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Two-Stage Dilute-Acid Pretreatment of Softwoods

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Abstract

Whole tree chips obtained from softwood forest thinnings were pretreated via single- and two-stage dilute–sulfuric acid pretreatment. Whole-tree chips were impregnated with dilute sulfuric acid and steam treated in a 4-L steam explosion reactor. In single-stage pretreatment, wood chips were treated using a wide range of severity. In two-stage pretreatment, the first stage was carried out at low severity to maximize hemicellulose recovery. Solubilized sugars were recovered from the first-stage prehydrolysate by washing with water. In the second stage, water-insoluble solids from first-stage prehydrolysate were impregnated with dilute sulfuric acid, then steam treated at more severe conditions to hydrolyze a portion of the remaining cellulose to glucose and to improve the enzyme digestibility. The total sugar yields obtained after enzymatic hydrolysis of two-stage dilute acid–pretreated samples were compared with sugar yields from single-stage pretreatment. The overall sugar yield from two-stage dilute-acid pretreatment was approx 10% higher, and the net enzyme requirement was reduced by about 50%. Simultaneous saccharification and fermentation using an adapted *Saccharomyces cerevisiae* yeast strain further improved cellulose conversion yield and lowered the enzyme requirement.

Index Entries: Softwood; pretreatment; acid hydrolysis; enzymatic hydrolysis; ethanol.

Introduction

This study is part of our ongoing effort at developing an effective process for converting softwood forest residues to fuel ethanol. In previous studies *(1,2)*, we began examining the feasibility of single-stage dilute-acid pretreatment followed by enzymatic hydrolysis and two-stage dilute–sulfuric acid hydrolysis of softwoods. The severe dilute-acid pretreatment

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conditions necessary to obtain high enzyme digestibility significantly degrade the hemicellulosic sugar. In two-stage, cocurrent, dilute-acid hydrolysis, although the hemicellulosic sugar recovery was in the 80–90% range, the maximum glucose yield was only 50%. In this study, we investigated the technical feasibility of further improving the glucose yield of a two-stage dilute-acid hydrolysis process by enzymatic hydrolysis of the remaining cellulose. One potential obstacle is that the residual cellulose after a two-stage dilute-acid hydrolysis (or pretreatment) may be recalcitrant to enzymatic attack. The objectives of this study were to determine the optimal conditions of single- and two-stage dilute-acid pretreatment for maximum sugar recovery from softwood forest thinnings, and to determine the potential ethanol yield via simultaneous enzymatic saccharification and fermentation.

Materials and Methods

Feedstock Preparation and Pretreatment

The Pacific Wood Fuels Company, Redding, CA, harvested a feedstock consisting of softwood forest thinnings from a site in the Quincy, CA, area. The trees were specifically chosen to represent the area and consisted of approx 70% White fir (*Abies concolor*) and 30% Ponderosa pine (*Pinus ponderosa*). The freshly harvested whole trees were immediately chipped on site into cardboard totes and shipped to the National Renewable Energy Laboratory. The wood chips were milled using a Mitts and Merrill rotary knife mill (Model 10 × 12; Reduction Technology, Leeds, AL) equipped with a ½-in. (12.7-mm) rejection screen. Chips passing through the rejection screen were mixed on large tarpaulins using a front-end loader, stored in double-sealed plastic containers and kept in –20°C freezer warehouses to prevent spoilage and loss of moisture. The moisture content of milled chips was 55% based on total wet wt. The wood chips were thawed to room temperature before acid impregnation. They were placed in a Hastelloy C-276 wire-mesh basket, and submerged in a 60°C circulating bath of dilute sulfuric acid for 4 h. The mass ratio of liquid (including water in the original wood chips) to dry wood is approx 12:1. Acid titration results obtained in previous soaking experiments (data not shown) indicate that the acid concentration inside the chips is approximately the same as in the bulk liquid after soaking for 3 h at 60°C. One hour was added to the soaking procedure to ensure thorough acid penetration inside the chips. After overnight draining, the acid-soaked chips were pressed to approx 43% solids using a large custom-made hydraulic press (25-cm-diameter × 30.5-cm-high cylinder) at an internal pressure of approx 600 psi. Pretreatment of the acid-impregnated and pressed feedstock for single- and first-stage experiments and production runs were performed using a 4-L steam explosion reactor *(1)* under conditions ranging from 180 to 215°C, 0.6 to 2.4% H_2SO_4 (in soaking liquid), and 100- to 240-s residence times. The liquors from the first-stage, pretreated materials were obtained by pressing in the large hydraulic press

and used for fermentation studies. The pressed solids were washed extensively (4 × 10 vol of water) with 50°C water, and reimpregnated with dilute acid by mixing 10% (w/w) slurries for 3 h in 30-gal 114-L plastic barrels. The solids were collected on a large (60-cm) Buchner funnel. The collected acid-impregnated solids were pressed to approx 50% solids using the hydraulic press before second-stage pretreatment. The conditions for second-stage pretreatment were 210°C, 2.5% acid concentration, and 100- to 120-s residence times.

