

Annual Report
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**EVALUATION OF ALTERNATE PRETREATMENT AND BIOMASS
FRACTIONATION - AMMONIA RECYCLED PERCOLATION PROCESS**

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TABLE OF CONTENTS

ABSTRACT	i
I. INTRODUCTION	1
II. MATERIALS AND METHODS	5
III. AMMONIA RECYCLED PERCOLATION PROCESS	10
IV. FERMENTABILITY/TOXICITY TESTS ON ARP EFFLUENTS.....	27
V. SIMULTANEOUS SACCHARIFICATION AND FERMENTATION.....	39
VI. CONCLUSIONS	48
VII. NREL SAMPLE ANALYSIS WORK.....	49
VIII. APPENDIX	
A. AMMONIA-HYDROGEN PEROXIDE PRETREATMENT	50
B. AUTOHYDROLYSIS.....	60
C. DILUTE-ACID PROCESS PLUS ARP.....	67

ABSTRACT

An ammonia-based pretreatment method termed Ammonia Recycled Percolation (ARP) was developed for pretreatment of herbaceous biomass, namely corn cobs/stover mixture (CCSM) and switchgrass. The process involves treatment of biomass with aqueous ammonia through a percolation reactor (packed-bed flow-through type) at a temperature between 160 °C to 200 °C. The effects of process parameters such as temperature, reaction time, and ammonia concentration were studied. The extent of delignification in the ARP process was in the range of 60-80% for CCSM and 65-85% for switchgrass. The ARP process solubilized significant amounts of the hemicellulose fraction into the pretreatment effluent yet left most of the glucan fraction intact. The experimental data on CCSM and switchgrass show that the ARP is a highly effective pretreatment method. Near complete conversion of cellulose to glucose was obtained by enzymatic hydrolysis of ARP treated solid samples of CCSM, while conversion was slightly lower for switchgrass. The rate of enzymatic hydrolysis of ARP treated samples was substantially higher than that of α -cellulose. The scanning electron microscope examination of the solid samples revealed that the ARP process induced considerable morphological changes reflected by an increase in pore size and porosity. The ARP effluents were evaluated for fermentability/toxicity by the xylose fermenting yeast *Pichia stipitis* (NRRL Y-11545). The adaptability of ARP treated solid samples to simultaneous saccharification and fermentation (SSF) was tested for ethanol production using cellulase enzymes and the yeast *Saccharomyces cerevisiae* (D₅A). Modified ARP processes aimed at achieving full biomass fractionation were investigated and included in the appendix. The modified ARP processes included ARP in conjunction with hydrogen peroxide treatment, autohydrolysis, and dilute-acid process. Ammonia material balance based on Kjeldahl

nitrogen analysis indicated that the actual of ammonia was 0.02g NH₃/g dry biomass.

I. INTRODUCTION

Pretreatment is an essential prerequisite in bioconversion of lignocellulosic material to fuels and chemicals. The primary purpose of pretreatment is to open the structure of the lignocellulosic materials making it accessible to cellulase enzymes. An ideal pretreatment would accomplish fractionation of biomass into three main streams: cellulose, hemicellulose, and lignin. It should be environmentally friendly and economically acceptable. Various pretreatment techniques have been developed in the last decade to achieve the above goals. Among these treatments, the dilute acid process has been extensively investigated and accepted as a mature pretreatment technique. This process is effective in solubilization of the hemicellulose and provides a solid residue that is easily hydrolyzed by cellulase enzymes.

As an alternative pretreatment method to the acid process, ammonia recycled percolation process (ARP), has been developed in our laboratory. The ARP process when applied to woody biomass (hybrid poplar) brought about not only pretreatment effects but also a partial fractionation of biomass into cellulose, hemicellulose, and lignin. The rate and extent of enzymatic saccharification of the ARP treated samples was substantially higher than those of alpha-cellulose. Lignin generated from the ARP process was sulfur- and sodium-free, unlike the lignin generated from conventional pulping processes. It is quite conceivable that the uncontaminated lignin can be a marketable by-product, enhancing the overall economics of the bio-conversion process. The volatile ammonia is easily recovered and reused, minimizing the operating cost and eliminating the potential environmental problem.

In the subcontract work performed this year, we extended our investigation of the ARP process as an alternative pretreatment method to cover herbaceous biomass feedstocks including corn cobs/stover mixture and switchgrass. The scope of this work included the technical factors

concerning the operating conditions, effectiveness as a pretreatment, fermentability/toxicity tests of the effluents and adaptability to simultaneous saccharification and fermentation (SSF) for ethanol production.

In the ARP process, we found that more than half of the original hemicellulose fraction was solubilized along with the lignin, and thus separating the hemicellulose portion into streams. This complicates the utilization of hemicellulose sugars. An attempt was made to modify the ARP process to completely separate the hemicellulose and lignin from the biomass leaving the solid residue which was primarily composed of cellulose. One of the potential benefits of such process is that it can lower the enzyme loading and increase the rate of hydrolysis resulting in shorter hydrolysis and fermentation times. Although these additional tasks were not included in the statement of work, we think that they are important and relevant to this project. The results are summarized in the appendix of this report.

Although the features of the ARP process have been described in the last annual report, for the reader's convenience and better understanding of the idea of the ARP process, a simple introduction of the ARP process and its main features are repeated in this year's report.

The concept behind the Ammonia Recycled Percolation process is this: aqueous ammonia is used as a pretreatment agent and is pumped through a percolation reactor containing biomass to remove the lignin and hemicellulose. Ammonia is continuously regenerated and recycled to the percolation system during the process.

Ammonia has a number of characteristics which make it suitable for use as a pretreatment agent. It is a proven delignification agent and a cellulose swelling agent. It also causes significant changes in the biomass other than delignification. For example, ammonia increases the accessibility to carbohydrates by hydrolyzing glucuronic acid ester cross links. Ammonia is

known to cleave the bonds between lignin and hemicellulose, as well as the C-C and C-O bonds of the lignin macromolecule. It also causes ammonolysis of the uronic acid ester groups in hemicellulose. Ammonia also changes the cellulose fiber structure from cellulose I to cellulose III. Use of ammonia causes not only pretreatment effects, but also fractionates the biomass, especially separating lignin from biomass. This is an important attribute of ammonia pretreatment for several important reasons. First, the lignin content of the pretreated biomass can be lowered to any desired level, thereby increasing the efficiency in enzyme usage during hydrolysis. Second, as is the case with most alkaline pretreatments, ammonia pretreatment does not cause significant loss of carbohydrates. Third, the lignin generated in this process is sulfur free, unlike the lignin generated in conventional pulping process. It is therefore of high quality and may command high byproduct credits. High volatility of the ammonia in comparison to water makes it easy to be separated from an aqueous mixture. A straight batch evaporation is sufficient to remove all of ammonia content in the mixture. Therefore the unbound ammonia is easily recovered and recycled, a key feature in the ARP process scheme. Ammonia, although generally considered toxic in the form of vapors, is not an acute health hazard. In fact it is one component that exists in human body. There has been no evidence of harmful byproducts from ammonia-lignin-carbohydrate interaction at elevated temperatures. Ammonia is also one of the most heavily used industrial commodity chemicals. It is not very expensive, the current price of ammonia is \$108/ton, although sulfuric acid is only \$75/ton. Nevertheless, on a molar basis, ammonia costs only one fourth the price of sulfuric acid.

The potential problems associated with the ARP process will now be addressed. From a process viewpoint, there is a concern as to the high pressure condition that may develop due to the high volatile nature of ammonia. However, within the range of expected reaction conditions

of 140-180°C and 5-10% (wt/wt) NH_3 , the upper limit of the pressure is about 20 kg/cm³ or 290 psi. This is somewhat higher than a normal pulp mill digester pressure, but certainly within a manageable range. Ammonia is far less corrosive than sulfuric acid at high temperatures. Overall, it does not present a major technical problem to design and operate a process of this nature.

The concept of percolation process applies well to this pretreatment design. The distinctive feature of the percolation process in comparison to a straight batch process is that the process stream is continuously fed and withdrawn from the reactor. In connection with the biomass pretreatment, this offers a unique advantage because the lignin and other extraneous components are separated from the biomass structure. This prevents recondensation of lignin within the biomass. It may also eliminate the need for washing of pretreated biomass which will have a significant bearing in the operation cost.

II. MATERIALS AND METHODS

Materials

Corn Cobs/Stover Mixture (CCSM) and switchgrass feedstocks were supplied from NREL (National Renewable Energy Laboratory). The CCSM was milled and screened to the nominal size of 0.25 -2.00 mm. Switchgrass was used as supplied (the size being very fine - 10 mm). The initial composition of CCSM and switchgrass are reported in Table 1. The cellulase enzyme, Cytolase CL, Lot No. 17-92262 - 09 was obtained from Environmental Biotechnologies, Inc., Santa Rosa, CA. The average specific activity of the enzyme as determined by the supplier is as follows: Filter paper activity = 95.9 FPU/mL, β -glucosidase activity = 80.6 p-NPGU/mL, Endoglucanase activity = 613 CMCU/mL. A culture of xylose fermenting yeast *Pichia stipitis* (NRRL Y-11545) was obtained from the USDA and was used for fermentability and toxicity studies on ARP effluents. A culture of *Saccharomyces cerevisiae* (D₅A strain) obtained from NREL was used for SSF studies on ARP treated solid substrates for ethanol production and α -cellulose used as a reference in the digestibility tests and SSF studies was also supplied from NREL.

Experimental Setup and Operation

The schematic diagram of an entire ARP process is shown in Figure 1. In our experiments, the ammonia was not recycled to use, the actual experimental setups is shown in Figure 1a. The system consists of stock solution reservoirs, a pump, a temperature programmable oven, a packed-bed reactor (percolation reactor), and a liquid holding tank which also served as a back

Table 1.

Initial Composition of CCSM and Switchgrass

Components Identified*	Percentage	
	CCSM	Switchgrass
Glucan	38.10	35.20
Xylan	20.00	17.76
Arabinan	3.10	3.72
Galactan	1.20	1.92
Mannan	0.60	3.40
Klason lignin**	15.95	19.88
Acid soluble lignin	3.10	3.30
Ash	4.29	5.25
Extractives	6.70	6.09
Others	6.96	3.48

*Based on oven-dry untreated biomass.

**For Klason lignin determination, biomass was treated with 95% ethanol to remove extractives.

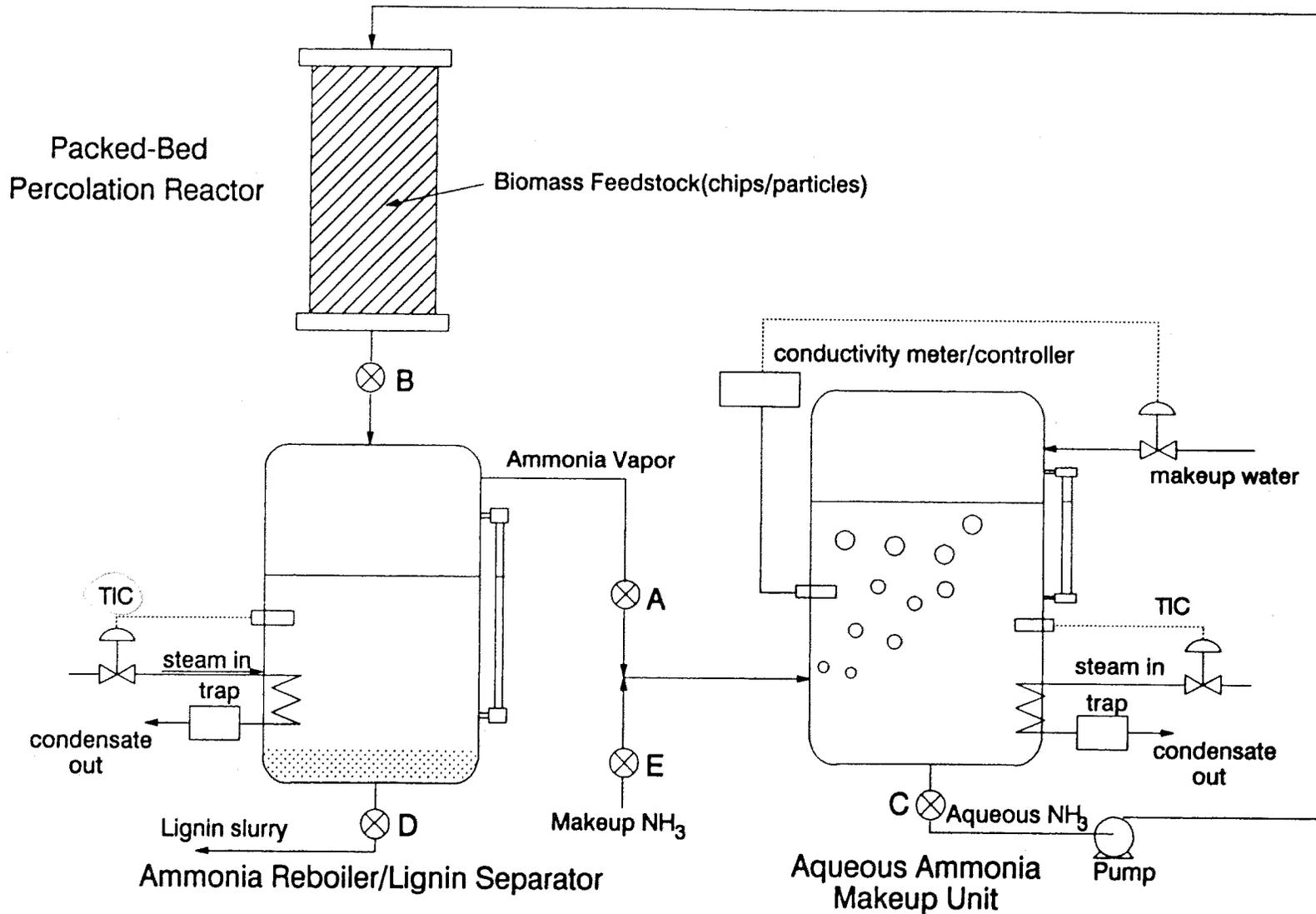


Figure 1. Schematics of Proposed Aqueous-Ammonia Percolation Pretreatment Reactor and Ammonia Recycle System

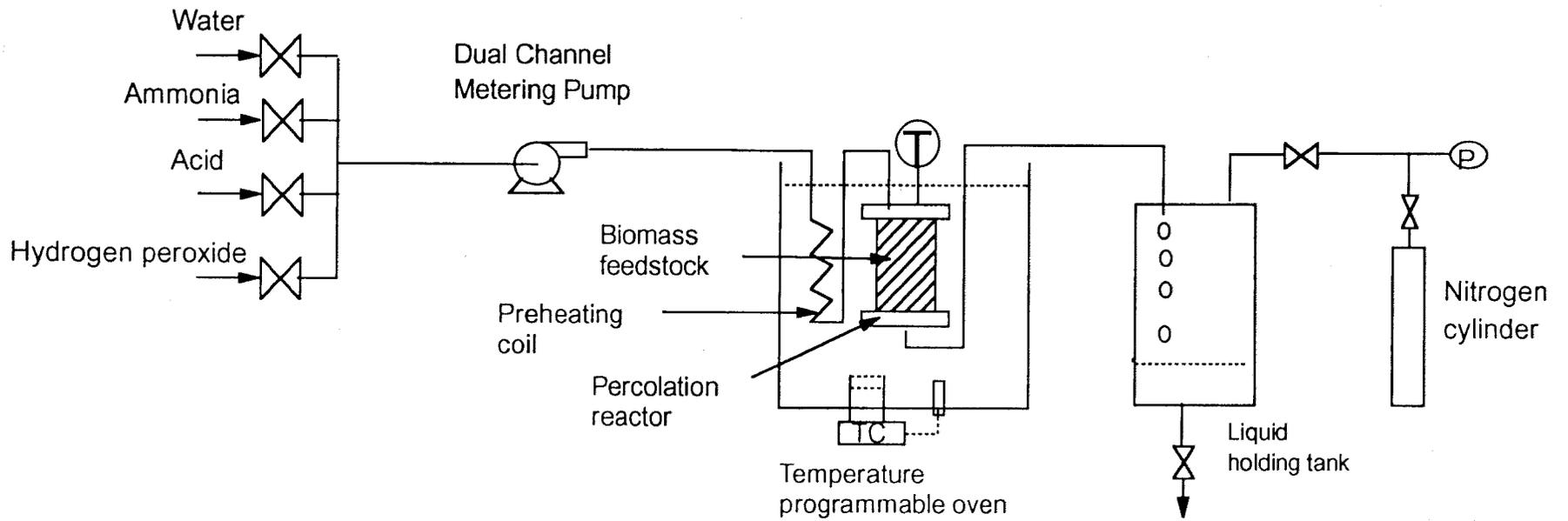


Figure 1a. Schematic Diagram of Percolation Reaction System

pressure vessel. The reactor was constructed out of ss 316 tubing, 5/8 inch OD x 6 inch length (33 cm³ empty reactor volume). It was flanged and screen sealed at both ends. An autoclave (600 mL, Parr Instrument) was used as a liquid holding tank. All the connecting lines and fittings were of ss 316 grade. The reaction temperature was controlled by the oven temperature. The pretreatment agents (aqueous ammonia, water, hydrogen peroxide etc.) were pumped by a displacement duplex pump (Metering Minipump, Milton Roy) to a packed-bed reactor through a preheating coil. The flow rate of pretreatment agents was controlled by the pump speed and monitored by a flow gauge.

