

## ERIP/DOE Quarterly Report #5 & 6

October 1, 1998 through March 30, 1999

### A Pilot Plant Scale Reactor/Separator for Ethanol from Cellulosics

ERIP DOE Project DE-FG01-97EE15958

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#### **Project Aim**

The objective of this project is to develop and demonstrate a continuous, low energy process for the conversion of cellulosics to ethanol. This process involves a pretreatment step followed by enzymatic release of sugars and the consecutive simultaneous saccharification/fermentation (SSF) of cellulose (glucans) followed by hemi-cellulose (pentosans) in a multi-stage continuous stirred reactor separator (CSRS).

During the 5th and 6th quarters, we have continued tests of our steep delignification process for the pretreatment of straw, corn stover, and hardwood sawdust/shavings. Correlations for soluble lignin as a function of OD were determined to allow a quick determination of the level of lignin solubilized by the SDP. Levels of xylose/hemicellulose determined during Q4 and Q5 seemed lower than expected. Samples of hardwood sawdust from TVA and NREL with known composition were obtained to allow us to standardize our analysis for cellulose and hemicellulose. Analyses of the steep liquor showed some xylans, accounting for some of the missing xylose. SSF experiments and pretreatment trials continued.

During Q8, a 130 L process scale unit will be operated to demonstrate the process using straw or cornstalks. During Quarter 5 and 6, we have acquired two 300 L stainless steel jacketed tanks for steeping delignification pilot operation, a regenerative blower for gas stripping ethanol separations from the pilot plant, four stainless steel 6" ID by 6' columns for ethanol and ammonia recovery operations, and built a steel rack/ framework for the columns/ CSRS system. The magnetic stirring system for the CSRS pilot reactor was tested in January.

## **DISCLAIMER**

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### **Project Objectives**

There are three basic objectives to this ERIP project, 1) some basic research on co-production and/or recycling of cellulosic enzymes and development of pretreatment processing which allows enzymatic breakdown of the biomass 2) a lab scale development phase where the process is operated on a small scale, and process modeling, design, and economics, and finally, 3) a demonstration scale CSRS being built and operated.

### **General Approach**

Cellulosics, or biomass sources, are a great internal renewable resource for fuels and chemicals for the USA. In this project, we will be using corn stover and straw as biomass sources to produce ethanol. Ethanol production from cellulose is currently hampered by several major difficulties, most notably 1) the difficulty in reducing biomass to fermentable sugars economically, and 2) the difficulty in fermenting xylans, or five carbon sugars which are the breakdown monomer of hemicellulose, which constitutes between 15 and 25% of most cellulosics. There are two basic methodologies for breaking down cellulosics to constituent sugars, acid and enzymatic (cellulase) processes. In our work, we will be focussing on an enzymatic process which should require much milder pH's and reduced capital costs. However, if enzymes are purchased, the enzyme cost alone can cause the biomass based ethanol to cost more than the current market price for ethanol.

The goals of this project are thus to make progress towards reducing three basic barriers to commercialization of biomass to ethanol via enzymatic hydrolysis, 1) co-production and/or recycling of cellulase (and hemicellulases) will be examined closely, 2) a low temperature/low pressure chemical/ chemical recycle process for biomass pretreatment, and 3) successive fermentation of cellulose/glucose followed by hemicellulose/xylose in a new bioreactor, the continuous stirred reactor/ separator (CSRS).

Ethanol production from cellulosics can be improved by various means:

- 1) Develop a high density of cells within the reactor so as to convert sugars to ethanol quickly
- 2) Combine enzymatic conversion of cellulose and hemi-cellulose polymers with fermentation so as to keep sugar levels low, improving enzymatic conversion rates
- 3) Convert both xylose and glucose to ethanol
- 4) Either co-produce crude cellulase enzyme or recycle the enzymes so as to reduce enzyme costs.
- 5) Separate ethanol from the reactor broth so as to keep reaction rates high

A central effort of this project is to develop and demonstrate a continuous stirred reactor separator for the successive fermentation of xylans and glucans which will incorporate all the above design parameters.. Biomass generally consists of about 25-30% lignin, 25-30% hemicellulose, and 30-45% cellulose. The ideal process for biomass conversion to useful products must utilize each of these components. We are developing a delignification/pretreatment, followed by enzymatic release of xylans and glucans from the hemicellulose and cellulose respectively. Combining reaction (fermentation) with enzymatic release of sugars (Simultaneous Saccharification and Fermentation or SSF) improves the enzyme kinetics due to reduction of product inhibition. The basic process flows of the CSRS process for cellulose as we currently envisage it are shown in Figure 1.

