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Optimization of Reverse-Flow, Two-Temperature, Dilute-Acid Pretreatment to Enhance Biomass Conversion to Ethanol

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ABSTRACT

A reverse-flow, two-temperature dilute-acid prehydrolysis process of commercial yellow poplar sawdust using two percolation reactors was designed to simulate countercurrent flow of the biomass solids and prehydrolysis liquor, and to exploit the xylan biphasic kinetics. Lower temperatures (150–174°C) are initially applied to hydrolyze the easily hydrolyzable xylan, and higher temperatures (180–204°C) are applied to hydrolyze the remaining xylan. Two reactors were used to optimize each temperature range, using varying concentrations of sulfuric acid from 0.073–0.73 wt% and reaction times. Yields of soluble xylose, as high as 97% of theoretical, expressed as monomeric and oligomeric xylose, have been achieved with only 2.9% of the xylan being degraded to furfural, at concentrations of total potential sugar between 2.4 and 3.7 wt% before flashing. Depending on the combined severity of the acid concentration, residence time of the solids and liquor, and temperature of prehydrolysis, 81–100% of the hemicellulose, 3–32% of the glucans, and up to 46% of the Klason lignin could be solubilized. The lignocellulosic substrate produced from the pretreatment is readily converted to ethanol at a yield of approx 91% of theoretical, with ethanol concentrations of up to 4.0 wt% in 55 h via a simultaneous saccharification and fermentation (SSF) process. In terms of xylose recovery and ethanol production level and rate, the present results are far superior to those previously reported using a single-temperature, dilute-acid pretreatment.

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

INTRODUCTION

Energy sources alternative to petroleum, especially those that are renewable, have been a subject of intense research interest in recent years. Ethanol production from lignocellulosic biomass is one area that has received considerable attention. Ethanol can be used as a transportation fuel in at least three forms: as hydrous

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ethanol (95% ethanol and 5% water), in ethanol–gasoline blends (e.g., 10% ethanol and 90% gasoline), and as a feedstock material for producing the high-octane, oxygenated fuel additive ethyl tertiary butyl ether (ETBE) *(1)*. These uses may displace the use of petroleum-derived gasoline, boost the octane number of gasoline, improve the quality of automobile emissions, and result in a net reduction in the current CO_2 accumulation in the atmosphere. These effects appear to be particularly important for the US because:

1. With the adoption of the 1990 Clean Air Act Amendments (CAAA) that mandate many urban areas to have oxygenates added to gasoline, ethanol can be used to meet the CAAA requirements;
2. Enough ethanol might be produced from lignocellulosic biomass to replace all gasoline used *(2)*;
3. Energy use by the transportation sector accounts for more than 60% of the total petroleum consumption; and
4. Imported oil accounts for a significant fraction of the US foreign trade deficit *(3)*.

Lignocellulosic biomass consists of cellulose fibers embedded in a sheath of hemicellulose and lignin. Dense binding among cellulose chains presents additional constraints to the hydrolysis of cellulose. To convert the carbohydrates in lignocellulosic biomass to ethanol, a dilute-acid pretreatment effectively removes the lignin-hemicellulose protective shield and renders the remaining cellulose amenable to enzymatic conversion to glucose *(4–11)*. The acid-catalyzed removal of hemicellulose in hardwoods and agricultural residues follows biphasic kinetics *(4,10,12–14)*. As a result, xylan in lignocellulosic biomass probably consists of an easy-to-hydrolyze and a hard-to-hydrolyze fraction *(4,12–15)*, and can thus be modeled as two parallel pseudo-first-order reactions of xylan-solubilization yielding oligomeric xylose. These are followed by xylose formation with subsequent degradation, and have been modeled accordingly *(16,17)*. The biphasic nature of hardwood xylan solubilization suggests using a reactor configuration that exploits these kinetics, which should produce higher yields of the solubilized carbohydrates. Additionally, because the hydrolysis reactions are all pseudo-first-order, countercurrent operation will produce higher yields of the desired carbohydrates than does a cocurrent mode of operation *(18)*.

Researchers have reported that yields of solubilized xylose equivalents of 92% of theoretical can be achieved by applying a nonuniform temperature policy to address the biphasic xylan *(11,19,20)*. In this study, further improvements and optimization of the performance parameters using percolation reactors in a "reverse-flow" mode of operation to simulate countercurrent flow was used to increase further the solubilized xylose equivalent yield and concentration. The effects of particle size, dry vs wet packings before prehydrolysis, temperature, acid concentrations, and residence time of the acid catalyst and the solids were all studied to optimize the process in terms of sugar yield and concentration in the prehydrolyzate.

MATERIALS AND METHODS

Materials

Liriodendron tulipifera L. (yellow poplar) sawdust substrate was provided by Eric Miller of Saw Miller Inc., Haydenville, OH, and was shipped in 1-t parcels from

Lannes Williamson Pallets, Inc., Southside, WV, as fresh green sawdust with a moisture content of 43 wt% when received at the National Renewable Energy Laboratory (NREL). The sawdust was then double-bagged in plastic and stored frozen at –20°C. The chemical composition of a representative sample of the sawdust after being milled to pass through a 2-mm screen (*see below* for a description of the mill) was 41.9 wt% glucan, 15.5 wt% xylan, 2.7 wt% mannan, 0.8 wt% galactan, 0.6 wt% arabinan, 23.4 wt% Klason lignin (determined on unextracted sawdust), 3.2 wt% acid-soluble lignin, 0.6 wt% ash, 3.4 wt% acetyl groups, and 7.9 wt% others (most likely extractives and uronic acids). For pretreatment runs, the sawdust was thawed at room temperature and separated into two size fractions using a portable sieve shaker (Tyler Industrial Products, Mentor, OH) equipped with a 4-mm mesh screen (USA Standard Testing Sieves). The material that passed through a 4-mm screen (87% of the feed material) was set aside to be mixed with the milled overs. The overs (13% of the feed material) were milled using a laboratory knife mill (Thomas-Wiley Laboratory Mill, Arthur H. Thomas Co., Philadelphia, PA) equipped with a 6-mm rejection screen. These milled overs were manually mixed with the 4-mm screened material and used for experiments without further processing; thus, 100% of the commercial sawdust was thus used.