In the operation of ARP, 5 -7 g of an air-dried biomass sample was packed into the reactor and soaked with ammonia solution of the same concentration as the experimental run reactant concentration overnight or more than 4 hours at room temperature. The ratio of liquid to solid inside the reactor was kept in the range of 7-10. Nitrogen back pressure was applied to the reaction system at 325 psi to prevent ammonia vaporization . Reaction was carried out in a temperature programmable oven with an initial temperature setting of 10 °C higher than the desired reaction temperature, and then the oven temperature was reduced to the required reaction temperature when the reactor temperature reached within 10 °C to the final desired temperature. This operation reduced the pre-reaction heating time to 15 minutes. The flow of the reagent was started at the beginning of the preheating process. After the reaction, water was pumped through to remove residual sugars and ammonia trapped in biomass. The reactor was then flushed with nitrogen to remove the excess water. The biomass samples discharged from the reactor were divided into two portions. One portion of the wet solid residue was oven dried at 105°C overnight to measure moisture content and then subsequently the weight loss on pretreatment. The other remaining portion of solid residues was stored in a walk-in cool room (4°C) under wet

condition for carrying out the digestibility test or chemical composition analysis. The ARP effluent collected in a holding tank was transferred into an airtight sample bottle and stored in the cool room for composition analysis.

Digestibility Test

Enzymatic hydrolysis of pretreated substrates was performed at 50°C and pH 4.8 (0.05 M sodium citrate buffer) on a shaker bath (New Brunswick Scientific) agitated at 150 rpm. The enzyme loading was 60 IFPU/g-glucan and the initial glucan concentration was 1% (w/v). Samples were taken periodically and analyzed for glucose and cellobiose content using HPLC. The total glucose content after 72 hours of hydrolysis was taken to calculate the enzymatic digestibility (as % of theoretical). Untreated switchgrass and CCSM and α -cellulose (from Sigma) were used as controls and as a reference.

Analytical Methods

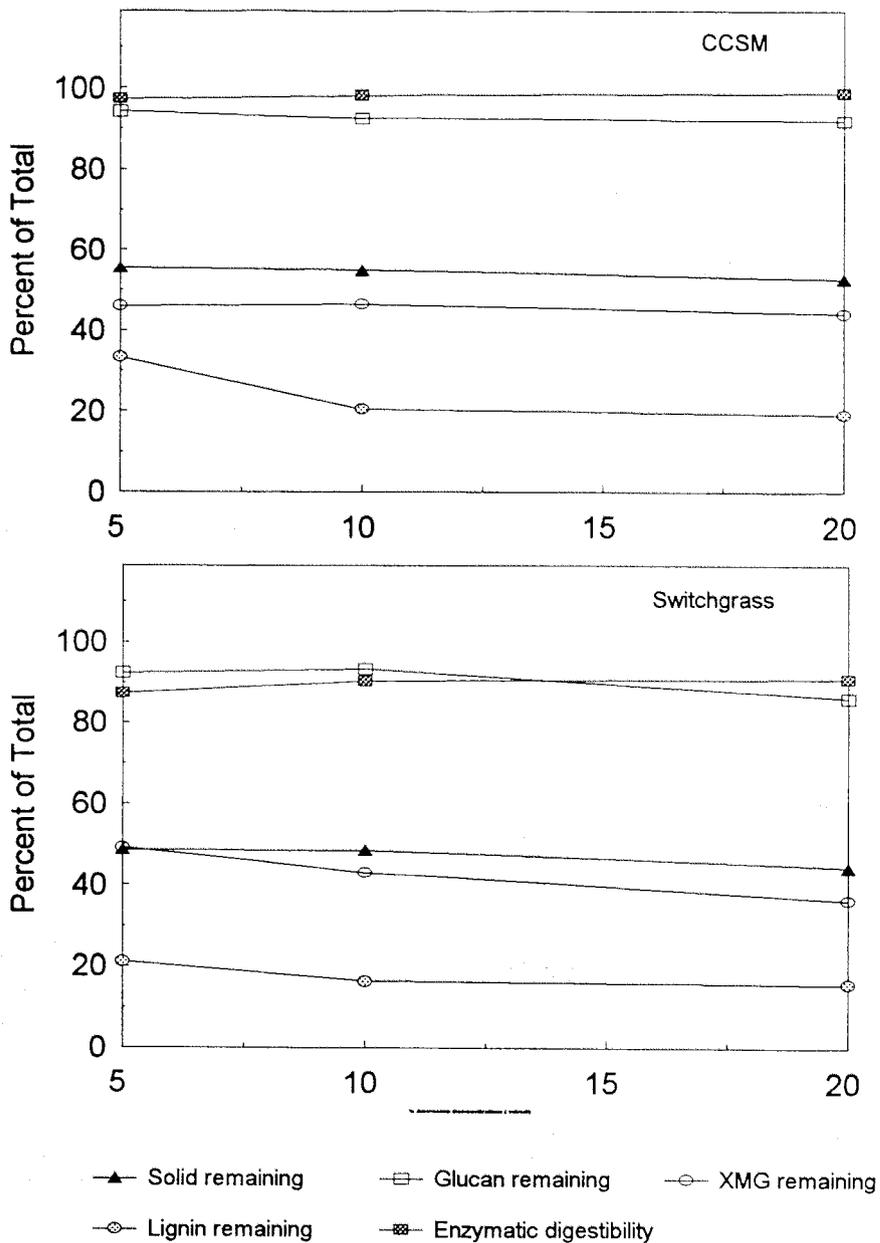
Samples of the wet biomass were weighed with a 4-digit analytical balance and dried in an oven for 24 hour at 105 °C to determine the exact moisture content. This value was used to calculate the weight remaining of the biomass after pretreatment reaction. The determination of sugar and Klason lignin content in the solid samples were followed the procedures described in NREL-CAT Standard Procedures # 002 - 003. The effluents from the ARP process were boiled until all free ammonia was evaporated. Since most of the sugars contained in the effluents were oligomers, a secondary hydrolysis was carried out at 121°C with 4% (wt/wt) sulfuric acid for one hour of reaction time to convert oligomers into monomers. Sugars, acids, and decomposition products were measured by HPLC using a Bio-Rad, Aminex HPX-87H column. The total amount

of xylan, mannan, arabinan, and galactan was used to represent the hemicellulose content in this study. The fermentation samples were analyzed using the H-column and/or a YSI 2300 Stat Plus sugar analyzer. The lignin in the ARP effluent was determined by UV spectrophotometric method at 280 NM. Indulin (from Westvaco) dissolved in aqueous ammonia solution and 10% (wt/wt) ammonia were used as a standard and a reference. Surface structure of solid residues was also observed by a scanning electron microscope (SEM).

III. AMMONIA RECYCLED PERCOLATION PROCESS

Effect of Ammonia Concentration

Ammonia concentrations were varied over 2.5-20 % (wt/wt) keeping other conditions at 170°C, reaction time of 1 hr, flow rate = 1 mL/min. Figure 2 shows the weight remaining, amounts of glucan, hemicellulose, lignin remaining and cellulose enzymatic digestibility upon the ARP pretreatment. During the ARP process, CCSM lost 43-47% of its weight. The weight loss for switchgrass was slightly higher at 49-56%. Lignin and hemicellulose accounted for the major part of the weight loss. In the case of CCSM, as high as 56% of the original amount of hemicellulose was extracted into ARP effluent, while less than 8% of the total glucose content was extracted. These numbers were slightly higher for switchgrass, 64% and 14% respectively. The amount of glucan and hemicellulose solubilized remained essentially constant showing little effect of ammonia concentration over this range. The lignin remaining, however, decreased from 48% - 19% for CCSM (32% - 16% for switchgrass) as the concentration of ammonia was increased from 2.5% to 20%. The increase in ammonia concentration beyond 10% had negligible



Pretreatment condition: 170C, 60 min, 325 psi, reagent flow rate = 1 mL/min.
 Enzymatic hydrolysis condition: 60 IFPU/g cellulose, pH = 4.8, 50C.
 XMG: the total amount of xylan, mannan, and galactan.

Figure 2. Compositional changes and cellulose enzymatic digestibility in ARP at various ammonia concentrations.

effect on the degree of delignification. Fig. 2 also shows that the enzymatic digestibility of ARP samples exceed 90% for CCSM and 87% for switchgrass in all cases. The digestibility generally increased as the ammonia concentration was increased. However, above 5% for CCSM, the increase in digestibility was insignificant. Results were similar for switchgrass except that the leveling off occurred at 10%. An ammonia concentration of 10% is therefore regarded as a near optimum level for pretreatment of switchgrass. For CCSM, an ammonia concentration of 5% was sufficient to achieve high digestibility, but considering that an ammonia concentration of 10% gives a higher degree of delignification (79%) and that all of the ammonia is recycled, it was taken as the optimum level for CCSM as well.

Effect of Temperature

ARP on CCSM retained 44% of the hemicellulose (43% for switchgrass) at 170°C, 10% ammonia, treatment time of 60 min., flow rate = 1 mL/min (Fig. 2). Complete retention or complete removal of the hemicellulose (depending on the subsequent fermentation strategy) is desirable for efficient utilization of hemicellulose sugars. Increased solubilization of the hemicellulose is expected with an increase in the reaction temperature. Ensuing runs were made over 160°C to 200°C for CCSM, maintaining other conditions at 10% ammonia, 1 hour reaction time, flow rate = 1 mL/min. Table 2 summarizes the composition data for the hydrolyzate and the remaining solid residue after pretreatment. From the data on CCSM, it is seen that the amounts of glucan and hemicellulose solubilized increased from 8% to 15% and 46% to 80% respectively as the pretreatment temperature was increased from 160°C to 200°C. However, the maximum amount of hemicellulose recovered as oligomers in the ARP effluent was only about 72% of the original amount and it occurred at 200°C. The ARP treatment of CCSM gave a near complete

Table 2.

Effect of Reaction Temperature on Composition of Hydrolyzates and Solid Residues in ARP*

Temperature (deg. C)	% Lignin (Klason)	% Glucan			% Hemicellulose		
		Solid	Liquid	Total	Solid	Liquid	Total
CCSM							
Untreated	15.95	38.10		38.10	24.90		24.90
160	5.03	35.10	1.89	36.99	12.53	11.04	23.57
170	3.26	35.30	2.04	37.34	11.01	12.65	23.66
180	3.10	34.78	2.11	36.89	9.51	14.53	24.04
190	5.32	33.41	2.36	35.77	7.96	16.51	24.47
200	5.63	32.18	2.39	34.57	5.09	18.07	23.16
Switchgrass							
Untreated	19.88	35.20		35.20	26.80		26.80
160	4.84	33.34	1.58	34.92	13.24	12.26	25.50
165	4.26	32.02	1.28	33.30	11.60	13.30	24.90
170	3.26	32.88	1.27	34.15	10.94	14.34	25.28
175	3.53	31.94	1.11	33.05	8.51	15.40	23.91

*All sugar and lignin contents based on the oven-dry untreated biomass.

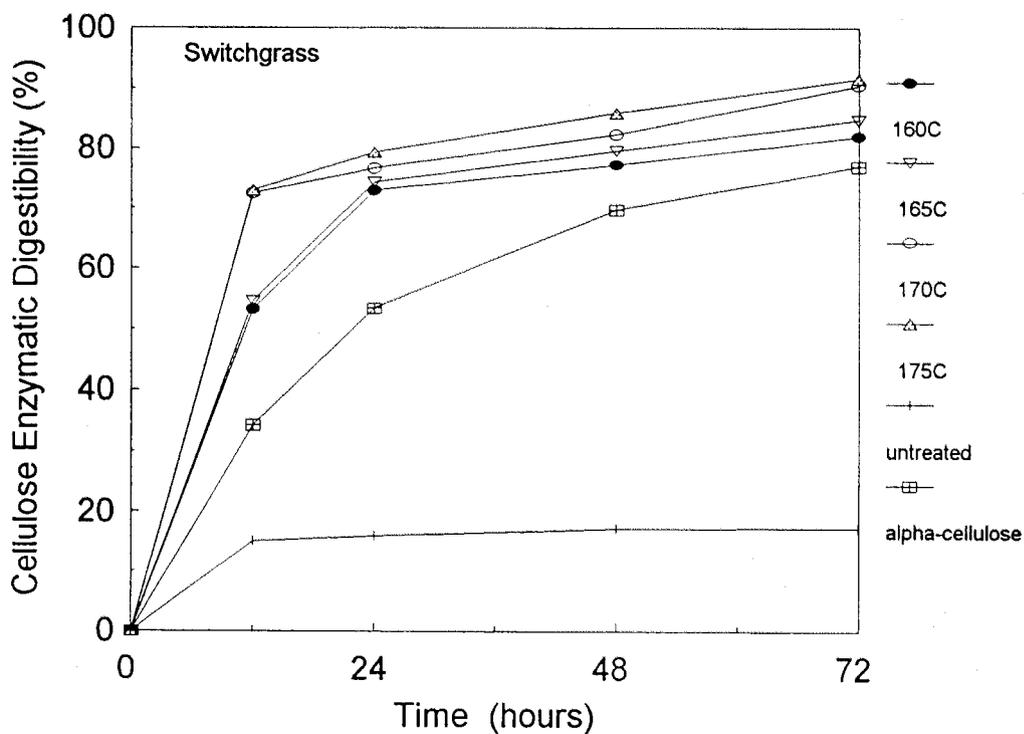
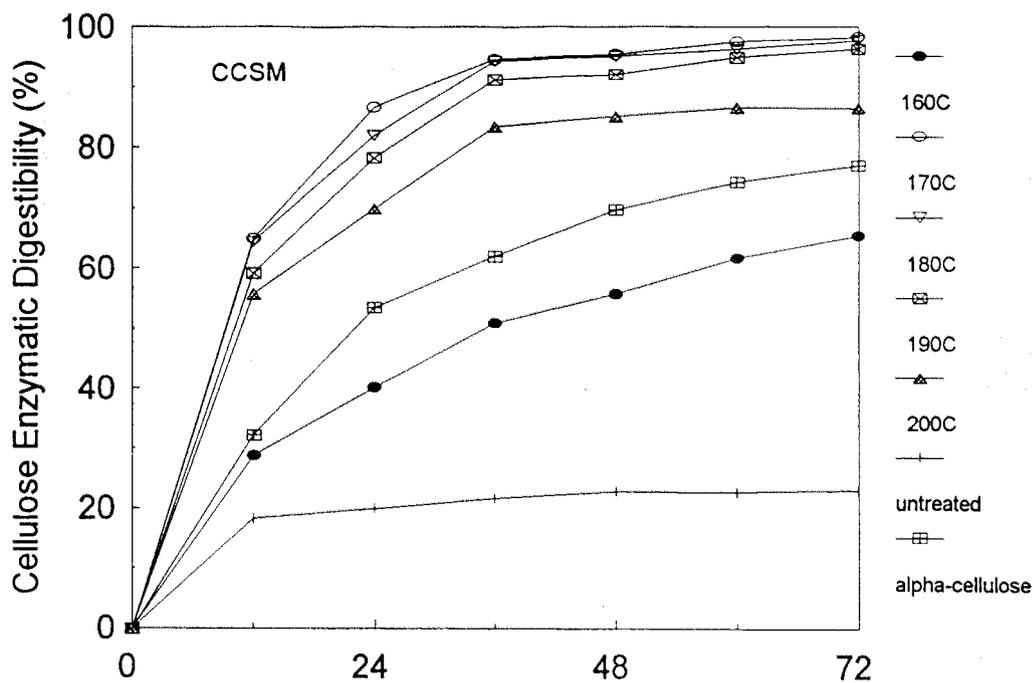
Hemicellulose: the total amount of xylan, mannan, arabinan, and galactan.

