Hybrid Process for the Conversion of Lignocellulosic Materials

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ABSTRACT

Because of the recalcitrant nature of lignocellulosic materials, it is important to pretreat the biomass in order to obtain a suitable material for the bioconversion. In this study, two different types of pretreatments were performed. The first experiment used a 2-gal Parr reactor operated at 140, 150, 160, and 170°C with sulfuric acid concentrations varying from 0.5 to 2%. A second pretreatment was performed with a two-stage low-temperature process. The first-stage pretreatment was performed at 100 or 120°C with sulfuric acid concentrations of 0.5, 2, and 5% followed by a second-stage pretreatment at 120°C with 2% acid concentration. The best residues for enzymatic hydrolysis and simultaneous saccharification and fermentations (SSF) came from the higher temperature pretreatment with the Parr reactor. However, a large portion of the xylose fraction was degraded to furfural and glucose was degraded to HMF. On the contrary, the two-stage low temperature pretreatment resulted in a very low percentage of xylose degradation, and no glucose degradation. The residues from this two-stage pretreatment performed satisfactorily toward the production of ethanol by SSFs. This study discusses the results obtained from these experiments.

Index Entries: Biomass; lignocellulosic materials; ethanol fermentation; simultaneous saccharification and fermentation (SSF); *Saccharomyces cerevisiae*; pretreatment; enzymatic hydrolysis.

INTRODUCTION

The production of ethanol from lignocellulosic materials is not a new concept to mankind. This has been done since the early 1900s using a per-

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colation process for the hydrolysis of biomass to molasses and fermentable sugars. However, because of the postwar economics and the technological advances in oil refining and other processes, energy could be generated much more easily and economically. Not until the oil embargo in the late 1970s did people realize the insecurity of our economy to be based largely on foreign petroleum. Since the Middle East countries hold the majority of the oils, the US economy and daily life are very vulnerable to the effects of its foreign policies. As a result, alternative energy sources are necessary. One of these alternatives is the production of ethanol from biomass. The technology has advanced a lot since the percolation hydrolysis of biomass at the turn of the century. With the development of the enzymatic hydrolysis process and other fermentation processes, the economical production of ethanol from biomass could become a reality in the near future. It is estimated that over 40 billion gallons of ethanol can be theoretically produced from all biomass per year *(1)*. This is more than sufficient to replace imported oils for the US economy.

Besides improving the nation's security toward energy sources, there are numerous other advantages in reducing its dependency on foreign oils. First, the CO_2 released by the combustion of fossil fuels is a greenhouse gas associated with global warming. It is estimated that a doubling of gases corresponds to an increase of 1.5–4.5°C in earth temperature *(2)*. In addition, other pollutants are produced during the combustion of fossil fuels. Second, since most of the imported oils are consumed by the transportation sector, gasohol can be used to reduce oil consumption and improve the efficiency of automobiles. However, this is only limited to certain states in the American Midwest because corn is the only feedstock used in this effort at present. When biomass is used as the feedstock, ethanol can be produced without any geographical preferences. Third, besides woody crops, municipal solid waste (MSW) is also a good source of fermentable materials for ethanol production. Because paper waste has a high cellulose content, it is an excellent starting material for the acid hydrolysis process because of its ease of operation. Fourth, when ethanol is produced from carefully planed woody crops and herbaceous crops, new businesses can be opened for farmers and this can improve local economies.

The Tennessee Valley Authority (TVA) at Muscle Shoals, AL has been conducting research for over 15 yr to develop technology for the conversion of renewable biomass resources to fuels and chemicals. This paper discusses the most recent efforts on the bioconversion of lignocellulosic materials to ethanol using a hybrid process combining dilute acid hydrolysis and simultaneous saccharification and fermentation (SSF). The focus of this study is on the bioconversion of southern mixed hardwoods because of their abundance in the southern US states. It is estimated that there are over 6,463,000 t of biomass available for alternative uses per year in the 201 counties in the Tennessee valley and 74% of these are hardwoods *(3)*.

MATERIALS AND METHODS

Materials

The feedstock for this study was southern mixed hardwoods. They were used in other previous studies on bioconversion to ethanol using the dilute acid process *(4–6)* and hybrid process *(7)*. The biomass was milled with a Thomas-Wiley mill to less than 3 mm. The fines were removed by filtering through a 60-mesh screen. Cellulose powder (α-cellulose), used as a control substrate, was obtained from Sigma Chemical Company (Cat. no. C-8002) (St. Louis, MO). A second control material was a generous gift from NREL (Golden, CO). The yeast, *S. cerevisiae* strain D_5A, was obtained from NREL and the cellulase Cytolase CL was obtained from Environmental Biotechnogies (Menlo Park, CA). All chemicals used are of reagent grade.

Enzyme Activities

The filter paper activity of the cellulase was measured using the Whatman no. 1 filter paper according to IUPAC Biotechnology Commission Recommended Methods *(8)* and the β-glucosidase activity was measured using p-nitrophenyl-β-D-glucoside as substrate.

Analytical Methods

The dry weight of the biomass was determined at 105°C, according to the NREL Chemical Analysis and Testing Standard Procedure No. 001 (NREL). The carbohydrate content was determined using a two-stage sulfuric acid method according to the NREL procedure No. 002. In addition, Klason lignin, acid-soluble lignin, and ash were determined according to the NREL procedures No. 003 to 005, respectively. Sugars (primarily glucose, xylose, mannose, arabinose, and galactose), acetic acid, furfural, and HMF were determined using a Thermo SP HPLC system equipped with a Bio-Rad Aminex HPX-87P or HPX-87H column (Bio-Rad, Hercules, CA). Ethanol was analyzed with gas chromatography. Occasionally, glucose was analyzed with the YSI 2700 Analyzer (YSI, Yellow Springs, OH).

EXPERIMENTAL

Pretreatment I

This first pretreatment study used 500 g bone-dry-biomass at 10% solids level. The sulfuric acid concentration was varied from 0.5, 1, 1.5, to 2% and the temperature was varied from 140, 150, 160, to 170°C at each acid level. The biomass was weighed into a 2-gal Parr reactor (Parr Instrument, Moline, IL) made of carpenter 20Cb-3 stainless steel with complete instrumentation controls. Appropriate amount of water was added

so that when concentrated sulfuric acid at 72% was injected into the reactor, a correct acid concentration could be obtained. The reactor was sealed and heated to the appropriate temperature before acid was injected into the reactor with an HPLC pump. Samples were removed at regular intervals for about 30 min. Then the heat was shut off and the entire reactor was immersed in a water tank to quench the reaction. After the temperature and pressure had reached a safe level, the reactor was removed from the water tank and the contents were emptied into a stainless steel filter layered with a sheet of linen. The prehydrolyzate was suction removed and stored at 4°C until further analysis. Before the prehydrolyzates were analyzed with HPLC, they were neutralized with calcium carbonate and filtered through a 0.22 μm syringe filter. Pretreated residues were washed with water until a final pH of about 5 was obtained, and were stored at 4°C for further studies.

Pretreatment II

The scheme of this second pretreatment study was outlined in Fig. 1. First, 450 g biomass was boiled in distilled water for about 30 min at 20% solids. Then the residues were filtered through Whatman no. 1 filter paper and resuspended in distilled water. They were rinsed with running distilled water until the water became clear. Second, the washed residues were dried at 40°C overnight and stored at room temperature.