A liquid cellulase preparation (CPN International, Jupiter, FL) was stored at 4°C until used. The specific activities of the cellulase and β-glucosidase enzymes were 88 filter paper units (FPU)/mL and 230 IU/mL, respectively *(21)*. The yeast used in the SSF experiments was *Saccharomyces cerevisiae* D_5A *(22)*. Cellulose powder (α-cellulose, Sigma Chemical Co., St. Louis, MO) was used as a control substrate. The remaining chemicals were purchased from national laboratory supply houses.

Analytical Methods

Compositional analyses of the pretreated biomass and the prehydrolyzate were carried out by standard methods as previously described *(4-7,11,23–25)*. Before enzymatic saccharification and SSF testing of the pretreated sawdust, the particle size had to be reduced for maximum conversion of the glucan to glucose *(26,27)*. Thawed pretreated chips and 3 vol of deionized (DI) water were blended for 5 min in a single-speed laboratory Waring blender, which reduced the chips to a very fine slurry. The solids were separated from the water by filtration.

Enzymatic hydrolysis was performed as previously described *(4,5,9)*, except that cellulase enzyme loading was approx 60 FPU/g cellulose with β-glucosidase at approx 157 IU/g cellulose. This level of cellulase is higher than the FPU/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 provide additional information on the fermentability of the pretreated substrate. SSF was carried out in 250-mL flasks outfitted with stoppers constructed to vent CO_2 through a water trap as previously described *(28)*, with minor modifications. The cellulase preparation was used at a concentration of 25 FPU/g cellulose (16.8 FPU/g bone dry pretreated wood) with a substrate loading of approx 8 wt% cellulose (11.9 wt% solids). All SSF experiments were run at 38°C, in a laboratory rotating incubation at 150 rpm, an initial pH of 5.0, and with an α-cellulose control. Glucose and ethanol concentrations were monitored by using a YSI model 2700 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Ethanol levels were confirmed by gas chromatography as previously described *(28)*.

EXPERIMENTAL

The present study was undertaken (1) to further optimize process parameters for scale-up of a previously described two-temperature, dilute-acid percolation pretreatment of hardwood sawdust *(11)*, and (2) to investigate a reported alternative reactor configuration (reverse-flow, two-stage, dilute-acid prehydrolysis), which has been shown through modeling to generate higher concentrations of xylose equivalents in the prehydrolysis liquor and in higher sugar yields *(29)*. We sought to address concerns of the limited size of the previous pretreatment reactor (2 in length × 1 in diameter), the small particle size (–60 mesh to +80 mesh), and the limited amount of pretreated biomass that could be generated from our prior reactor system. We therefore chose a reactor of a length/diameter (L/D) ratio of 6 with reactor volume 24-fold larger than our previous reactor, and used particle sizes (–4 mm mesh) that would require less energy for milling to obtain more realistic data to be used in the NREL biomass-to-ethanol economic analysis model *(30)* to assess the viability of this process. Before pretreatment experiments could begin, we initiated a study to quantify the flow behavior of the acid catalyst through the sawdust bed as a function of temperature and liquid flow rate. We then assessed the quality of the pretreated residue by both enzymatic saccharification and converting the glucan to ethanol via SSF.

Reverse-Flow, Two-Temperature Percolation Reactor and Liquor Collection System

The design of the percolation reactor (Fig. 1) is a modified 2 × 12-in. Hastelloy C276 Modcolumn (Anspec Inc., Ann Arbor, MI) with titanium dispersion (60-μ pore size) and retention (20-μ pore size) frits. Three 1/8-in. thermocouples (Hastelloy C sheaths, Omega Engineering Co., Stamford, CT) were installed equidistant along the length of the reactor to monitor temperature. Hastelloy C276 tubing (1/8-in. [1.6-mm] od × .03-in. [0.8-mm] id, 1/4-in. [1.6-mm] od × .03-in. [0.8-mm] id) was used to connect the reactor with other components of the system as well as for the preheating coil (Colorado Springs Windustrial Co., Colorado Springs, CO). The preheating coil was long enough to allow the incoming prehydrolysis liquor at a flow rate of 200 mL/min to be at the desired prehydrolysis temperature. The three-way Hastelloy C valves were obtained from Valco Valve Corporation, Houston, Texas (the third port of the three-way valves was not used in the current study). Teflon-lined 304 stainless steel collection cylinders (1- and 1/2-gal capacities) were obtained from Denver Valve and Fitting, Denver, CO. A schematic of the flow system and equipment design used for the reverse-flow experiments is shown in Fig. 2. For the flow characteristic studies, only one reactor was used as previously described *(11)*.

To determine the void volume of the packed bed, the reactor was charged with 191.7 g (114.7 g bone dry basis) of thawed, 4°C refrigerated screened sawdust, and the void volume was determined as previously described *(11)* using a Dynamax Model SD-1 HPLC pump (Rainin Instrument, Inc., Ridgefield, NJ). The total void volume (interparticle and intraparticle) of the water-saturated, deaerated, packed reactor was 470 mL.