Enzymatic Hydrolysis

Extensively washed (4 × 10 vol of 40°C H_2O) pretreated softwood first- and second-stage pretreatment residues were tested for enzymatic digestibility using Iogen cellulase enzyme (lot no. BRC 191095; Iogen, Ottawa, Ontario, Canada) at a loading equivalent to 60 filter paper units (FPU)/g of cellulose. Enzymatic digestions were carried out in 125-mL screw-capped Erlenmeyer flasks. The prewarmed (50°C) 75-mL reaction cocktails in each flask contained solids equivalent to 1% (w/v) cellulose; 50 m*M* citrate buffer, pH 4.8; 40 μg/mL of tetracycline; and 30 μg/mL of cycloheximide to minimize contamination. Control flasks contained 1% α-cellulose (C-8002, lot no. 107F-0674; Sigma, St. Louis, MO). All flasks were incubated at 50°C in a shaking incubator at 150-rpm rotation. Five-milliliter samples were withdrawn at timed intervals, and the samples were centrifuged at 5000*g* for 10 min. The flasks were vigorously shaken before sampling to obtain homogeneous samples. Glucose concentrations in the sample supernatants were determined using a YSI Model 2700 Select Biochemistry Analyzer equipped with an immobilized glucose oxidase membrane (Yellow Springs Instruments, Yellow Springs, OH) calibrated with YSI-supplied 2.5 g/L of glucose calibration standard. Linearity of the instrument was checked with YSI-supplied 2, 5, and 9 g/L standards.

A second set of enzymatic digestions was carried out using selected washed second-stage pretreated solid residues and α-cellulose control. The screw-capped 125-mL Erlenmeyer flasks containing the 75-mL reaction cocktails consisted of 10% insoluble solid substrates; 50 m*M* citrate buffer, pH 4.8; 40 μg/mL of tetracycline; and 30 μg/L of cycloheximide to minimize contamination. Enzyme-loading experiments for each substrate were carried out in duplicate at 25, 45, and 60 FPU/g of cellulose. The glucose concentrations of samples taken at timed intervals were determined using the YSI instrument.

A third enzymatic hydrolysis experiment was carried out to explore the possibility that a harshly pretreated first-stage residue contains more than the 3.4% glucan, which was reported following a Klason lignin determination. The lignin composition of this pretreated solid residue was reported as 96%. The mass balance closure around the pretreatment indicated that the glucose closure was 34% (i.e., 66% of glucan could not be unaccounted for) and the lignin closure was near 129%. The discrepancy was postulated to be owing partly to the formation of pseudo-lignin from

degradation products of cellulose and extractives. In this hydrolysis experiment, enzyme doses as high as 400 FPU/g of cellulose were performed in duplicate in 125-mL Erlenmeyer flasks, with a cocktail volume of 75 mL, with 50 m*M* citrate buffer, pH 4.8, and incubated at 50°C and 150 rpm. The solids were adjusted to give 1% (w/v) cellulose based on the assumed higher glucan content. The glucose concentrations of samples taken at timed intervals were determined using the YSI instrument. Since the enzyme contains a small amount of glucose, in calculating the quantities of glucose released from enzymatic hydrolysis, the glucose concentrations at time zero (immediately after enzyme was added to the flasks) were subtracted from subsequent concentrations.

Simultaneous Saccharification and Fermentation

Simultaneous saccharification and fermentation experiments were carried out in shake flasks containing slurries of 10% (w/v) insoluble solids. The shake flasks were 125-mL Delong flasks with stainless Morton closures (Bellco Glass, Vineland, NJ). They contained 60% of their volume (75 mL) as hydrolysate culture slurry. The saccharification was run at enzyme loadings of 15 and 25 FPU/g of cellulose using filter-sterilized Iogen enzyme. A 30% inoculum of a *Saccharomyces cerevisiae* yeast strain, which had been adapted to softwood hydrolysate, was used. The cells for inocula were taken from mid- to late-log phase culture. The estimated cell mass concentration in the SSF whole slurry when the experiment started was about 0.7 ± 0.1 g of dry solids/L. The yeast adaptation, culture development and cell mass determination methods were as reported previously *(2)*.