Pretreatment conditions: 10% (wt/wt) ammonia, 60 min, flow rate = 1 mL/min.

Secondary hydrolysis conditions: 121C, 45 min, 4% sulfuric acid.

sugar balance at temperatures below 200°C indicating sugar decomposition is insignificant. With regard to the delignification, the temperature effect gave a complicated picture. As the temperature was increased from 160 °C to 180 °C, lignin remaining in the solid decreased to 19% of the original. As the temperature was increased from 180-200°C, however, the lignin remaining increased to 35%. We do not have a clear explanation for this unusual behavior at this time. The data in Tables 2 & 3 show that the material balance of the treated solids do not close out because of the fact that ash content, extractables, and gaseous degradation components (if any) were not measured and are thus unaccounted for. The enzymatic digestibility was investigated for all samples of CCSM treated at various temperatures (Fig. 3). The maximum cellulose enzymatic digestibility after 72 hrs was 98% of the theoretical maximum for CCSM and it occurred with the ARP conducted at 170°C. With only one exception (CCSM at 160°C), all treated samples are seen to exhibit high digestibility, most of them showing above 90% in 36 hours. At the 36 hr point, the digestibility of α -cellulose was only 56%. Although the reaction temperatures of both 170°C and 180°C offer good pretreatment performance for CCSM, a reaction temperature of 170°C would be preferred to 180°C from a process view point.

A 10 °C increment was used in the study of temperature effect on CCSM. After a finding that the optimum temperature for CCSM is near 170 °C, a 5 °C increment was then used for switchgrass, applying 4 levels over 160-175 °C. The composition data of treated switchgrass are also included in Table 2. The enzymatic digestibilities for ARP treated switchgrass samples are shown in Fig. 3. As the temperature was increased from 160 °C to 175 °C, there was a substantial increase in hemicellulose removal. However, the lignin content in the solid remained essentially constant. The glucan removal was below 10% for all the temperatures. Again the initial rate of hydrolysis of ARP treated samples was much higher than that of α -cellulose and the digestibility



pretreatment condition: 10 wt% ammonia, 1 hour, 325 psi, flow rate = 1 mL/min.
 enzymatic hydrolysis condition: 60 IFPU/g cellulose, pH = 4.8, 50C.

Figure 3. Enzymatic Digestibility of ARP Treated Biomass at Different Temperatures.

at 72 hours of the best ARP treated switchgrass was above 90% as opposed to 77% for α -cellulose. From the data in Table 2 and Fig. 3, 170-175°C is judged to be the optimum region for pretreatment of switchgrass in ARP.

Effect of Reaction Time

The time effect on the ARP process was first studied by taking on-line samples of the ARP effluent of switchgrass. The conditions were: 10% ammonia, 170°C, reaction time of 1 hour, flow rate = 1 mL/min. These samples were analyzed for sugars and lignin. Lignin contents were determined by UV @ 280 nm using 10% ammonia solution as a reference. The data indicates that most of the hemicellulose removal and delignification occurs within the first 30 min of the ARP operation (Fig. 4). In the ensuing runs, reaction time was varied over 15 min to 60 min for CCSM keeping other conditions at 10% ammonia, 170°C, flow rate = 1 mL/min. The composition data for the hydrolyzate and the remaining solid residues after the ARP pretreatment and the enzymatic digestibility for both switchgrass and CCSM are presented in Table 3. From the data, it is quite evident that the reaction time of 15 min. is sufficient to increase the 72-hr digestibility from the control level of 23% to 94% for CCSM. For switchgrass, a reaction time of 30 min. was required. The digestibilities were somewhat lower at 17.3% for control and 87.3% for treated samples. Further increases in reaction time showed only a marginal increase in digestibility for both CCSM and switchgrass. As the reaction time was increased from 15 min to 60 min for CCSM (30 min to 60 min for switchgrass) the remaining lignin decreased from 26% to 20% of original for CCSM (29% to 16% for switchgrass). However the hemicellulose and glucan remained relatively constant (Table 3). Data in Table 3 collectively indicate that the acceptable range of reaction time under the previously described reaction conditions is 15 - 60 min.

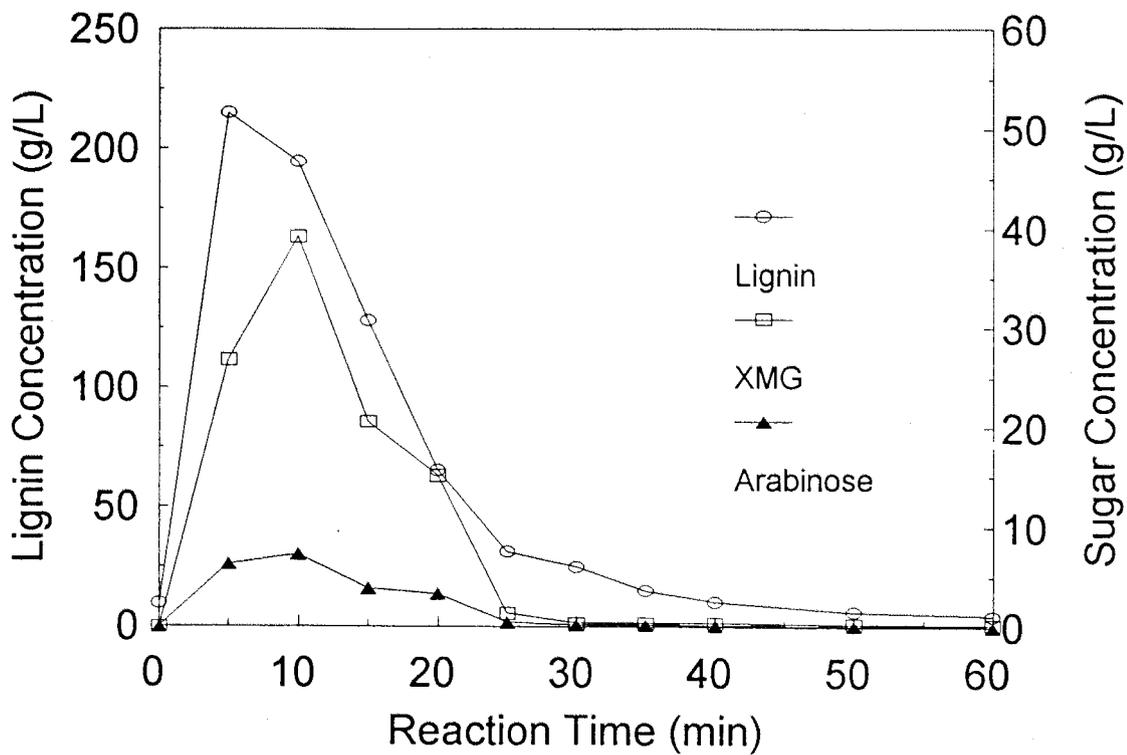


Figure 4. Time-course of Lignin and Sugar in ARP Effluent of Switchgrass.

Pretreatment condition: 170C, 10 wt% ammonia, 325 psi, flow rate = 1.0 mL/min.

XMG: Total amount of Xylose, Mannose, and Galactose.

Table 3.

**Effect of Reaction Time on Composition of Hydrolyzates, Solid Residues,
and Enzymatic Digestibility in ARP.***

Enzymatic hydrolysis conditions: 50C, pH 4.8, 60 IFPU/g glucan.

Reaction Time (min.)	% Lignin (Klason)	% Glucan			% Hemicellulose			% Digestibility (72 hr)
		Solid	Liquid	Total	Solid	Liquid	Total	
CCSM								
Untreated	15.95	38.10		38.10	24.9		24.90	22.90
15	4.16	35.48	1.82	37.30	11.95	12.61	24.56	94.10
30	3.82	34.43	1.88	36.31	11.15	13.01	24.16	95.28
45	3.44	34.98	2.16	37.14	11.39	13.35	24.74	96.45
60	3.26	35.30	2.04	37.34	11.01	12.65	23.66	98.30
Switchgrass								
Untreated	19.88	35.20		35.20	26.80		26.80	17.30
30	5.76	31.07	1.88	32.95	12.88	12.72	25.60	87.30
60	3.26	32.88	1.27	34.15	10.94	14.34	25.28	90.46

Notes are same as table 2.

ARP Effluent and Lignin Separation

ARP treatment of CCSM and switchgrass solubilized about 80% of the original lignin into the ARP effluent at the pretreatment condition of 170°C, 1 hr, 10% ammonia, 1 mL/min. At the same time, about 55% of the original hemicellulose was extracted into the ARP effluent, mostly in the form of oligomers. The ARP effluents were boiled until all the free ammonia evaporated (or until pH reached 7.0 from 11.5) and then subjected to a secondary acid hydrolysis. The acidic condition of secondary hydrolysis induced precipitation of lignin from the ARP effluent. The effluent was cooled to room temperature and filtered. The precipitated lignin was further washed with DI water and then dried at 105°C overnight and weighed. During the pretreatment, 80% of the original Klason lignin was extracted into the liquid effluent for CCSM (84% for switchgrass), however, only 50-60% of the original Klason lignin was precipitated and recovered. The balance of the lignin is believed to be broken down into low molecular weight lignin fragments and remained soluble even under acidic condition.

We made a lignin balance of switchgrass in the ARP process including the acid soluble lignin (ASL). The pretreatment conditions were: 170 °C, 10% ammonia, 30 min. The lignin contents of switchgrass before and after the ARP treatment are summarized in the table 4. As seen in the table 4, the ARP process solubilized 85.6% of the total lignin (Klason + ASL) in the feedstock. Upon the secondary acid hydrolysis, 42.1% of total lignin (or 48.7% of Klason lignin) was recovered as precipitated lignin and 12.8% of the total lignin as the ASL. A large fraction of total lignin (30.8%) was unaccounted for in our analysis. We believe it is was broken down to small molecules that are not detectable with UV@205 nm.

Effect of Reagent Flow Rate

Table 4. Lignin Balance of Switchgrass in ARP*.

		Klason Lignin %	A. S. Lignin %	Total %
Untreated		20.94	3.3	24.24
ARP	Solid Residue	3.19	0.29	3.48
	Hydrolyzate	10.2**	3.1	13.3

*All sugar and lignin contents based on the oven-dry untreated biomass.

** lignin precipitated from the acid hydrolyzed ARP effluent.

A. S. Lignin = acid soluble lignin.

Pretreatment conditions: 170C, 10% ammonia, 30 min, flow rate = 1 mL/min.

Secondary hydrolysis conditions: 121C, 45 min, 4% sulfuric acid.

Flow rate of the ammonia solution was varied over 1 mL/min to 5 mL/min at a preselected reaction condition of 170°C, 1 hr, 10% ammonia and pressure = 325 psi. The data in Table 5 show the composition data for the hydrolyzate and the remaining solid residue after pretreatment. Increase in a flow rate from 1 mL/min to 5 mL/min, increased the degree of delignification from 79% to 88%, also the solubilization of hemicellulose and corresponding recovery in the effluent (in the form of oligomers) increased from 55.8% to 64.7% and 50.8% to 62.4% of the original amount respectively, However, no appreciable changes in enzymatic digestibility were observed for the pretreated samples. Although the flow rate of 5 mL/min seems better if we consider lignin removal, digestibility of cellulose and xylose recovery in the ARP effluent, it may not be advisable to operate at such high flow rates. Not only the pumping cost will be higher; also the concentration of the sugars obtained will be lower and substantial energy input to boil off ammonia may be needed (in the ammonia regeneration step) which will have a direct bearing upon the operation costs. Therefore, the flow rate of 1 mL/min can be concluded as the preferred flow rate for treating biomass in the given reactor system using ARP process.

Effect of Pre-reaction Soaking Time

One of the main features of the ARP process is that the reactor runs under a unique solid liquid contact pattern termed percolation whereupon liquid passes through the stationary solid. However, non-uniform contact between the liquid reactant and solid might occur due to bypassing or channeling. Hence the biomass is presoaked with water or ammonia before carrying out the reaction. The effect of pre-reaction soaking time on the composition of solid residue and hydrolyzate was studied at the preselected reaction condition of 10% ammonia, 170°C, 15 min, 1 mL/min, 325 psi. The relevant data is shown in Table 6. From the data, it is seen that sugar

Table 5. Effect of Reagent Flow Rate on CCSM in the ARP*.

Pretreatment conditions: 10 wt% ammonia, 170C, 1hr, 325 psi.

Secondary hydrolysis: 121C, 45 min, 4% sulfuric acid.

Enzymatic hydrolysis conditions: 50C, pH 4.8, 60 IFPU/g glucan.

Flow rate (mL/min)	%Solid Remaining	% Lignin (Klason)	% Glucan			% Hemicellulose			% Digestibility (72 hrs)
			Solid	Liquid	Total	Solid	Liquid	Total	
Untreated	100	15.95	38.10		38.10	24.90		24.90	
1.08	54.9	3.26	35.30	2.04	37.34	11.01	12.65	23.66	98.30
3.47	52.48	2.63	35.01	2.44	37.45	9.94	14.50	23.44	97.98
5.12	51.1	1.92	34.68	2.58	37.26	8.79	15.55	24.34	99.20

*All sugar and lignin contents based on the oven-dry untreated biomass.

Hemicellulose: the total amount of xylan, mannan, arabinan, and galactan.

Table 6. Effect of pre-reaction soaking time on CCSM in the ARP*.

Pretreatment conditions: 10 wt% ammonia, 170C, 1hr, 325 psi.
 Secondary hydrolysis: 121C, 45 min, 4% sulfuric acid.

Soaking Time (hour)	%Solid Remaining	% Lignin (Klason)	% Glucan			% Hemicellulose		
			Solid	Liquid	Total	Solid	Liquid	Total
Untreated	100	15.95	38.10		38.10	24.90		24.90
0	60.12	8.77	34.28	1.85	36.13	13.13	11.71	24.84
2	57.19	5.41	33.39	1.79	35.18	12.02	12.20	24.22
4	55.84	4.32	34.61	1.84	36.45	12.16	12.77	24.93
Overnight -1	56.07	4.16	35.48	1.88	37.31	11.95	13.01	24.96
Overnight -2	55.62	4.25	35.12	1.92	37.04	12.24	12.50	24.74

*All sugar and lignin contents based on the oven-dry untreated biomass.
 Hemicellulose: the total amount of xylan, mannan, arabinan, and galactan.
 Overnight -1: soaked in ammonia for 12 hours or greater.
 Overnight -2: soaked in DI-water for 12 hours or greater.

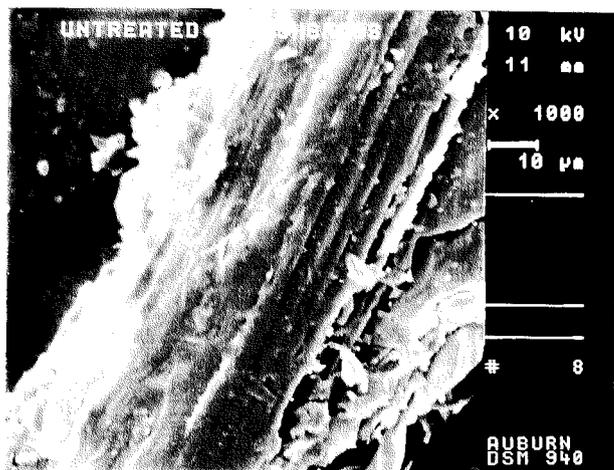
contents, in both the hydrolyzates and in the solid residues, remained practically constant showing no effect of pre-reaction soaking time. The lignin remaining after pretreatment, however was seen to decrease as the pre-reaction soaking time was increased from zero to 12 hours or greater, indicating some effect of pre-reaction soaking on delignification. From the data, it can be concluded that pre-reaction soaking time of at least 4 hours is needed for maximum delignification. All the previous data on CCSM and switchgrass were collected when CCSM and switchgrass were soaked with aqueous ammonia and left overnight under wet condition.

