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Two-Temperature Dilute-Acid Prehydrolysis of Hardwood Xylan Using a Percolation Process

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ABSTRACT

A novel two-temperature dilute-acid prehydrolysis of hybrid poplar xylan that exploits the xylan biphasic kinetics at moderate temperatures is described. A lower temperature (140°C) is applied to hydrolyze the easily hydrolyzable xylan, and a higher temperature (170°C) is subsequently applied to hydrolyze the remaining xylan. Using a bench-scale percolation reactor, yields of soluble xylose expressed in monomeric xylose equivalents as high as 92% of theoretical have been achieved with only 2% of the xylan being degraded to furfural. The lignocellulosic substrate produced from the pretreatment is readily converted to ethanol at a yield of 94% of theoretical via a simultaneous saccharification and fermentation process in 48 h. In terms of both yield of xylose equivalents and ethanol production level and rate, these improvements are far superior to those previously reported using a single-temperature dilute-acid pretreatment.

Index Entries: Hemicellulose; dilute-acid pretreatment; ethanol, xylan kinetics; percolation reactor.

INTRODUCTION

Using biotechnology to convert the energy stored in lignocellulosic biomass to ethanol that can be used as an octane booster, fuel extender, or neat liquid fuel has received considerable attention over the years. The inherent recalcitrance of the lignocellulosic feedstock to bioconversion

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requires a pretreatment of the biomass to render the cellulose fraction amenable to enzymatic conversion to glucose, which then can be utilized for ethanol production. Physical, chemical, and biological means, used alone or in combination, have been reported in the literature (*1–3*) to be effective pretreatment techniques. For the past several years, the National Renewable Energy Laboratory (NREL) has studied and developed a dilute-acid pretreatment technique that cooks the biomass feedstock, causing the hemicellulose fraction to solubilize (*4–11*). The use of dilute (0.5–1.0 wt%) sulfuric acid prehydrolysis for times ranging from minutes to hours at relatively mild temperatures (120–180°C) for hardwoods (*4–8*), herbaceous crops (*7,9*), and agricultural wastes (*4,5,8,10,11*) causes dissolution of the hemicellulose fraction. This technique was found to significantly enhance the enzyme digestibility of the biomass studied, presumably by creating pores in the biomass large enough to allow access by cellulase enzymes to the additional cellulose surface area (*12*).

Kinetic studies have shown that the dilute-acid solubilization of hemicelluloses in hardwoods and some agricultural residues (e.g., wheat straw) exhibits a biphasic phenomenon when xylan remaining in the solid residue is plotted vs time on a semilog scale; i.e., in such a plot, two distinctly different slopes are observed (*4,11,13–15*). As a result, it has been proposed that xylan in lignocellulosic biomass is composed of two fractions—one easy to hydrolyze and one hard to hydrolyze (*4,13–16*). Some have further proposed that, rather than there being two distinct polymeric species of xylan, the slow rate may be the result of a portion of xylan being embedded within or attached to the lignin via lignin–carbohydrate bonds (*17*). Two parallel pseudo first-order reactions of xylan solubilization yielding oligomeric xylose, followed by xylose formation with subsequent degradation, have therefore been proposed as follows (*17,18*):

$$\begin{matrix} \text{Xylan}_f \\ \quad \searrow \\ \quad\quad \text{Oligomeric xylose} \rightarrow \text{Xylose} \rightarrow \text{Furfural and other degradation products} \\ \quad \nearrow \\ \text{Xylan}_s \end{matrix} \qquad (1)$$

where xylan_f and xylan_s represent the easy to hydrolyze (fast-hydrolyzing) and hard to hydrolyze (slow-hydrolyzing) xylan fractions, respectively.

Currently published engineering plant designs for the prehydrolysis unit operation use a single-temperature, batch-wise, or cocurrent flow operation for the prehydrolysis of xylan to soluble sugars (*6,19*). Although yields of soluble xylose equivalents (i.e., hydrolyzed xylan in oligomeric or monomeric form expressed in total xylose units) on a molar basis of 70–80% have been achieved (*4*), further yield increases are limited because of kinetic constraints. Recently (*20,21*), it has been reported that, by using a two-temperature prehydrolysis regime, with one (lower) temperature addressing the fast-reacting xylan (about 60–70% of the total xylan) and

the other addressing the remaining xylan, yields of xylose equivalents approaching 92% in the prehydrolyzate can be achieved. Thus, we propose that the prehydrolysis step be designed and optimized as two separate unit operations to address the two xylan fractions.

The current study has been undertaken to demonstrate further in the laboratory the higher xylose yields of the two-temperature pretreatment technique and to produce other pertinent data, including digestibility and fermentability of the pretreated wood, related to this novel design. A percolation reactor (packed-bed up-flow-through-type) configuration has been chosen in the present study because it is one of the reactor types most suitable for biomass pretreatment in that the sugar product is removed from the reactor as it is formed. This enables the process to attain high sugar yield by minimizing sugar decomposition. Furthermore, the sugar product from a packed-bed-type reactor is obtained at a high concentration level because of the relatively high solid-to-liquid ratio that prevails in such a reactor.