A measured amount of as-produced, thick slurry hydrolysate was adjusted to pH 5.0 ± 0.1 with powdered calcium hydroxide in a smooth, round-bottomed polyethylene mixing bowl using an electric eggbeater (type M09, 5-speed Model 62530; Hamilton Beach/Proctor-Silex, Washington, NC). Small amounts of calcium hydroxide were sprinkled over a large fraction of the material. The mixture was blended so it was uniform throughout, at medium speed for 75–90 s, allowing the slurry to fold over itself during mixing. The bowl was covered with snug-fitting Parafilm M (American National Can, Greenwich, CT) to reduce evaporation. The pH was measured by placing the pH probe over a thick portion of the slurry paste and gently pressing the surrounding material over the probe. The process was repeated rapidly until the pH was within range.

The weight of slurry required for each flask was added aseptically to sterile, tarred flasks, and reweighed. The amount of makeup, distilled deionized water required to bring the flask contents to 75 mL was calculated and 50% of it was added, rinsing down any slurry on the flask wall. The flasks were then closed using previously autoclaved rubber stoppers fitted with sterile, empty water traps, each with a 5.0-mL capacity, reweighed, autoclaved for 20 min, cooled, and reweighed. The moisture loss from each flash was recorded. The nutrients and the other 50% of the makeup, distilled deionized water, plus any moisture lost on autoclaving

were then added to the flasks. The final broth contained 1% (w/v) yeast extract, 2% (w/v) peptone, 1% (v/v) clarified corn steep liquor, and 0.75% (w/v) ammonium sulfate.

The flasks were incubated at 33°C at 160 rpm in a rotary incubator (Innova model 4000; NBS, Edison, NJ) for 15 min. Then the experiment began by adding the enzyme and yeast inoculum to each flask, 60 s apart. The flasks were swirled vigorously for 5 s by hand to mix well, and then 3.0 mL was transferred, using wide-mouth 10-mL pipets, into tubes packed in ice. The flasks were weighed and reincubated immediately after the last flask was sampled. The flasks were usually sampled again at 8, 12, 24, 48, 72, 96, 168, and 192 h. Each flask was shaken vigorously before sampling to obtain a homogeneous slurry sample. No pH adjustment was made during the course of the experiment.

In another set of SSF experiments, we replaced the distilled deionized water with liquor from the first-stage hydrolysate to make up the 10% insoluble solids slurry of second-stage solids. The first-stage liquor was obtained by pressing out the liquid from a slurry of first-stage hydrolysate. This liquor simulates the conditions one would obtain from a countercurrent extractor envisioned for a two-stage dilute-acid pretreatment in commercial operation.

Analysis of Wood, Water-Insoluble Solids, and Liquor

Procedures for analyzing chemical composition of feedstock (lignin and individual carbohydrate components) and hydrolysate liquor (monomeric and oligomeric sugars, organic acids, furfural, and hydroxymethylfurfural) were reported previously *(1)*.

Results and Discussion

Single-Stage Dilute-Acid Pretreatment Results

Table 1 presents the chemical composition of the acid-impregnated mixed softwood forest thinnings. The acid-soaking step removed a significant portion of ash and a small amount (<3%) of carbohydrates from the original feedstock. All process sugar yields from pretreatment and enzymatic hydrolysis were calculated from the basis of the composition of carbohydrates in the acid-impregnated feedstock.

Comparisons of sugar yields obtained from various pretreatment conditions and processes were simplified with the use of the severity concepts of combining time, temperature, and acid concentration *(3–5)*. Chum et al. *(4)* proposed the concept of combined severity (CS) factor, $\log_{10}(\text{Ro}) - \text{pH}$, to correlate data for the efficiency of removing carbohydrates and lignin from lignocellulosic materials in acid-catalyzed aqueous and nonaqueous media. For this study, we applied this CS concept as follows:

1. The CS is given by

$$\text{CS} = \log_{10}(\text{Ro}) - \text{pH}$$

Table 1
Composition of Acid-Impregnated Mixed Softwood Forest Thinnings (wt%)

Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin	Ash	Unidentified
39.9	6.0	2.7	<0.1	10.4	34.9	0.3	5.7

Table 2
CS of Single-Stage Dilute-Acid Pretreatment

Run no.	T (°C)	t (s)	Feed SC[a] (%)	AC in feed (%)[b]	AC in reactor (%)	pH	Log_{10}(Ro)	CS
1	190	180	43	0.80	0.55	0.95	3.13	2.18
2	205	100	42	0.65	0.43	1.06	3.31	2.25
3	205	140	42	0.65	0.43	1.06	3.46	2.40
4	215	100	42	0.65	0.43	1.05	3.61	2.56
5	215	140	42	0.65	0.43	1.05	3.75	2.70
6	205	100	43	2.38	1.59	0.48	3.31	2.83
7	210	120	42	1.50	0.99	0.69	3.54	2.85
8	210	120	42	1.50	0.99	0.69	3.54	2.85
9	205	140	43	2.38	1.59	0.48	3.46	2.98
10	215	100	43	2.38	1.56	0.49	3.61	3.12
11	215	140	43	2.38	1.56	0.49	3.75	3.26

[a]Solids content of acid-impregnated wood chips before pretreatment.
[b]Sulfuric acid concentration (wt%) in liquid present in acid-impregnated wood chips before pretreatment.

