-Value Fuels, Feed, Nutraceuticals, And Chemicals: Pilot-Scale Research And Development." Corn Utilization Technology Conference (CUTC), Dallas, TX, June 5-7, 2006. K. Beery and C.A. Abbas.

"Advanced Confocal Imaging of Corn Fiber Using Starch- and Cellulose-Specific Fluorescent Probes". 28th Symposium for Biotechnology for Fuels and Chemicals. Nashville, TN April 2006. S. Porter, Q. Xu, S.Y. Ding, K. Beery, C. A. Abbas and M. E. Himmel.

"Progress for DOE-Supported Corn Fiber Project." Corn Utilization Technology Conference (CUTC), Indianapolis, IN, June 7-9, 2004. K. Beery

"Detection Of Sterol, Stanol, Lipid, And Carbohydrate Components In Corn Fiber Products 13 C And 1H NMR And Chromatographic Methods." 25th Symposium for Biotechnology for Fuels and Chemicals, Breckenridge, CO, May 4-7, 2003. J. Franz, A. Rammelsberg, K. Beery, C.Abbas, N. Stair, D. Muzatko, A. Schmidt, T. Werpy, R. Orth, M. Alnajjar, G. Mendenhall, N. Dannielson and R. Shunk.

"**Application Of The Biorefinery Concept To Corn Processing.**" Corn Utilization Technology Conference (CUTC), Kansas City, Kansas, June 3-5, 2002. C. Abbas, K. Beery, A. Rammeslberg, M. Alnajjar, J. Franz, R. Orth, A. Schmidt, T. Werpy, R. Landucci, B. Sedlacek and R. Shunk.

"**Separation And Conversion Of Corn Fiber**." 225th ACS National Meeting, New Orleans, LA, March 23rd, 2003. K. Beery, C. Abbas, T. Werpy, A. Schmidt and R. Orth.

"Thermochemical Hydrolysis of Corn Fiber." 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, April 28th-May1st, 2002.Abbas C.A., K.E. Beery, A. Rammelsberg, M. Alnajjar, J. Franz, R. Orth, A. Schmidt, T. Werpy, and R. Shunk.

"Application of Biorefinery Concept To Corn & Soybean Processing." 23rd Symposium on Biotechnology for Fuels and Chemicals. Breckenridge, Colorado, May 6-9, 2001. C. Abbas, K. Beery and M. Cheryan.

Serial Journal Articles:

Porter, Stephanie E., Bryon S. Donohoe, Kyle E. Beery, Qi Xu, S.-Y. Ding, Todd B. Vinzant, Charles A. Abbas, and Mike E. Himmel. Microscopic Analysis of Corn Fiber Using Starch- and Cellulose-Specific Molecular Probes. Biotechnology and Bioengineering (2007) In Press

Abbas, C.; Beery, K.; Dennison, E.; and Corrington, P. (2004) "Thermochemical Treatment, Separation, and Conversion of Corn Fiber to Ethanol." In Saha, B.; and Hayashi, K. (eds.) "Lignocellulosic Degradation." ACS Symposium Series 889. American Chemical Society, Washington, D.C. pp. 84-97.

Articles:

"**Profitable to the last drop**". R. Orth, C. Abbas and R. Shunk. Resource Magazine (2003) 10:5-6.

Patents:

"**Method of producing compounds from plant material"** Werpy, T.A.; Schmidt, A.J.; Frye, Jr. J.G.; Zacher, A.H.; Franz, J.A.; Alnajjar, M.S.; Neuenschwander, G.G.; Alderson, E.V.; Orth, R.J.; Abbas, C.A.; Beery, K.E.; Rammelsberg, A.M.; Kim. C.J. U.S. Patent 6,982,328, 2006