### **Progress Report**

This project is divided into three phases, 1) a basic phase consisting of laboratory studies on enzyme production, enzyme performance and enzyme recycling in cellulose breakdown, and pretreatment effectiveness, 2) a lab development stage consisting of operating batch bench scale saccharification fermentations (SSF) trials and 3) an applied or demonstration phase focusing on construction and operation of a 130 L or larger CSRS for the production of ethanol. During quarters 5 and 6, we have continued development of our non-acid pretreatment process, testing performance on several different biomass substrates, then completed SSF experiments of hard wood sawdust to compare with our previous work with straw, paper mill sludge, and corn stover.

During quarters 5 & 6 , we have been focussing on

- 1) refinement of analytical procedures to explain low levels of xylans/hemicellulose found in our earlier work.
- 2) pretreatment studies (Task 1. b) as applied to hardwood sawdust,
- 3) simultaneous saccharification/fermentation of pretreated biomass,
- 4) acquisition of pilot plant materials for the 130 Liter pilot plant
- 5) begun contacting companies interested in enzymatic cellulose to ethanol.

**1. Basic Research-**The basic research is being performed at BPI's laboratory in W. Lafayette, IN.

*Task 1. a) Cellulase/hemicellulase production and performance comparison-* The enzymatic conversion of cellulose is often the rate limiting step during the simultaneous saccharification and fermentation. During quarters 1 and 2, we demonstrated that recycled fermentation broth retained a high degree of enzymatic ability. Thus, our preliminary focus is now on recycling enzymes rather than the co-production in our upcoming pilot plant efforts. In the CSRS, temperatures stay low during the ethanol separation, so there is no destruction of the enzymes as would occur during a standard distillation process.

Co-production of enzymes does however, would have a major beneficial effect on economics, so we will continue to pursue this line of research. During Q4, our work with one strain of *Trichoderma reesei* showed little cellulase activity. We are waiting on three strains from the NRRL collection. We currently are using a cellulase commercially available from Genecor for our work.

During the Q5 and Q6 we used our own proprietary low temperature steeping delignification/ extraction pretreatment for biomass to solubilize lignin. Lignin concentration as a function of absorbance is shown in Figures 1, 2 and 3 for straw, hardwood, and corn stover respectively. We are working on improving the performance of the SD process on the hardwood currently.