The reaction ordinate is given by

$$\text{Ro} = t_r \cdot \exp[(T_r - 100)/14.75]$$

in which t_r is the reaction time in minutes and T_r is the reaction temperature in degrees Celsius.

2. The pH value is calculated from the sulfuric acid concentration inside the steam reactor. We use this calculated pH value because the true pH of the reaction mixture inside the reactor probably changes with time and is difficult to measure. Furthermore, we assume that sulfuric acid fully dissociates under the pretreatment conditions in this study, and the acid is diluted by the theoretical steam condensate formed at the reaction temperature. The theoretical steam condensate varies with moisture content of input material and reaction temperature.

Using these assumptions, the pretreatment conditions are expressed as CS factors, as shown in Table 2.

Figure 1 presents the soluble sugar recovery yields (monomers and oligomers) after single-stage pretreatment. The hemicellulosic sugars include mannose, galactose, xylose, and arabinose. The total sugars include all of these sugars and glucose. The hemicellulosic sugar recovery appears

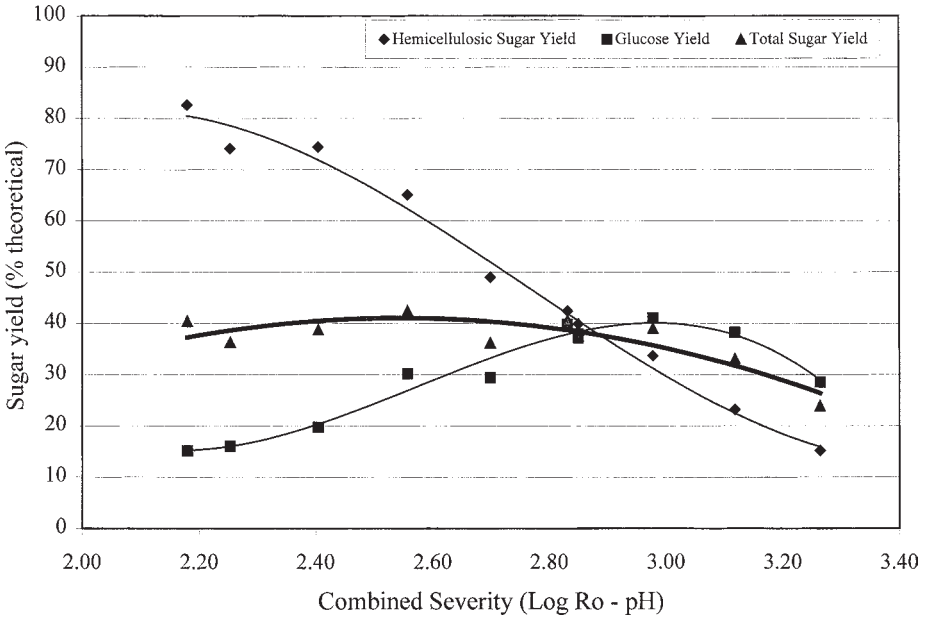

Fig. 1. Soluble sugar recovery from softwood forest thinnings after single-stage dilute-acid pretreatment.

to peak at a CS of <2.2. The total sugar recovery yield is about 40% theoretical over a wide range of severity. As the pretreatment severity increases, the degradation of hemicellulose increases, but the glucose yield also increases.

Two-Stage Dilute-Acid Pretreatment Results

Table 3 presents the pretreatment conditions and soluble sugar yields of two sets of two-stage pretreatment experiments. Table 4 presents the compositions of the hydrolysate liquors. Although the glucose concentration in the liquor of second-stage run no. 2 was higher than that of second-stage run no. 1, the glucose yield for run no. 1 was higher (as shown in Table 3) because a higher volume of liquor was obtained from run no. 1. The dilution of sugar in run no. 1 was from the higher amount of steam condensate formed at longer reaction time. The same water-washed, first-stage pretreated material was used as feedstock for the two second-stage pretreatment runs. Hemicellulosic sugar (excluding glucose) yield from first-stage pretreatment was 79% of theoretical. The glucose recovery after the first stage was 21%. Assuming a ratio of 1 U of glucose for every 3 U of mannose in galactoglucomannan, approx 40% of the glucose released in the first stage comes from hemicellulose, and the remainder from cellulose. The glucose yield from cellulose would then be approx 13%. The second-stage pretreatment hydrolyzes the remaining hemicellulose and 35–38% of the original glucan content. It is conceivable that higher glucose yields may be achieved with increasing the pretreatment severity, but the harsher pretreatment may also reduce the enzyme digestibility.

