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Comparison of Yellow Poplar Pretreatment Between NREL Digester and Sunds Hydrolyzer

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ABSTRACT

Single-stage cocurrent dilute acid pretreatments were carried out on yellow poplar *(Liriodendron tulipifera)* sawdust using an as-installed and short residence time modified pilot-scale Sunds hydrolyzer and a 4-L bench-scale NREL digester (steam explosion reactor). Pretreatment conditions for the Sunds hydrolyzer, installed in the NREL process development unit (PDU), which operates at 1 t/d (bone-dry t) feed rate, spanned the temperature range of 160–210°C, 0.1–1.0% (w/w) sulfuric acid, and 4–10-min residence times. The batch pretreatments of yellow poplar sawdust in the bench-scale digester were carried out at 210 and 230°C, 0.26% (w/w) sulfuric acid, and 1-, 3-, and 4-min residence times. The dilute acid prehydrolysis solubilized more than 90% of the hemicellulose, and increased the enzymatic digestibility of the cellulose that remained in the solids. Compositional analysis of the pretreated solids and liquors and mass balance data show that the two pretreatment devices had similar pretreatment performance.

Index Entries: Biomass; ethanol; dilute-acid pretreatment; bioconversion.

INTRODUCTION

The enzymatic utilization of lignocellulosic biomass for ethanol production requires steps to make the recalcitrant cellulose more accessible to cellulase enzymes. This may be accomplished by chemically hydrolyzing the bonds in the lignin–hemicellulose matrix by dilute acid hydrolysis, which allows the subsequent enzymatic conversion of cellulose to glucose to proceed at reasonable rates. The glucose formed can then be fermented

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into useful products. Prehydrolysis of lignocellulosic biomass with dilute sulfuric acid, at temperatures higher than 160°C, effectively hydrolyzes the glycosidic bonds in the hemicellulosic component, and increases the enzymatic digestibilities of the cellulosic component in the remaining pretreated residues *(1–6)*.

Dilute acid prehydrolysis of yellow poplar *(Liriodendron tulipifera)* sawdust was carried out in a series of experiments using the Sunds CD-300 Laboratory Hydrolyzer (Sunds Defibrator, Norcross, GA) installed in the NREL PDU. To conduct experiments at residence times shorter than the unit was designed for, the Sunds hydrolyzer was modified with a custom-built displacement cylinder. High-temperature, short-residence-time pretreatments were investigated at low acid concentrations that would result in savings in catalyst and lime costs, and lower costs for materials of construction for the reactor. It would also reduce the problems and costs associated with handling the gypsum formed during neutralization. Because of the considerable investment in human resources, operating expenses, and quantities of feedstock involved in operating the Sunds hydrolyzer *(7)*, a 4-L-batch steam-explosion reactor (NREL Digester) was designed and installed for conducting optimization experiments at a smaller scale. A series of experiments to test the pretreatment performance of the NREL digester were conducted to compare the possibility of scale-up of this reactor to the pilot-scale Sunds hydrolyzer. Applicability of the bench-scale pretreatment results to the larger-scale reactor would result in considerable savings in resources and feedstock.

This paper reports the compositional analysis of hydrolysate liquors and solids, xylose and carbon mass balance closures, and enzymatic digestibility results from several experimental runs that used dilute acid prehydrolysis of yellow poplar sawdust pretreated in the pilot-scale Sunds hydrolyzer and the NREL digester.

MATERIALS AND METHODS

Feedstock Preparation

Yellow poplar sawdust was obtained from SawMiller (Haydenville, OH) and stored at −20°C in plastic-lined cardboard totes of approx 1000 lbs (~50–55% solids). The totes were thawed for several days before being pretreated in the Sunds hydrolyzer. Thawed totes of sawdust were dumped into a feedhopper bin and metered onto a weighbelt for feedback control of the feed, acid, water, and steam addition rates to the Sunds hydrolyzer. The sawdust used in the NREL digester pretreatment experiments was collected by combining and mixing several buckets-full each of material removed from totes during the experimental runs for the Sunds hydrolyzer.