MATERIALS AND METHODS

Materials

The *Populus eugeneii* (hybrid poplar) DN 34 substrate was provided by the University of Minnesota at Crookston. It was harvested in the fall of 1992, manually debarked, and coarsely chipped using a Formost mobile knife chipper (''Brush Bandit''). The chips were milled further using a laboratory knife mill (Thomas-Wiley laboratory mill, Arthur H. Thomas Co., Philadelphia, PA) equipped with a 1-mm rejection screen. Milled material was further separated into a −60 to +80 mesh (0.18–0.25 mm) fraction by using a portable sieve shaker (Tyler Industrial Products, Mentor, OH) equipped with USA Standard Testing Sieves.

A liquid cellulase preparation (Genencor Laminex cellulase, San Francisco, CA), stabilized by the addition of glycerol, was stored at 4°C until it was used. The specific activity of the enzyme was approx 64 international filter paper units (FPU)/mL (22). β-glucosidase activity in this preparation was approx 82 international units (IU)/mL (22). Fungal β-glucosidase (250 IU/mL [22], Novozyme 188, NOVO Lab Inc., Wilton, CT) was used to supplement the cellulase preparation such that the ratio of β-glucosidase to cellulase was 3:1 in all experiments. The yeast used in the simultaneous saccharification and fermentation (SSF) experiments (*see* Analytical Methods *below* for a description of SSF) was *Saccharomyces cerevisiae* D_5A (23). The remaining chemicals were purchased from national laboratory supply houses. Cellulose power (α-cellulose), used as a control substrate, was obtained from Sigma Chemical Co. (St. Louis, MO).

Analytical Methods

The dry weight of all solids and the ash content of the native feedstock were determined by standard methods (*24*). Lignin and other acid-insoluble components were determined as Klason lignin (*25*). Acid-soluble lignin was determined by using an aliquot from the Klason lignin filtrate (*26*). Uronic acids, acetyl groups, and furfural were determined as described previously (*7*). The carbohydrate composition of biomass solids was determined by a modification of the two-stage sulfuric acid hydrolysis (*25*) followed by determination of monomeric sugars by ion-moderated partition (IMP) chromatography (the slight modification was the use of a 2-h incubation of the substrate in 72 wt% sulfuric acid instead of a 1-h in order to solubilize the glucan completely). The prehydrolyzates (prehydrolyzate is defined as the liquid phase resulting from an acid pretreatment run) obtained from pretreatment experiments were neutralized by calcium carbonate and filtered. The carbohydrates could then be analyzed directly. If the presence of oligomeric sugars was suspected in the neutralized filtrate, the prehydrolyzates were adjusted to 4 wt% sulfuric acid and autoclaved at 121°C for 1 h (*25*), neutralized, and analyzed by IMP chromatography using Aminex HPX 87XP and HPX-87C columns (Bio-Rad, Richmond, CA), deionized water as eluant, and refractive index (RI) detection.

Enzymatic hydrolysis was performed in batch mode at 50°C, pH = 4.8 using a 0.05*M* sodium citrate buffer, in gently rotated 20-mL glass scintillation vials at approximately a 45° angle as previously described (*4,5,7*). Cellulase enzyme loading was approx 42 FPU/g cellulose and supplemented with fungal β-glucosidase at approx 126 IU/g cellulose. This level of cellulase loading has been shown previously (*4*) to be at saturating levels of activity when using α-cellulose as a standard. This is above the IFPU/g cellulose used in the SSF experiments described below.

In addition to testing the pretreated substrate for the efficacy of the pretreatment in terms of rates and the extent of enzymatic saccharification, an SSF protocol was used to give additional information on the quality of the pretreated substrate as to its rate and the extent of convertibility of its glucan content to ethanol. Extensive research has demonstrated that SSF, the simultaneous saccharification (hydrolysis) of cellulose to glucose and fermentation of glucose to ethanol, improves the kinetics (*27*) of biomass conversion through circumvention of enzyme inhibition by hydrolysis products, minimization of contamination risk because of the presence of ethanol, and reduction of capital equipment requirements.

Shaker flask SSFs were carried out in 250-mL flasks outfitted with stoppers constructed to vent CO_2 through a water trap as previously described (*27*), with minor modifications. The cellulase preparation was employed at a concentration of 25 FPU/g cellulose for both the standard α-cellulose and the pretreated poplar wood, and supplemented with β-glucosidase at approx 3 IU β-glucosidase to 1 FPU cellulase. This cellulase

loading translates into 21.4 FPU/g native bone-dry poplar hybrid. Ethanol concentrations in the supernatants were measured by gas chromatography as previously described (27).

EXPERIMENTAL

The experimental approach used in the present study was first to construct a percolation reactor that was of sufficient dimensions so as to hold enough raw and pretreated biomass for analytical work, but still be very manageable. A reactor 2 in. (51 mm) in length by 1 in. (25 mm) in diameter was used. The particle size, −60 mesh to +80 mesh (0.18–0.25 mm), was chosen to minimize dispersion of the acid catalyst in the flow-through reactor. Before dilute-acid pretreatment experiments could begin, a flow characteristic study was initiated to quantify the flow behavior of the catalyst as a function of temperature and flow rate. Once the flow characteristics were quantified, the pretreatment experiments were conducted. The quality of the pretreated residue was assessed by both enzymatic saccharification and the conversion of the glucan to ethanol by the SSF protocol.