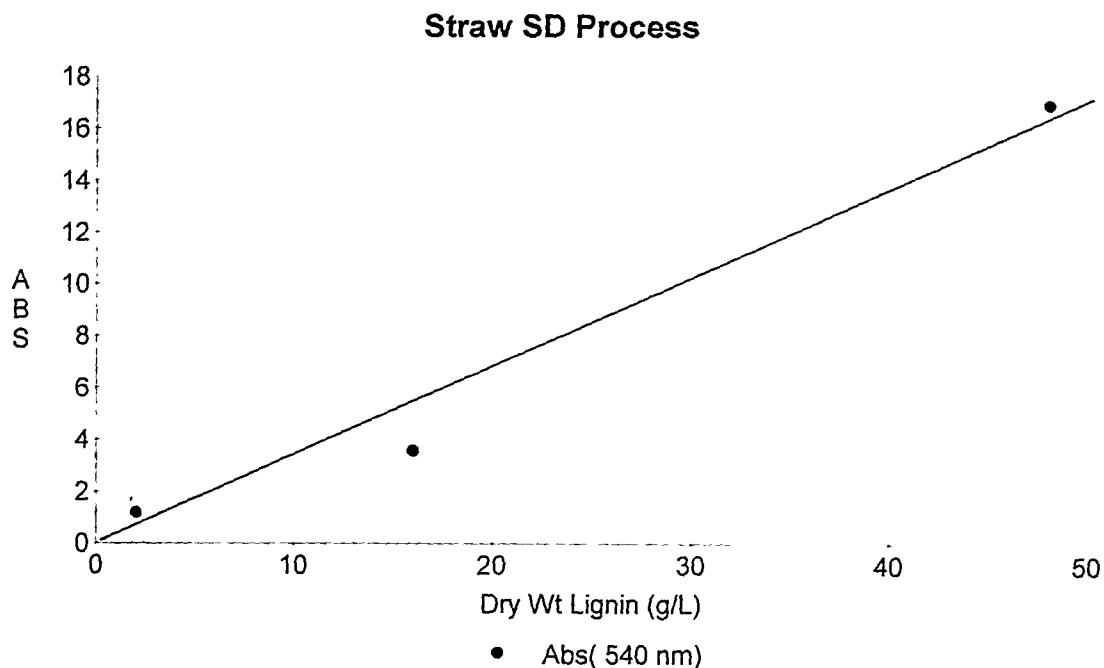


Figure 1. Absorbance as a measure of lignin concentration in the steeping process. The highest concentration represents our first contact, and the lowest, the third contact.

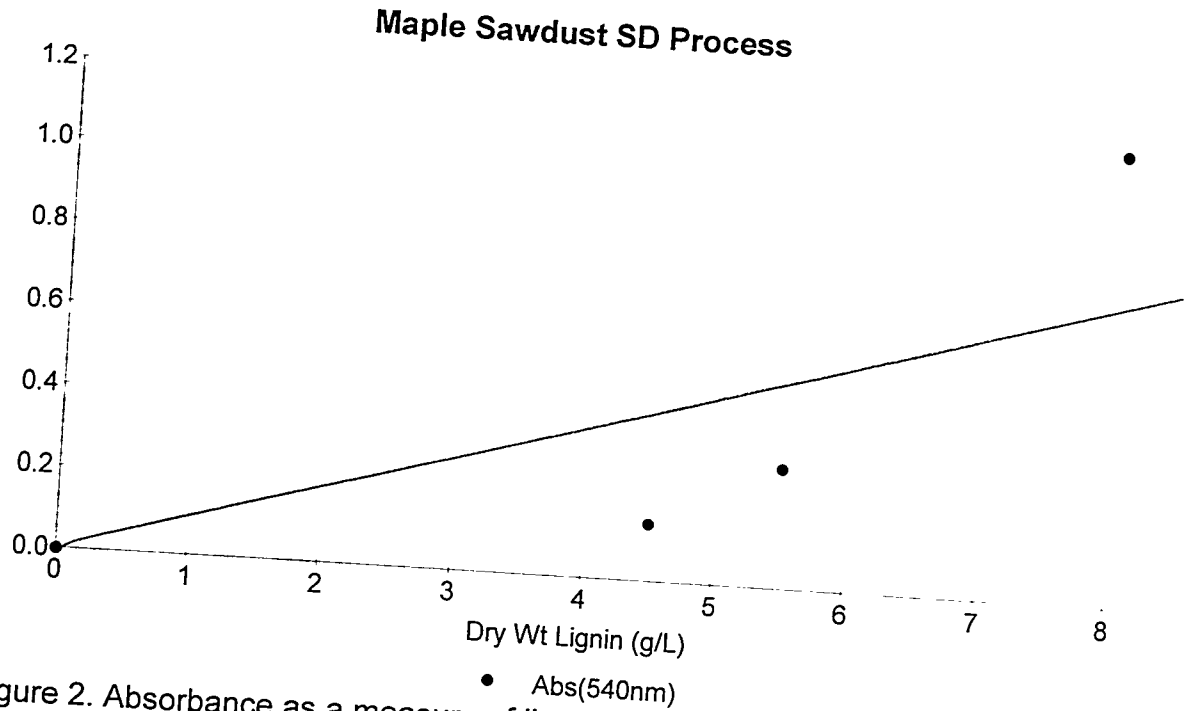


Figure 2. Absorbance as a measure of lignin concentration in the steeping process for hardwood sawdust. The highest concentration represents our first contact, and the lowest, the third contact.