Large chips, twigs, and branches in the poplar sawdust collected for pretreatment experiments with the NREL digester were removed by

screening through a 7.4-mm screen. The screened sawdust was soaked in 0.3% (w/w) sulfuric acid solution at 60°C for 3 h, then drained overnight to approx 38% solids before pretreatment. The concentration of acid that remained in the chips to be pretreated was determined by titration. A small sample of the impregnated chips was placed in deionized water and blended with a Waring blender for 30 min. Titrations with standardized NaOH solutions (J. T. Baker, Phillipsburg, NJ) gave 0.26% (w/w) sulfuric acid concentration in the chips, as reported in the tables.

Pretreatment Reactors

The Sunds CD-300 Laboratory Hydrolyzer used in the pretreatment section of the NREL PDU can process as much as 1 t/d (bone-dry t) of various lignocellulosic feedstocks, using dilute acid prehydrolysis at temperatures as high as 230°C and residence times as long as 60 min (*7*). Acid and water are added in a stainless steel pug-mill mixer to achieve the desired acid and solids concentration in the hydrolyzer. A stainless steel plug-flow screw feeder compresses the acid-wetted biomass feed into a plug solid enough to resist the steam pressure (maximum 400 psig). A blow-back preventer valve is also present, in case the biomass plug fails. Liquid stream produced during the compression process (squeezate) is pumped back into the vertical impregnator section of the hydrolyzer to maintain the desired solids and acid concentrations. The acid impregnated feedstock stream in the reactor is heated by direct steam injection with ports near the bottom and top of the hydrolyzer. Temperature is measured near the bottom, middle, and top, with feedback control via temperature or pressure sensors. Noncondensable gases entrained in the feedstock are bled off the top of the reactor with volatile components (furfural, HMF, and acetic acid), condensed, and analysis performed for use in mass-balance calculations. Residence time is a function of the various feed rates and level within the hydrolyzer, and is controlled by a level sensor that controls the operation of a double-reciprocating valve isolating the high pressure of the reactor from the low-pressure (5 psig) flash tank. The pretreated biomass is flash-cooled in the flash tank, and flash vapor is condensed and components analyzed. A high-volume open-faced centrifugal pump (Discflo, San Diego, CA) is used to recirculate the pretreated slurry (approx 25% total solids) between the bottom and top of the flash tank, to keep the solids in the slurry suspended. Lime may be added to the flash tank for either neutralization or overliming of the slurry. Metering off the recirculation loop allows the pretreated slurry to enter the fermentation train.

The NREL digester consists of a 4-L-batch steam-jacketed reactor equipped with a 4-in (10-cm) top and 2-in (5-cm) bottom ball valve, two direct steam injection ports near the top and bottom, with K-type thermocouples inserted near the top and bottom of the reactor used for temperature measurements. At the end of pretreatment, the contents of the reactor are discharged into a cooled flash tank and the flash vapor is condensed

in a separate condenser, as is the case with the Sunds pretreatments. Noncondensable gases are scrubbed by a dilute alkali solution prior to exhausting to atmosphere. Noncondensable gases can be vented from the top of the digester during pretreatment, if temperature and resulting steam pressure differ significantly.

Pretreatment

Pretreatment experiments with the Sunds hydrolyzer were carried out at temperatures that ranged from 160 to 210°C, acid concentrations of 0.1–1%, and residence times of 4–10 min. The feed rate of sawdust into the Sunds hydrolyzer was set at 37 or 40 bone-dry kg/h. The higher feed rate was needed to consistently achieve 4-min residence times. The reactor was brought to steady state, residence times measured, and then operated for a minimum of 1 h before being sampled. Before the sample was taken, the flash tank was emptied, and material was allowed to collect for at least 15 min with recirculation before sampling. The solids loadings in the Sunds hydrolyzer and flash tank were controlled to approx 20–25% total solids. Steady-state conditions could be changed 2–3× in a long day.