Percolation Reactor and Liquor Collection System

The design of the percolation reactor (Fig. 1) is basically patterned after pressure chromatography columns with valving at both ends and a dispersion frit at the entrance followed by a retention frit. A retention frit was also installed at the exit. Because of the height-to-diameter ratio of the reactor (Fig. 1), channeling could have been a problem if the dispersion frit were ineffective. A control residence time distribution (RTD) experiment was run in which a dummy entrance head plate was drilled out forming a conical shape to allow the entering liquor to disperse over the entire surface area of the biomass. If channeling was a problem, the RTD function for the nonconical head plate would have shown more nonideal flow characteristics than the drilled-out head plate. This, however, was not the case (data not shown). Therefore, channeling was not a problem. A threaded hole is located at the midpoint of the reactor length through which a thermocouple is installed to monitor temperature. The thermocouple head can be bent to be located at almost any point in the interior of the reactor. For all experimental runs reported in this article, the thermocouple head was located at the center of the reactor. Wherever possible, Carpenter 20Cb-3 stainless steel was used as the material of construction for the reactor. The dispersion frit (1-in. [25 mm] diameter, 2 μ pore size, made of titanium) and the retention frits (1-in. [25-mm] diameter, 60 μ

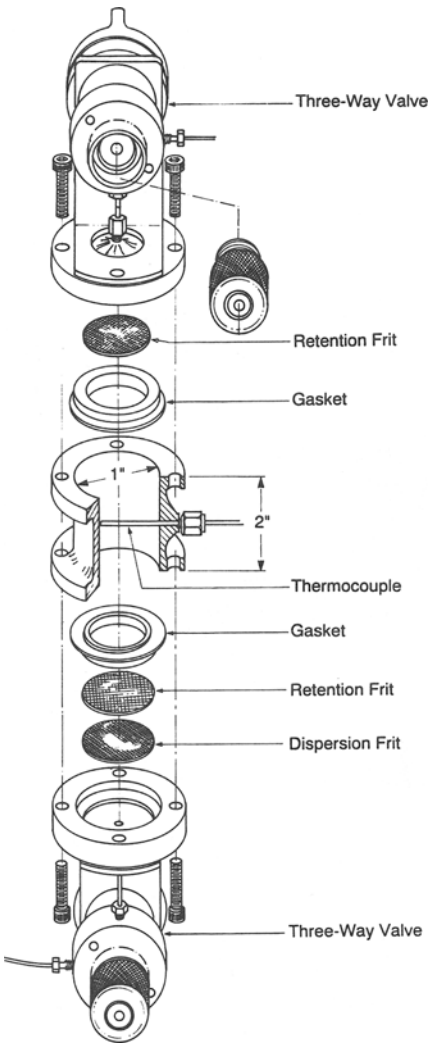

Fig. 1. Schematic diagram of the percolation reactor.

pore size, made of Inconel) were obtained from Mott Metallurgical, Farmington, CT. Titanium tubing (1/16-in. [1.6-mm] outside diameter × .03-in. [0.8-mm] inside diameter) used to connect the reactor with other components of the system was obtained from Anspec, Ann Arbor, MI. The 2-in. (51-mm) bar stock (used for the head plates of the reactor) and the 1-in. (25-mm) inside diameter tubing (used for the body of the reactor), which were both made of Carpenter 20Cb-3 stainless steel, were obtained from Carpenter Technology, Dallas, TX, and Marmon Keystone, Denver, CO, respectively. The three-way Hastelloy C valves were obtained from Valco Valve Corporation, Houston, TX (the third port of the three-way valves was not used in the current study). The reactor was fabricated and assembled by Falcon Fabrication, Arvada, CO.

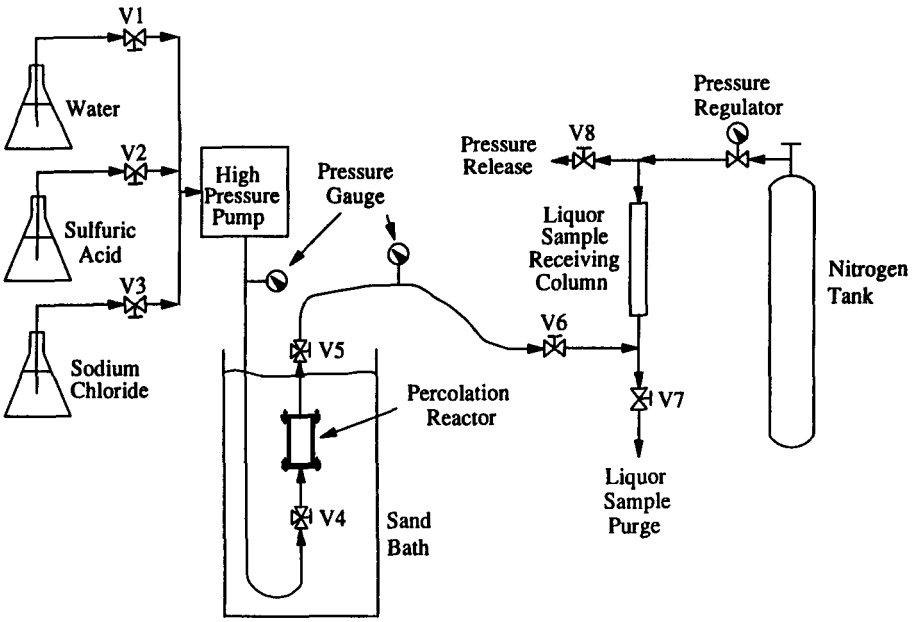

Fig. 2. Schematic diagram of the laboratory two-temperature percolation reactor pretreatment apparatus.