The first-stage pretreatment studies involved the use of 10% solids in a total of 100 mL acid in a 125-mL serum bottle equipped with rubber stoppers and aluminum crimp tops. Appropriate amount of biomass was weighed into the individual bottles, then 100 mL acid at 0.5, 2, and 5% was added. They were mixed by gentle shaking and rolling until all the biomass was carefully rinsed into the acid solution before they were placed inside the autoclave (Vernitron Medical Products, Carlstadt, NJ). The first set of experiments were autoclaved at 100°C for 30 min and a second set of experiments were autoclaved at 120°C for 30 min. At the end of the process, they were cooled at room temperature until safe to the touch. Then they were filtered through Whatman no. 1 filter papers. The prehydrolyzates were collected and neutralized with calcium carbonate. They were clarified with centrifugation at 15000 rpm (27,000g) for 10 min at 4°C using a Sorvall RC5C centrifuge equipped with the SS-34 rotor (DuPont, Wilmington, DE). The supernatants were stored at 4°C and filtered through a 0.22 μm syringe filter before analyzed for sugars and degradation products with HPLC. The residues were washed with distilled water until the wash water became neutral (tested with pH paper). They were stored at 4°C until further studies. Two replicates were performed for this first-stage pretreatment. The total solids/moisture were determined for both replicates, but only the first replicate was
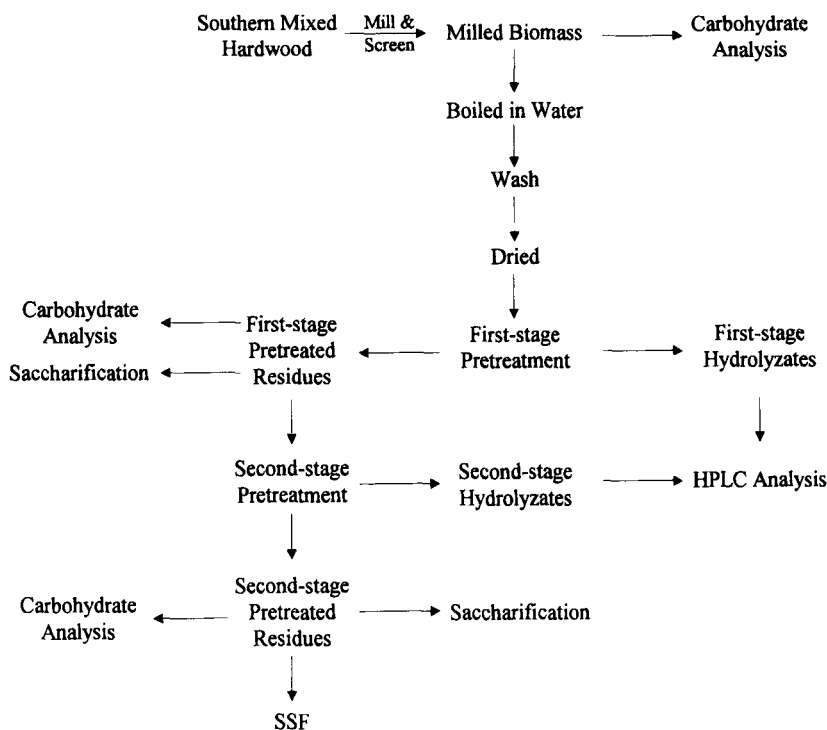
Southern Mixed → Mill & → Milled Biomass → Carbohydrate Analysis
Hardwood Screen

↓

Boiled in Water

↓

Wash

↓

Dried

↓

Carbohydrate Analysis ← First-stage Pretreated Residues ← First-stage Pretreatment → First-stage Hydrolyzates

Saccharification ←

↓

Second-stage Pretreatment → Second-stage Hydrolyzates → HPLC Analysis

↓

Carbohydrate Analysis ← Second-stage Pretreated Residues → Saccharification

↓

SSF

Fig. 1. Scheme of pretreatment II studies.

used for carbohydrate determination. The second replicate was used for a second-stage pretreatment.

The second-stage pretreatment was similar to the first-stage pretreatment except that the residues from the first pretreatment were used and the acid concentration, temperature, and retention time were fixed at 2%, 120°C, and 30 min, respectively. The residues from both pretreatments were used for enzymatic hydrolysis reactions; whereas only the residues from the second-stage pretreatment were used for SSF studies.

Enzymatic Hydrolysis Reactions

Enzymatic hydrolysis reactions were carried out at 1% cellulose concentration in 20 mL screw cap glass vials. Appropriate amounts of pretreated biomass were first weighed into the vials. Then 5 mL 0.1M sodium citrate buffer at pH 4.8 was added. In addition, appropriate amount of deionized distilled water was added so that the final volume was 10 mL after the addition of 400 µg tetracyline (10 mg/mL in 70% ethanol) and cellulase (79 FPU/ml and 89 β-NPGU/mL). Cellulase at a loading of 60 FPU/g cellulose was used with no additional β-glucosidase added. The vials were placed inside a Lab-Line Shaker/Incubator (Lab-Line Instruments, Melrose Park, IL) at a 45° inclination at 50°C with gentle shaking for 7 d. Samples (200 µ) were removed for analysis of glucose using the YSI 2700 Analyzer and the final samples at 168 h were analyzed with HPLC for various sugars.

The samples were boiled inside tightly capped 1.7-mL microcentrifuge tubes or screw cap test tubes for 5 min and cooled to room temperature in a water bath. The microcentrifuge tube samples were centrifuged at 12,000 rpm (11,750g) for 10 min, whereas the screw cap test tube samples were centrifuged with the Sovall Centrifuge at 4000 rpm (1,935g) for 10 min. The supernatants were stored at 4°C or –20°C until analysis. The glucose percent yield or digestibility was calculated as:

$$\text{Yield}(\%) = \frac{[\text{Glucose}] + 1.053[\text{Cellobiose}]}{1.111 \ f \ [\text{Biomass}]} 100\%$$

where [Glucose] = glucose concentration (g/L); [Cellobiose] = cello-biose concentration (g/L); [Biomass] = dry biomass concentration at the beginning of the reaction (g/L); and f = cellulose fraction in dry biomass (g/g).

Simultaneous Saccharification and Fermentation (SSF)

SSF experiments were performed using the pretreated materials from Pretreatment I and the second-stage pretreated materials from Pretreatment II. The fermentation conditions were as follows: 3% cellulose, 10% yeast inoculum, 1% yeast extract, 2% peptone, 25 FPU/g cellulose enzyme loading, pH 5.0 ± 0.2. The biomass was weighed into a 250-mL baffled Erlenmeyer flask. Then appropriate amount of deionized distilled water was added so that a final volume of 100 mL was obtained for residues from Pretreatment I and 40 mL for residues from Pretreatment II. The pH was adjusted to 5.0 ± 0.2 with concentrated phosphoric acid. They were autoclaved at 121°C, 15 psig for 20 min. The 10 X YP (10% yeast extract and 20% peptone) at pH 5.0 was prepared flesh and sterilized by autoclave. In addition, the water traps were also sterilized by autoclave. After the biomass solutions were cooled to room temperature, 10% (v/v) 10X YP, 10% (v/v) inoculum, and appropriate amount of cellulase were added. The water traps were installed to allow CO_2 escape. They were incubated at 38°C for 7 d at 150 rpm in a Lab-Line Environmental Shaker. Samples were withdrawn at regular intervals and centrifuged at 4000 rpm (1,935g) for 10 min, the supernatants were stored in closed vials at –20°C until analysis for carbohydrates with HPLC and ethanol with GC. The ethanol yield was calculated as:

$$\text{Yield}(\%) = \frac{[\text{Ethanol}]_{\text{final}} + [\text{Ethanol}]_{\text{initial}}}{0.568 \ f \ [\text{Biomass}]} 100\%$$

where [Ethanol]$_{\text{final}}$ = final ethanol concentration (g/L); [Ethanol]$_{\text{initial}}$ = initial ethanol concentration (g/L) determined as 10% of the ethanol concentration in the inoculum for SSFs; [Biomass] = dry biomass concentration at the beginning of the reaction (g/L); and f = cellulose fraction in dry biomass (g/g).

RESULTS AND DISCUSSION

Pretreatment I

Figures 2A–D show the concentration of glucose, xylose, furfural, and HMF (5-hydroxymethylfurfural) present in the prehydrolyzates at different pretreatment conditions. At 140°C (Fig. 2A), the glucose and xylose concentrations increased from 0.9 to 2.8 g/L and 14.5 to 16.8 g/L, respectively as the acid concentration increased from 0.5 to 2%. The furfural concentration also increased from 0.2 to 1.5 g/L. However, the HMF concentration was very low. At 150°C (Fig. 2B), the glucose concentration increased from 1.9 to 5.1 g/L from 0.5% to 2% acid. Because of the severity of the conditions, more xylose was degraded to furfural. As a result, the xylose concentration was decreased from 17.5 g/L at 0.5% acid to 12.5 g/L at 2% acid with the concomitant increase of furfural from 0.7 to 3.1 g/L. The HMF during this treatment was still fairly low. At both 160 and 170°C, a much lower concentration of xylose was detected at higher acid concentration compared to that at 150°C. The xylose concentrations were 2.0 and 0.4 g/L at 2% acid at 160 and 170°C pretreatment, respectively. At 160°C (Fig. 2C), the glucose concentration increased from 1.25 g/L at 0.5% acid to 4.0 g/L at 2% acid. The HMF concentration also increased from 1.6 to 5.5 g/L under the same pretreatment conditions. At 170°C (Fig. 2D), a similar trend was observed. The glucose concentration was increased from 5.8 to 9.5 g/L when the acid concentration was increased from 0.5% to 2%. Similar concentrations of HMF were obtained as the 160°C pretreatment. It should be noted that the furfural and HMF concentrations do not reflect a stoichiometric conversion of xylose and glucose, respectively. This is probably because of the further degradation of furfural and HMF to other products.