Physical Characteristics of ARP Solid Samples

The untreated and ARP treated switchgrass and CCSM were observed by SEM (Fig. 5 & 6). Figure 5 shows the side-view of untreated switchgrass and the ARP treated switchgrass. The commercial alpha-cellulose was also included as a reference. As can be seen from Figure 5, the ARP treatment increases the pore size and porosity. In the cross-section view (Fig.6), the cell structures of switchgrass and CCSM are seen to be collapsed owing to the pressing action of the knife blade cutting during the sample preparation. From here it can be seen that the mechanical strength of the biomass was greatly reduced after the lignin and hemicellulose were removed in the ARP process.

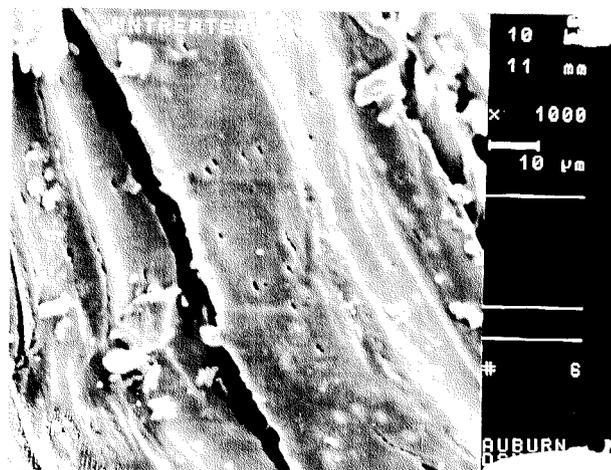
Untreated Switchgrass

X: 1000



Untreated CCSM

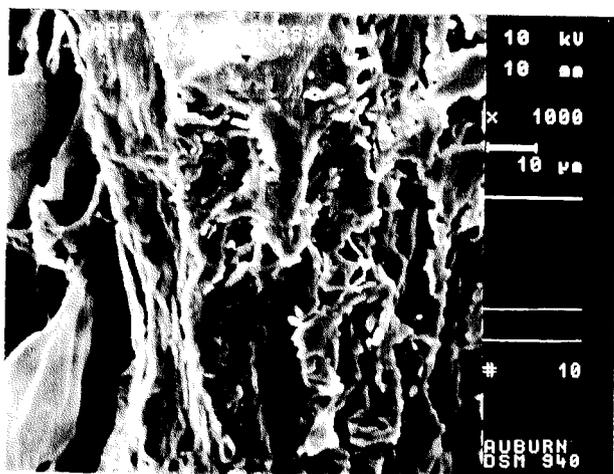
X: 1000



ARP Switchgrass

X: 1000

Pretreatment Conditions: 170°C, 10 wt% NH₃, 30 min.



ARP CCSM

X: 1000

Pretreatment Conditions: 170°C, 10 wt% NH₃, 30 min.

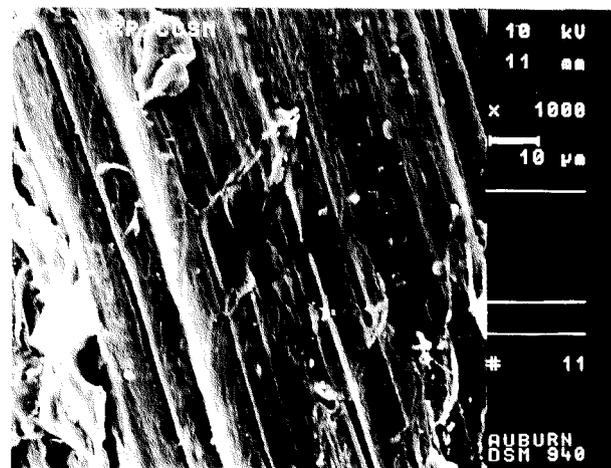


Figure 5. SEM Micrographs (side view) of Various Biomass Samples.

Continuation of
Figure 5 SEM Micrographs of Untrated α -cellulose.

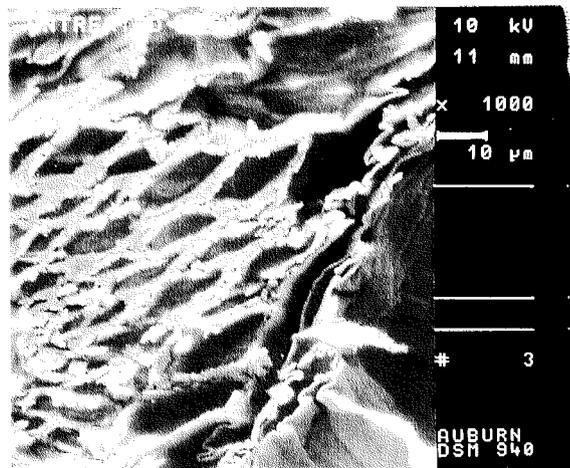
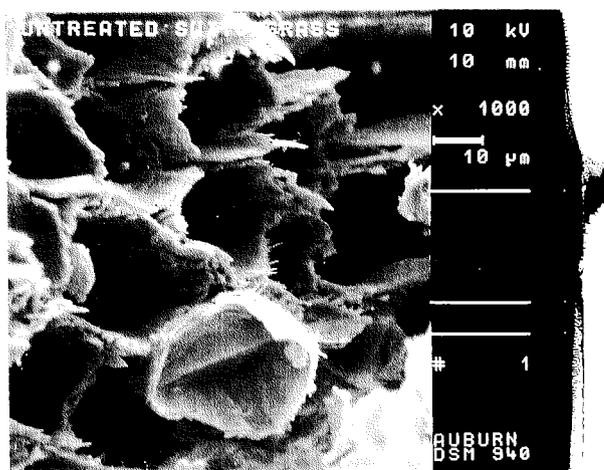


Untreated Switchgrass

X: 1000

Untreated CCSM

X: 1000



ARP Switchgrass

X: 1000

ARP CCSM

X: 1000

Pretreatment Conditions: 170°C, 10 wt% NH₃, 30 min.

Pretreatment Conditions: 170°C, 10 wt% NH₃, 30 min.

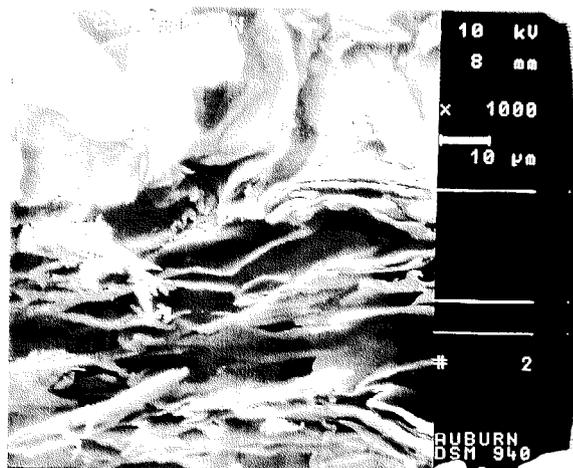
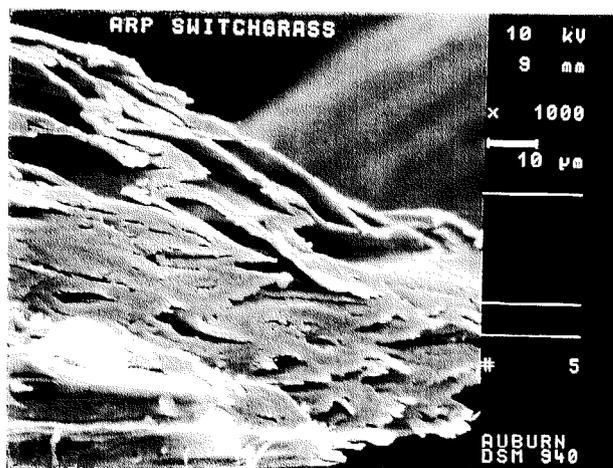


Figure 6. SEM Micrographs (cross-section view) of Various Biomass Samples.

VI. FERMENTABILITY/ TOXICITY TESTS ON ARP EFFLUENTS

ARP treatment of CCSM and switchgrass under the best conditions solubilized nearly 50-56% of original amounts of the hemicellulose into ARP effluent mostly in the form of xylose oligomer. The extraneous components such as extractives, acetic acid, furfural and dissolved lignin components present in the hydrolyzates along with xylose are potential inhibitors of microbial xylose fermentation. Therefore we tested the toxicity/fermentability of the ARP effluent to ethanol. No attempt was made to optimize the yield of ethanol. The test of hybrid poplar effluent also was included in this year's report.

Fermentability/Toxicity tests of ARP effluents containing xylose were performed following the procedure obtained from the USDA. The fermentation was performed at 30 °C, pH 6.0 with 1% yeast extract and 3% (wt/v) xylose.

Microorganism and Preparation for Fermentation

A culture of *Pichia stipitis* (NRRL Y-11545) was received from the USDA. The culture was streaked and incubated at 25 °C, on the recommended YM agar and observed after 3 days. No contamination was noted and the plates were stored in the cold room at 4 °C.

The effluents of ARP treatment on CCSM, switchgrass, and hybrid poplar at different conditions were collected for the fermentation tests. The effluents were boiled to remove all free ammonia. Since ARP effluent contained xylan mainly in oligomeric form, a secondary hydrolysis at 4% sulfuric acid, 121 °C, 60 min, was needed to convert all xylan into monomers. This process had also precipitated lignin and the liquor was filtered to remove lignin and was stored in the cold

room. The pretreatment conditions and concentration of effluent streams in g/lit are shown in Table 7. The concentration can be varied depending on the extent of boiling to remove ammonia. However, concentration in Table 7 is closer to the actual concentration of untreated ARP effluent including washing and hence was used as is to test fermentability/toxicity using *P. stipitis* culture.

All experiments were carried out in duplicate in 250 mL shake flasks stoppered with dry foam plugs. The final culture volume was made 100 mL in all cases. Effluent loading was varied from 0% to 80% of culture volume but the final xylose concentration and yeast extract were made about 3% (w/v) and 1% (w/v) respectively in all cases. The effluent loading means the percentage of the ARP effluent to the total fermentation liquor. Concentrated inoculum was prepared and fermentation flasks were inoculated to achieve a cell concentration of 5 g DCM/L. The final pH was adjusted to 6.0 using 1 N NaOH or 5% H₂SO₄ and was monitored and periodically adjusted to pH 6.0 when samples were taken every 24 hrs.

The ARP effluents contained arabinose which overlapped with a xylitol peak in our HPX-87H column and hence a HPX-87C Column was used in conjunction with the H-column for the analysis of the effluents.

Determination of Fermentation Parameters

A yield (g/g) was calculated as the ratio of maximum ethanol concentration detected minus the concentration of ethanol at zero time to the initial concentration of sugars (glucose + xylose) present. Volumetric productivity (g/L-hr) was calculated as the maximum ethanol concentration detected minus the concentration of initial ethanol divided by the fermentation time. Although fermentation time is normally taken as the time when both glucose and xylose are depleted, in this study it was defined as the time at which ethanol reaches maximum concentration

Table 7. Concentration of ARP Effluents after Secondary Hydrolysis.

Pretreatment conditions: 10 wt% ammonia, 170C, 325 psi, reagent flow rate = 1mL/min.
Secondary hydrolysis conditions: 121C, 45 min, 4% sulfuric acid.

Substrate	pretreatment Conditions	glucose (g/lit)	xylose (g/lit)	mannose (g/lit)	galactose (g/lit)	arabinose (g/lit)	acetic acid (g/lit)	HMF (g/lit)	furfural (g/lit)
CCSM - 1	170C, 10%, 15 min.	1.51	8.37	0.10	0.71	2.33	3.36	trace	0.14
CCSM - 2	170C, 10%, 30 min.	1.00	4.40	0.10	0.40	1.24	1.34	trace	0.12
Switchgrass	170C, 10%, 30 min.	1.68	12.34	0.48	1.27	2.64	2.62	trace	trace
Hybrid Poplar	180C, 10%, 60 min.	0.63	7.95	1.4	0	0.43	1.62	trace	0.24

All values are calculated after secondary hydrolysis and expressed as grams per 1000 mL of hydrolyzates.

because ethanol concentration had started to decrease before the glucose and xylose were completely depleted. Specific productivity (g/g-hr) was calculated as the ratio of maximum ethanol concentration detected minus concentration of ethanol initially present to average cell concentration (average of initial cell mass and final cell mass concentrations after 72 hours) divided by 72 hours.

Results and Discussion

The data of fermentability/toxicity of ARP effluents of CCSM generated at two different conditions are shown in Tables 8 and 9. All experiments are run in duplicate. The concentration of ethanol generally increased with fermentation time, but for the effluent loadings of 0%, 40%, and 60%, concentration of ethanol decreased after reaching a maximum, probably due to consumption of ethanol by *P.stipitis* when xylose and glucose were depleted. A similar trend was also noticeable for acetic acid. There was no indication of consumption of arabinose by *P.stipitis*. Xylitol, which is one of the intermediates through which xylose is fermented to ethanol, was present only in very low amounts.

Final cell mass concentration and yield, volumetric productivity and specific productivity calculated, are reported in Table 10. Fermentation of pure xylose, i.e. 0% effluent loading (control) gave a yield of 0.4 g/g which is 78.2% of the theoretical yield and effluent loading of 40% and 60% approached that of the control while the fermentation of 80% effluent loading was incomplete after 72 hours in both cases (pretreatment time of 15 and 30 min.). High acetic acid concentration (a known inhibitor) at 80% loading may be one of the reasons for it. Hence it was felt that dissolved lignin products in the ARP effluent may not be toxic to xylose fermentation by *P.stipitis*.

Table 8. Fermentation Data of ARP Effluent of CCSM at Different Dilutions (#1)

Pretreatment conditions: 10 wt% ammonia, 170C, 15 min, 325 psi,
reagent flow rate = 1 mL/min.

Effluent loading (% of culture volume)	Fermentation time (hrs)	glucose (g/lit)	xylose (g/lit)	arabinose (g/lit)	acetic acid (g/lit)	xylitol (g/lit)	alcohol (g/lit)
0 % (control)	0	0.00	29.50	0.00	0.03	0.03	1.01
	24	0.00	1.21	0.00	0.84	0.38	13.05
	48	0.00	0.00	0.00	0.37	0.38	10.40
	72	0.00	0.00	0.00	0.31	0.40	9.50
40%	0	0.61	29.40	0.98	1.28	0.00	1.08
	24	0.00	6.80	0.92	1.03	0.08	12.40
	48	0.00	0.00	0.96	0.46	0.11	12.00
	72	0.00	0.00	0.94	0.12	0.12	10.79
60%	0	0.88	29.80	1.25	2.01	0.00	0.92
	24	0.19	15.00	1.29	1.43	0.09	7.76
	48	0.00	0.26	1.24	1.15	0.12	12.30
	72	0.00	0.00	1.27	0.38	0.12	11.90
80%	0	1.15	29.30	1.76	2.80	0.00	1.05
	24	0.23	18.10	1.72	1.87	0.08	4.87
	48	0.00	10.12	1.74	1.34	0.12	6.93
	72	0.00	7.80	1.71	1.01	0.16	8.51

Table 9. Fermentation Data of ARP Effluent of CCSM at Different Dilutions (#2)

Pretreatment conditions: 10 wt% ammonia, 170C, 1hr, 325 psi,
reagent flow rate = 1 mL/min.