Table 3
Soluble Sugar (monomers + oligomers) Recovery from Two-Stage Dilute-Acid Hydrolysis[a]

Stage	Temperature (°C)	Time (min)	AC in feed (%)[b]	CS	Hemicellulosic sugar yield (% theoretical)	Glucose yield (% theoretical)	Total soluble sugar yield (g/100 g feedstock)
First stage	180	4.0	2.66	2.41	79	21	26.0
Second-stage run no. 1	210	2.0	2.50	3.03	5	38	17.7
Second-stage run no. 2	210	1.5	2.50	2.91	5	36	16.9
First-stage + second-stage run no. 1	180/210	4/2	2.66/2.5	2.41/3.03	84	59	43.7
First-stage + second-stage run no. 2	180/210	4/1.5	2.66/2.5	2.41/2.91	84	57	42.9

[a]All yield values are based on carbohydrate contents of wood input.
[b]Sulfuric acid concentration (wt%) in liquid present in acid-impregnated wood chips before pretreatment.

Table 4
Concentration of Sugars in First- and Second-Stage Hydrolysate Liquors (g/L)[a]

Sample	CEL	G	X	GA	A	M	AC	HMF	FL
First stage[b]	0 (0)	47.2 (49.8)	24.9 (23.8)	12.6 (13.4)	7.4 (5.9)	42.3 (47.8)	7.5	2.4	1.8
Second-stage run no. 1[c]	4.4 (0)	84.0 (90.1)	1.1 (1.2)	1.0 (0.6)	1.1 (0.9)	2.8 (2.4)	0.7	4.1	0.4
Second-stage run no. 2[d]	5.5 (0)	87.2 (99.7)	1.4 (1.2)	1.3 (0.7)	1.2 (1.1)	3.6 (2.8)	0.6	3.0	0.3

[a]Hydrolysate liquor is the liquid obtained from pressing the hydrolysate slurry. Values in parentheses were obtained after 4% sulfuric acid hydrolysis of the liquor at 121°C for 1 h. CEL, cellobiose; G, glucose; X, xylose; GA, galactose; A, arabinose; M, mannose; AC, acetic acid; HMF, 5-hydroxymethyl-2-furaldehyde; FL, furfural.

[b]Acid hydrolysis conditions: 180°C, 4 min, 2.66% H_2SO_4, CS = 2.41. Total solids content of first-stage hydrolysate slurry = 37.7 wt%.

[c]Acid hydrolysis conditions: 210°C, 2 min, 2.5% H_2SO_4, CS = 3.03. Total solids content of second-stage hydrolysate slurry run no. 1 = 31.6 wt%.

[d]Acid hydrolysis conditions: 210°C, 1.5 min, 2.5% H_2SO_4, CS = 2.91. Total solids content of second-stage hydrolysate slurry run no. 2 = 34.3 wt%.

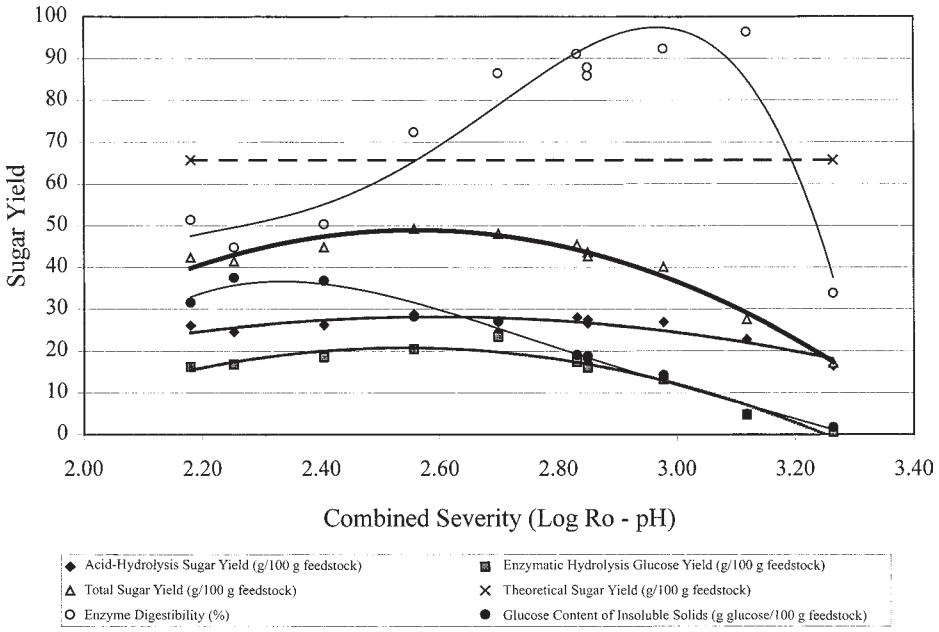

Fig. 2. Sugar yields from single-stage dilute-acid pretreatment of softwood forest thinnings followed by enzymatic hydrolysis.