The compositions of the pretreated residues are shown in Fig. 3. The potential xylose in the raw material before pretreatment was 17.7%. After the 140°C and 0.5% acid pretreatment, the potential xylose remained in the residue was only 7.6%. As the severity increased, the potential xylose decreased with a complete potential xylose removal starting at 160°C and 1.5% acid.

Enzymatic Hydrolysis

The results from the enzymatic hydrolysis reactions are shown in Fig. 4A to D. The glucose concentrations in these experiments were obtained using the YSI 2700 Analyzer. The final samples at 168 h were also analyzed by HPLC for glucose, cellobiose, xylose, and galactose. The final glucose concentrations determined by HPLC were very close to the YSI values. They were within about 2% of each other. Hence, the following discussion is based on the YSI values only. From HPLC, it was found that no more than 0.2 g/L of cellobiose was obtained in all cases, and in some cases less than 0.1 g/L was obtained. As a comparison, the percent conversion using α-cellulose and NREL pretreated materials were also shown in these fig-
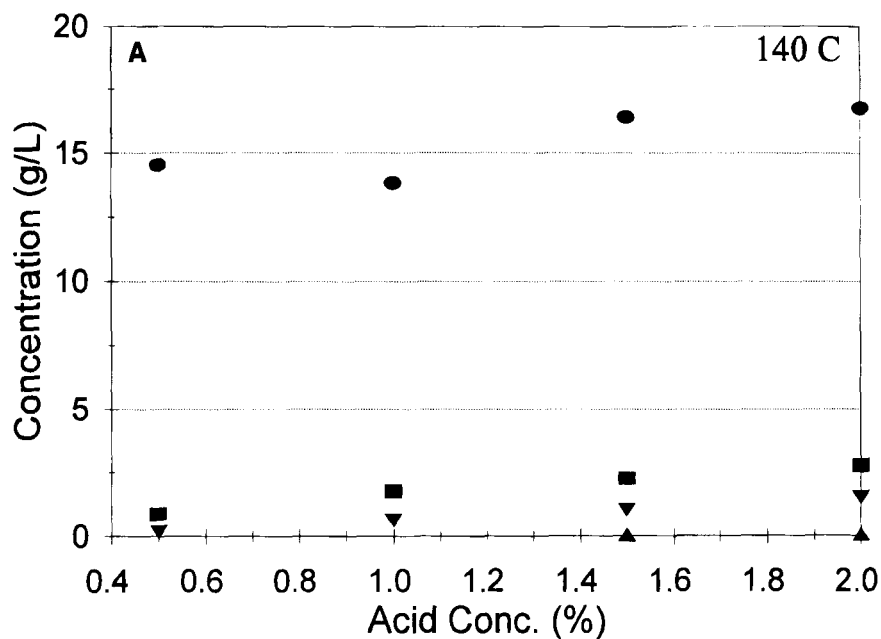
Fig. 2A. Sugar and degradation product concentration of prehydrolyzates at 140°C.
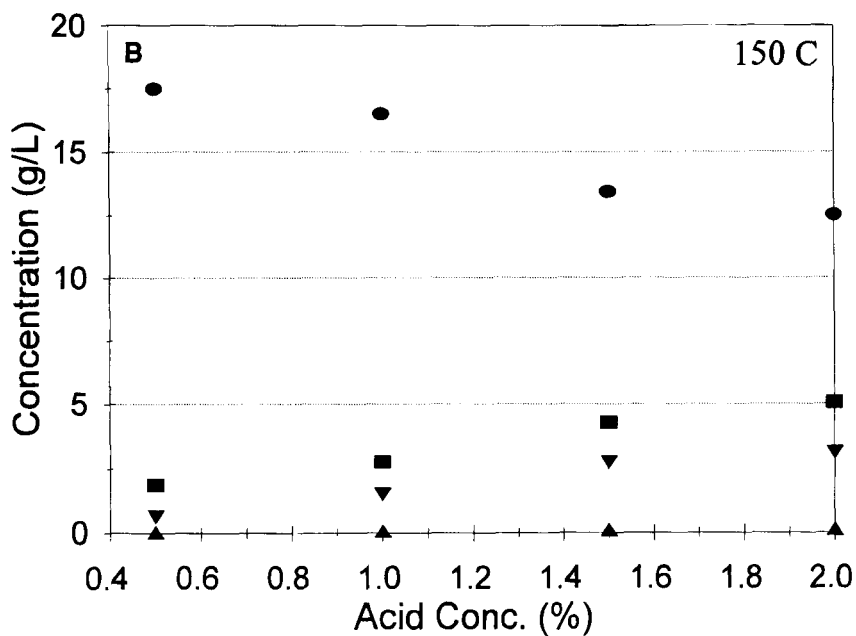■ Glucose, ● Xylose, ▼ Furfural, ▲ HMF.

Fig. 2B. Sugar and degradation product concentration of prehydrolyzates at 150°C.
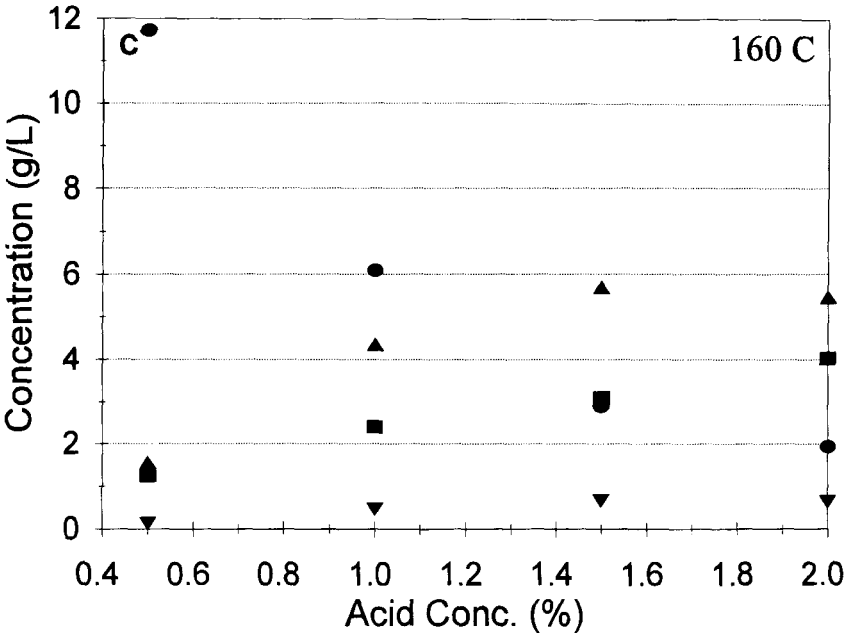■ Glucose, ● Xylose, ▼ Furfural, ▲ HMF.