Effluent loading (% of culture volume)	Fermentation time (hrs)	glucose (g/lit)	xylose (g/lit)	arabinose (g/lit)	acetic acid (g/lit)	xylitol (g/lit)	alcohol (g/lit)
0 % (control)	0	0.00	29.50	0.00	0.03	0.03	1.01
	24	0.00	1.21	0.00	0.84	0.38	13.05
	48	0.00	0.00	0.00	0.37	0.38	10.40
	72	0.00	0.00	0.00	0.31	0.40	9.50
40%	0	0.41	29.70	0.49	0.55	0.00	0.15
	24	0.00	3.10	0.47	0.85	0.09	11.90
	48	0.00	0.00	0.46	0.79	0.12	11.07
	72	0.00	0.00	0.47	0.68	0.13	9.50
60%	0	0.61	29.40	0.78	0.85	0.00	0.12
	24	0.00	6.40	0.76	0.82	0.14	11.50
	48	0.00	0.00	0.78	0.61	0.15	11.10
	72	0.00	0.00	0.75	0.55	0.16	10.70
80%	0	0.81	29.80	0.98	1.10	0.12	0.15
	24	0.00	15.30	0.94	1.16	0.13	7.20
	48	0.00	6.40	0.95	1.06	0.13	10.10
	72	0.00	3.10	0.94	1.03	0.18	10.50

Table 10. Other Pertinent Data on Fermentation of ARP Effluents of CCSM

Effluent loading (% of culture volume)	Final cell mass conc. (g DCM/L)	Yield (g/g)	Vol. Productivity (g/L-hr)	Specific productivity (g/g-hr)
pretreatment condition: 10 w% ammonia, 170 C, 1 hr, flow rate 1 mL/min.				
0 % (control)	8.62	0.40	0.50	0.024
40%	8.71	0.38	0.47	0.022
60%	8.57	0.37	0.23	0.017
80%	7.68	0.24	0.10	0.016
pretreatment condition: 10 w% ammonia, 170 C, 1 hr, flow rate 1 mL/min.				
0 % (control)	8.62	0.40	0.50	0.024
40%	8.22	0.39	0.49	0.024
60%	8.31	0.38	0.47	0.023
80%	7.34	0.33	0.14	0.023

DCM: dry cell mass.

*: represents the maximum.

The yield at 40% and 60% effluent loading for pretreatment condition of 170°C, 15 min, 10% ammonia, flow rate = 1 mL/min. was essentially the same as that of the control (0% loading). The volumetric productivity, however, had decreased significantly with effluent loading of 60%. With the pretreatment condition of 170°C, 1 hour, 10% ammonia, flow rate = 1 mL/min, the yield and volumetric productivity of both 40% and 60% effluent loading were quite close to that of the control. In terms of effluent fermentation alone, the latter pretreatment condition would obviously be judged as better between the two. However, the concentration of sugars obtained in the effluent is lower than that at a pretreatment condition of 15 minutes. Hence, the optimum condition has to be determined by the overall process economics.

The results of fermentation/toxicity test of ARP effluents of switchgrass and hybrid poplar were quite similar to those of CCSM. The data are summarized in Tables 11-13. The ethanol value increases with fermentation time to a maximum point and then declines. This would be an indication that the microorganism (*P. stipitis*) consumes ethanol when xylose is depleted. A similar pattern was also noticed for acetic acid. For switchgrass and CCSM, the fermentation of xylose was incomplete after 72 hours when the effluent loading was 80%. With hybrid poplar, xylose was completely consumed at 72 hr with 80% loading. The ethanol concentration, however, was lower than those obtained with low effluent loading. The observed yield of ethanol (at the maximum point) was in the range of 0.43-0.46 g/g xylose for switchgrass (0.34 -0.4 g/g-xylose for hybrid poplar), which is 86-92% of theoretical for switchgrass (68 - 80% of theoretical for hybrid poplar). The data of acetic concentration and pH show that *P. Stipitis* produces a low amount of acetic acid along with alcohol, but it consumes acetic acid when xylose and glucose are depleted. As the effluent percentage was increased from 0% to 80%, the maximum ethanol yield decreased slightly. The fermentation time to attain maximum level of ethanol, however, increased

Table 11. Fermentation of ARP effluent of switchgrass at different dilutions.

Pretreatment conditions: 170C, 10 wt% ammonia, 4 mL/min, and 325 psi.

Effluent loading (% of culture volume)	Fermentation Time (hrs)	Glucose g/L	Xylose g/L	Arabinose g/L	Acetic acid g/L	xylitol g/L	Ethanol g/L	pH
0% (control)	0	0.00	23.02	0.00	0.00	0.00	0.00	6
	24	0.00	0.65	0.00	0.65	0.11	10.41	4.4
	48	0.00	0.00	0.00	0.12	0.11	5.59	6.7
	72	0.00	0.00	0.00	0.00	0.11	4.47	6.20
20%	0	0.33	25.06	0.48	0.00	0.00	0.00	6.00
	24	0.00	0.75	0.48	0.63	0.08	11.63	4.65
	48	0.00	0.00	0.26	0.00	0.11	9.65	6.70
	72	0.00	0.00	0.00	0.00	0.16	8.91	6.35
40%	0	0.67	26.09	1.07	1.28	0.00	0.00	6.00
	24	0.00	6.23	1.13	1.45	0.06	9.65	4.85
	48	0.00	0.70	0.26	0.00	0.11	11.52	6.40
	72	0.00	0.00	0.00	0.00	0.17	10.37	7.10
60%	0	1.12	24.73	1.65	1.79	0.00	0.00	6.00
	24	0.00	10.99	1.66	2.29	0.08	8.54	4.80
	48	0.00	1.69	1.10	1.45	0.11	11.92	6.00
	72	0.00	0.10	0.38	0.51	0.16	10.46	7.10
80%	0	1.34	24.84	2.13	1.92	0.00	0.00	6.00
	24	0.00	12.92	2.12	2.78	0.11	5.20	4.85
	48	0.00	4.04	1.13	1.55	0.11	6.21	6.00
	72	0.00	0.35	0.86	1.15	0.22	8.23	6.80

Table 12. Fermentation of ARP effluent of hybrid poplar at different dilutions.

Pretreatment conditions: 180C, 10 wt% ammonia, 1hr.

Effluent loading (% of culture volume)	Fermentation Time (hrs)	Glucose g/L	Xylose g/L	Arabinose g/L	Acetic acid g/L	xylitol g/L	Ethanol g/L	pH
0% (control)	0	0.00	24.50	0.00	0.00	0.00	0.00	6
	24	0.00	11.69	0.00	0.24	0.12	8.75	4.12
	48	0.00	0.00	0.00	0.48	0.10	7.82	4.9
	72	0.00	0.00	0.00	0.24	0.10	6.70	6.26
20%	0	0.04	24.60	0.04	0.30	0.00	0.00	0.00
	24	0.00	12.43	0.02	0.34	0.07	6.37	4.45
	48	0.00	0.40	0.00	0.18	0.11	9.96	4.58
	72	0.00	0.00	0.00	0.15	0.13	8.70	6.12
40%	0	0.67	24.63	0.07	0.61	0.00	0.00	6.00
	24	0.00	13.70	0.05	0.42	0.06	5.26	4.88
	48	0.00	0.22	0.00	0.36	0.12	9.68	5.11
	72	0.00	0.08	0.00	0.18	0.15	8.37	6.18
60%	0	1.12	24.40	0.11	0.91	0.00	0.00	6.00
	24	0.00	16.68	0.07	0.50	0.08	4.65	5.19
	48	0.00	1.21	0.03	0.36	0.12	9.18	5.45
	72	0.00	0.10	0.00	0.12	0.17	8.42	6.25
80%	0	1.34	24.80	0.14	1.22	0.00	0.00	6.00
	24	0.00	17.80	0.10	0.74	0.11	3.54	5.14
	48	0.00	2.96	0.30	0.48	0.13	8.45	5.65
	72	0.00	0.13	0.30	0.21	0.18	8.47	6.43

Table 13. Other pertinent data on fermentation of ARP effluents of switchgrass and hybrid poplar

Pretreatment conditions (for switchgrass): 170C, 10 wt% ammonia, 30 min, and 325 psi.

Pretreatment conditions (for poplar): 180C, 10 wt% ammonia, 1 hour, and 325 psi.

Effluent loading % of culture volume	Final cell mass conc g DCM/L	Yield* g/g	Vol. Productivity* g/L-hr	Specific productivity* g/g-hr
Switchgrass				
0% (control)	10.37	0.45	0.43	0.0189
20%	8.24	0.46	0.48	0.0191
40%	8.53	0.43	0.24	0.0090
60%	8.62	0.46	0.25	0.0100
80%	8.22	0.31	0.14	0.0044
Hybrid poplar				
0% (control)	9.46	0.34	0.36	0.0142
20%	10.14	0.4	0.21	0.0083
40%	10.32	0.39	0.2	0.0081
60%	11.36	0.38	0.19	0.0079
80%	11.05	0.34	0.12	0.0047

DCM: dry cell mass.

*: represents the maximum.

from 24 hr to 72 hr. The volumetric and specific productivities thus declined with an increase of the effluent loading. The net effect of ARP effluent is that it does not influence the product yield, but slows down the fermentation process.

The results of fermentation of xylose in the ARP effluent indicate that the components in the effluent are not very toxic to the xylose fermenting microorganism *P.stipitis*. The result is not very surprising because, although, the ARP process solubilized 74-84% of the original lignin into the ARP effluent, the majority of lignin solubilized from CCSM, switchgrass and poplar by ARP treatment was precipitated and recovered, leaving only a small amount of dissolved lignin in the effluent. Also, furfural, another major inhibitor was detected only in low amounts in the ARP effluent after a secondary hydrolysis.

V. SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

The adaptability of ARP treated solid samples for ethanol production by SSF was determined by following NREL-CAT Standard Procedure No.008. The SSF conditions are: 38 °C, pH 5.0, 3% (wt/v) glucan, and enzyme loading 25 IFPU/g-glucan. No attempt was made to optimize an ethanol yield.

Microorganism

A culture of *Saccharomyces cerevisiae* (D₅A strain) was obtained from NREL and was used throughout the study. It was stored at 4°C on the recommended YPD (yeast extract, peptone, dextrose) agar.

Results and Discussion

Two near optimum pretreatment conditions for CCSM were chosen for this study viz. 170 °C, 10 wt%, 15 min, flow rate=1 mL/min and 170 °C, 10 wt%, 60 min, flow rate=1 mL/min. Five runs were made to collect solid samples of the SSF. The composition data of solid residues after pretreatment (based on oven-dry pretreated biomass) are summarized in following table:

Reaction Time (min)	Glucan %	Xylan %	Arabinan %	Mannan %	Galactan %
15	65.27	22.32	2.25	trace	trace
60	68.76	21.18	2.12	trace	trace

The SSF data of ARP treated solids at two different conditions along with filter paper and α -cellulose (controls) are summarized in Table 14. The data shows that some xylan and arabinan

Table 14. SSF data of ARP treated CCSM.

SSF conditions: 38C, pH 5.0, 150rpm, 3 % cellulose, and enzyme loading=25 IFPU/g-cellulose
 Inoculum information: end pH=4.5, cell conc =5.20 g/L, and inoculation level=10% of reaction volume.
 *corn cobs/stover mixture pretreated at 170C, 10 wt% ammonia, 15min, flowrate= 1 mL/min.
 **corn cobs/stover mixture pretreated at 170C, 10 wt% ammonia, 30min, flowrate= 1 mL/min.

Fermentation Time (hrs)	Substrate	cellobiose (g/L)	glucose (g/L)	xylose (g/L)	arabinose (g/L)	lactic acid (g/L)	glycerol (g/L)	acetic acid (g/L)	ethanol (g/L)	%Yield (theoretical)
0	CCSM*	0.00	0.024	0.00	0.00	0.013	0.015	trace	1.92	0.00
	CCSM**	0.00	0.024	0.00	0.00	0.013	0.015	trace	1.92	0.00
	alpha-cellulose	0.00	0.024	0.00	0.00	0.013	0.015	trace	1.92	0.00
	filter paper	0.00	0.024	0.00	0.00	0.013	0.015	trace	1.92	0.00
24	CCSM*	1.42	0.45	4.81	0.77	0.085	0.650	0.47	12.61	62.72
	CCSM**	1.21	0.41	4.64	0.79	0.092	0.610	0.49	13.35	67.07
	alpha-cellulose	0.35	0.32	0.76	0.00	0.044	0.520	0.17	7.90	35.08
	filter paper	0.57	0.36	0.00	0.00	0.043	0.580	0.00	10.20	48.58
48	CCSM*	0.52	0.28	4.45	0.00	0.079	0.720	0.16	15.80	81.44
	CCSM**	0.49	0.26	4.28	1.07	0.085	0.690	0.18	16.10	83.20
	alpha-cellulose	0.31	0.22	0.74	1.19	0.042	0.560	0.19	12.07	59.55
	filter paper	0.37	0.24	0.00	0.00	0.037	0.610	0.08	14.51	73.87
72	CCSM*	-	0.15	3.94	0.84	0.11	0.8/22	0.28	16.10	83.20
	CCSM**	-	0.18	3.86	0.96	0.095	0.850	0.32	16.32	84.50
	alpha-cellulose	0.26	0.19	0.99	0.00	0.057	0.540	0.69	13.50	67.95
	filter paper	0.21	0.12	0.00	0.00	0.052	0.640	0.24	15.80	81.44

Contd.....

continuation of
Table 14 SSF Data of ARP Treated CCSM

Fermentation Time (hrs)	Substrate	cellobiose (g/L)	glucose (g/L)	xylose (g/L)	arabinose (g/L)	lactic acid (g/L)	glycerol (g/L)	acetic acid (g/L)	ethanol (g/L)	%Yield (theoretical)
96	CCSM*	-	0.095	3.97	1.02	0.081	0.76	0.57	15.70	80.86
	CCSM**	-	0.085	4.12	1.11	0.091	0.81	0.63	15.82	81.56
	alpha-cellulose	0.22	0.150	0.63	0.00	0.046	0.52	0.97	14.40	73.23
	filter paper	0.21	0.075	0.00	0.00	0.045	0.66	0.27	16.21	83.85
120	CCSM*	-	0.068	3.91	1.08	0.082	0.86	0.41	16.20	83.79
	CCSM**	-	0.056	4.05	1.14	0.089	0.83	0.65	16.35	84.67
	alpha-cellulose	0.15	0.053	0.88	0.00	0.050	0.52	1.11	15.40	79.10
	filter paper	0.13	0.028	0.00	0.00	0.047	0.67	0.34	16.75	87.02
144	CCSM*	-	0.055	3.97	0.96	0.082	0.89	0.43	16.40	84.96
	CCSM**	-	0.049	3.99	0.99	0.091	0.94	0.55	16.52	85.67
	alpha-cellulose	0.09	0.025	0.79	0.00	0.054	0.51	1.24	16.00	82.62
	filter paper	0.10	0.021	0.00	0.00	0.046	0.66	0.34	17.60	92.01
168	CCSM*	-	0.068	3.83	0.85	0.092	0.81	0.35	16.38	84.85
	CCSM**	-	0.059	3.97	0.89	0.099	0.95	0.51	16.48	85.43
	alpha-cellulose	0.01	0.023	0.55	0.00	0.071	0.54	1.31	16.20	83.79
	filter paper	0.01	0.013	0.14	0.00	0.057	0.68	0.41	17.81	93.24

- data could not be obtained due to peak overlap in the chromatogram.

are also hydrolyzed besides glucan and the maximum xylose and arabinose concentration (when expressed as xylan and arabinan equivalent) corresponded to 40-45% and 89-100% of the original amount respectively. Also, minor fluctuations in the concentrations of xylose and arabinose after reaching the maximum were observed. Cellobiose and glucose concentrations generally decreased with fermentation time. While lactic acid and formic acid were detected in trace amounts, glycerol and acetic acid were produced in measurable quantities.

The ethanol yield (as % of theoretical) calculated at different fermentation times is shown in Fig. 7. The theoretical yield is calculated based on the total glucan content of treated biomass input into fermentation. The ethanol yield (after 7 days) for pretreated CCSM reached 84-85% of theoretical which was essentially the same as that of α -cellulose, but was substantially lower than that of filter paper. However, pretreated CCSM gave an ethanol yield of 62-67% of theoretical in just 24 hours (1 day) as opposed to 35% for α -cellulose and 49% for filter paper (refers Fig. 7). Since the two pretreatment conditions rendered the same ethanol yields, the one with the less reaction time (15 min), 170°C, 10 wt% ammonia, pressure = 325 psi, flow rate = 1 mL/min (for the present reactor system) is the preferred condition for ARP treatment of CCSM.