Enzymatic Hydrolysis and SSF Results

Figure 2 presents the enzyme digestibility of washed, single-stage pretreated materials. Also shown are the sugar recovery yields from pretreatment and cellulose content of the washed residual solids. At low CS (i.e., <2.4), the enzyme digestibility was about 50%. The enzyme digestibility reached about 90% when the severity increased to 2.8–3.1. The pretreated material became recalcitrant to enzymatic hydrolysis at further increase in pretreatment severity. Increasing the severity of the pretreatment slightly from 215°C, 2.4% H_2SO_4, and 100 s (CS = 3.12) to 215°C, 2.4% H_2SO_4, and 140 s (CS = 3.26) decreased the digestibility from about 90 to 34%. This dramatic decrease in digestibility suggests that the cellulose in the residue is undergoing a rapid conversion to a substrate that is not recognizable as cellulose to the cellulase enzyme complex. The cellulose may be covered with a condensed-lignin coating that prevents access to the substrate by the enzyme *(6)*, or the cellulose is degraded to some other product *(7)*.

Based on the amount of glucose released (data not shown) in a hydrolysis experiment in which very high enzyme loading was used on harshly pretreated material (CS = 3.26), we determined that the traditional cellulose content analysis method (72% sulfuric acid digestion) gives a slightly low value (3.4%). The enzyme digestion test indicates a glucan content of approx 5% assuming all the cellulose was hydrolyzed by enzyme.

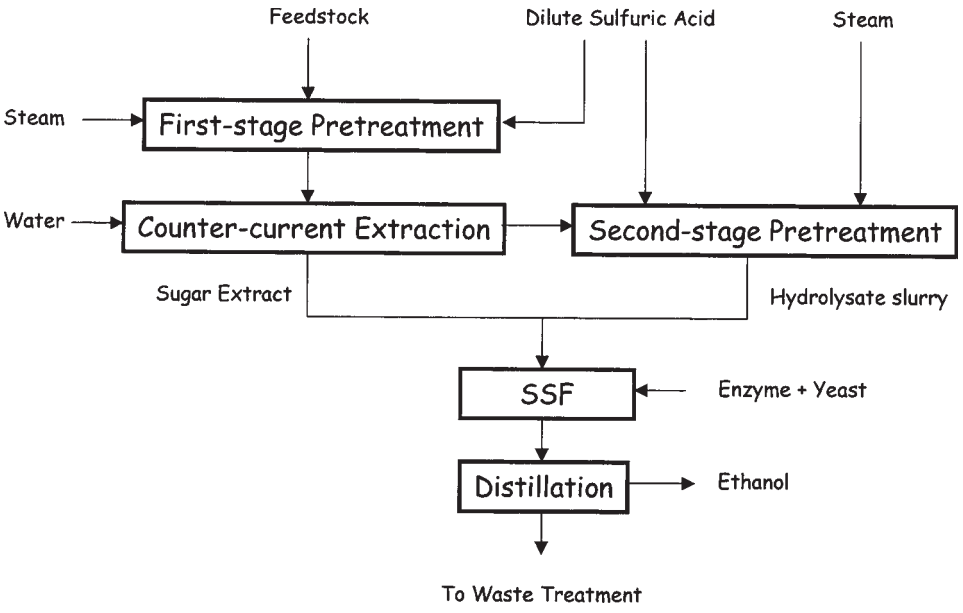

Fig. 3. Block flow diagram of a two-stage dilute-acid pretreatment process.

Figure 2 also illustrates the drawback of single-stage batch dilute-acid pretreatment of softwood in that to obtain high digestibility, the pretreatment severity must be high, to a degree that also causes significant degradation of the carbohydrates. The dry matter recovery for severely treated material (CS > 3.2) was in the 80–85% range. The maximum enzymatic sugar yield does not coincide with the maximum digestibility because the cellulose content of the residual solids rapidly decreases as the severity increases. The maximum total sugar recovery yield (from acid and enzymatic hydrolysis) for this set of pretreatment conditions occurred at a combined severity of about 2.56, which happens to be approximately the peak for both acid hydrolysis and enzymatic hydrolysis sugar yields. The maximum total sugar yield was 49 g/100 g of dry wood input, or about 75% of theoretical. At this maximum, the hemicellulosic sugar yield was only 65%.

To explore various options for enzymatic conversion of the residual solids after second-stage dilute-acid pretreatment, we performed a series of enzymatic hydrolysis and SSF experiments to determine the effect of enzyme loading, insoluble solid loading, and combining first- and second-stage hydrolysate. For a fermentation or SSF process coupled with a two-stage dilute-acid pretreatment, the first-stage hydrolysate would be a liquid and the second-stage hydrolysate would be in slurry form. Figure 3 presents a block flow diagram of a two-stage dilute-acid pretreatment followed by SSF in which both hydrolysates are combined.