A schematic of the flow system and equipment design is seen in Fig. 2. For all flow characteristic studies and dilute-acid pretreatment runs, the following protocol was used. The reactor was charged with 3.80 g (3.53 g bone-dry basis) of ambient-air-dried biomass, and the reactor head plates bolted shut. Once the reactor was closed, the inlet line was attached to the water line from the high-performance liquid chromatograph (HPLC) pump (Beckman Instruments, model 110B, Fullerton, CA), and the exit line was left open to the atmosphere. Water was pumped through the reactor at ambient temperature at 1.1 mL/min for at least 4 h to ensure total wetting of the biomass. The reactor was then submerged in a 90°C sand bath (SB series General Laboratory Fluidized Bath equipped with a temperature controller, Cat #W3280-3 VWR Scientific, Denver, CO) with water being pumped through the reactor at 1.1 mL/min until the center of the reactor reached 88°C. The reactor was then heated for an additional 10 min to allow the trapped air in the wood pores to escape. The reactor temperature was then changed either for flow characteristics experiments or pretreatment experiments as described below.

The total void volume of a water-saturated, deaerated, packed reactor was determined by quantitatively removing the entire contents of the reactor and immediately recording the weight, followed by drying the reactor contents at 105°C. The weight difference between the wet and dried reactor contents is a measure of the water present in the reactor or of the total void volume of the reactor, which was determined to be 21.4 mL.

Flow Characteristics Studies

In order to define the prehydrolysis time, we needed to address the flow characteristics in the reactor (i.e., how the observed flow varies from ideal plug flow). We employed the method of residence-time distribution (RTD) determination (*28*) in a homogeneous reactor operated isothermally. In this part of the investigation, conditions (of tracer and temperature) were selected so that reactions did not occur. The RTD method assumes that the linear axial velocity, u, and the tracer concentration are uniform across the diameter. In our packed-bed percolation reactor, we further assume that the total void volume is distributed evenly throughout the length of the reactor. Accordingly, the deviation from ideal plug-flow conditions can be determined from the tracer concentration in the effluent in response to a step change in the feed tracer concentration. The governing equation is:

$$[(C / C_0)]_{step} = \tfrac{1}{2}\{1 - erf[\tfrac{1}{2}(uL / D_L)^{1/2} \quad ((1 - \theta / \bar{\theta}) / (\theta / \bar{\theta})^{1/2})]\} \qquad (2)$$

where C and C_0 are the tracer concentrations in the effluent and feed, respectively; L is the reactor length; D_L is the axial effective diffusivity; θ is the elapse time after the step change in the feed tracer concentration; $\bar{\theta}$ is the mean residence time; and *erf* is the error function (*29*). As such, the response in the effluent, C/C_0, is a function of D_L/uL (the reciprocal of the Peclet number), and the greater the group D_L/uL, the greater the flow in the reactor deviates from ideal plug flow.

For the RTD studies, the reactor was charged with biomass and deaerated as described above. The reactor inlet was connected to the HPLC pump, and the exit port was connected to an RI detector (Altex model 156). Three reservoirs were available for liquid flow (Fig. 2): 10 m*M* sodium chloride, deionized water, and 0.4 wt% sulfuric acid. At time zero, either the NaCl or H_2SO_4 reservoir was brought on line and replaced the water reservoir for the step-change tracer experiments. A strip chart recorder (Recordall, Fisher Scientific, Denver, CO) was connected to the RI detector, which responded to both the NaCl and H_2SO_4 solutions. For the 140°C tracer studies, the reactor was submerged in a 140°C sand bath and connected on line through the RI detector with the valve (V6 in Fig. 2) to the receiving column (Omni glass chromatography column assemblies rated at 150 psig [1030 kPa gage]) closed. Once the reactor reached 140°C, nitrogen gas was used to charge the receiving column and match the reactor pressure. When the entire system was at equal pressure, V6 was opened (the HPLC pump was then switched on to allow water, and later [at time zero] NaCl or H_2SO_4, to flow through). The entire transient response in the reactor effluent after the feed was step-changed to NaCl, or H_2SO_4 was recorded. The experimental reproducibility was verified by conducting two runs on two different days using two different biomass packings (data not shown).

Dilute-Acid Pretreatment Studies

In the present study, the two-temperature pretreatment using a percolation reactor packed with hybrid poplar wood flour was investigated (*see* Fig. 2 for the experimental setup). In order to define better the experimental conditions that would give high xylose-equivalent yields, we used the mathematical modeling of a percolation process using the two-temperature pretreatment of hybrid poplar xylan studied by Auburn University (through an NREL subcontracted study). It has been demonstrated through mathematical simulations (*30–32*) that after the biomass is cooked at temperatures between 135 and 150°C using 0.73 wt% sulfuric acid for residence times necessary to hydrolyze 60% of the xylan, a step-change increase in prehydrolysis temperature of between 25 and 35°C to hydrolyze the remaining xylan results in maximum yields of xylose in the prehydrolyzate. Furthermore, using 170°C as the upper prehydrolysis limit (which happens to be the practical upper limit of the current experimental percolation system), maximum yields of xylose are obtained by using 140°C as the lower prehydrolysis temperature and residence times of 34 min, 20 s, and 21 min, 51 s at the lower and higher temperatures, respectively, as well as a total volume of hydrolysis liquor equaling two total voids (*32*). Although the wood substrate used in the modeling work had a slightly different chemical composition than that used in the present study (data now shown), the above residence times and temperatures were nonetheless used in the present experimental protocol.