The switchgrass used in this study was an unscreened mixture with the size ranging from very fine powders to about 1 mm length. Thus the SSF experiments for switchgrass were carried out using two different size switchgrass, switchgrass mixture and a screened one. The screened switchgrass was the portion that passed through No. 14 mesh and was retained by No. 40 mesh. All switchgrass samples were presoaked with ammonia solution overnight. The ARP pretreatment was carried out in a large reactor with volume of 72 mL to collect enough biomass for the SSF test. The reaction conditions were: 170 °C, 10 wt% ammonia, 45 minutes. The composition of screened and unscreened switchgrass after the ARP pretreatment are summarized in the following

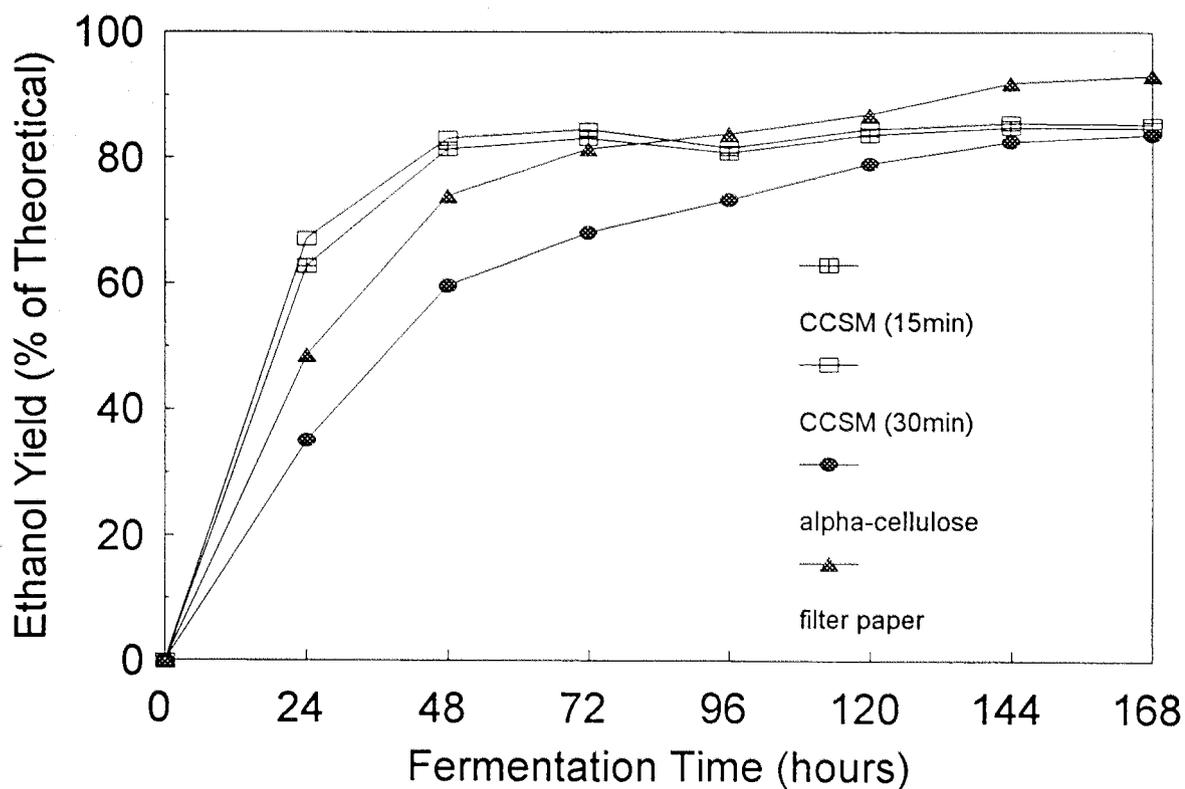


Figure 7. Ethanol Yield in SSF of CCSM after ARP Treatment.

Pretreatment conditions: 170C, 10 wt% ammonia, 45min.
 SSF conditions: 38C, pH 5.0, 150 rpm, 3 (w/v)% cellulose content, and
 enzyme loading =25 IFPU/g-cellulose.

table:

	%Solid remaining	K. Lignin %	Glucan %	Xylan %	Arabinan %	Ash %
unscreened switchgrass	46.94	6.8	69.59	17.80	1.71	4.41
screened switchgrass	45.87	6.0	71.08	18.45	1.98	4.19

Note: solid remaining based on the untreated biomass. The composition data based on the treated biomass

Composition data of these two samples are almost identical, indicating that the size of switchgrass within the range selected in this experiment is not a factor influencing the pretreatment.

The results of SSF data are shown in Table 15 and Figure 8. The results of SSF tests were quite similar to what we observed in CCSM.

It is seen that the glucose content is extremely low for the entire SSF process indicating that the process is limited by the enzymatic hydrolysis, not by the microbial action. Cellobiose was detected only in early samples (24-hr sample) for Switchgrass. With α -cellulose, however, cellobiose was present at a measurable level throughout the SSF process. Xylan and Arabinan are also hydrolyzed into xylose and arabinose. There was a slight decrease of xylose during the SSF. Lactic acid and acetic acid were detected in trace amounts only, whereas glycerol was produced in measurable quantities. At the 24 hr point, the ethanol yield for pretreated Switchgrass was above 50%, much higher than that of α -cellulose. As shown in Figure 8 (the time course of ethanol), the yield for Switchgrass leveled off at the 72 hr point whereas the yields for α -cellulose increased to the final point where it eventually reached maximum. The terminal yield of ethanol from Switchgrass averaged at 90.8% of theoretical (for the two SSF runs), substantially higher than the terminal yield from α -cellulose (82.7%). The ethanol data in Figure 8 reaffirms that the

Table 15. SSF data of ARP treated switchgrass.

Pretreatment conditions: 170C, 10 wt% ammonia, 45min, flowrate=4mL/min, and reactor volume=72mL.
 SSF conditions: 38C, pH 5.0, 150rpm, 3 % cellulose, and enzyme loading=25 IFPU/g-cellulose
 Inoculum information: end pH=4.5, cell conc =5.20 g/L, and inoculation level=10% of reaction volume.

Fermentation Time (hrs)	Substrate	cellobiose (g/L)	glucose (g/L)	xylose (g/L)	arabinose (g/L)	lactic acid (g/L)	glycerol (g/L)	acetic acid (g/L)	ethanol (g/L)	pH	%Yield (theoretical)
0	alpha-cellulose	0.000	0.024	0.000	0.000	0.013	0.015	trace	1.920	5.00	0.00
	Switchgrass I	0.000	0.000	0.000	0.000	0.015	0.177	trace	2.306	5.00	0.00
	Switchgrass II	0.000	0.000	0.000	0.000	0.015	0.177	trace	2.306	5.00	0.00
24	alpha-cellulose	0.350	0.320	0.760	0.000	0.044	0.520	0.170	7.900	4.95	34.62
	Switchgrass I	1.350	0.381	4.729	0.825	0.061	0.749	0.042	11.206	5.30	52.27
	Switchgrass II	1.700	0.358	4.859	0.850	0.062	0.783	0.036	11.480	5.35	53.87
48	alpha-cellulose	0.310	0.220	0.740	0.000	0.042	0.560	0.190	12.070	4.85	59.10
	Switchgrass I	0.000	0.136	4.017	0.838	0.250	0.885	0.060	14.079	4.80	69.13
	Switchgrass II	0.000	0.127	4.049	0.838	0.395	1.157	0.039	14.811	4.85	73.42
72	alpha-cellulose	0.260	0.190	0.990	0.000	0.057	0.540	0.690	13.500	4.65	67.49
	Switchgrass I	0.000	0.403	3.433	0.838	0.255	0.953	0.089	16.800	4.75	85.09
	Switchgrass II	0.000	0.269	3.563	0.838	0.321	1.089	0.089	17.140	4.75	87.09
96	alpha-cellulose	0.220	0.150	0.630	0.000	0.046	0.520	0.970	14.400	4.65	72.77
	Switchgrass I	0.000	0.107	3.348	0.796	0.302	1.297	0.149	17.140	4.70	87.09
	Switchgrass II	0.000	0.143	3.431	0.838	0.458	1.453	0.179	17.361	4.70	88.39
120	alpha-cellulose	0.150	0.053	0.880	0.000	0.05	0.520	1.110	15.400	4.60	78.64
	Switchgrass I	0.000	0.118	0.319	0.795	0.077	1.310	0.158	17.642	4.55	90.04
	Switchgrass II	0.000	0.101	0.327	0.838	0.482	1.632	0.182	17.560	4.75	89.55
144	alpha-cellulose	0.092	0.025	0.790	0.000	0.054	0.510	1.240	16.000	4.45	82.16
	Switchgrass I	0.000	0.087	0.311	0.795	0.083	1.343	0.173	17.600	4.54	89.79
	Switchgrass II	0.000	0.102	0.325	0.838	0.464	1.701	0.198	17.876	4.75	91.41
168	alpha-cellulose	0.008	0.023	0.550	0.000	0.071	0.540	1.310	16.200	4.40	83.33
	Switchgrass I	0.000	0.071	0.306	0.795	0.073	1.352	0.178	17.598	4.50	89.78
	Switchgrass II	0.000	0.092	0.317	0.838	0.221	1.800	0.209	17.950	4.72	91.84

Note:

Switchgrass I: the mixture of all sizes of switchgrass.

Switchgrass II: the screened switchgrass of size between 40 to 14 mesh.

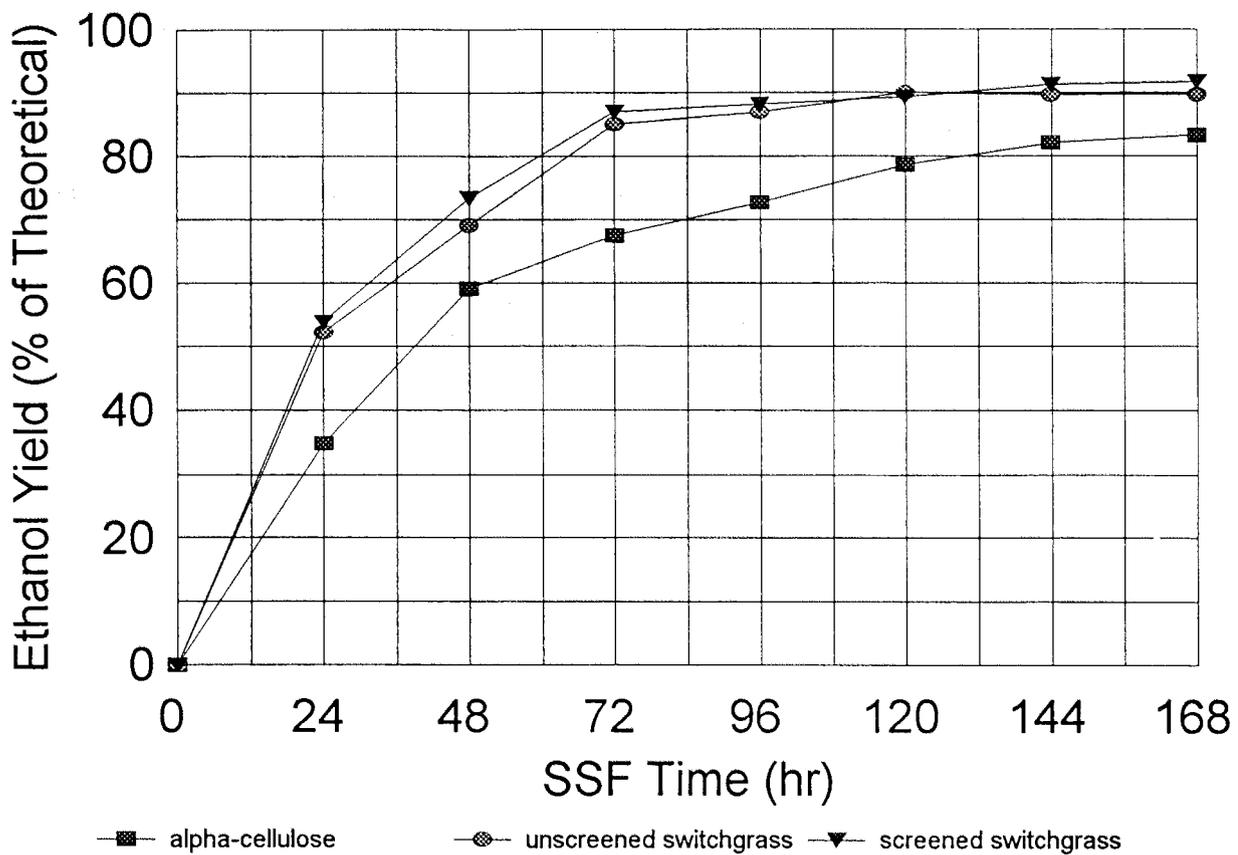


Figure 8. Yield of Ethanol of ARP Treated Switchgrass and Cellulose.

Pretreatment conditions: 170C, 10 wt% ammonia, 45min.

SSF conditions: 38C, pH 5.0, 150 rpm, 3 (w/v)% cellulose content, and enzyme loading =25 IFPU/g-cellulose.

size of switchgrass has negligible effect on the SSF.

The SSF results on the ARP treated CCSM and switchgrass are deemed quite satisfactory in view of the fact that nearly 10% of initial glucose is used for the growth of cells.

VI. CONCLUSIONS

ARP as tested against CCSM and switchgrass is a highly effective pretreatment method. The enzymatic digestibility of the pretreated substrates were found to be in the vicinity of 90%. Hemicellulose and lignin removal and modification of physical structure attribute to the increase of the enzymatic hydrolysis. The optimum condition identified for ARP on CCSM is: 170°C, 10% (wt/wt) ammonia concentration, flow rate = 1 mL/min, and reaction time 15-60 min. For switchgrass, it is: 170°C, 10% (wt/wt) ammonia concentration, flow rate = 1 mL/min, and reaction time 30-60 min. The Extent of delignification was 74-80% for CCSM and 71-84% for switchgrass. Under the optimum conditions, 50-56% of the total hemicellulose in CCSM and switchgrass whereas less than 8% of the total glucan in CCSM (10% for switchgrass) were solubilized. The toxicity of the ARP effluent is such that its loading up to 60% could be employed without adversely affecting the ethanol yield. ARP treated solid samples of CCSM and switchgrass are easily adaptable to SSF for ethanol production.

VII. NREL Sample Analysis Work

Solid Analysis: Includes 5 carbohydrates, klason lignin, acid-soluble lignin, ash, and some include extractives.

2-1-94 to 2-1-95

102 samples (in duplicate)

2-1-95 to 8-15-95

65 samples (in duplicate)

Total (2-1-94 to 8-15-95)

167 solid samples (in duplicate)

Liquid Analysis: Includes 5 carbohydrates (before and after secondary hydrolysis), pH, acetic acid, HMF, furfural, and some include acid-soluble lignin.

2-1-94 to 2-1-95

119 samples

2-1-95 to 8-15-95

63 samples

Total (2-1-94 to 8-15-95)

182 liquid samples

Enzymatic Digestibility Analysis

2-1-94 to 2-1-95

18 solid samples (in duplicate)

2-1-95 to 8-15-95

75 solid samples (in duplicate)

2 liquid samples (in duplicate)

Total (2-1-94 to 8-15-95)

95 samples (in duplicate)

APPENDIX

A. AMMONIA-HYDROGEN PEROXIDE TREATMENT (ARPH)

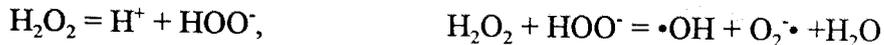
We have modified the ARP process by adding hydrogen peroxide to enhance the extent of hemicellulose and lignin removal. We refer to this process as **ARPH**, **H** standing for hydrogen peroxide. The ARPH process includes two steps: first the biomass was pretreated partially with an ARP process to remove some hemicellulose and lignin, then hydrogen peroxide was added into the ammonia stream to remove the remaining hemicellulose and lignin.

Hydrogen peroxide is a well-known delignification agent. It is used in the bleaching stage of pulp & paper process to remove the lignin residues. There have been a number of pretreatment studies also based on the hydrogen peroxide in which a high degree of delignification and enzymatic digestibility were claimed. The effect of process parameters such as temperature, time, concentration of ammonia and hydrogen peroxide, and the flow patterns of reaction agents were investigated.