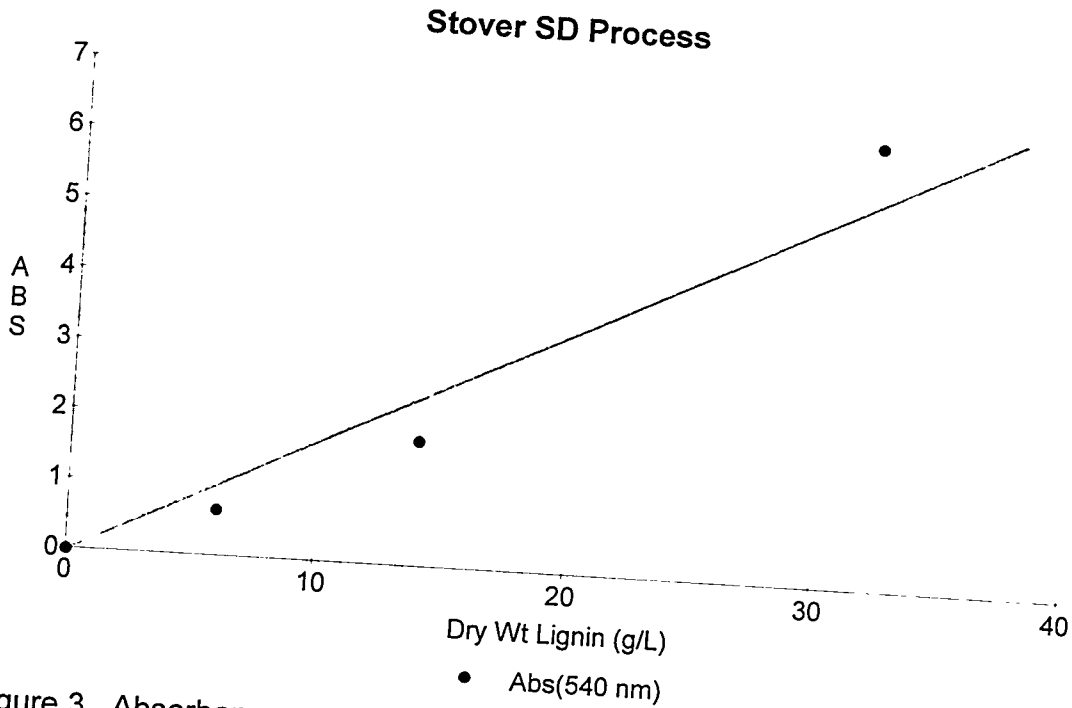


Figure 3. Absorbance as a measure of lignin concentration in the steeping process with corn stover. The highest concentration represents our first contact, and the lowest, the third contact.

## Analytical Work

Xylose levels measured during our work with SSF of straw and corn stover seemed lower than we would have expected. During Q5 and Q6, we attempted to determine the cause for these low levels. We completed two basic studies, 1) an examination of the steep liquid from the pretreatment for xylans/xylose, and 2) a study of our analytical techniques to determine if our methodology for determination of hemicellulose/xylans is faulty.

**1) Steep Liquid Analysis-** Our Steep Delignification process is designed to dissolve the lignin from the biomass, thus allowing enzymatic attack of the cellulose and hemicellulose polymers during the SSF process. We have demonstrated excellent results using straw and stover (see previous project reports) using our SD process. However, the xylose concentrations noted in the SSF's of straw seemed low. Published determinations of composition of straw are shown in Table 1.

**Table 1. Composition of Straw**

Reference	Cellulose	Hemicell.	Lignin	
1. Dale et al. (1995)	39.5	19.1	23.4	
2. Gould & Freer (1983)	36.2%	(45%)*	13.6%	*acid det. extract
3. Bjerre et al (1995)a.	40.8	35.5	10.5.	
b.	38.0	32.8	8.9	
4. Papat. et al (1992)	40.2	29.2	21.8	

There is a considerable variation, which may be due to different varieties of straw, or differing methods of analysis. We are using NREL standard methods for determination of cellulose and hemicellulose as given in Appendix 1.

To check our methods, samples of NREL hardwood poplar, and TVA hardwood were obtained. We then ran our analysis of these samples to compare our results. These are shown in Table 2.