Figure 4 shows the effect of enzyme loading on glucose yield from 1% slurry of washed second-stage solids (CS = 3.03). The low substrate loading was selected for comparison with the digestibility of the single-

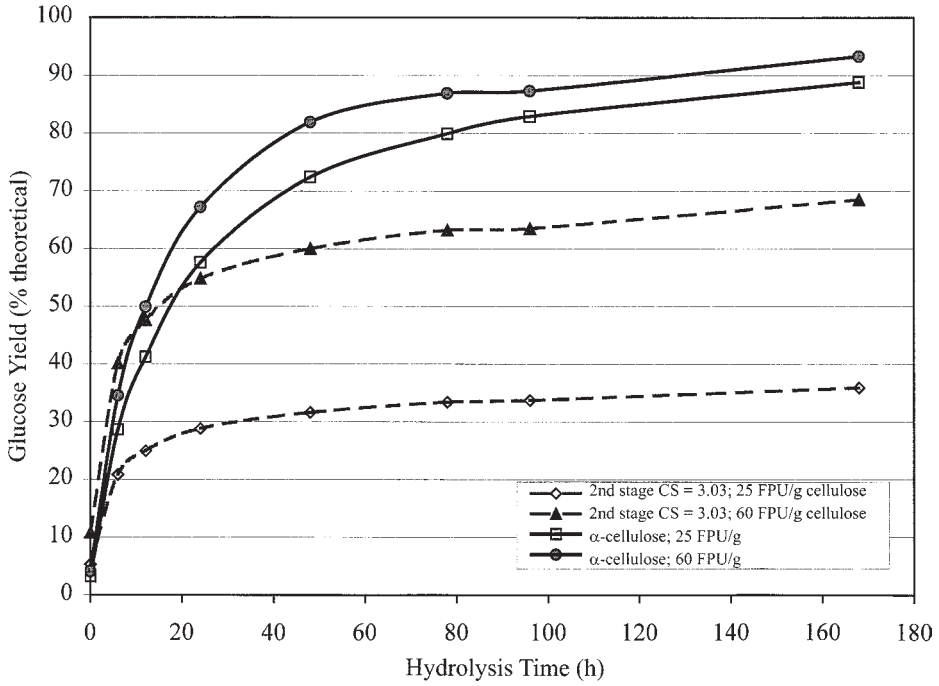

Fig. 4. Effect of enzyme loading on glucose yield (1% cellulose loading).

stage pretreated material. The digestibility of the second-stage pretreated material was 69%, or just slightly lower than the digestibility of the optimal single-stage pretreated material (CS = 2.56). However, in commercial operation, the initial substrate loading would likely be maintained at about 10% insoluble solids or higher to keep the energy requirement for ethanol recovery at an acceptable level. At 1% cellulose concentration, at which the end product inhibition is minimal, the pretreated wood is less digestible than α-cellulose, as clearly shown in Fig. 4.

Figure 5 shows the effect of enzyme loading at 10% (w/v) slurry. In comparison to α-cellulose, the washed, second-stage pretreated material requires higher enzyme loading (45 FPU/g of cellulose or higher) to yield similar digestibility. At 10% cellulose concentration, at which end product inhibition is significant, the glucose yield for both α-cellulose and pretreated wood is reduced to about the same level even at 60 FPU/g of cellulose.

Figure 6 compares the SSF of unwashed second-stage hydrolysate and combination of unwashed second-stage hydrolysate and first-stage hydrolysate liquor (obtained from a 24% slurry). It appears that combining the two hydrolysates improves the ethanol yield slightly. The reported ethanol yields were based on total available hexose (including potential glucose from cellulose in the fibers and soluble hexose). A small portion of the increase (<5%) in ethanol yield probably comes from the hydrolysis of additional oligomers in the first-stage hydrolysate. The overall addi-