Fig. 2C. Sugar and degradation product concentration of prehydrolyzates at 160°C.
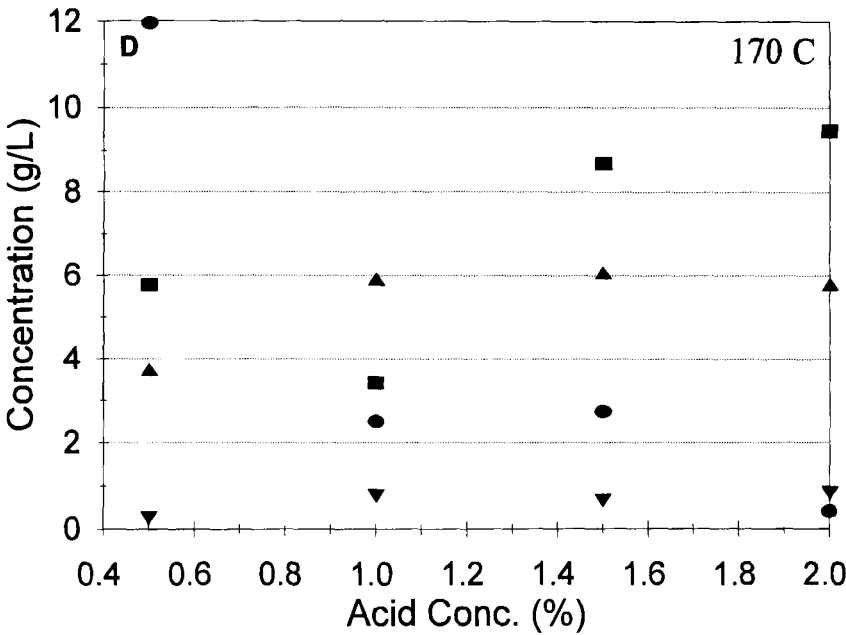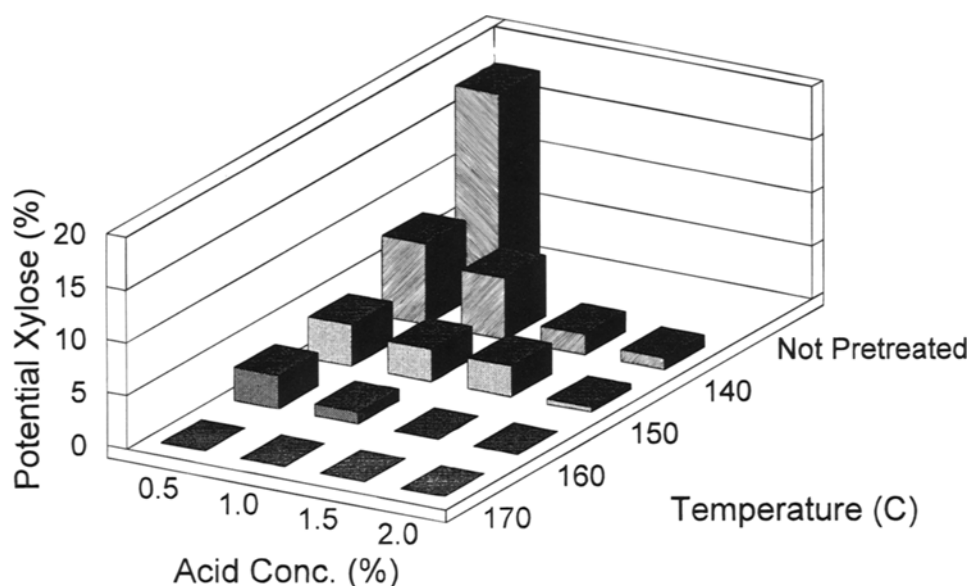■ Glucose, ● Xylose, ▼ Furfural, ▲ HMF.

Fig. 2D. Sugar and degradation product concentration of prehydrolyzates at 170°C.
■ Glucose, ● Xylose, ▼ Furfural, ▲ HMF.

Fig. 3. Percent of potential xylose present in pretreated residues.

ures. In general, the percent conversion increases as the severity increases. At 140°C (Fig. 4A), the conversion increased from 36.4 to 75.8% from 0.5 acid to 2% acid pretreatment. Less than 40% of the cellulose was converted to glucose and cellobiose at 0.5% acid, indicating a very ineffective pretreatment condition. At 150°C (Fig. 4B), the conversion varied between 77.6 and 82.8% from 0.5 to 1.5% acid concentration, but an almost theoretical conversion was obtained at 2% acid. At 160°C (Fig. 4C), 82.1% conversion was obtained at 0.5% acid, higher than 90% conversion was obtained at 1 and 2% acid, and more than theoretical conversion was obtained at 1.5% acid. At 170 °C (Fig. 4D), higher than theoretical conversions were obtained at all acid concentrations indicating that the pretreatment conditions were very effective. When the conversions at 48 h were compared, eight of the conditions used produced a residue of which more than 80% could be converted to glucose. These eight conditions were as follows: 150°C, 2% acid; 160°C at 1–2% acid; and 170°C at all acid concentrations. The results for enzymatic hydrolysis reactions at 48 h and 168 h are summarized in Tables 1 and 2, respectively. They show the pretreatment conditions under which the percent enzymatic conversion was obtained.

SSFs

Only the 1 and 1.5% acid pretreated residues were used in the SSFs, and the sugar concentrations were determined with the HPLC. The glucose and cellobiose concentrations for the SSFs at 1% acid are shown in
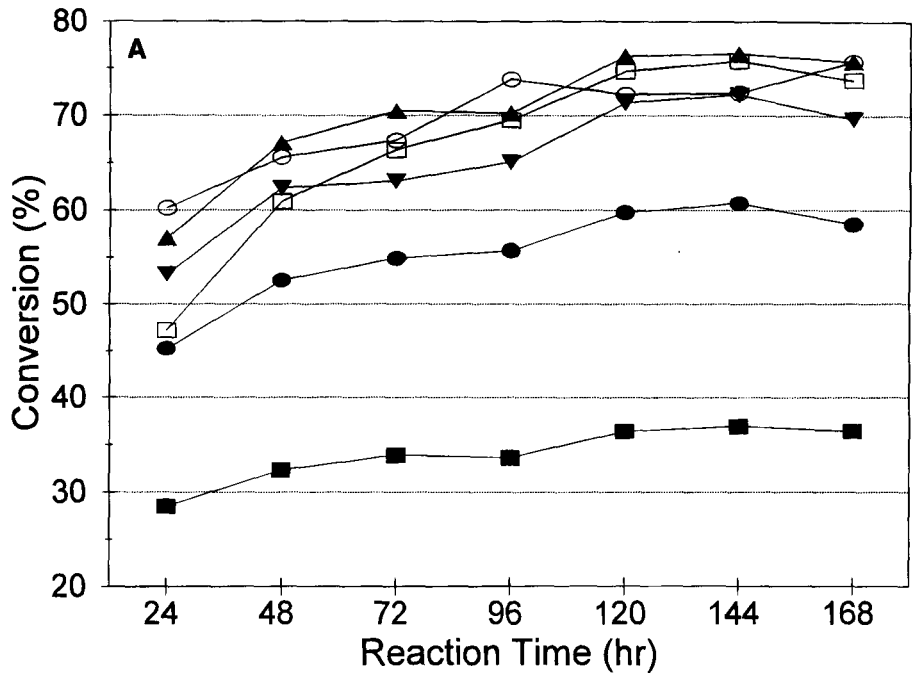
Fig. 4A. Enzymatic Conversion for 140°C Pretreatment Residues. ■ 0.5% acid, ● 1% acid, ▼ 1.5 acid, ▲ 2% acid, □ NREL Material, ○ Alpha-Cellulose.
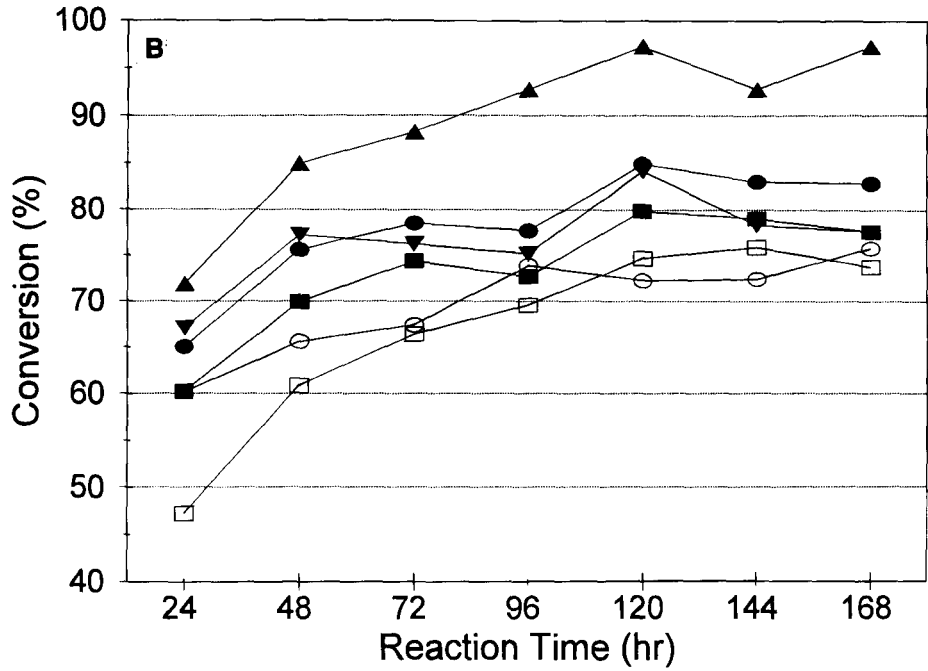