NREL Hardwood	Cellulose (Glucose)	Hemicellulose (Xylose)	Xylose/Glucose Ratio
TVA analysis No. 1	44.53	14.9	0.33
No. 2	44.8	15.8	0.35
No. 3	43.5	13.4	0.31
No. 4	45.6	14.2	0.31
BPI analysis No. 1	35.4	11.9	0.33
No. 2	35.5	11.9	0.33
TVA Hardwood			
TVA Analysis No. 1	42.7	18.7	0.43
No. 2	40.9	15.9	0.39
No. 3	42.3	16.4	0.38
BPI Analysis No. 1	34.7	14.3	0.42
No. 2	28.5	12.6	0.44

### Analysis of Steep Liquor

The possibility that hemicellulose is being solublized and lost in our pretreatment process was investigated. A chromatogram of the single strength lignin liquor after precipitation of the lignin is shown in Figure 4. A large peak at 6.4 minutes is a tri or higher degree saccharide. Enzyme addition to this liquor gave the chromatogram shown in Figure 5. The multi saccharide has been degraded to three monosaccharides, unknown (mannose?), glucose, and largest, xylose at a concentration of 3.6 g/L. In our process design, these sugars would carry over to the CSRS, thus accounting for a large fraction of the xylose which we were missing.

### 2.1 Bench Scale SSF of Maple Shavings

There are two parts to our laboratory process development phase: 1) Bench scale testing of the saccharification/fermentation process, and 2) Process modeling and economic optimization/costing.

During our work with hardwood sawdust over the last two quarters, we have noted somewhat less success with our pretreatment, indicating a more resistant structure in the hardwood. We have been modifying our process to try to improve these results.

STRAW SQUEEZE 1 STOPPED & DEWATERED  
 ALL CHROMATOGRAMS WILL BE REPLACED  
 INK LOW - CHANGE PRINT CARTRIDGE AND PRESS [CTRL-SHIFT-C]  
 LOW BATTERY