Pretreatment with the batch NREL digester was accomplished by prewarming the reactor to the desired pretreatment temperature, then loading it with a batch of pre-weighed, acid-soaked chips. Steam was introduced at the desired pressure and pretreatment allowed to proceed for the desired residence time. At the end of pretreatment, the contents of the reactor were discharged into the flash tank. Typically, two 4-L batches are pretreated at each temperature, after which the contents of the flash tank were emptied and cone-blended, and the slurry subjected to analysis. The flash tank surfaces were rinsed down and the rinsate collected separately for analysis. Portions of the pretreated solids were stored at −20°C for later digestibility and fermentation assays. The pretreated material (approx 20–25% total solids) was processed into a liquor fraction by vacuum filtration (Whatman No. 1 paper), and a water-insoluble fraction (after washing with 10 vol of H_2O) followed by chemical analysis of each fraction.

Analysis of Wood and Solid Residues

Dry wt (by oven drying at 105°C to constant weight) *(8)* and Klason acid-soluble and acid-insoluble lignin were determined by standard methods *(9)*. Anhydrosugars in the whole-wood and pretreated solids were determined by a procedure slightly modified from that developed at the US Forest Products Laboratory *(4,10)*. Ash in the wood and pretreated solid residues were analyzed by standard gravimetric methods *(11)*.

Analysis of Liquid Residues

Organic acids, glycerol, hydroxymethyl furfural (HMF), and furfural in the filtrate and rinsate fractions were determined by high-performance

liquid chromatography (HPLC), using Bio-Rad Aminex HPX-87H columns (Bio-Rad, Hercules, CA) *(4,10,12)*. Monomeric sugars were also determined by HPLC using Bio-Rad Aminex HPX-87P columns *(4,10,12)*. Subsequent to the HPLC analysis above, the oligomeric sugars in the liquors and rinsate fractions were converted to monomers using 4% H_2SO_4 hydrolysis at 121°C for 1 h, and the monomeric sugars analyzed using the Bio-Rad HPX-87P column, and corrected for sugar losses, using sugar recovery standards *(10,12)*. Acetic acid released by hydrolysis of the oligomeric xylan in the liquor fraction, following the 4% posthydrolysis analysis, was determined by HPLC with the Bio-Rad HPX-87H column.

Enzymatic Analysis

Extensively washed (10 vol of H_2O) biomass residues were tested for enzymatic digestibilities with Iogen cellulase (Iogen, Ottawa, ON, Can) enzyme (lot no. BRC 191095, assayed at 91 FPU/mL and 198 IU/mL β-D-glucosidase activity) *(13)* at a loading equivalent to 60 FPU/g of cellulose in prewarmed (50°C) 10-mL reaction cocktails that contained solids equivalent to 1% (w/v) cellulose, 50 m*M* citrate buffer, pH 4.8, and 40 μg/mL tetracycline and 30 μg/mL cycloheximide, to minimize contamination *(6)*. Duplicate reaction vials were incubated at 50°C with 120 rpm rotation at a 45-degree angle, and compared to controls that contained 1% (w/v) Solka Floc, grade NF-FCC (Fiber Sales and Development, Urbana, OH), and enzyme blanks. One-half mL samples were removed and glucose concentrations determined with a YSI Model 2700 Select Biochemistry Analyzer equipped with an immobilized glucose oxidase membrane (Yellow Springs Instruments, Yellow Springs, OH) calibrated with YSI-supplied 2.50 g/L glucose calibration standards. Samples were centrifuged at 12,000*g* for 5 min, with the supernatant diluted to keep the glucose readings below the 2.50 g/L level used for calibrating the YSI instrument.