Flow Characterization Studies

To characterize the flow in the reactor (i.e., how the observed flow varies from ideal plug flow), we used the NaCl step-change method and recorded the resi-

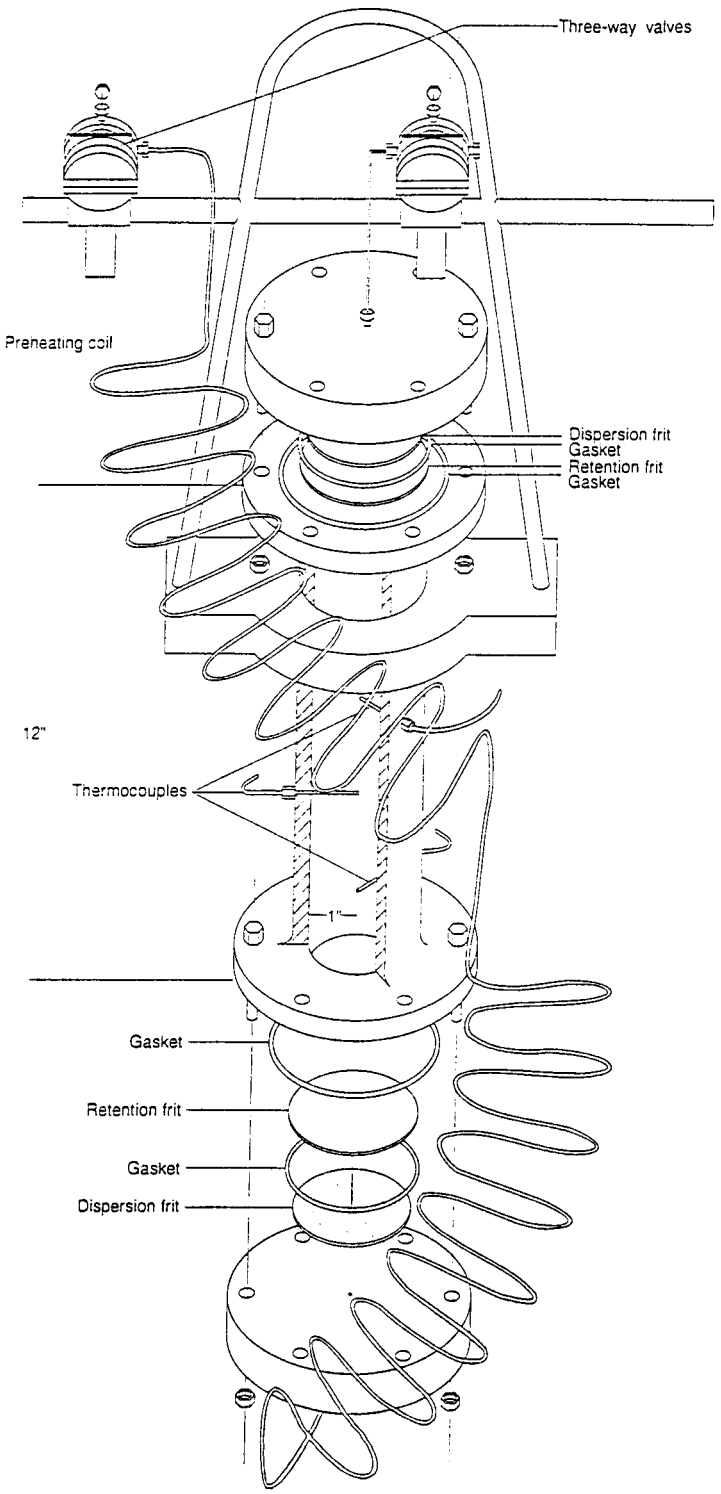

Fig. 1. Schematic diagram of the percolation reactor.

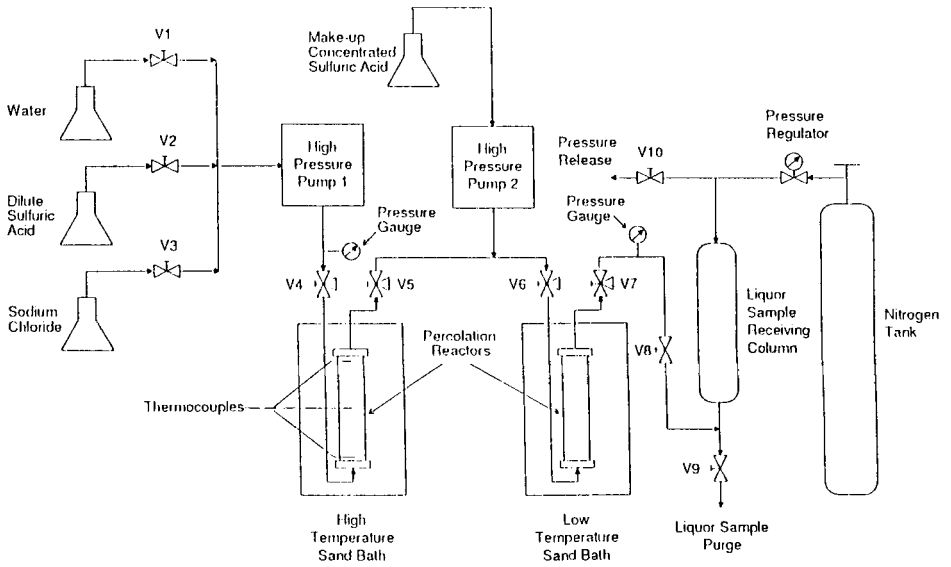

Fig. 2. Schematic diagram of the laboratory reverse-flow, two-temperature pretreatment reactor apparatus.

dence-time distribution (RTD) of the eluant NaCl in a similar manner as previously described *(11)*. We used 160°C and a flow rate of 100 mL/min measuring the NaCl (0.25*M*) RTD response via a density differential (Biotage model mPDS, Charlottesville, VA).