Effect of Hydrogen Peroxide on Delignification and Hemicellulose Recovery

The reaction of hydrogen peroxide with lignin contains a complex mechanism. However, the net reaction can be expressed as the reaction between lignin and two lignin oxidizing species, hydroxy radical ($\bullet\text{OH}$) and perhydroxy anion (HO_2^-) formed during the decomposition of H_2O_2 . The perhydroxyl anion is a mild oxidant which is not strong enough for prompting delignification, whereas the hydroxyl radical is a powerful lignin oxidant. The latter also nonspecifically attacks carbohydrates, resulting in depolymerization of carbohydrates. The formation of both species can

thus be expressed by the following two reactions:



Both reactions are strongly pH-dependent. Alkaline condition promotes formation of perhydroxyl anion by neutralizing the H^+ ions, whereas the perhydroxyl anion accelerates the formation of a hydroxy radicals through the second reaction. Table 16 shows various reaction schemes for ARP-H treatment of corn cobs/stover mixture (CCSM) under a fixed level of H_2O_2 loading. The control reaction, No. 1, was conducted at the reaction condition of 170°C , 10 wt% ammonia without H_2O_2 . From this run about 60% hemicellulose removal and 85% delignification were achieved, which indicates that the ARP treatment itself is quite effective in the delignification of CCSM. To further improve delignification, only ammonia solution was pumped initially and then H_2O_2 was added into the ammonia stream to remove the residual lignin (Runs No. 2 ~ 4). This scheme has improved the fractionation to achieve 77% hemicellulose removal and a phenomenal 98% delignification. From Runs 2 ~ 4, it seems clear that ammonia concentration is an important variable in hemicellulose recovery. In Runs 5 - 7, H_2O_2 was added from the beginning of the experiment. This has increased hemicellulose recovery to 81% and delignification very close to 100%. It is to be noted that the solubilization of the cellulose portion increases slightly with an increase in hemicellulose recovery. In view of the fact that commercial α -cellulose contains considerable amounts of xylan (about 6%), it seems to be extremely difficult too selectively fractionate all of the xylan content. We consider 80% hemicellulose recovery as upper the limit of fractionation in ARP-H.

Effect of Reaction Parameters on Hemicellulose Recovery and Delignification

The temperature effect on ARP-H was investigated over the range of $165 \sim 180^\circ\text{C}$. The

Table 16

Effect of ammonia concentration and hydrogen peroxide feeding mode on ARPH treatment of CCSM*.

No.	reaction time (min)	ammonia conc. (wt%)	duration of hydrogen peroxide input	(1)	glucan (%)	xmg**(%)	delignification (%)	solid remaining (%)
1***	90	10	no addition	L	2.6	13.1	85.1	49.2
				S	34.9	8.6		
2	90	10	60-90	L	3.0	13.8	96.2	40.6
				S	33.5	7.6		
3	90	15	60-90	L	3.4	15.3	95.3	39.9
				S	32.3	6.3		
4	90	20	60-90	L	3.6	16.7	98.2	38.6
				S	31.8	5.1		
5	60	15	0-90	L	3.5	15.7	97.9	37.5
				S	32.2	5.6		
6	60	20	0-90	L	3.9	16.9	98.6	38.5
				S	32.1	5.0		
7	90	20	0-90	L	4.0	17.6	98.3	37.1
				S	30.7	4.3		

*All sugar contents are based on oven-dry untreated biomass and expressed as glucan, xylan, mannan and galactan equivalents.

Reaction condition: 170C, cumulative hydrogen peroxide input = 0.84 g/g dry biomass, ammonia stream = hydrogen peroxide stream = 1 mL/min.

**xylan + mannan + galactan.

***flow rate of ammonia solution = 2 mL/min.

results are summarized in Table 17. The isolated effect of temperature was such that the solubilization of cellulose and delignification were insensitive to temperature over this range remaining relatively constant at 4% and 98%, respectively. The hemicellulose recovery also increased slightly as temperature was increased from 165°C to 170°C. A further increase in temperature did not result in any improvement in hemicellulose recovery. For this reason, the reaction temperature of 170°C was chosen in most of the subsequent experiments.

In order to reduce total liquid input in the ARP-H process, the reaction time was reduced from 90 minutes to 30 and 60 minutes. The results are shown in Table 18. The degree of delignification with lower reaction times was almost at the same level as that of 90 minutes reaction time. The hemicellulose recovery, however, has decreased from 81% to 70% (30 minutes) and to 77% (60 minutes). For a process where delignification is the primary purpose, it seems logical to choose a reaction time of 30 minutes. However, if the process aims at the maximum fractionation and recovery of biomass components, the reaction time should be kept above 90 minutes.

An increase of H_2O_2 from 0.84 g/g to 1.12 g/g caused a slight increase in solubilization of hemicellulose and cellulose, but no significant change in delignification (Table 19). A decrease of H_2O_2 from 0.84 g/g to 0.28 g/g again showed little effect on delignification and only a slight decrease in hemicellulose recovery. Further decrease of H_2O_2 down to 0.14 g/g-biomass caused a slight decrease in delignification, but significant reduction in hemicellulose recovery. These results collectively indicate that 0.28 H_2O_2 g/g biomass is the minimum loading for maximum delignification and hemicellulose recovery within this study.

Table 17

Effect of temperature on the composition of CCSM hydrolyzate and solid residues*.

temp. (C)	(1)	glucan (%)	xmg** (%)	delignification (%)	solid remaining (%)
165	L	4.0	17.1		
	S	32.4	4.8	98.6	38.9
170	L	4.0	17.6		
	S	30.7	4.3	98.3	37.1
175	L	4.0	17.4		
	S	30.8	4.2	98.7	35.4
180	L	4.1	17.3		
	S	30.5	4.4	98.6	34.6

*All sugar contents are based on oven-dry untreated biomass and expressed as glucan, xylan, mannan and arabinan equivalents. Reaction condition: 90 min, hydrogen peroxide input = 0.84 g/g dry biomass, ammonia stream = hydrogen peroxide stream = 1 mL/min.

**xylan + mannan + galactan.

(1) L: hydrolyzate after secondary hydrolysis.

S: solid residue.

Table 18

Effect of reaction time on the composition of CCSM hydrolyzate and solid residues*.

reaction time (min)	(1)	glucan (%)	xmg** (%)	delignification (%)	solid remaining (%)
30	L	3.5	15.2		
	S	33.7	6.6	96.8	42.2
60	L	3.8	16.8		
	S	31.8	4.8	98.0	38.5
90	L	4.0	17.6		
	S	30.7	4.3	98.3	37.1

*All sugar contents are based on oven-dry untreated biomass and expressed as glucan, xylan, mannan, and arabinan equivalents. Reaction condition: 170C, hydrogen peroxide input = 0.84 g/g dry biomass, ammonia stream = hydrogen peroxide stream = 1 mL/min.

**xylan + mannan + galactan.

Table 19

Effect of hydrogen peroxide input on the composition of CCSM hydrolyzate and solid residues*.

hydrogen peroxide input (g/g dry biomass)	(1)	glucan (%)	xmg** (%)	delignification (%)
1.12	L	4.4	17.9	
	S	29.3	3.9	98.2
0.84	L	4.0	17.6	
	S	30.7	4.3	98.3
0.56	L	4.2	17.3	
	S	29.6	4.3	98.8
0.28	L	4.2	17.4	
	S	29.7	4.5	99.1
0.14	L	3.5	16.2	
	S	32.9	5.7	97.8
0.06	L	2.7	13.5	
	S	34.9	8.1	95.9

*All sugar contents are based on oven-dry untreated biomass and expressed as glucan, xylan, mannan and arabinan equivalents.

Reaction condition: 170C, 90 min, ammonia stream = hydrogen peroxide stream = 1 mL/min.

**xylan + mannan + galactan.

(1) L: hydrolyzate after secondary hydrolysis.

S: solid residue.

Application of ARP-H Treatment to Switchgrass and Hybrid Poplar

The performances of the ARP-H process were further tested using additional substrates of switchgrass and hybrid poplar. For these substrates, the ARP-H runs were conducted at 170°C, 90 min. and 0.28 g H₂O₂/g-biomass. The performance indices are depicted in Figure 9. The hemicellulose recovery obtained from switchgrass was almost the same as that of CCSM. The degree of delignification, however, was lower. The ARP-H treatment was less effective on the woody substrate on both accounts, hybrid poplar yielding 50% hemicellulose removal and 80% delignification. Interestingly, the cellulose solubilized in the ARP-H effluent of hybrid poplar was only about one-fifth of that solubilized in the herbaceous substrates. This may be explained by the fact that wood cellulose in general has more rigid structure, higher crystallinity, and higher molecular weight than cellulose in herbaceous feedstock.

Enzymatic Digestibility

The enzymatic digestibility of ARP-H treated CCSM, switchgrass, hybrid poplar, untreated filter paper (Whitman No. 1), α -cellulose and CCSM are shown in Figure 10. The 72 hr digestibilities of ARP-H treated CCSM and switchgrass were 95% and 93%, respectively. These values were much higher than that of α -cellulose digestibility which stands at 83%, and essentially in the same range as filter paper which is at 94%. The digestibility of hybrid poplar was 90%. The digestibility of the treated CCSM represents a 3 ~ 4 fold increases over the untreated one.

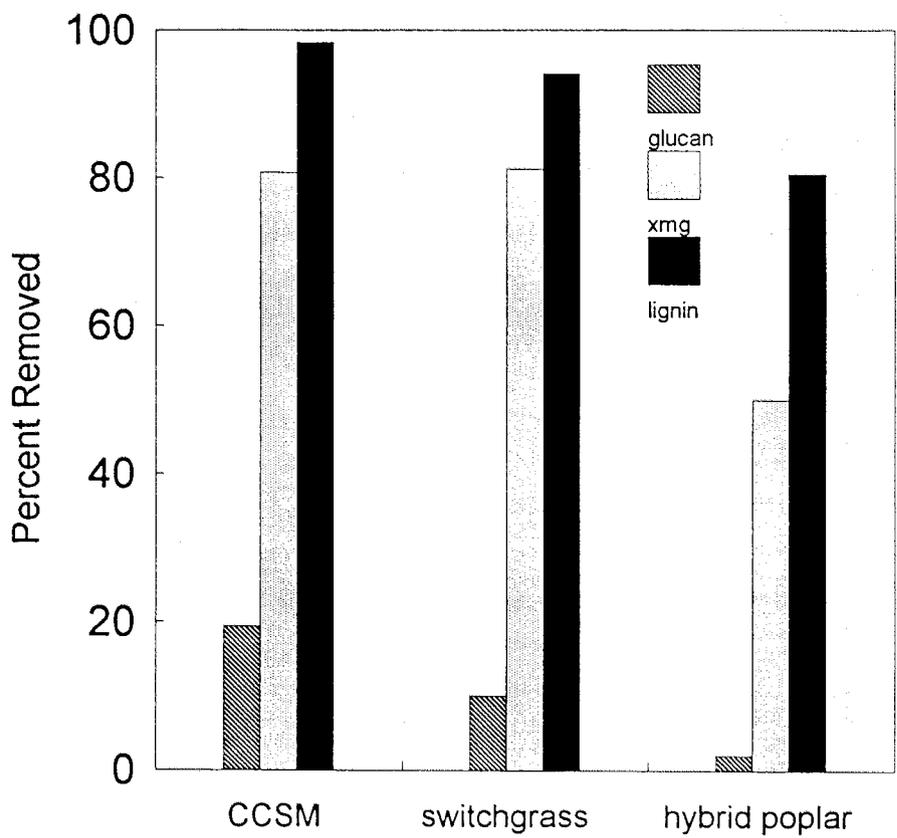


Figure 9. Sugars and Lignin Removal from Biomass after ARPH treatment.

Pretreatment conditions: 170C, 20% (wt/wt) ammonia, 90 min., the H₂O₂ loading 0.28g/g-biomass, the flow rate of ammonia stream = H₂O₂ stream = 1 mL/min.

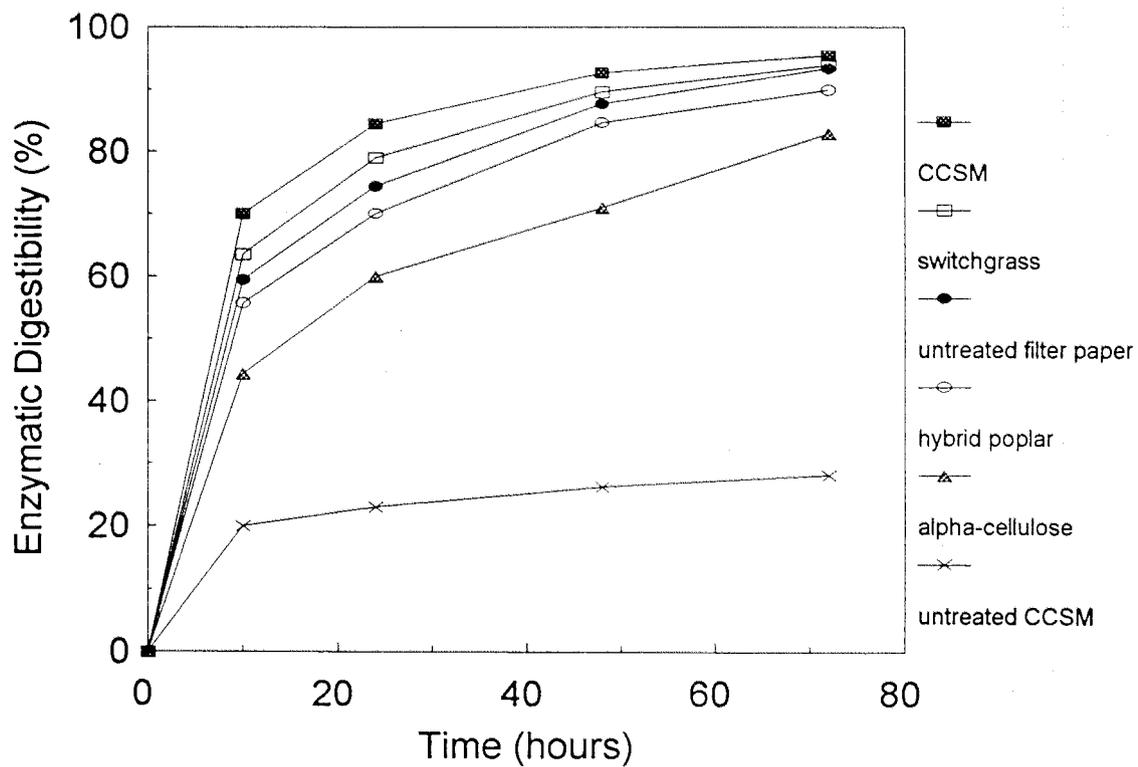


Figure 10. Enzymatic Digestibility of ARPH Treated and Untreated Biomass.

Pretreatment conditions: 170C, 20% (wt/wt) ammonia, 90 min., the H₂O₂ loading 0.28g/g-biomass, the flow rate of ammonia stream = H₂O₂ stream = 1 mL/min.

Enzymatic hydrolysis conditions: 60 IFPU/g-glucan, 50C, pH 4.8.

B. AUTOHYDROLYSIS

It has long been recognized that when biomass is heated to high temperatures with pressured water, acids formed by the solubilization of the acidic components of hemicellulose will catalyze the hydrolysis of the hemicellulose, a process called autohydrolysis. However, the acids mainly are formic acid, acetic acid, and glucuronic acid etc. All these acids are weak organic acids and are not strong enough to hydrolyze cellulose, therefore autohydrolysis can selectively remove hemicellulose. With this understanding, we applied autohydrolysis as a supplementary step before the ARP process to extract and recover all of the hemicellulose from the biomass. The remaining solid residue is further through passed the ARP process to remove the lignin and finally achieve the desired fractionation of biomass.