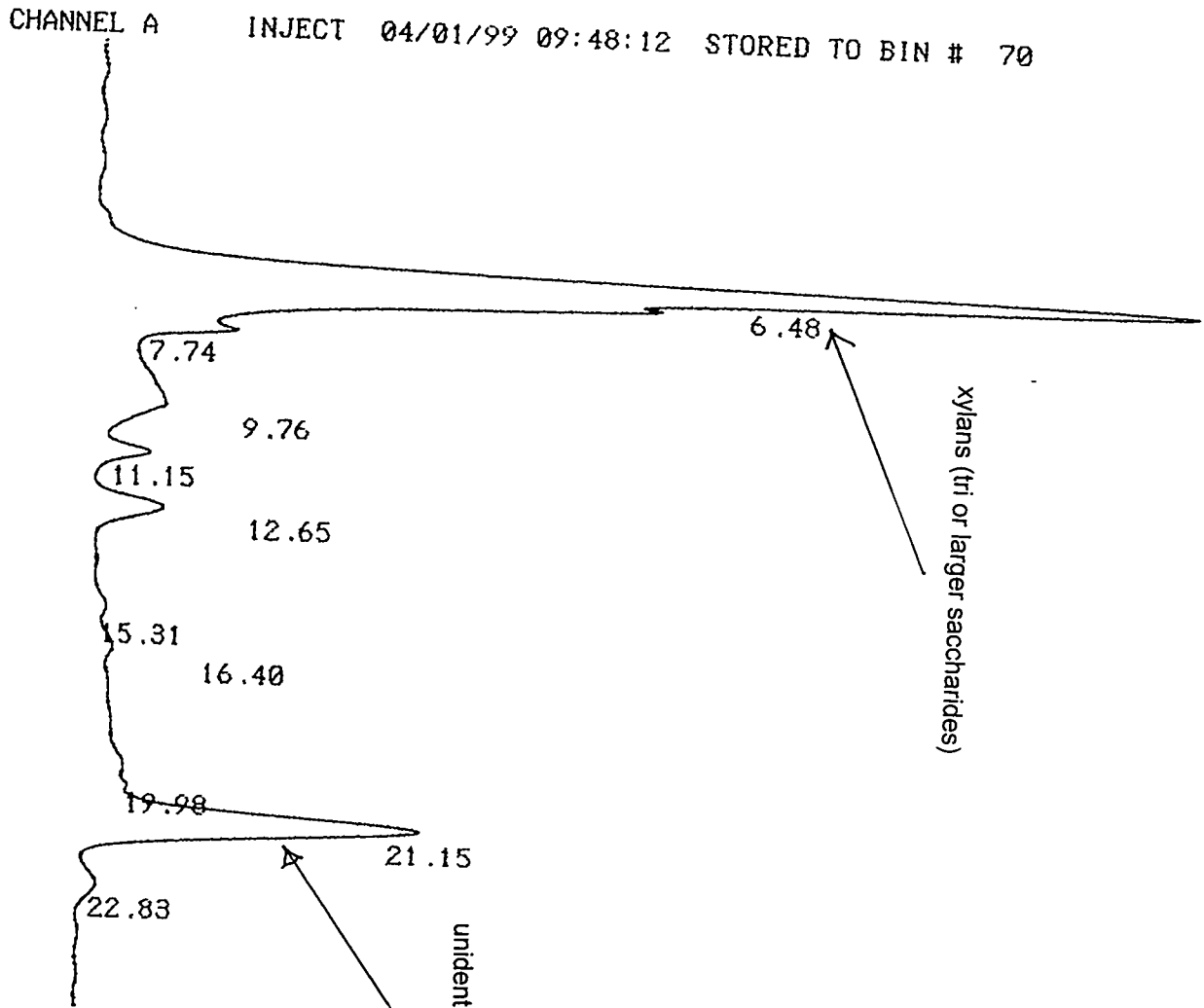


Figure 4. Steep Liquor Before Hydrolysis (full strength)

DATA SAVED TO BIN # 70

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FILE 1. METHOD 0. RUN 70 INDEX 70 BIN 70

PEAK#	AREA%	RT	AREA	BC
1	66.651	6.48	4795791	02
2	2.669	7.74	192016	03
3	3.777	9.76	271789	02
4	1.493	11.15	107408	03
5	3.216	12.65	231440	01
6	0.356	15.31	25631	02
7	0.488	16.4	35113	03
8	1.075	19.98	77333	02
9	19.391	21.15	1395283	03
10	0.884	22.83	63611	01

TOTAL 100. 7195415

C10 Hydrolysis of straw glucose 1 STR + de. lysed U=90 L  
 INK LOW - CHANGE PRINT CARTRIDGE AND PRESS [CTRL-SHIFT-C]  
 LOW BATTERY

CHANNEL A INJECT 04/02/99 09:01:54 STORED TO BIN # 74

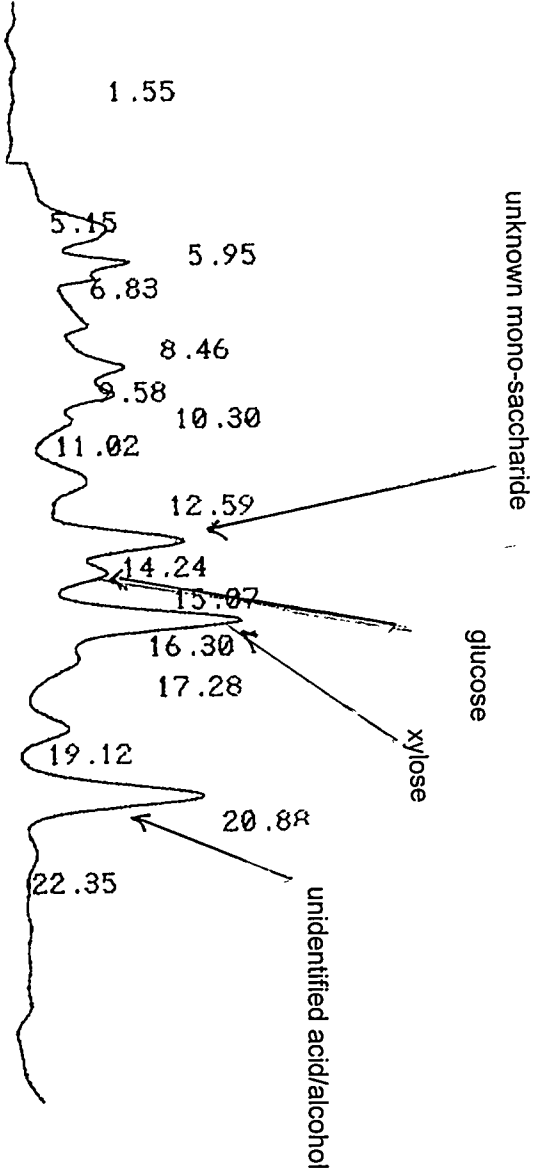


Figure 5. Steep Liquor after Enzyme Hydrolysis (1/2 strength)