A computer reproduction of a tracing of the 0.25*M* NaCl concentration distribution as a function of void volumes pumped is shown in Fig. 3. Significant deviation from a plug flow plot is observed, indicating nonideal dispersion of the salt front. This plot was used to calculate the make-up acid pumping rate in the reverse-flow dilute-acid pretreatment runs described below under step 2 (*see* more detailed explaination under step 2: "Steady-State" Reverse-Flow, Two-Temperature Pretreatment).

Dilute-Acid Pretreatment Studies: Three Steps in Generating Simulated Steady-State, Reverse-Flow, Two-Temperature Samples

Step 1: Generating Partially Pretreated Sawdust at Low Temperature to be Used for the Reverse-Flow High-Temperature Reactor (Fig. 4)

Two percolation reactors were charged with biomass and placed in two sand baths with valves to the reactors closed. (Note: The packed bed was not prewet or deaerated to pretreat the biomass.) One sand bath was set at the "low" temperature mode (150 or 174°C), and the other at the "high" temperature mode (180, or 204°C). Once the reactors were at the "low" temperature, reactor 1 was connected on line as previously described *(11)*, whereas reactor 2 was set aside. The entire system was overpressurized by 10–20 psig above the steam pressure. Three voids of acid (0.735, 0.404, or 0.0735 wt%, one void to wet the reactor contents, and two voids for collection) were pumped through reactor 1 and collected in the desired solids residence

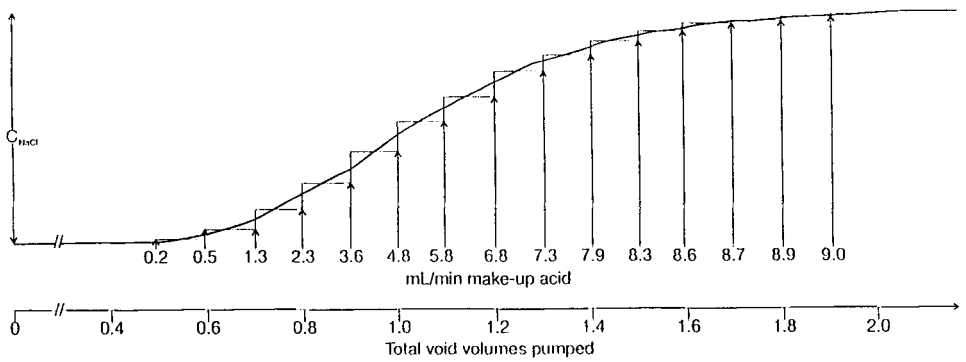

Fig. 3. Percolation flow characteristics using density detection and NaCl as tracer at 160 C and a flow rate of 100 mL/min as a function of total void volumes pumped. Also depicted is a representative step change make-up acid pump rate as a function of total void volumes pumped.

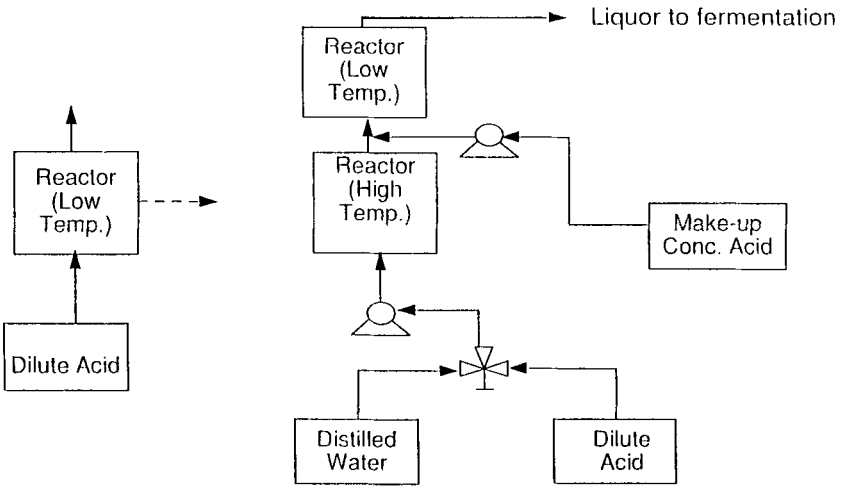

Fig. 4. Schematic of the concept of reverse-flow, two-temperature pretreatment.

time. This prehydrolysis liquor (step 1 liquor) was expelled from the collection column and saved for compositional analysis. V5 was then closed, and V10 opened to release all pressure downstream of reactor 1.

Step 2: "Steady-State" Reverse-Flow, Two-Temperature Pretreatment (Fig. 4)

Reactor 1 was moved to the high-temperature sand bath, while reactor 2 was placed in the low-temperature sand bath and the system assembled (Fig. 2). The entire system was over-pressurized by 10–20 psig. One void of acid (0.735, 0.404, or 0.0735 wt%, depending on the experiment) was then pumped into reactor 1 in 1/3 residence time of the solids (i.e., one void of liquor was pumped into reactor 1 in 3.33 min for the 10-min solids cook; in 5 min for the 15-min solids cook, and 6.66 min for the 20-min solids cook), which simultaneously wetted reactor 2 with the displaced acid from reactor 1, since the reactors are in series (Fig. 2). Two voids of DI water were then pumped through reactor 1 in 2/3 residence time of the solids (i.e. two voids of liquor were pumped into reactor 1 in: 6.66 min for the 10-min solids cook, 10 min for

the 15-min solids cook, and 13.3 min for the 20-min solids cook) to totally wash the contents of reactor 1. To the diluted acid eluant from reactor 1 was added concentrated make-up acid (0.649, 1.224, 4.595, 6.49, or 12.24 wt% sulfuric acid, depending on the experiment) using increased step change pump rates dictated by the RTD curve (an example of pump rates is seen in Fig. 3), in which after about 0.48 voids of water had been pumped into the high-temperature reactor, the effluent from this reactor, which fed directly to the low-temperature reactor, had to have 0.2 mL/min of concentrated make-up acid added at the "T" to keep the concentration of acid being fed to the low-temperature reactor constant. Then after approx 0.6 voids of water had been added to the high-temperature reactor, 0.5 mL/min of concentrated make-up acid was added to keep the concentration of acid being fed to the low temperature reactor constant. This procedure of increasing the pumping rate of make-up acid as a function of total voids of wash water pumped through the high-temperature reactor was continued until 2.0 voids of wash water had been pumped through the system). Thus, reactor 2 during the entire solids residence time always saw a constant concentration of acid catalyst. The two total voids of liquor collected from this "reverse-flow" stage were expelled from the collection column and set aside for compositional analysis (step 2 liquor). Both reactors were isolated by valving, and reactor 1 was disconnected and cooled. The sugar concentrations and yields in this liquor fraction represented the actual "steady-state" liquor from a semicontinuous countercurrent reactor system.