RESULTS

Composition of hydrolysate liquors from the various pretreatments are shown in Table 1. The data show that, as expected, at harsher pretreatment conditions (i.e., higher temperatures and acid concentrations), more glucose is produced from the cellulosic portion of the biomass. The data indicate that at low acid concentrations (below 0.21%) and short residence times, the Sunds hydrolyzer incompletely hydrolyzes the hemicellulose to monomeric sugars, even at temperatures of 200 and 210°C (rows 4 through 8). Xylose concentrations of 30–34 g/L result from pretreatments at temperatures of 200°C and higher in both reactors; concentrations between 39 and 40 g/L are found at 160–170°C (rows 9 and 10). Because the furfural concentrations are comparable for the various pretreatment conditions listed, xylose is apparently converted to other products that are not measured (*see* losses of xylose listed in Table 3). The lower acid concentrations

Table 1
Compositional Analysis of Hydrolysate Liquors of Pretreated Yellow Poplar

Experiment number	Reactor	Temperature °C	Acid (%-w/w)	Residence time (min)	Cellobiose (g/L)	Glucose (g/L)	Xylose (g/L)	Mannose (g/L)	Acetic acid (g/L)	Furfural (g/L)	HMF (g/L)
1	Digester	210	0.26	3	0.9	13.9 (15.8)[a]	34 (33.9)[a]	7.5 (8.9)[a]	10.5 (14)[a]	1.8	0.4
2	Digester	210	0.26	4	0.8	14.6 (16.4)	31.1 (30)	7.2 (8.2)	10.5 (14.2)	2.8	0.7
3	Digester	230	0.26	1	1.1	15.2 (17.9)	31.7 (32)	6.7 (8.7)	8.3 (13.5)	1.3	0.4
4	Sunds[b]	161	1.01	9.2		11.7 (11.7)	39.4 (39.7)	6.4 (9.8)	12.5	2	nd[d]
5	Sunds[b]	170	0.72	10.5		9.2 (9.7)	38.6 (39.6)	6.7 (8.2)	12.9	1.7	nd
6	Sunds[c]	190	0.2	4	4.5	3.2 (6.7)	14.5 (35.3)	2.7 (7.6)	3.8 (12.1)	0.4	0.1
7		200	0.1	4	4.2	1.6 (5.8)	6.9 (32.2)	1.4 (6.5)	2.9 (11.1)	0.4	0.3
8		200	0.15	4	4.7	2.8 (6.5)	13 (34.5)	2.6 (7.5)	4 (12.9)	0.6	0.3
9		200	0.21	4	3.9	3.2 (6.1)	14.6 (31.1)	2.9 (6.9)	3.7 (11.6)	0.7	0.2
10		210	0.1	4	4	1.7 (5.7)	7.4 (30.9)	1.6 (6.3)	3.2 (11.5)	0.4	0.3
11	Sunds[c]	200	0.32	4.6	0.9	8.9 (10.7)	32.2 (33.6)	6.7 (9.9)	10.5	2.1	0.5

[a] Parenthesis indicates component analysis following 4% hydrolysis at 121°C for 1 h.
[b] As-installed Sunds hydrolyzer.
[c] Sunds hydrolyzer modified with displacement cylinder.
[d] nd = not determined.

(rows 4 through 8) result in lower furfural concentrations, presumably because the solubilized oligomeric xylans are partially protecting the xylose incorporated in the oligomers. HMF concentrations (not shown) were less than 0.8 g/L. Galactose concentrations (not shown) are near 3.5–4 g/L, and arabinose concentrations were near 2.2 g/L for all the liquors. The galactose and arabinose were usually hydrolyzed to monomeric sugars, except for the low-acid concentration experiments with the Sunds hydrolyzer, in which they were mixtures with monomer/total sugar ratio of approx 50%. Mannose concentrations, except in the low-acid concentration pretreatment experiments in the Sunds hydrolyzer (in which they are associated with the solubilized oligomeric xylan in a monomer/total sugar ratio of approx 50%), were in the range of 6.4–9.9 g/L. Acetic acid concentrations varied between 8 and 14 g/L, except at the low-acid concentration pretreatments in the Sunds hydrolyzer (rows 4 through 8). The total solids of the pretreated material and the compositional analysis of the liquors are similar, which suggests that the two reactors have similar performance under similar pretreatment conditions.