Fig. 4B. Enzymatic conversion for 150°C pretreatment residues. ■ 0.5% acid, ● 1% acid, ▼ 1.5 acid, ▲ 2% acid, □ NREL Material, ○ Alpha-Cellulose.
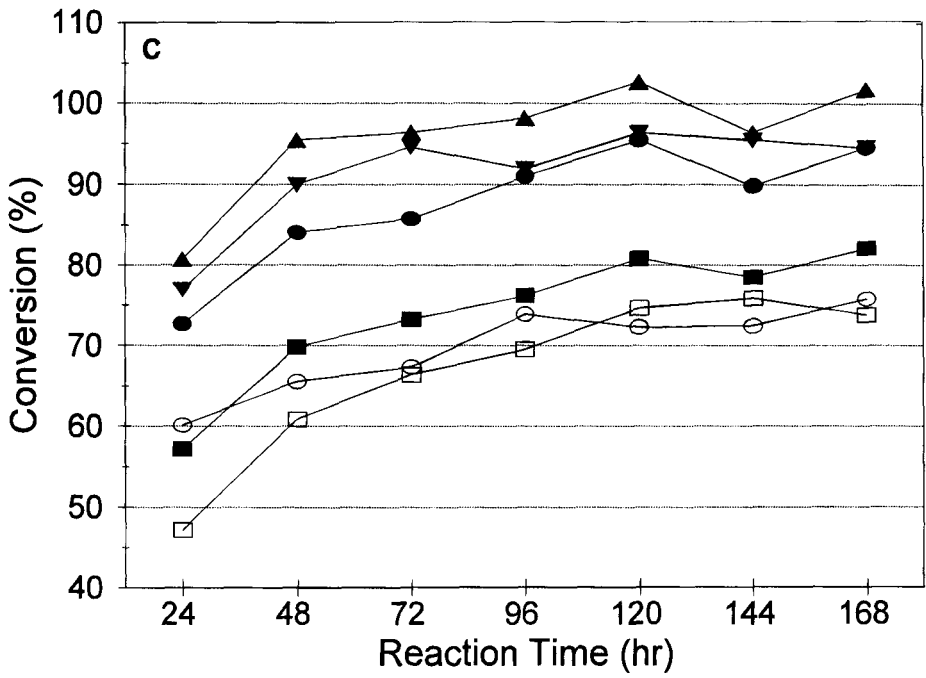
Fig. 4C. Enzymatic conversion for 160°C pretreatment residues. ■ 0.5% acid, ● 1% acid, ▼ 1.5 acid, ▲ 2% acid, □ NREL Material, ○ Alpha-Cellulose.
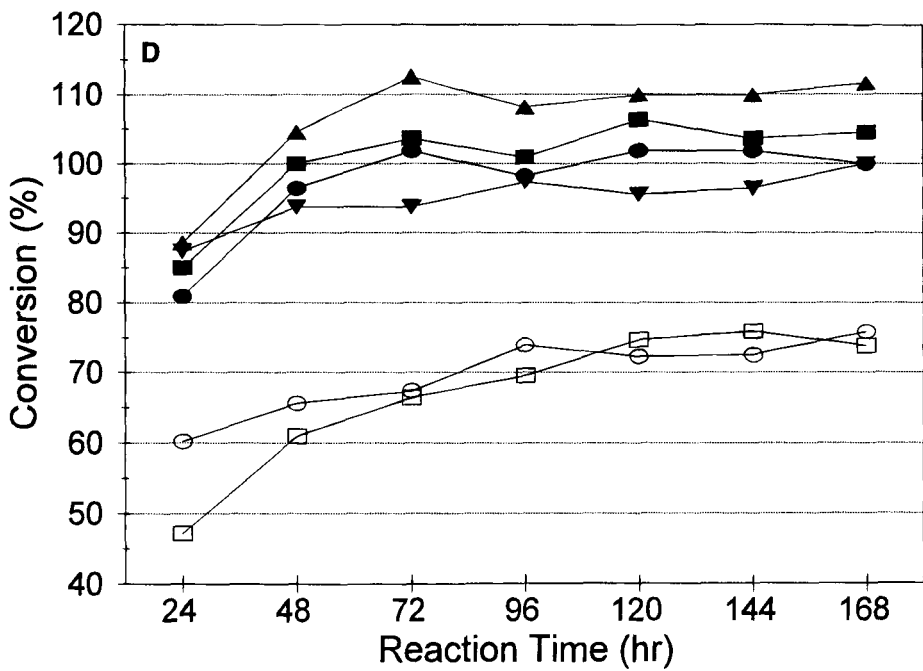

Fig. 4D. Enzymatic conversion for 170°C pretreatment residues. ■ 0.5% acid, ● 1% acid, ▼ 1.5 acid, ▲ 2% acid, □ NREL Material, ○ Alpha-Cellulose.

Table 1
Glucose Yield from Enzymatic Hydrolysis at 48 h

Percent Enzymatic Conversion:							
< 70 %		70 - 80 %		80 - 90 %		> 90 %	
Pretreatment Conditions:							
Temp (°C)	Acid (%)	Temp (°C)	Acid (%)	Temp (°C)	Acid (%)	Temp (°C)	Acid (%)
140	all	150	0.5 - 1.5	150	2	160	1, 2
		160	0.5	160	1.5	170	all

Table 2
Glucose Yield from Enzymatic Hydrolysis at 168 h

Percent Enzymatic Conversion:							
< 70 %		70 - 80 %		80 - 90 %		> 90 %	
Pretreatment Conditions:							
Temp (°C)	Acid (%)	Temp (°C)	Acid (%)	Temp (°C)	Acid (%)	Temp (°C)	Acid (%)
140	0.5, 1, 1.5	140	2	150	1, 2	160	1, 1.5, 2
		150	0.5, 1.5	160	0.5	170	all

Figs. 5A and B. Figure 5A shows that the glucose concentration increased slightly during the first 24 h of fermentation indicating the release of glucose from the residues by the action of the cellulases. Then the glucose started to decline because of the uptake of glucose by the yeast for ethanol production under the anaerobic condition. A final concentration of less than 0.4 g/L of glucose was obtained. A similar trend was obtained for the cellobiose concentration and the final cellobiose concentration in these cases was less than 0.2 g/L (see Fig. 5B). Similar results were obtained for the SSFs with 1.5% acid pretreated materials (data not shown).

The ethanol production yields for the SSFs are shown in Figs. 6A and B for the 1 and 1.5% acid pretreatment, respectively. At 168 h, the percent ethanol yields of the residues pretreated at 140°C were 60.1 and 69.5% for 1 and 1.5% acid, respectively. More than 70% yield was obtained from the residues at both 150 and 160°C pretreatment. At 170°C, 1% acid pretreatment, the percent ethanol yield was 91.2% and a theoretical conversion was obtained for the 170°C, 1.5% acid condition. In comparison, final ethanol yields of 69.5 and 62.4% were obtained from the NREL material and α-cellulose respectively. The results are summarized in Table 3 at 168 h fermentation. Its shows the pretreatment conditions under which the percent ethanol yield was obtained.
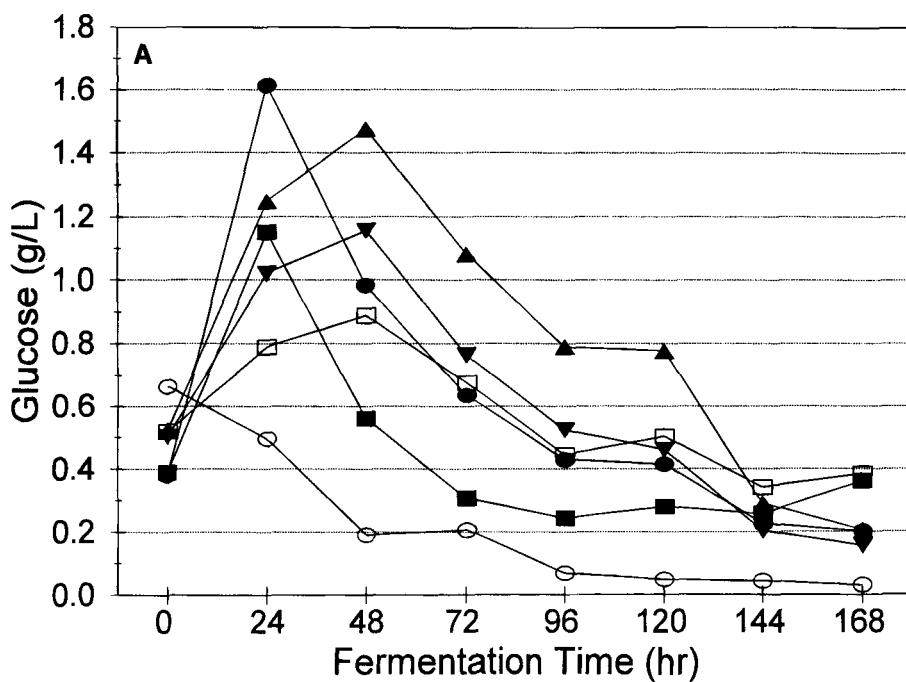
Fig. 5A. Glucose concentration during SSFs for 1% acid pretreated residues.
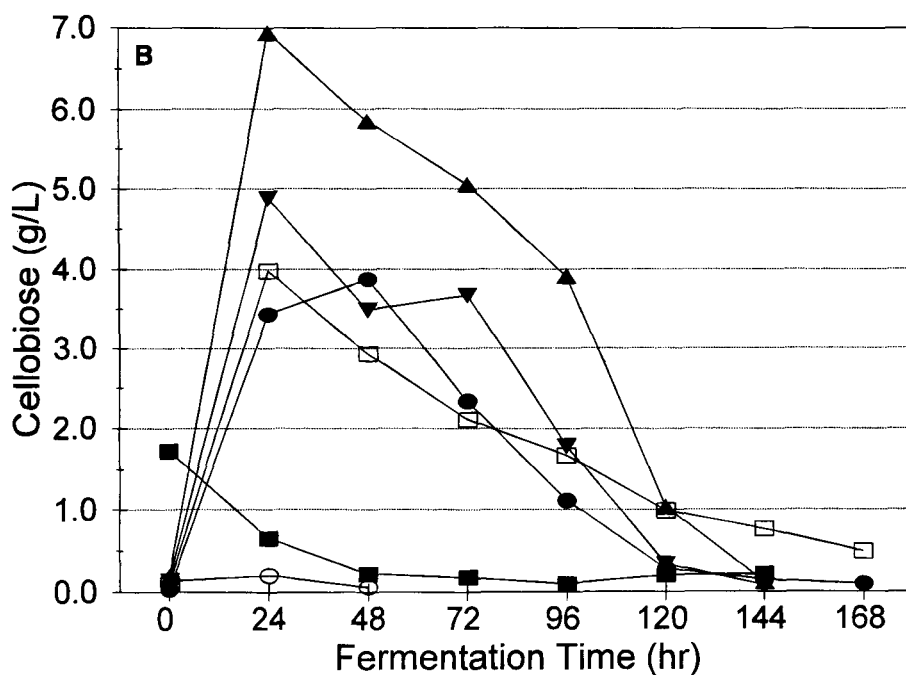■ 140°C, ● 150°C, ▼ 160°C, ▲ 170°C, □ NREL Material, ○ Alpha-Cellulose.