Step 3: Completing the Prehydrolysis of Reactor 2 for Mass Balance Closure

Reactor 2 was submerged in the high-temperature sand bath and connected as in step 1 above (without the addition "T"). One void of dilute acid was pumped through reactor 2 in 1/3 residence time for the solids, followed by two voids of DI water in 2/3 residence time for the solids to wash out all the acid and solubilized sugars. Reactor 2 was disconnected and cooled.

The liquor from step 3 was saved for analysis. Solid contents of both reactors were expelled, combined, and collected in a glass-sintered funnel of medium porosity. The solid residue was then weighed and chemically analyzed; the three liquor fractions were individually analyzed for sugars, hydroxyl-methyl-furfural (HMF), furfural, acetic acid, and acid-soluble lignin. The additive quantities of the components from all three liquors were then used for total mass balance calculations.

RESULTS AND DISCUSSION

In the present study, a reverse-flow, two-temperature pretreatment using two percolation reactors (which simulates a semicontinuous, countercurrent mode of operation) packed with commercial yellow poplar sawdust was investigated. To define better the experimental conditions that would give high xylose equivalent yields, we used the mathematical modeling of the reverse-flow percolation process using the two-temperature pretreatment of hybrid poplar xylan studied by Auburn University, Auburn, AL (through an NREL subcontracted study). Mathematical simulations *(29)* and our prior study *(11)* have demonstrated 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 (92% of theoretical xylose equivalents) in the prehydrolyzate. We sought to optimize these results not only in terms of total yield, but also in terms of concentration of fermentable sugar in the prehydrolyzate using the concept of reverse flow. The biomass is first treated at a low temperature in the percolation mode. Then it is treated again at a higher temperature. The throughput stream from the high-temperature treatment is again put through a percolation reactor packed with fresh biomass at low temperature. The reacted residue from this low-temperature reactor is then treated with fresh acid at high temperature (Fig. 4). This process is repeated and simulates countercurrent movement of the solids relative to the liquor *(29)*. A further refinement of the process uses process water to wash the acidic medium containing solubilized sugars from the high-temperature reactor after the desired extent of acidic prehydrolysis. To the effluent from the high-temperature reactor (before entering the low-temperature reactor) is added make-up acid to the desired concentration for prehydrolyzing the biomass in the low-temperature reactor. Thus, the contents of the high-temperature reactor (which have been washed *in situ*) can now be added directly to a fermentation vessel to produce ethanol.

The flow characteristics of the catalyst in percolation reactors, which influence observed rates of reactions and thus yields of desired products, are a function of temperature, packing density, flow rate, particle size, and porosity, as well as other parameters, such as moisture content. Additionally, since in a reverse-flow mode of operation with a water-wash step in the high-temperature stage, a make-up acid loop before the low-temperature stage would be required, it was essential to have a method available to quantitate the amount of make-up acid to add to achieve constant acid concentration throughout the lower-temperature stage. We therefore initiated our laboratory investigation with a study of the flow characteristics in the packed-bed reactor under experimental prehydrolysis conditions. However, because solubilized sugar yields and flow patterns are influenced by sawdust particle size, we conducted a literature search to define the largest particle size that could be used without incurring significant losses in sugar yield.

Particle Size Selection

Among the most important parameters to be considered when scaling up from our previous reactor was the selection of the nominal screened chip size to be used in the experiments. Milling sawdust to a size amenable for the enzymatic conversion of the glucan fraction of pretreated hardwood sawdust *(26,27)* requires substantial energy *(31)*. Therefore, our approach was to use the largest critical chip size possible for which diffusion of the solubilized sugar products out of the porous wood will not contribute to degradation losses.

Additionally, bulk packing density was a concern, as was the rate of "wetting" or impregnation of the hydrolyzing liquor as dictated by diffusion and capillary action. Capillary absorption proceeds significantly faster than diffusionary liquid penetration in wood *(32)*, and the rate of diffusion for a solute in wood in the longitudinal direction is more than an order of magnitude greater than either in the radial or in the tangential direction *(32)*. Also, for hybrid poplar, the critical chip size for diffusing hydrolyzed sugars is from 0.29–0.4 cm for two-temperature pretreatments that use 150 or 140°C as the lower temperature, respectively *(33)*. Additionally, the pulping industry uses wood chips that are 3–6 mm thick *(34)*, which agrees well with the above-mentioned critical chip size. Thus, based on the above considerations and

on the fact that 87% of the commercial sawdust passed through a 4-mm laboratory screen and thus only 13% overs would require milling (data not shown), sawdust passing through a 4-mm screen was therefore used for all experiments.