The solids compositional analysis (based on a 105°C dry wt) of the pretreated solids and the starting yellow poplar sawdust is shown in Table 2. The values for yellow poplar sawdust are averages of two separate determinations of representative sawdust samples. Data for glucan and xylan components (presented as glucose and xylose) in the pretreated solids show that, at the lower temperatures (161 and 170°C), the percent glucan is near 60%, and the xylan remaining is near 2–3%; thus about 90% of the xylan is solubilized. The pretreatment at 0.21% acid and 200°C in the Sunds hydrolyzer leaves about 4% of the residue composed of xylan, which represents less solubilization (~87%). Increasing the acid concentration to 0.32% at 200°C increased the solubilization and decreased the amount of xylan in the solid residue to 0.7%, and decreased the percentage of mannose in the residue from 0.68 to 0.33%. Galactose and arabinose (not shown) in the sawdust were 0.25 and 0.7%, respectively, but very little of the sugars were found in the pretreated residues (less than 0.06%). Klason lignin in the sawdust was approx 25% of the sawdust, which increased to approx 33 to 35% of the water-insoluble solids following pretreatment. The acid-soluble lignin decreased to approx 1.5% following pretreatment, except in the lower temperature, higher-acid concentration experiments, in which approx 4.4–4.8% of the pretreated residue was acid-soluble lignin. Total ash in all cases decreased from 0.9% in the sawdust to 0.5% in the pretreated solids.

The xylose and carbon closures *(14)* for the various pretreatments are shown in Table 3. Conditions of low acid (rows 6 and 7) and lower temperatures (rows 4 and 5) result in 9 and 15%, respectively of the pretreated solids composed of xylan. In addition, low temperatures (rows 4 and 5) result in higher percentages of xylose recovered in the hydrolysate liquor, in which recoveries near 85% are possible. Xylose closure, based

Table 2
Solids Compositional Analysis of Yellow Poplar[a]
Components (% dry wt)

Experiment number	Reactor/ feedstock	Temperature °C	Acid conc. (%-w/w)	Residence time (min)	Glucose	Xylose	Mannose	Klason lignin	Acid soluble lignin	Total ash
Yellow poplar sawdust					46.3	20.2	3.6	23.5	2.6	0.9
1	Digester	210	0.26	3	69.2	0.4	0.08	34.2	1.5	0.5
2	Digester	210	0.26	4	69.3	0.5	0.11	34.8	1.5	0.5
3	Digester	230	0.26	1	67.5	0.4	0.05	35.3	1.6	0.5
4	Sunds	161	1.01	9.2	61	3	0	33.1	4.4	nd[b]
5	Sunds	170	0.72	10.5	62.6	2.4	0	34.2	4.8	nd
9	Sunds	200	0.21	4	65.7	3.9	0.68	31.4	1.7	0.5
11	Sunds	200	0.32	4.6	65.8	0.7	0.33	35.2	1.3	0.5

[a] Sawdust and water-washed pretreated sawdust solids.
[b] nd = Not determined.

Table 3
Xylose Recovery and Cellulose Digestibility

Experiment number	Reactor	Temperature °C	Acid conc. (% w/w)	Residence time (min)	% Xylose in solids	% Xylose in liquor	% Xylose to furfural	% Xylose mass closure	% Carbon mass closure	Digestibility[a] %
1	Digester	210	0.26	3	0.38	21.9	3.2	25.5	72	79.8
2	Digester	210	0.26	4	0.5	66.3	11.1	77.9	101	80.5
3	Digester	230	0.26	1	0.36	38.7	1.5	40	89	83.9
4	Sunds	161	1.01	9.2	9.9	84.8	9.8	101.5	94.6	nd[b]
5	Sunds	170	0.72	10.5	9	74.8	11.1	88.9	98.6	nd
7	Sunds	200	0.1	6.6	13.8	66.5	12.2	86.4	93.2	nd
9	Sunds	200	0.21	4	15.4	50.2	7	76.7	105.4	67.6
11	Sunds	200	0.32	4.6	2.3	60.5	25.8	81	107	82.9