Fig. 5B. Cellobiose concentration during SSFs for 1% acid pretreated residues.
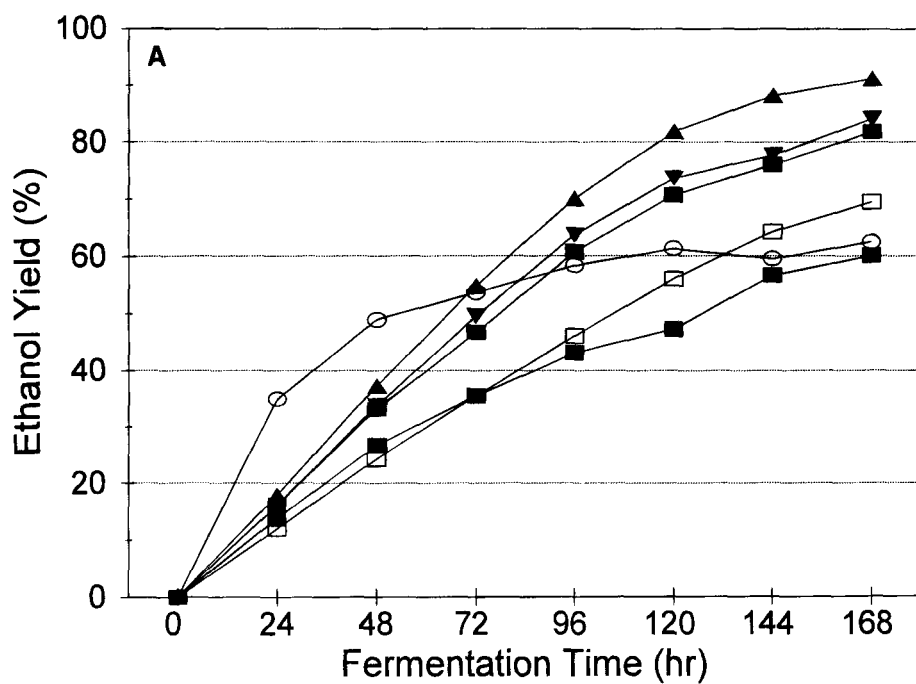■ 140°C, ● 150°C, ▼ 160°C, ▲ 170°C, □ NREL Material, ○ Alpha-Cellulose.

Fig. 6A. Ethanol production yield (%) for 1% acid pretreated residues. ■ 140°C, ● 150°C, ▼ 160°C, ▲ 170°C, □ NREL Material, ○ Alpha-Cellulose.
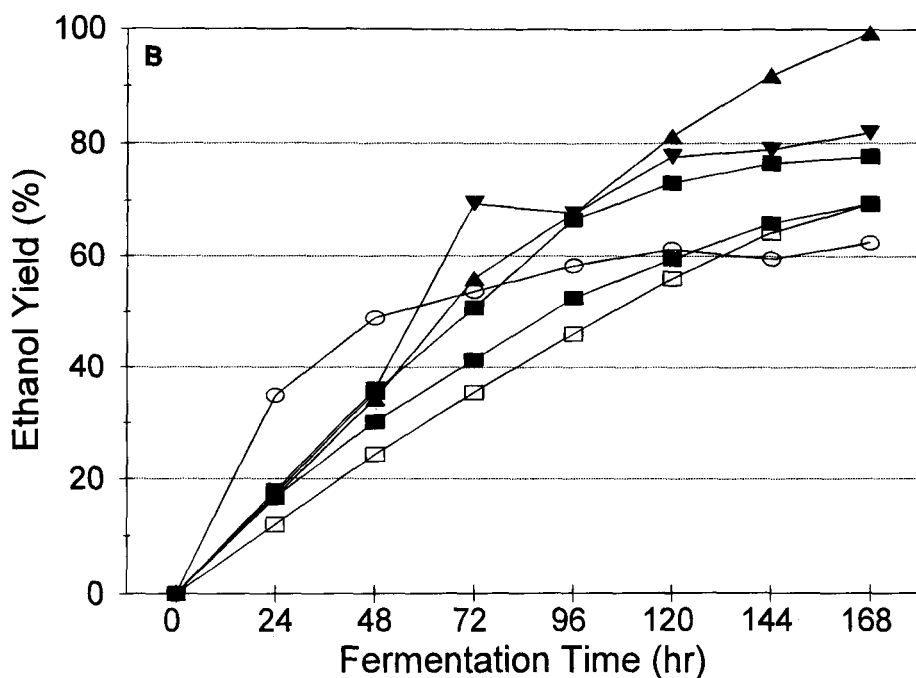

Fig. 6B. Ethanol production yield (%) for 1.5% acid pretreated residues. ■ 140°C, ● 150°C, ▼ 160°C, ▲ 170°C, □ NREL Material, ○ Alpha-Cellulose.

Table 3
Ethanol Yields (%) of SSFs at 168 h

Ethanol Yield:							
< 70 %		70 - 80 %		80 - 90 %		> 90 %	
Pretreatment Conditions:							
Temp (°C)	Acid (%)	Temp (°C)	Acid (%)	Temp (°C)	Acid (%)	Temp (°C)	Acid (%)
140	1	140	1.5	150	1	170	1,1.5
		150	1.5	160	1,1.5		
NREL Material							
Alpha-Cellulose							

Pretreatment II

The composition of the prehydrolyzates from the first-stage and two-stage pretreatments are shown in Figs. 7A and B, respectively. Both figures show the concentrations of glucose, xylose, and acetic acid. At the 100°C first-stage pretreatment, there were no measurable glucose, furfural, and HMF present in the prehydrolyzates; the xylose concentration increased from a low of 0.08 g/L (average of 2 runs) at 0.5% acid to 9.3 g/L at 5% acid. As the temperature was increased to 120°C, glucose concentrations at 0.72 and 1.3 g/L were obtained from 2 and 5% acid concentrations, respectively. This was probably a result of the hydrolysis of the glucan fraction of the hemicellulose instead of the cellulose. At the same time, the xylose concentration increased from 3.5 g/L at 0.5% acid to 11.7 g/L at 5% acid. There was only a very small amount of furfural (0.74 g/L) at 5% acid. Moreover, there was still no measurable amount of HMF. As the severity increased, the acetic acid concentration increased to 4.6 g/L at 120°C and 5% acid. Figure 7B shows the results of the prehydrolyzates when the residues from the first-stage pretreatment were pretreated again at 120°C and 2% acid for 30 min. At a first-stage pretreatment temperature of 100°C, the xylose fraction was almost untouched at 0.5% acid, and there was a high concentration of xylose (15.0 g/L). As more potential xylose was removed from the first-stage pretreatment, the amount of xylose from the second-stage decreases. Similar results were obtained for the 120°C first-stage pretreated residues. The compositions of the residues after the pretreatments are shown in Table 4.