Flow Characteristics in the Percolation Reactor

It has been previously shown that NaCl at low concentrations (10 m*M*) can be used instead of sulfuric acid as a tracer in RTD studies up to 140°C to determine the flow characteristics of the H_3O^+ ion *(11)*. Because our lower prehydrolysis temperatures were 150 and 174°C at flow rates from 91–141 mL/min, we chose to measure the RTD function at 160°C at a flow rate of 100 mL/min (Fig. 3) using 0.25*M* NaCl, which gave a good response using a densitometer to measure the concentration profile. However, the Na^+ ion (at elevated temperatures) causes partial hydrolysis of polymeric carbohydrates *(35)*, and therefore might not be considered an inert tracer. We therefore performed a prehydrolysis at 160°C for 10 min using 0.25*M* NaCl and a dry-packed column and passing three void volumes of liquor through in 10 min to quantitate the catalytic effect of Na^+ ions. Under these conditions, 54% of the xylan is hydrolyzed along with 30% of the lignin, with only 2% of the glucan being hydrolyzed. Therefore, indeed, the observed RTD curve reflects a partially hydrolyzed bed. This may be a fortuitous degree of hydrolysis to simulate what rates of make-up acid to add in the reverse-flow mode, since the xylan in the lower temperature reactor is partially hydrolyzed with one void of acid, which totally wetted the column, and an additional 0.5 void of acid before make-up acid needed to be added (step 2, Materials and Methods). Therefore, although this method of calculating the amount of make-up acid is not totally quantitative, it offers a reasonable approximation of the required mode of addition of make-up acid. An affirmation that the RTD curve is reasonably close to what is experimentally required is the fact that the measured pH of the effluent of the reverse-flow mode of operation (liquor from step 2, Materials and Methods) was nearly identical to that measured from the liquor from step 1 (Materials and Methods) in which no make-up acid was added. The dispersion effects seen in the present study using –4-mm screened material were comparable to those in the previous study using –60 to +80 mesh material.

Scale-Up Effects from Our Prior Reactor

Several concerns from our prior study were addressed in scaling up the reactor 24-fold in total capacity. First, we chose an L/D ratio of 6 in the present reactor, instead of 2, to examine whether a different L/D ratio affected the flow characteristics and channeling. Despite the different particle size (–4 mm instead of –60 to + 80 mesh), we obtained similar RTD curves with the larger reactor packed with larger mesh particles (data not shown). Therefore, wall effects and channeling do not significantly affect the flow patterns in the packed sawdust bed; rather, the flow characteristics are predominantly dictated by the intraparticle flow.

Second, we not only sought to reproduce the previously published results using the larger reactor, but also to compare pretreatment results using a dry-packed bed (just before adding acid) to results using a totally water-saturated and deaerated bed. Using a dry-packed bed allows higher sugar concentrations in the prehydrolysis liquor, which is desirable for fermentation and downstream processing. Table 1 lists the prehydrolysis conditions used to reproduce our prior results and further optimize the process parameters, and Table 2 tabulates the mass balance results of

Table 1
Prehydrolysis Parameters

Run, (code)	Temp 1, C°	Temp 2, C°	Reaction time 1, min	Reaction time 2, min	Acid concentration, wt%
Scale-up reproducibility					
1 (inhpop 01) (water-saturated)	140	170	30	20	0.735
2 (inhpop 05) (dry-packed)	140	170	30	20	0.735
Factorial optimization of reverse-flow pretreatment					
3 (inhpop 08)	150	180	10	10	.0735
4 (inhpop 15)	150	200	10	10	.0735
5 (inhpop 13)	150	180	20	20	.0735
6 (inhpop 09)	150	200	20	20	.0735
7 (inhpop 14)	150	180	10	10	0.735
8 (inhpop 12)	150	200	10	10	0.735
9 (inhpop 11)	150	180	20	20	0.735
10 (inhpop 10)	150	190	15	15	.4015
11 (inhpop 16)	150	190	15	15	.4015
Verification of optimization					
12 (inhpop 17)	174	204	10	10	0.0735

the scale-up study. As can be calculated from Table 2, the mass balance closure on the xylan fraction (%soluble + %insoluble + %furfural) from both run 1 (the water-saturated, deaerated reactor that represents the method used in our prior study *[11]*) and run 2 (dry chip loading) averages only 91.5%, which is well below our average closures of 99.7% from runs 3–12 in Table 2. Nevertheless, similar amounts of xylan are solubilized irrespective of the wetting mode (86.5% for the water-saturated reactor and 83.0% for the dry packing). Moreover, the 86.5% xylose equivalents solubilized is similar to the previously reported 92% of the xylose equivalents solubilized using the small reactor. This demonstrates that under reaction conditions, the acid catalyst reaches sites of reaction via capillary or diffusion mechanisms at rates that cannot be differentiated in the two experimental setups.

Reverse-Flow, Two-Temperature Pretreatment Optimization Experiments

To increase the solubilized sugar concentrations and reduce the required reaction times of the above pretreatment regime, a reverse-flow, two-temperature mode of operation (Fig. 4), which simulates countercurrent operation *(29)*, was employed using 150°C as the low prehydrolysis temperature. A factorial experimental design involving nine runs was developed. Acid concentrations varied from 0.0735–0.735 wt%, the high prehydrolysis temperature from 180–200°C, and the solids residence time in each stage from 10–20 min, as shown in Table 1 (runs 3–11 under Factorial optimization of reverse-flow pretreatment). As shown in Table 2, all three major polymers are solubilized to varying extents, with xylan being preferentially hydro-