The reactor was charged with biomass and deaerated as described above. Immediately following deaeration, the HPLC pump was turned off and the reactor was connected to the collection system (Fig. 2) with V6 closed. The reactor was then submerged in the 140°C sand bath. Once the reactor reached the prehydrolysis temperature, the system was pressure-equalized as described above, the pump turned on, 0.73 wt% sulfuric acid pumped to the reactor at the rate of 8.8 mL/min for 1 min, 30 s (the time for the acid to first appear at the exit end of the reactor), and the effluent liquor discarded (all subsequent prehydrolyzate was collected for analysis). The acid was then pumped at 8.8 mL/min for an additional 2 min, 30 s to saturate the reactor totally with acid (which was determined from flow characteristics studies). The pumping rate was then adjusted to equal just under one total reactor void volume over the desired prehydrolysis time to hydrolyze approx 60% of the xylan (flow rate = 0.62 mL/min for 30 min, 15 s). The reaction was quenched by pumping water to the reactor at the rate of 8.8 mL/min for a total of 6 min, 10 s, which was the time to wash all the acid out of the reactor completely. The pump was then shut off and V6 closed. With the reactor remaining in the sand bath, the temperature was raised to 170°C. Once the reactor's thermo-couple indicated that the biomass had reached 170°C, the system pressure was equalized, V6 was opened, and the liquor pumping sequence mentioned above was repeated (except that the pumping rate and time used to send

just over one total void volume through was 1.35 mL/min for a total of 17 min, 46 s). After quenching the reaction as described above, the reactor was cooled to ambient temperature and the solid contents collected in a glass-sintered funnel of medium porosity. The solid residue was then weighed and chemically analyzed; the combined prehydrolyzate (from 140 and 170°C prehydrolyses) was analyzed for xylose and other components of interest as described above.

RESULTS AND DISCUSSION

In a percolation reactor, the understanding of the flow characteristics is essential for designing experiments. We therefore initiated our laboratory investigation into the dilute-acid prehydrolysis of hybrid poplar xylan with a study of the flow characteristics in the packed-bed reactor. Once the flow patterns were defined, the two-temperature dilute-acid study could begin. In order to address the efficacy of the pretreatment, three criteria were investigated:

1. The yield of equivalent xylose;
2. The rate and extent of enzymatic digestibility of the pretreated substrate; and
3. The rate and extent of the conversion of the glucan in the pretreated substrate to ethanol using the SSF protocol.

Flow Characteristics in the Percolation Reactor

The flow characteristics in the packed-bed percolation reactor were initially determined by a step-change in the feed from deionized distilled water to dilute sulfuric acid at ambient temperature and a flow rate of 8.8 mL/min (linear flow rate of 2.09 cm/min), which was the fastest flow rate used in the present study. Two closely matched curves are shown in Fig. 3A: one is the trace of the sulfuric acid concentration in the reactor effluent monitored by RI detection, and the other is that of pH. The reason the effluent was detected both by an RI detector and by pH was to ensure that the hydrogen ions and sulfate ions travel through the reactor at the same speed, meaning that no significant ion-exchange or otherwise adsorption effects of H^+ take place. In the prehydrolysis reactions, H^+ is the catalyst and its speed is more of interest than that of SO_4^{-2}. pH monitoring does not apply to inert tracer studies (using NaCl as described below), so the remaining flow characteristics studies, regardless of the tracer species, were conducted using the RI detection technique. Figure 3A also shows that, although precautions were taken in designing the percolation reactor and the biomass particle size distribution was narrowed to between −60 and +80 mesh (0.18–0.25 mm) to reduce flow dispersion, some deviations from ideal plug-flow characteristics occurred.