As predicted from the xylose concentration in the prehydrolyzates, the potential xylose content decreased as the severity of pretreatment increased. With the first-stage pretreatment, only the condition at 120°C and 5% acid concentration removed all the potential xylose from the biomass. With a second-stage pretreatment, only a slight amount of potential xylose was present in the residues at 0.5% acid pretreated materials at both 100 and 120°C. The rest of the pretreatment removed all the potential xylose from the residues.
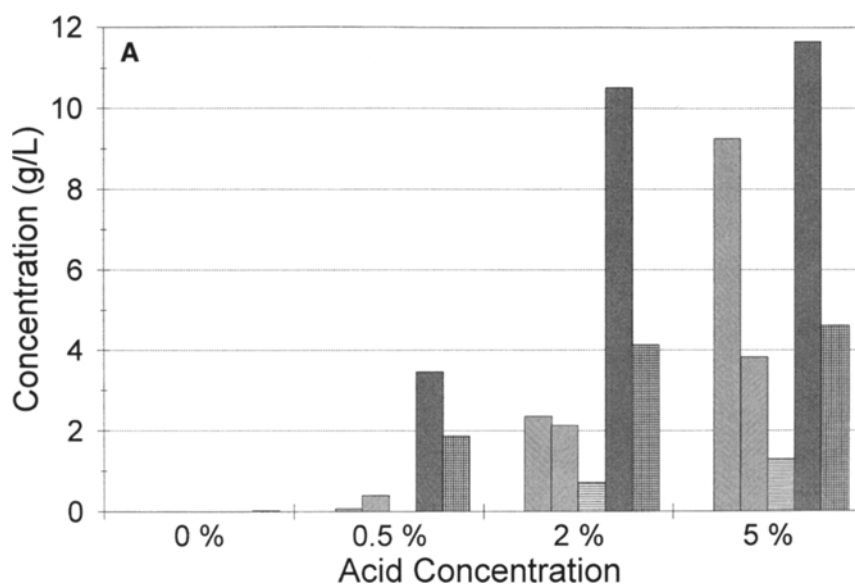
Fig. 7A. Prehydrolyzates from first-stage pretreatment. ▨ Xylose (First-stage at 100°C), ▨ Acetic Acid (First-stage at 100°C), ▦ Glucose (First-stage at 120°C), ■ Xylose (First-stage at 120°C), ▩ Acetic Acid (First-stage at 120°C).
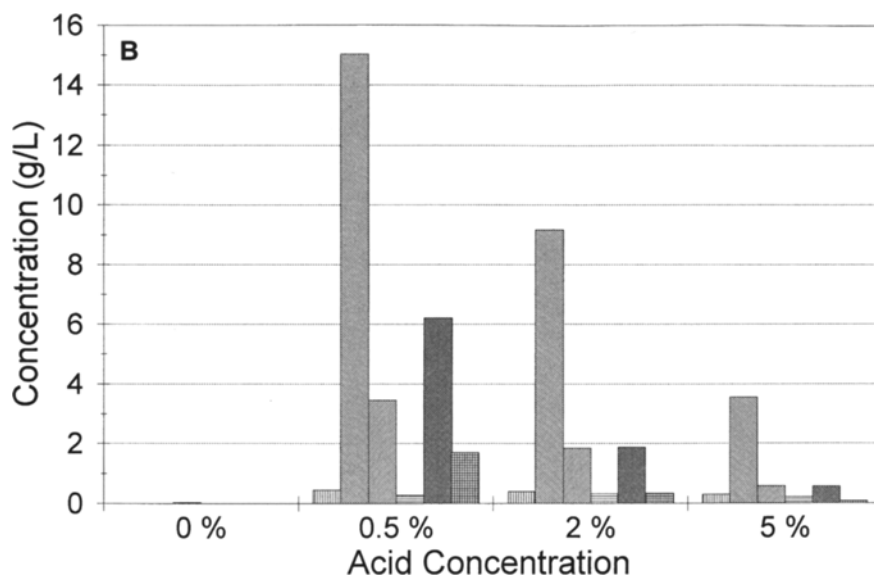

Fig. 7B. Prehydrolyzates from second-stage pretreatment. ▥ Glucose (First-stage at 100°C), ▨ Xylose (First-stage at 100°C), ▨ Acetic Acid (First-stage at 100°C), ▦ Glucose (First-stage at 120°C), ■ Xylose (First-stage at 120°C), ▩ Acetic Acid (First-stage at 120°C).

Table 4

Compositions of Residues from Two-Stage Pretreatment

Before Pretreatment			Temp (°C)	[Acid] (%)	After First-stage Pretreatment			After Second-stage Pretreatment		
Glucose (%)	Xylose (%)	Klason Lignin (%)			Glucose (%)	Xylose (%)	Klason Lignin (%)	Glucose (%)	Xylose (%)	Klason Lignin (%)
41.44[*]	17.72[*]	25.94	100	0	43.48	17.36	ND			
41.53	16.96									
43.13[#]	17.44[#]	ND	100	0.5	45.78	20.17	25.51	63.28	5.02	30.89
			100	2.0	51.56	16.16	28.13	55.76	0	31.58
			100	5.0	58.17	7.25	33.79	58.28	0	32.13
			120	0	44.68	18.09	ND	44.72	18.19	ND
			120	0.5	51.64	13.43	28.3	63.87	4.61	31.6
			120	2.0	62.63	6.31	31.71	60.90	0	32.44
			120	5.0	59.74	0	33.13	60.57	0	33.84

ND; Not determined; [*]before the initial boiling step, average of two runs; [#]after the initial boiling step.

Based on the concentration of the glucose and xylose in the prehydrolyzates and the potential sugars remaining in the residues, the percent conversion of glucan and xylose were computed. Table 5 shows the conversion during the first-stage pretreatment. It is observed that no glucan was converted to glucose at 100°C conditions. The conversion increases from 0.44% at 0.5% acid to 53.34% at 2% acid. At 120°C conditions, there was no glucan conversion at 0.5% acid and only very small conversions at 1 and 2% acid. The xylan conversion increased from 19.95% at 0.5% acid to 70.16% at 2% acid.

The conversion during the second-stage pretreatment is shown in Table 6. The conversion of glucan and xylan depends on the amount remaining after the first-stage pretreatment, and there are good reasons to believe that the first-stage pretreatment might remove the fast-reacting xylan leaving the slow-reacting xylan *(9,10)*. As a result, a higher conversion was observed for the lower acid concentration pretreatment at both temperatures during second-stage pretreatment. Since there were more xylan remaining at 100°C than 120°C, the conversion at 100°C was higher than those at 120°C. There was no potential xylose obtained for the residues at 120°C and 2% acid. This is probably because of the degradation of the minute amount of xylose to furfural during the carbohydrate determination. For the second-stage pretreatment, the conversion of glucan to glucose remained low.

The overall conversion for the two-stage pretreatment was also shown in Table 6. This is computed as: first-stage percent conversion + (100%-first-stage percent conversion) × second-stage percent conversion. It is shown that a two-stage pretreatment at 100°C is better than the two-stage pretreatment at 120°C at 0.5 and 1% acid concentrations. A theoretical conversion of the xylan to xylose was obtained from the two-stage pretreatment at 120°C

Table 5
Percent Conversion of Glucan and Xylan
During First-stage Pretreatment

Temp (°C)	Acid Conc. (%)	Percent Conversion* (%)	
		Glucan	Xylan
100	0	0	0
100	0.5	0	0.44
100	1	0	13.55
100	2	0	53.34
120	0	0	0
120	0.5	0	19.95
120	1	1.70	60.89
120	2	3.03	70.16

*Average of two runs except those at 0 % acid concentration.