Table 2
Mass Balance Data

	Xylan				Glucan				Lignin	
Run,code	%Insoluble	%Soluble	%Monomer	%Furfural	%Insoluble	%Soluble	%Monomer	%HMF	%Insoluble	%Soluble
Scale-up reproducibility										
1 (inhpop 01) (water saturated)	0.8	86.5	92.0	4.4	90.1	10.3	96.6	0.2	59.7	35.8
2 (inhpop 05) (dry packing)	0.6	83.0	95.2	7.7	87.5	12.2	95.2	0.5	64.6	41.0
Factorial optimization of reverse-flow pretreatment										
3 (inhop 08)	12.6	86.0	32.1	1.8	88.7	2.8	88.9	0.1	71.2	40.8
4 (inhpop 15)	4.6	86.1	58.5	2.8	94.8	6.0	56.9	0.1	66.2	29.7
5 (inhpop 13)	13.3	80.8	44.3	1.0	98.4	3.2	36.8	0	73.6	26.3
6 (inhpop 09)	2.1	94.0	47.4	3.6	89.6	8.1	87.9	0.3	60.9	46.5
7 inhpop 14)	6.2	90.4	N.R.[a]	4.9	89.2	12.3	100	0.2	69.8	32.9
8 (inhpop 12)	0	98.4	73.4	4.4	61.8	31.7	91.9	1.5	65.1	52.5
9 (inhpop 11)	0.7	101.3	79.6	5.8	72.9	17.7	92.3	0.6	68.6	40.6
10 (inhpop 10)	1.0	91.8	63.8	3.6	83.2	14.0	78.4	0.5	59.8	46.3
11 (inhpop 16)	2.4	94.4	68.2	3.7	81.7	14.0	91.0	0.3	68.1	33.3
Verification of optimization										
12 (inhpop 17)	0.5	97.0	36.0	2.9	91.6	9.2	77.0	0.3	57.7	35.7

[a]N.R. not reported.

lyzed. Mass balance closures for each component (%insoluble + %soluble + % as a degradation product) varies from run to run and does not necessarily depend on the combined severity *(36)* of pretreatment. Mass balance closures for xylan vary from a low of 93.5% to a high of 107.8%, for glucan from 91.2–101.7%, and for lignin from 95.9–117.6%. The respective means and standard deviations of these closures are 99.7 ± 4.3%, 97.1 ± 4.0%, and 105.8 ± 6.6%, indicating that experimental reproducibility was at a satisfactory level in this study. Additionally, when pretreatment conditions are severe, as in run 8, we have observed lignin closures routinely above 100%, probably because of insoluble complexes of humic substances and sugar degradation products, which are quantitated as Klason lignin.

As shown in Table 2, when the combined severity was relatively low (runs 3–7), only 3–12% of the glucan (recovered both as glucose and oligomeric glucose in the prehydrolyzate) was hydrolyzed. This correlates well with the reported 10% of the cellulose that is assumed to hydrolyze at 50 times the rate of the rest of the cellulose *(37)*. However, as the combined severity increased, up to 32% of the glucan fraction was hydrolyzed (run 8), but with very low conversion to HMF. Except for two runs (run 4 and 5), most hydrolyzed glucan was recovered in the monomeric form. Lignin solubilization in all runs was relatively high, varying from 26% (run 5) to a high of approx 40%–46% (run 6 and 10), depending on which substrate (insoluble vs soluble fractions) is quantitated. This is significantly higher than that previously reported for batch dilute-acid pretreatment *(4–7)*, but similar to the hydrothermolysis experiments using a percolation reactor *(38)*. Not only would this solubilized lignin be very toxic to fermentative organisms *(39)*, but the lignin would have to be recovered for its energy content either by producing methane in the waste-water treatment facility or by separating, drying, and burning to generate power in the proposed NREL wood-to-ethanol plant design *(30)*.

One promising aspect of this pretreatment configuration is the very high yields of xylose equivalents recovered in the prehydrolysis liquor. Yields of xylose equivalents (released as oligomers and monomers) range from 81% (run 5) to approx 100% (run 9), with yields of more than 90% being common (run 6, 7, 8, 10, and 11). The concentration of total sugar equivalents in these liquors vary from 2.4 wt% for run 3, to 3.7 wt% for run 8 before any process flashing of the liquor would take place (data not shown). The high xylose yields obtained in the present study compared to those previously reported for a batch reactor *(4,5,7,8)* are highly desirable for maximizing ethanol yields. Also small amounts of furfural (5.8% of the xylan in the worst case, run 9), HMF (1.5% of the glucan in the worst case, run 8), and acetic acid (<0.4 wt%, data not shown) accumulated in the prehydrolyzate.

The fraction of monomeric xylose in the hydrolyzate relative to the oligomeric xylose, as shown in Table 2, varies from a low of 32.1% (run 3) to a high of nearly 80% (run 9). Further studies are needed to elucidate the degree of polymerization (DP) profile of the released oligomers. The impact of the presence of oligomeric xylose in the prehydrolyzate on its fermentability to ethanol needs to be addressed. The dimers and trimers, but not higher DP, of xylose can be fermented by certain microorganisms, such as ethanologenic strains of *Klebsiella oxytoca (40)*. Therefore, if higher DP values of xylose are obtained, a posthydrolysis of the prehydrolyzate must be performed. From a process standpoint, this could possibly be accomplished by holding the temperature below the hydrolysis temperature for a residence time necessary to form the fermentable low DP oligomers (monomers, dimers, and trimers) of xylose without further degrading the xylose. Alternatively, the use of xylanases can accomplish the same task.

Another promising aspect of this pretreatment configuration is that by selecting a set of different operational parameters (which all give high yields of xylose equivalents in the liquor), different ratios of the solubilized sugars in the prehydrolyzate can be achieved. This option of varying ratios of sugars can be extremely important in effectively converting the C_5 fraction to ethanol *(41)*.

Verification of Optimal Reverse-Flow Process

From the results of the factorial design, we sought to optimize the process around three criteria: low acid, minimal residence time of both the liquor and the solids, and high yield of xylose equivalents in the prehydrolysis liquor. We therefore chose a 10-min residence time of the solids at each stage, 0.0735 wt% sulfuric acid, 174°C for the lower temperature, and 204°C *(29,33)* for the higher temperature. Using these conditions, near-quantitative recovery (97.0%) of the xylose equivalents was seen in the prehydrolyzate with 36% hydrolyzed to monmers and only 2.9% of the xylan being recovered as furfural, and a total mass balance closure around xylan of 100.4% (run 12, Table 2). Slightly less than 10% of the glucan was solubilized and 36% of the lignin was solubilized (Table 2).