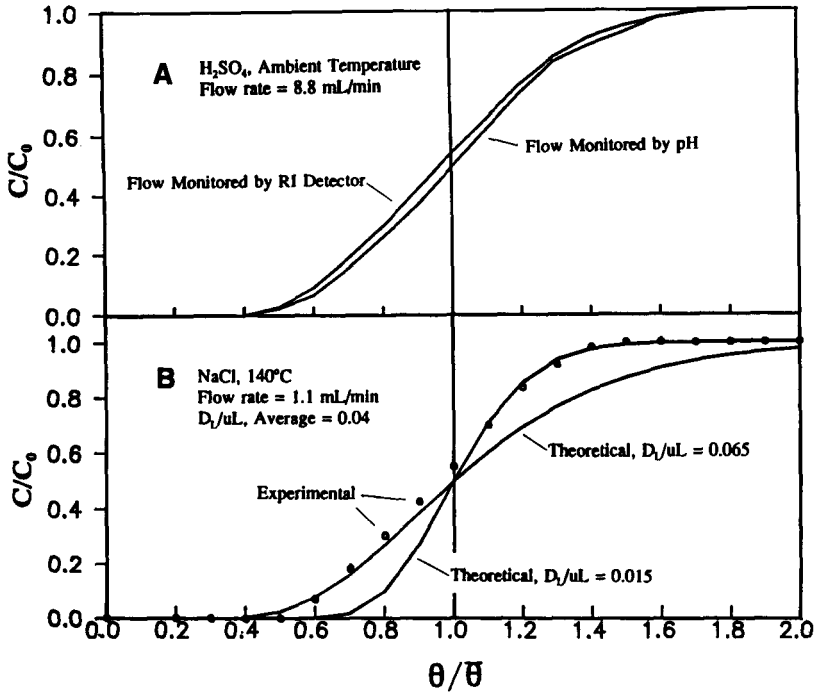

Fig. 3. Percolation flow characteristics: (A) flow profiles of H_2SO_4 at ambient temperature and 8.8 mL/min as measured by RI dectection and pH measurement, and (B) experimental flow profile (○) using RI dection and NaCl as tracer at 140°C and a flow rate of 1.1 mL/min (the solid lines are from two calculated D_L/uL values for predicted theoretical flow patterns).

Because the pretreatment experiments were to be conducted at elevated temperatures, it was desirable to study the flow characteristics under reaction conditions. Moreover, because it was preferred that reactions did not take place during the flow characteristics studies, we used NaCl as an inert tracer. To justify the use of NaCl in place of H_2SO_4, we pumped a NaCl solution and H_2SO_4 through the reactor at identical (20°C, 8.8 mL/min) conditions. The two effluent response curves were nearly identical (data not shown), indicating that the inert tracer (NaCl) and H_2SO_4 travel through the reactor with practically the same degree of deviation from ideal plug flow.

Although the highest temperature employed in the pretreatment tests was 170°C, a temperature of 140°C and a flow rate of 1.1 mL/min (linear flow rate of 0.26 cm/min) were used in the subsequent flow studies because of pressure limitations imposed by the flow cell in the RI detector. Figure 3B shows the RTD curve for NaCl under these conditions as well as the best-fit (or best-fit average) reciprocal Peclet number. From the data in Fig. 3B and additional data not shown, an extrapolated inverse Peclet number of 0.05 was obtained to approximate nonideal flow in the percolation reactor under the pretreatment conditions described previously.

Table 1
Chemical Composition of Debarked Hybrid Poplar DN-34 and the Pretreated Solid and Prehydrolyzate Fractions

	Native poplar DN-34, 100 g, bone dry basis	Pretreated biomass, normalized to 100 g of starting bone dry biomass	Prehydrolyzate, normalized to 100 g of starting bone dry biomass corrected for hydration
Klason lignin (unextracted biomass)	26.7	N.D.	N.D.
Soluble lignin	2.6	N.D.	N.D.
Ash	2.1	N.D.	N.D.
Glucan/glucan equivalents	41.7	35.7	5.8
Xylan/xylan equivalents	15.6	0.9	14.4
Galactan/galactan equivalents	1.0	0	1.0
Arabinan/arabinan equivalents	1.2	0	1.2
Mannan/mannan equivalents	3.0	0	3.0
Uronic acids	2.3	N.D.	N.D.
Acetyl groups	0.5	N.D.	N.D.

N.D. = not determined.

Although this extrapolated value had obvious limitations, it was used as a first approximation for conversion calculations of xylan to xylose for non-ideal flow in the percolation reactor. It should also be noted that the non-idealities in flow characteristics in the percolation reactor used in this study were treated using the approach for reactions in homogenous reactors. For the systems in this study, the reactions, involving a fluid and a solid phase, were heterogenous, and as the reactions proceeded, the void volume increased slightly because of the solubilization of hemicellulose fraction in the substrate. The increase in void volume (estimated to be a 3% increase), however, was rather limited.

Two-Temperature Pretreatment Experiments

The dilute sulfuric acid prehydrolysis of hybrid poplar wood using the two-temperature regime was conducted in triplicate with the averaged mass compositions of the starting biomass, the pretreated lignocellulosic residue, and the prehydrolyzate summarized in Table 1. (It should be noted that the process material balance with the data presented cannot be closed. Material balance closures are presented only for the carbohydrate fractions. This has been done to protect potentially proprietary information.) The native composition of the DN-34 debarked wood differs significantly in its Klason lignin, ash, and glucan contents from previously studied

hybrid poplar substrates and aspen wood from this laboratory (*4,7*). The reduced glucan content will affect the potential for ethanol yield on a wt% basis, whereas the increased lignin and ash contents will have plant design ramifications on cellulase adsorption (*33*), power generation, and waste disposal considerations in the proposed NREL wood-to-ethanol plant design (*34*).

As shown in Table 1, solubilization of 13.9% of the glucan (recovered both as glucose and oligomeric glucose in the prehydrolyzate) is comparable to the reported 10% of the cellulose that is assumed to hydrolyze at 50 times the rate of the rest of the cellulose (*35*). Actual recoveries of hemicellulosic sugars (released as oligomers and monomers) in the prehydrolyzate were 92.0 ± 1.6% for xylose (determined from three independent prehydrolysis runs), and 90–100% for galactose, mannose, and arabinose. Thus, xylose equivalents released into the prehydrolyzate using the two-temperature regime are extremely high. The high xylose yields obtained in the present study as compared to those previously reported for a batch reactor (*4,5,7,8*) are highly desirable from the standpoint of maximizing ethanol yields in a wood-to-ethanol process. The observation of high yields attributable to the two-temperature concept as compared to the use of a percolation reactor needs to be investigated further, because in percolation reactor operation, xylose released from xylan is removed from the reaction zone as it is released. In straight batch-wise pretreatments, xylose released from xylan stays in the system throughout the pretreatment time, thus resulting in greater xylose degradation. The two-temperature pretreatment experiments also resulted in small amounts of furfural (2% of the xylan), hydroxymethyl furfural (0.4% of the glucan), and acetic acid (ca. 0.04 wt%) accumulated in the prehydrolyzate.