Table 6
Percent Conversion of Glucan and Xylan During Second-stage
Pretreatment and Overall Conversion

First-stage Pretreatment[#]		Percent Conversion (%)		Overall Percent Conversion (%)[°]	
Temp (°C)	Acid Conc. (%)	Glucan	Xylan	Glucan	Xylan
Raw*		0	0	0	0
100	0.5	1.11	85.63	1.1	85.7
100	1	0.89	67.61	0.9	72.0
100	2	0.66	62.15	0.7	82.3
120	0.5	0.66	56.20	0.7	64.9
120	1	0.61	35.56	2.3	74.8
120	2	0.43	☆	3.6	100

[#]The temperature and acid concentration indicate those at first-stage pretreatment.

*Raw material pretreated at second-stage conditions only (120 °C and 30 min).

☆This can not be determined because the potential xylose content of the pretreated residue is zero. [°]Overall conversion from 2-stage pretreatment.

and 2% acid concentration. Even though a small amount of glucose was measured in the prehydrolyzates, this probably came from the glucan fraction of the hemicellulose rather than from the cellulose fraction.

Enzymatic Hydrolysis

Figure 8 shows the results from the enzymatic hydrolysis reactions performed at 50°C for 168 h. It was found that the conversion for the first-stage pretreated residues was very low. The highest conversion obtained
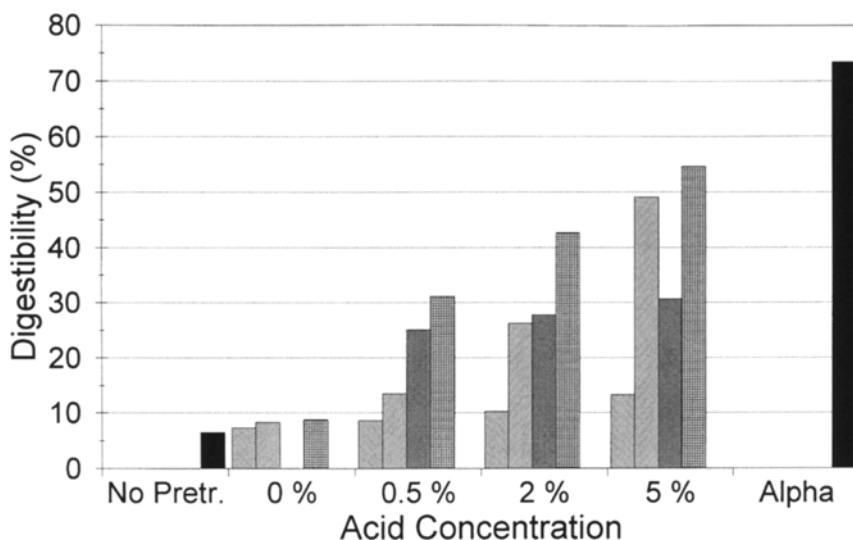
Fig. 8. Enzymatic conversion for first- and second-stage pretreated residues. ▨ First-stage at 100°C, ▨ First-stage at 120°C, ▨ Second-stage with First-stage at 100°C, ▨ Second-stage with First-stage at 120°C, ■ No Pretreatment.

was 49% at 120°C, 5% acid pretreatment. A slightly higher conversion was obtained for the two-stage pretreated residues. At first-stage pretreatment 42.6 and 54.6% conversions were obtained at 120°C and 2 and 5% acid, respectively. As a comparison, the conversion for the α-cellulose was 73.3%. The cellobiose in all cases was lower than detection limits (data not shown).

SSFs

The results from the ethanol production using SSFs are shown in Fig. 9. Since the residues from the first stage pretreatment did not perform very well on the enzymatic hydrolysis reactions, only the residues from the two-stage pretreated residues were used in the SSFs. At 168 h fermentation, the ethanol production yields for the residues from the initial pretreatment at 100°C, 0.5 to 5% acid were 49.3 to 55.8%. The percentage yields for the residues from the initial pretreatment at 120°C, 0.5, 2, and 5% were 53.4, 65.7, and 73.9%, respectively. In comparison, the percentage yield for the α-cellulose was 75.1%. As a result, at the highest severity of pretreatment, the percentage yield is almost the same as the α-cellulose. In all cases, the cellobiose concentration was less than 0.1 g/L.

CONCLUSIONS

The pretreatment performed at 140 and 150°C at 0.5, 1, 1.5, and 2% acid concentrations and 160°C at 0.5 and 1% acid concentration for 30 to 38 min removed most of the potential xylose from the southern mixed hard-
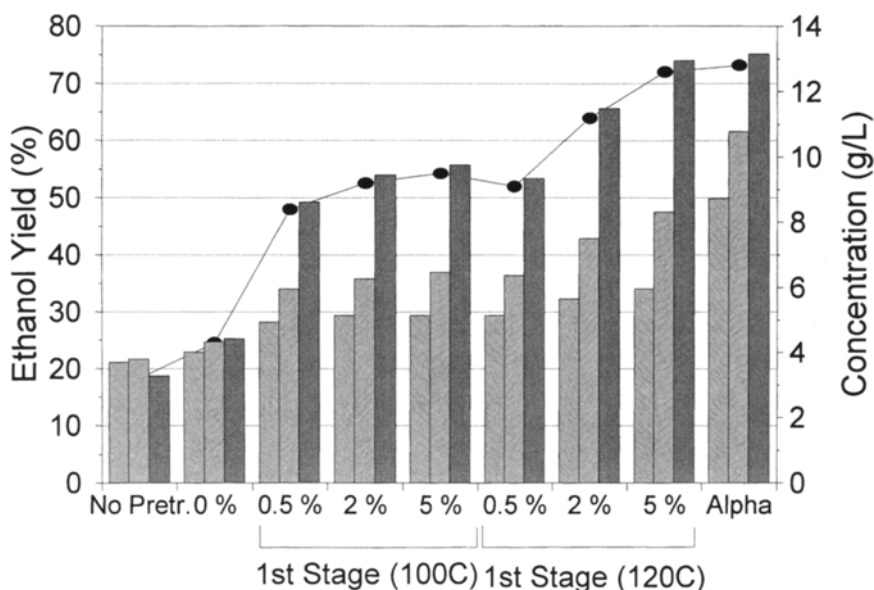
Fig. 9. Ethanol production yield (%) for Second-stage pretreated residues. ■ 24 h, ▨ 48 h, ■ at 168 h, ● Ethanol concentration at 168 h.

woods. The enzymatic conversion of these pretreated residues ranged from 32 to 84% at 48 h. Higher than 90% enzymatic conversion was obtained with the residues resulting from the 160°C at 1.5 and 2% acid concentration and 170°C at all acid concentration pretreatments. Hence, when the biomass was treated at 160 and 170°C with 1.5% acid and 0.5% acid, respectively, the residues were very effective substrates for enzymatic hydrolysis. The performance with these residues is summarized in Table 2. With low severity of pretreatment (140 and 150°C), less than 80% ethanol yield was obtained. Between 80 to 90% yield was obtained from the 160°C pretreatment. In addition, the pretreatment at 170°C (1 and 1.5% acid) was very effective for the production of ethanol using SSF.

For the two-stage pretreated biomass, complete potential xylose removal was obtained at:

1. First-stage pretreatment at 120°C and 5% acid;
2. Two-stage pretreatment with a first-stage pretreatment at 100°C with 2% acid, and second-stage pretreatment at 100°C with 2% acid;
3. Two-stage pretreatment with a first-stage pretreatment at 100°C with 5% acid, and second-stage pretreatment at 100°C with 2% acid;
4. Two-stage pretreatment with a first-stage pretreatment at 120°C with 2% acid followed by a second-stage pretreatment at 120°C with 2% acid.

The best enzymatic conversion of 54.6% was obtained from the residues resulted from 120°C with 5% acid at 168 h. Under SSF production of

ethanol, 65.7% yield was obtained from the two-stage pretreated biomass with first-stage at 120°C with 2% acid and second-stage at 120°C with 2% acid. A higher yield of 73.9% resulted from the two-stage pretreated biomass with first-stage at 120°C with 5% acid. This is almost equivalent to the percent yield produced by the α-cellulose. Even though the residues from this two-stage low-temperature pretreatment did not result in materials that are as good as the higher temperature pretreatment (Pretreatment I) for enzymatic hydrolysis and SSF, the advantage is that there are almost no degradation products from this two-stage low-temperature pretreatment. It is well known that in order to have economical production of ethanol using lignocellulosic materials, it is necessary to ferment both the cellulosic residues and the xylose fraction from the prehydrolyzates *(11,12)*. Since the presence of degradation products (furfural and HMF) can inhibit the fermentation to ethanol *(13,14)*, they can only be present at a very low concentration. Hence, the two-stage low-temperature pretreatment at low-acid concentration or one-stage low-temperature with slightly higher acid concentration is an alternative to other pretreatments.

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