Enzymatic Saccharification and SSF Performance of Pretreated Samples

The enzymatic digestibility of cellulose in the pretreated sawdust substrates reached 84–91% completion in 6 h, except for runs 3 and 5, and all substrates reached quantitative conversion (97.0% to 100% of theoretical) in 24 h (Fig. 5). In comparison, the α-cellulose control at 24 h was 76%. The two substrates that exhibited slower initial hydrolysis rates, but did ultimately digest to completion, had incomplete xylan hydrolysis (Table 2) compared to the rest of the substrates. This slow initial rate might be explained by the degree of porosity found in the two groups of substrates, i.e., the faster hydrolyzing substates could have had larger pores from the dilute-acid pretreatment, thus allowing the cellulase enzymes easier access to the cellulose backbone *(42)*.

To investigate whether this increased rate of digestibility would translate into an increased rate and extent of fermentation of the glucan into ethanol, we conducted an SSF investigation. In Fig. 6, the rates of ethanol production from selected pretreated sawdust substrates are compared to the performance of the control substrate, α-cellulose. The rates and conversion of the pretreated sawdust substrates were better than α-cellulose. With one substrate, run 12, 91% of the cellulose in the pretreated sawdust was converted to ethanol in 55 h at an ethanol concentration in the fermentation broth of 4.1 wt%. The other two substrates achieved slightly more than 90% conversion in 72 h at nearly identical ethanol concentrations of 4.0 wt%. These performance results, both in terms of yield and productivity, exceed those obtained with three other poplars pretreated using a batch reactor and similar cellulase and β-glucosidase loadings *(28)*. The straight enzymatic saccharification (Fig. 5) results agreed well with the SSF results in that more than 90% 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 reverse-flow, two-temperature pretreatment technique appears to present opportunities for other process improvements. It inherently produces a

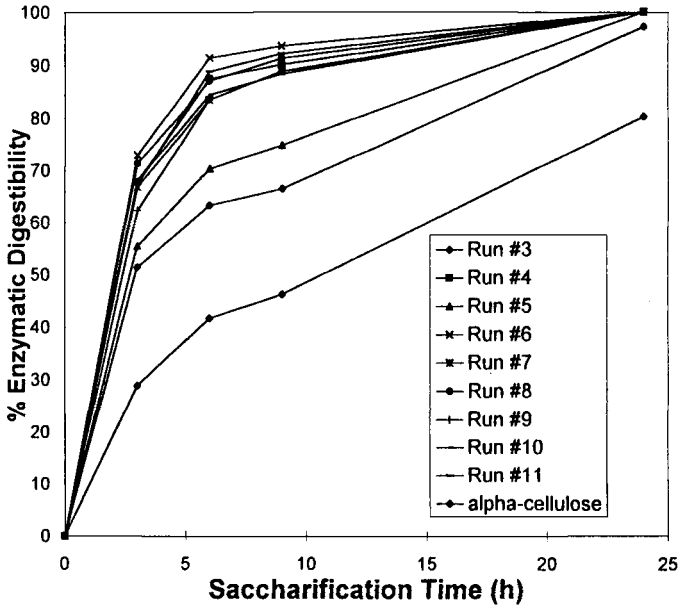

Fig. 5. Enzymatic digestibility of pretreated sawdust substrates as a function of time.

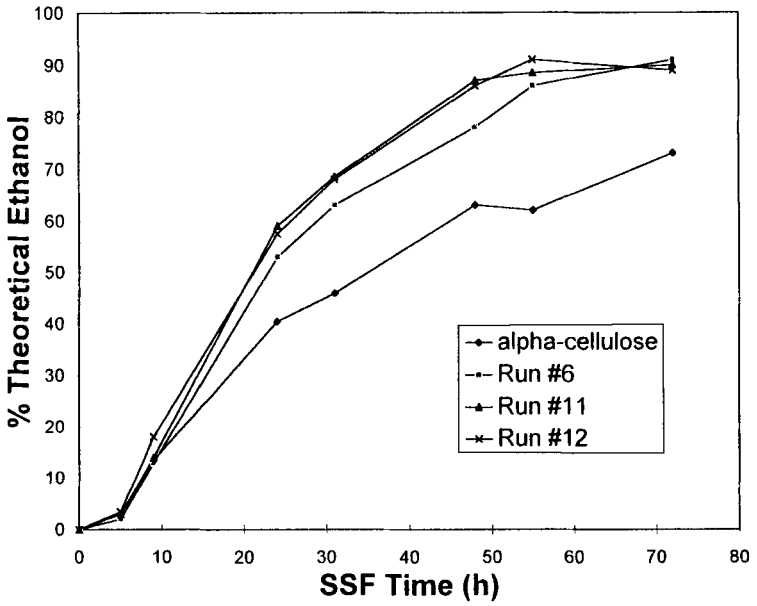

Fig. 6. SSF of selected pretreated sawdust substrates as a function of time.

xylose stream separate from the pretreated solid, with the pretreated solid being washed free of any fermentation inhibitors. Our SSF test, using pretreated wood discharged from the reactors without further washing, demonstrated the fermentation compatibility of the pretreated wood. Also, the byproduct gypsum produced from neutralizing the xylose stream can be removed readily to produce a xylose stream free of any solids. This stream may be more amenable to any detoxification process that addresses the fermentation inhibitors. Currently, we are looking closely into these potential benefits, and an engineering study is under way to project the

possible impact of the proposed pretreatment method on the economics of the NREL reference case biomass-to-ethanol process *(30)*.

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