In the present study as well as in our previous studies (*4,5,7,8*), we have chosen to use a particle size range in which mass and heat-transfer limitations would not affect the hydrolysis or degradation reactions. In scaling up of the percolation process for engineering studies, larger particles would need to be addressed. In addition, scaled-up percolation reactors would likely deviate further in nonideal flow characteristics. The effect of these factors will be investigated in our future studies.

The quantity of oligomeric xylose formation in the hydrolyzate relative to the monomeric xylose is seen in Fig. 4. The HPLC peak at 13.74 min in Fig. 4A, which has a relative peak height of 180, is the xylose determined directly from the neutralized prehydrolyzate. The HPLC peak at 13.70 min in Fig. 4B, which has a relative peak height of 320, is the xylose determined from a 4% sulfuric acid hydrolyzed aliquot of the original prehydrolyzate (posthydrolysis has been shown to hydrolyze all oligomeric carbohydrates to their respective monomers, while minimizing monomer degradation [*25*]). Thus, approx 44% of the hydrolyzed xylan is released into the prehydrolyzate as oligomers. Further studies are needed to elucidate the degree of polymerization (DP) profile of the released oligomers.

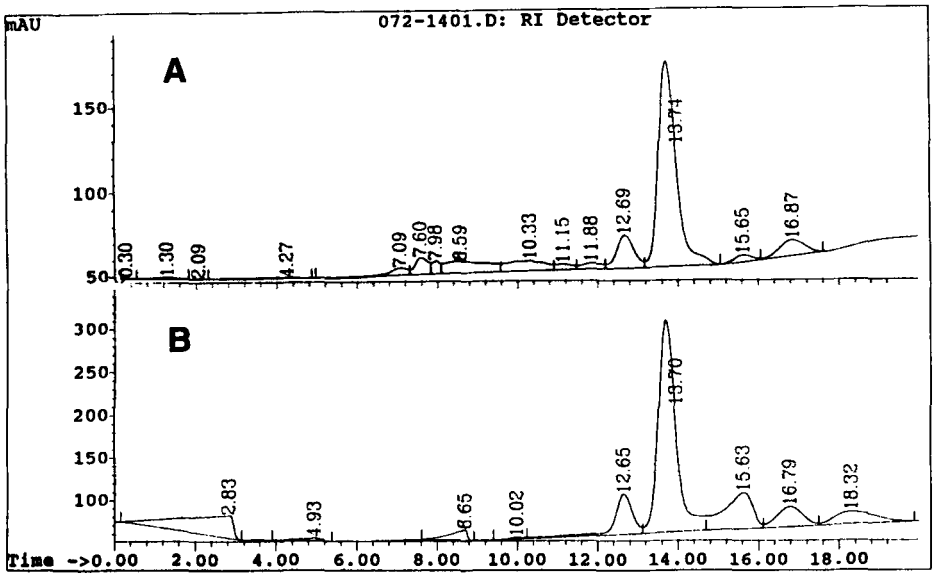

Fig. 4. HPLC chromatograms of sugar profiles from the prehydrolyzate: (A) the chromatogram from the neutralized prehydrolyzate, and (B) the chromatogram from the acid-treated prehydrolyzate.

The impact of the presence of oligomeric xylose in the prehydrolyzate on its fermentability to ethanol needs to be addressed. It has been reported that the dimers and trimers, but not higher DP, of xylose can be fermented by certain microorganisms, such as ethanologenic strains of *Klebsiella oxytoca* (*36*). Therefore, if higher DP values of xylose are obtained, a post-hydrolysis of the prehydrolyzate must be performed (unless a microorganism is found to convert xylose of higher DPs). From a process standpoint, this could possibly be accomplished by holding the effluent from the pretreatment reactor at a temperature below 140°C for a residence time necessary to form the fermentable low DP oligomers (monomers, dimers, and trimers) of xylose without the further degradation of xylose.

The one major drawback to a percolation reactor configuration, as compared to the NREL continuous-screw pretreatment reactor, is that a more dilute sugar stream is produced because a solids loading of higher than 15–16 wt% is probably not possible in a percolation reactor (15 wt% was used in the present study). In comparison, 35 wt% is used in the NREL design (*6*,*34*). In addition, the present study uses two total voids of hydrolysis liquor. However, because of the increased recovery of xylose and the other sugars in the prehydrolyzate, a sugar stream of approx 2.5 wt% was still produced.