Effect of temperature

The effect of pretreatment temperature of pure water hydrolysis in a batch mode on switchgrass was investigated over 160-190°C (170-200 °C for CCSM in a percolation mode). The composition data for switchgrass are summarized in Table 20 (CCSM in Table 21). It is seen in Table 20 that the percentage of hemicellulose extracted out varied from 23.3 to 89.2% and most of the hemicellulose was in the form of oligomer as the pretreatment temperature was increased from 160°C to 190°C. However, at 190°C, most of the hemicellulose was decomposed, which means 190°C, 1 hour is a condition too severe to recover hemicellulose sugars from switchgrass. The furfural content in the primary hydrolyzate increased only slightly over the pretreatment temperature range of 160°C to 180°C, whereas it nearly doubled over the range of 180°C to 190°C. Therefore, the optimum pretreatment temperature appears to be within the temperature of

Table 20. Effect of Pretreatment Temperature of Pure Water Batch Hydrolysis on Switchgrass.

Pretreatment conditions: 1 hour, solid/water = 1:5,
 Acid secondary hydrolysis conditions: 4.0% sulfuric acid, 121 C, 1 hour.

Pretreatment Temperature	Solid Residue			Primary Hydrolyzate			Hydrolyzate after Acid hydrolysis			
	%Lignin Removal*	%Glucan Removal*	%HC Removal*	pH	Oligomer (% of total HC**)	%Furfural Content***	%Glucan Content***	%HC Content***	%Formic acid Content***	%Acetic acid Content***
160 C	10.5	8.5	23.3	3.45	85.2	0	1.90	6.23	0.11	0.69
170 C	13.0	16.5	55.4	3.45	85.2	1.23	2.55	14.11	0.46	1.93
180 C	6.9	14.8	80.4	3.35	69.1	1.98	1.76	9.22	0.63	1.87
190 C	3.3	22.4	89.2	3.20	35.2	3.35	1.30	1.40	1.09	2.30

*: based on the its original content.

**: total HC means the total amount of hemicellulose in the primary hydrolyzate.

***: all data based on oven-dry untreated biomass.

HC = Hemicellulose refers the total amount of xylan, mannan, galactan, and arabinan.

Table 21. Effect of Pretreatment Temperature of Pure Water Hydrolysis in Percolation Mode on CCSM.

Pretreatment conditions: 1 hour, pure water, flow rate = 1 mL/min.,
 Acid secondary hydrolysis conditions: 4.0% sulfuric acid, 121 C, 1 hour.
 Enzymatic Digestibility: 50C, pH 4.8, enzyme loading 60 IFPU/g-glucan.

Pretreatment Temperature	Solid Residue				Hydrolyzate after Acid hydrolysis			
	%Lignin Removal*	%Glucan Removal*	%HC Removal*	%Digestibility (72 hr)	%Glucan Content**	%HC Content**	%Furfural Content**	%Acetic acid Content**
170C	28.60	9.40	71.40	80.77	3.08	15.11	0.32	2.77
180 C	36.60	8.40	88.30	81.30	2.73	17.03	0.92	3.16
190 C	38.00	9.20	93.00	91.20	3.26	16.00	1.32	3.61
200 C	48.70	10.50	95.60	82.26	2.94	13.34	1.59	3.56

*: based on the its original content.

**: all data based on oven-dry untreated biomass.

HC = Hemicellulose refers the total amount of xylan, mannan, galactan, and arabinan.

170-180°C for switchgrass.

Autohydrolysis of CCSM was performed in the percolation mode (Table 21). The response of CCSM to water pretreatment is quite similar to that of switchgrass. Because of the performance in the percolation mode, more hemicellulose and lignin removal, and less decomposition of sugars were observed within the experimental temperature. The digestibility of treated CCSM at 72 hr increased from 81% to 91% as the pretreatment temperature was increased from 170 to 190 °C. However, the digestibility decreased with further increase in temperature to 200 °C. We do not have explanation at this time.

Effect of Leaching Methods on Sugar Recovery

In the experiment, we found that the total amount of glucan in solid residue and liquid hydrolyzate was close to the total glucan in the original biomass and most of the glucan was left in solid residues. However, for hemicellulose, we were unable to close the sugar balance with respect to the original content in the biomass. We therefore felt that some oligomers extracted by water hydrolysis may have been trapped in the solid matrix which did not come out into the solution after discharging. We therefore have investigated various leaching methods as a way to maximize sugar recovery after the water hydrolysis (see footnotes in Table 22). Among the methods we have tested, the one that involved ultrasound bath and autoclaving (Method No. 5) has shown better sugar recovery than the other methods. In terms of the total sugar balance (sugar in liquid + sugar in solid), autoclaving the pretreated residue with addition of sulfuric acid (4%), a direct secondary hydrolysis (Method No.6) gave the best results.

Table 22. Effect of leaching method on sugar recovery of CCSM after pure water batch hydrolysis*.

Pretreatment Conditions	% Solid Remaining	% Glucan			% Hemicellulose			%Furfural (in primary hydrolyzate)
		Solid	Liquid	Total	Solid	Liquid	Total	
Untreated CCSM	100.00	38.10	0	38.10	24.90	0	24.90	-
170C, 1 hr (1)	63.50	33.50	2.88	36.38	10.52	8.48	19.01	0.39
170C, 1 hr (2)	61.65	32.76	2.58	35.34	12.60	8.64	21.24	0.69
170C, 1 hr (3)	60.32	33.19	2.51	35.70	11.71	8.49	20.20	0.7
170C, 1 hr (4)	61.75	33.29	2.63	35.92	11.91	7.97	19.88	0.72
170C, 1 hr (5)	62.40	34.60	2.49	37.09	12.15	9.25	21.40	0.78
170C, 1 hr (6)	55.80	33.81	3.41	37.22	19.30	3.51	22.81	0.79

* all data in the table based on the oven-dry untreated biomass.
sugar contents of hydrolyzate are calculated after secondary hydrolysis.
Hemicellulose = xylan + mannan + galactan + arabinan.

Leaching methods:

- (1) discharged solid was mixed with water at room temperature and allowed to stand for 2 hrs.
- (2) discharged solid was mixed with water at room temperature and allowed to stand for 1 hr, and then autoclaved for 45 min. at 121C.
- (3) discharged solid was mixed with water at room temperature and allowed to stand for 2 hrs, and then autoclaved for 45 min. at 121C.
- (4) solid was reacted with water at 170C for 1 hr and at 121C for 45 min and then it was discharged and mixed with water at room temperature and allowed to stand for 2 hrs.
- (5) discharged solid was mixed with water at room temperature and put in ultrasound bath for 45 min.
- (6) discharged solid was mixed with sulfuric acid to make 4% acid solution and autoclaved at 121C for 45 min.

Enzymatic Hydrolysis of Oligomers in Hydrolyzate

We have explored the use of cellulase enzymes in breaking down the oligomers of the primary hydrolyzates. The enzymatic hydrolysis of hydrolyzates was carried out at 50°C, pH 4.8, and 4.5 IFPU/mL hydrolyzate. The results of the enzymatic secondary hydrolysis are summarized in Table 23. The table shows that most of the oligomers are hydrolyzed into monomers after 6 hours of enzymatic hydrolysis. However, a small unidentified peak still appeared at the position of oligomers peak in the HPLC chromatogram after 24 hours of hydrolysis. A similar small peak also existed in the HPLC chromatogram of hydrolyzates after acid hydrolysis. For the convenience of data treatment, we counted unidentified component as the unhydrolyzed oligomer. It is interesting to note that the total amount of unhydrolyzed oligomers, glucose, xylose (including mannose), acetic acid in the solution (listed as A+B+C+D) were fairly constant throughout the enzymatic hydrolysis. This would be a positive indication that there is indeed no sugar decomposition during enzymatic hydrolysis. Therefore, this method could be used not only as a way to recover sugars from the hydrolyzate, but also as an analytical method to quantitatively determine the oligomer content of the hydrolyzate.

Autohydrolysis treatment was effective in selective solubilization of the hemicellulose fraction. Because only water was used in the treatment, it may be an economical supplementary step to other pretreatment techniques in order to fulfill the treatment tasks.

Table 23. Enzymatic Hydrolysis of the Hydrolyzates of Hybrid poplar of Pure Water Batch Hydrolysis.

Pretreatment conditions: 1 hour, solid/water = 1:5,
 Enzymatic hydrolysis conditions: 50C, pH 5.0, 4.5 IFPU/ml hydrolyzate.

Enzymatic hydrolysis time(hr)	%Glucose Content* A	%(Xyl+Man) Content* B	%Oligomer Content* C	%Acetic acid Content* D	A+B+C+D
Sample pretreated at 170 C					
0	0.22	1.24	-	1.21	
3	0.51	9.88	0.93	2.25	13.57
6	0.58	10.18	0.7	2.25	13.71
12	0.58	10.42	0.47	2.25	13.72
24	0.58	10.81	0.39	2.25	14.03
Sample pretreated at 180 C					
0	0.07	2.67	-	2.31	
3	0.69	7.64	0.74	2.64	11.71
6	0.69	8.16	0.62	2.81	12.28
12	0.76	8.83	0.54	2.97	13.1
24	0.76	8.60	0.47	2.97	12.8

*: based on oven-dry untreated biomass.
 Xyl: xylose, Man: mannose.
 Oligomer: equivalent as xylose.

C. DILUTE-ACID PROCESS PLUS ARP (DA-ARP)

In order to completely separate the hemicellulose fraction from the biomass, we are proposing a new scheme that combines the dilute-acid pretreatment and the ARP process. The rationale of this approach is quite simple. The dilute-acid process (DA) is quite effective in the recovery of hemicellulose whereas the ARP process is most effective in delignification. Combination of these, DA followed by ARP (DA-ARP), would essentially fractionate the biomass into the three main components. In terms of the equipment and the process setup only a minor modification is needed in combining these processes.

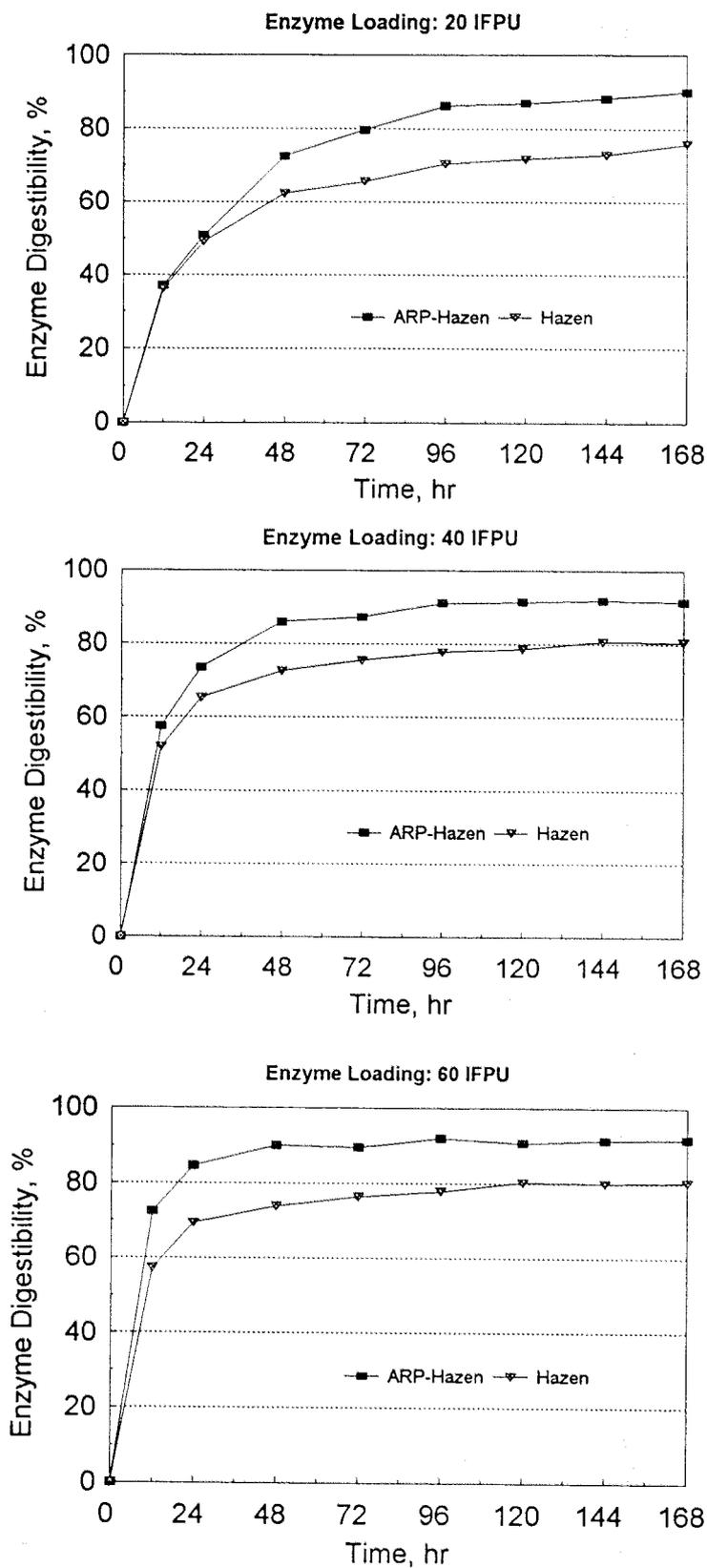
As a quick check of this concept, we took one of the dilute-acid pretreated samples (Hazen 2199-37) and put it through the ARP process. Permission/authorization to use Hazen 2199-37 solids sample for this part of the study was obtained from NREL beforehand. The ARP conditions were: 10 wt% ammonia, 180 °C, 1 hour. The composition data of the Hazen sample before and after ARP treatment are listed in the following table.

	%Solid Remaining	Glucan %	Xylan %	K. Lignin %	ASL %	Ash %
Hazen 2199-37	100	57.49	4.60	35.55	1.59	3.73
Hazen 2199-37 after ARP	58.38	48.53	2.26	7.58	0.37	1.64

Notes: All data based on oven-dried Hazen sample. ASL refers to acid soluble lignin.

As seen in the table, ARP again proves itself to be a very effective delignification method. It is noteworthy that 83% ($=48.53/58.38$) of the DA-ARP sample is glucan. The extent of additional delignification in the ARP stage is 79% ($=1-7.58/35.55$). This accounts for most of the weight loss. It was also observed that a substantial amount of glucan was solubilized into the ARP effluent. We think this can be reduced if we readjust the ARP condition. The present condition is

Figure 11. Enzyme Digestibility of Hazen 2199-37 and ARP-Hazen 2199-37 Substrates.



a typical one that we applied when ARP is the sole processing. When it is used as a supplementary process, we believe a condition much less severe (in terms of temperature, time and ammonia concentration) can be employed. We especially believe one can cut down on the reaction time and temperature.

The enzyme digestibilities of these samples (before and after ARP) were then measured using various levels of enzyme loading (20, 40, and 60 IFPU/g glucan). The enzyme hydrolysis tests were performed at 50 °C, 1% (w/v) glucan, pH 4.8. The results show that the supplementary ARP indeed increase the terminal enzymatic digestibility and shorten the reaction time (Fig. 11). The terminal digestibility of all ARP treated samples is 90-91% regardless of the enzyme loading whereas the digestibility of untreated Hazen samples is below 81%. At the enzyme loadings of 40 and 60 IFPU/g-cellulose, the enzymatic reaction for the ARP samples attained the highest conversion about 72 hours earlier than that of the untreated samples. The effectiveness of the supplementary ARP is more discernible at the enzyme loading of 20 IFPU. At this low enzyme loading (or more economically feasible enzyme loading), there is almost 15% increase in digestibility after the ARP treatment. An important point here is that the terminal digestibility of the untreated Hazen samples is dependent upon the enzyme loading. The 7-day digestibility of the untreated Hazen sample is only 75% at 20 IFPU of enzyme loading. It is questionable whether a digestibility of this level is acceptable from a process viewpoint. We think the difference in digestibility of these two samples is due primarily to the enzyme-lignin interaction. The interaction of cellulase enzymes with lignin is well documented. It has been reported that the cellulase enzymes were adsorbed into both the isolated lignin and the lignaceous residues remaining after a complete hydrolysis of the cellulose component. The rate of hydrolysis was shown to decrease when lignin was added to the hydrolysis reaction. These previous

findings, along with our results indicate that delignification is crucial and may even be a necessary element of pretreatment, if one is to maximize the efficiency of the cellulase enzymes.