[a] Digestibility determined with 60 FPU/g cellulose at 50°C, pH 4.8.
[b] nd = Not determined.

on xylose recoveries in the hydrolysate liquor, the solid residue, and xylose converted to furfural, are calculated to be 25–102% for the various pretreatments. Higher temperatures, lower acid concentrations, and shorter residence times (rows 6 and 7) decreased the recoveries. Presumably, the lost xylose is converted to furfural and other degradation products. Increasing the acid concentration in the Sunds hydrolyzer from 0.21 to 0.32% at 200°C, with a 4.6-min residence time, increases the amount of xylose converted to furfural (row 8). The results for the NREL digester pretreatment at 210°C, 4-min residence time (row 2) compares favorably with the Sunds hydrolyzer experiment carried out at 200°C and 4.6-min residence time (row 8). Increasing the temperature in the NREL digester to 230°C, with 1-min residence time (row 3), decreases the amount of xylose recovered in the liquor to approx 40%, and the %-xylose mass-balance closure to 40%, which suggests that these pretreatment conditions are severe. The total mass balance closures for xylose (25.5%) and carbon (72%) are low for the NREL digester experiment summarized in row 1, because of problems in recovering all of the hydrolysate liquor. With the above exception, the carbon mass-balance closure *(14)* for the other pretreatments ranged from 89 to 107%.

The enzymatic digestibility of washed pretreated solids in Table 3 was approx 80–84% after 24 h, at a loading of 60 FPU/g of cellulose and 50°C. The digestibility decreased to 55–60% at a loading of 25 FPU/g of cellulose and 37°C (data not shown). The digestibility of the NREL digester pretreated washed solids were near that obtained for the Sunds hydrolyzer. The high-temperature, short-residence-time, low-acid (0.21%) pretreatment in the Sunds hydrolyzer produced solids with a lower digestibility of approx 68% (row 7), which suggests the pretreatment conditions were not as severe.

CONCLUSIONS

The Sunds hydrolyzer very effectively solubilizes the hemicellulosic portion of biomass, and increases the enzymatic digestibility of the cellulose that remains in the solid residues for temperatures higher than 160°C, at which recoveries of xylose in the hydrolysate liquor approaches 85% (Table 3, rows 4 and 5). Increasing the temperature to 200°C with 0.32% (w/w) sulfuric acid concentrations, and the short residence time of 4.6 min, increases the amount of hemicellulose solubilized; however, a considerable amount of xylose is lost to unaccounted-for degradation products, and the amount of xylose recovered in the hydrolysate liquor decreases to approx 61%. Acid concentrations below 0.21% in the Sunds hydrolyzer pretreatments do not effectively solubilize the hemicellulose or increase digestibility of the cellulose in the residues, even at 200–210°C (Table 3, rows 6 and 7), which suggests that the ash within the sawdust is neutralizing a considerable amount of the acid catalyst. The pH measured between 2.3

and 2.9 for the low-acid experiments for hydrolysate liquors from the Sunds hydrolyzer (data not shown), which suggests that dilute acid hydrolysis with pH higher than 2.2, and temperatures of 200°C and 4.5-min residence times, is less effective for solubilizing hemicellulose in yellow poplar sawdusts. The results demonstrate that high-temperature (~200°C), short-residence-time, dilute-acid prehydrolysis with the Sunds hydrolyzer is an effective pretreatment that lowers the catalyst requirements (sulfuric acid) and gypsum formed when neutralizing the pretreated residues with lime, before they enter the fermentation train. The results presented for the NREL digester give similar levels of solubilization of hemicellulose and xylose recoveries in the liquors, compared to the Sunds hydrolyzer at short residence times. The results show only minor differences between the bench- and pilot-scale reactors in the pretreatment of yellow poplar sawdusts. The use of the bench-scale digester would result in shorter turnaround times for optimization experiments, and reduce requirements for feedstock and resources. The pretreatment parameters developed in the digester can then be applied to the Sunds hydrolyzer when pilot plant demonstration is required.

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