The enzymatic digestibility of cellulose in the pretreated hardwood as compared to an α-cellulose control reached 80% completion in 3 h and near-quantitative conversion (95.0% of theoretical) in 24 h (Fig. 5A). Although,

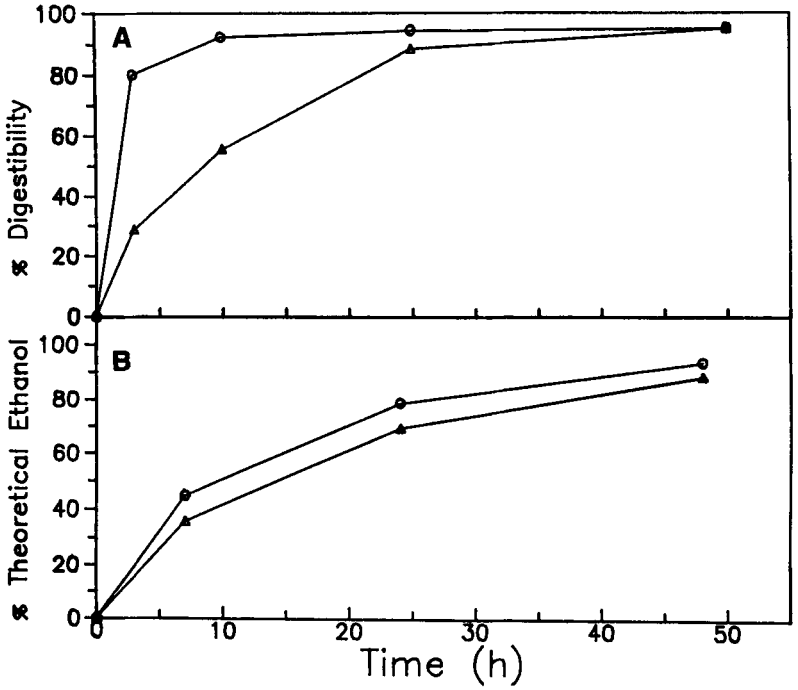

Fig. 5. Enzymatic digestibility and SSF results: (A) the enzymatic digestibility of α-cellulose (△) and pretreated hybrid poplar wood (○) as a function of time, and (B) % theoretical ethanol production from α-cellulose (⊕) and pretreated hybrid poplar wood (○) as a function of time.

as in our previous studies (*4,5,7,8*), a very high cellulase loading was used, the pretreated lignocellulosic residue showed a significantly faster rate of saccharification than either the α-cellulose control or any previously studied hardwoods (*4,5,7,8*). The increased rate was probably not a result of the extent of xylan removal because the composition of the pretreated residue was very similar to the Klason lignin, glucan, and xylan contents of previously studied hardwoods pretreated in a batch reactor (*4,5,7,8*). Therefore, additional reactions may have contributed to the development of porosity and, thus, enhanced cellulose accessibility during dilute-acid pretreatment (*12*).

In order to investigate if this increased digestibility would translate into an increased rate and extent of conversion of the glucan into ethanol, an SSF investigation was initiated. In Fig. 5B, the rates of ethanol synthesis from the pretreated wood are compared with the rate of the control substrate, α-cellulose. As can be seen, the rates and conversion of the pretreated wood were comparable to, if not better than, α-cellulose. In 48 h, 93.5% of the cellulose in the pretreated wood was converted to ethanol. These results, both in terms of yield and SSF time, are far superior to the results obtained from three other poplars pretreated using a batch reactor,

and using similar cellulase and β-glucosidase loadings (*27*). There was excellent agreement between the straight enzymatic saccharification (Fig. 5A) results and the SSF results in that in both studies, approx 94–95% of the available glucan in the pretreated substrate was converted in the former test to glucose and in the latter to ethanol.

In addition to the significantly increased xylose equivalent yield and SSF rate and yield, the two-temperature percolation reactor pretreatment technique appears to present opportunities for other process improvements. This pretreatment technique inherently produces a xylose stream separate from the pretreated solid with the pretreated solid being washed free of any fermentation inhibitors (because process water would be used at the end of the pretreatment to flush out xylose and, thereby, acid). Our SSF test, using pretreated wood discharged from the percolation reactor without further washing, in fact, has demonstrated this fermentation compatability of the pretreated wood. Another potential benefit is that the byproduct gypsum produced from neutralizing the xylose stream can be removed readily to produce a xylose stream free of any solids. This xylose stream may be more amendable to any process that addresses the fermentation inhibitors that may be present. Currently, we are looking closely into these potential benefits, and concurrently, an engineering study is just getting under way to project the possible impact of the proposed pretreatment technique on the NREL reference case wood-to-ethanol process (*34*).

CONCLUSIONS

The study reported in this article is still ongoing. Additional process information is being accumulated, and the process economics are yet to be investigated. Under the conditions studied, the following tentative concluding remarks can be made of the two-temperature percolation reactor pretreatment technique:

1. Despite the narrow particle size distribution (−60 to +80 mesh [0.18–0.25 mm]) of the biomass substrate used, the flow characteristics in the current NREL-designed percolation reactor show some deviation from ideal plug-flow conditions.
2. The two-temperature percolation reactor pretreatment results in xylose equivalent yields as high as 92% of theoretical.
3. Pretreatment with a percolation reactor using the two-temperature regime results in a significant fraction (approx 44%) of the solubilized xylan in the form of oligomers.
4. The enzymatic release of glucose from the two-temperature percolation reactor pretreated wood approaches a near quantitative yield of 95%.

5. Using an SSF protocol, a yield of 94% of theoretical conversion to ethanol is achievable from the two-temperature percolation reactor pretreated wood glucan in 48 h at 38°C.

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