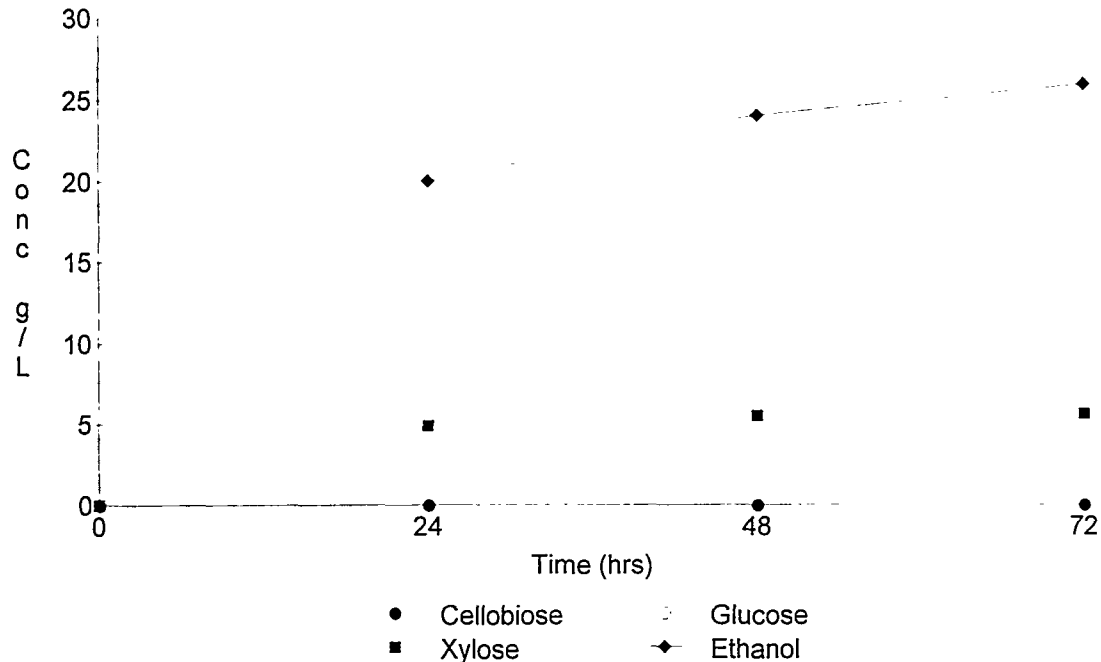
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1	0.917	1.55	58225	02
2	2.047	5.15	129957	02
3	8.137	5.95	516559	02
4	7.185	6.83	456136	02
5	6.687	8.46	424548	02
6	8.462	9.58	537207	02
7	5.604	10.3	355801	02
8	3.602	11.02	228658	02
9	7.29	12.59	462802	02
10	10.871	14.24	690173	02
11	1.070	15.07	200000	02

x 946 x 5 x 2 = 3.6

Figure 3. SSF of Maple Shavings



In Figure 3, we present one of our SSF experiments with the maple shavings/sawdust. We achieved about a 66% conversion of the initial solids (88 g/L) to sugars or ethanol in this trial compared to 90% and better with straw. We will be continuing this work in the next quarter.

### 3.0 Pilot/ Demonstration Scale CSRS

During Q8, a 130 L process scale unit will be operated to demonstrate the process using straw or cornstalks. During Quarter 5 and 6, we have acquired two 300 L stainless steel jacketed tanks for steeping delignification pilot operation, a regenerative blower for gas stripping ethanol separations from the pilot plant, four stainless steel 6" ID by 6' columns for ethanol and ammonia recovery operations, and built a steel rack/ framework for the columns/ CSRS system. The magnetic stirring system for the CSRS pilot reactor was tested in January.