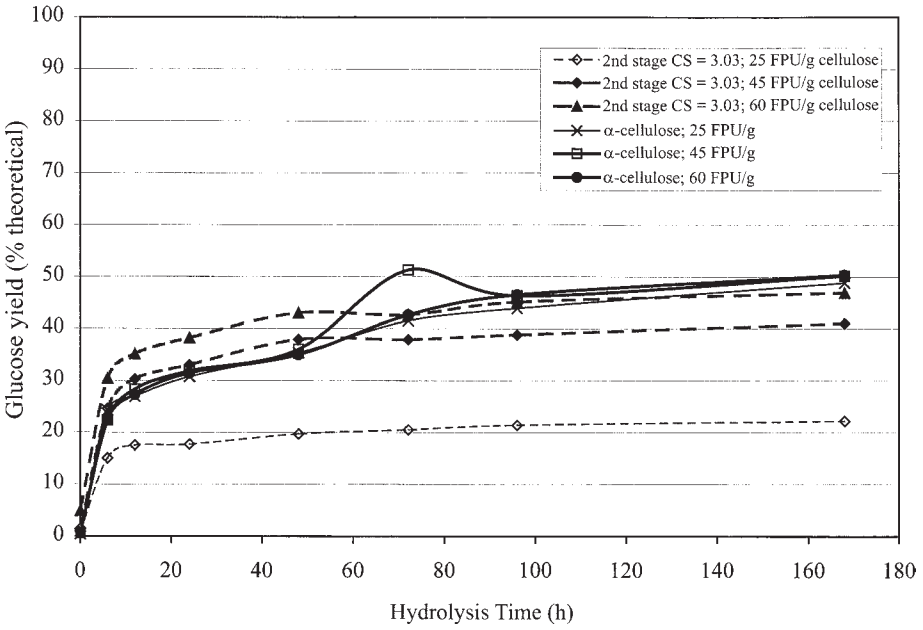

Fig. 5. Effect of enzyme loading on glucose yield (10% insoluble solids loading).

tion of fermentable sugars also likely improves the performance of the yeast. We had difficulty in determining low mannose concentrations in the fermentation broth accurately because of coelution of mannose with ethanol in the high-performance liquid chromatography chromatograms. As a result, the concentrations of hexose below 10 g/L were slightly overestimated.

Figure 7 compares the SSF of two second-stage hydrolysates. Assuming a fermentation efficiency of 85%, the enzymatic glucose yield is approx 85% for the CS = 2.91 sample and 65% for the CS = 3.03 sample. Previously, the digestibility of the CS = 3.03 sample was determined to be 69% using an enzyme loading of 60 FPU/g of cellulose (Fig. 4).

Figure 8 summarizes the total sugar yields of the single- and two-stage pretreatments followed by enzymatic hydrolysis. The sugar yield from the two-stage pretreatment process can potentially be further improved by optimizing the first-stage pretreatment to increase the sugar yield (i.e., lower the CS factor). In calculating the percentage of theoretical sugar yield, it is assumed that enzyme is purchased for the process. The yield values would be lower if enzyme were produced on-site using a fraction of the pretreated substrate.

For softwood forest thinnings, the sugar yield obtained from a two-stage pretreatment process is appreciably higher than that from single-stage pretreatment. Furthermore, the net enzyme requirement is reduced by as much as 50% because the cellulose content in the second-stage solid residues is less than half that in the first-stage solids.

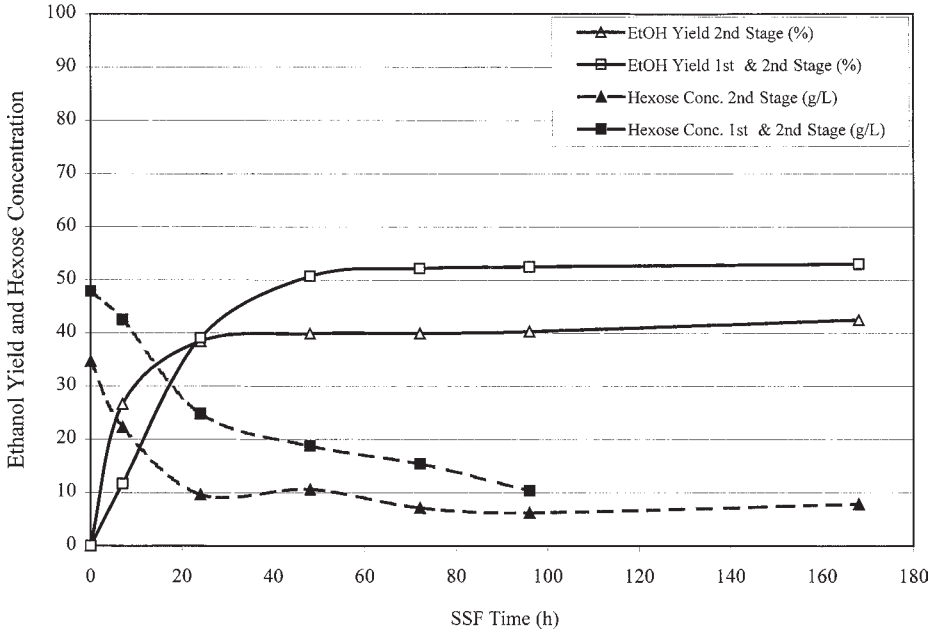

Fig. 6. SSF of second-stage hydrolysate and combined first- and second-stage hydrolysates (15 FPU/g cellulose; 10% insoluble solids; first-stage CS = 2.41; second-stage CS = 3.03).

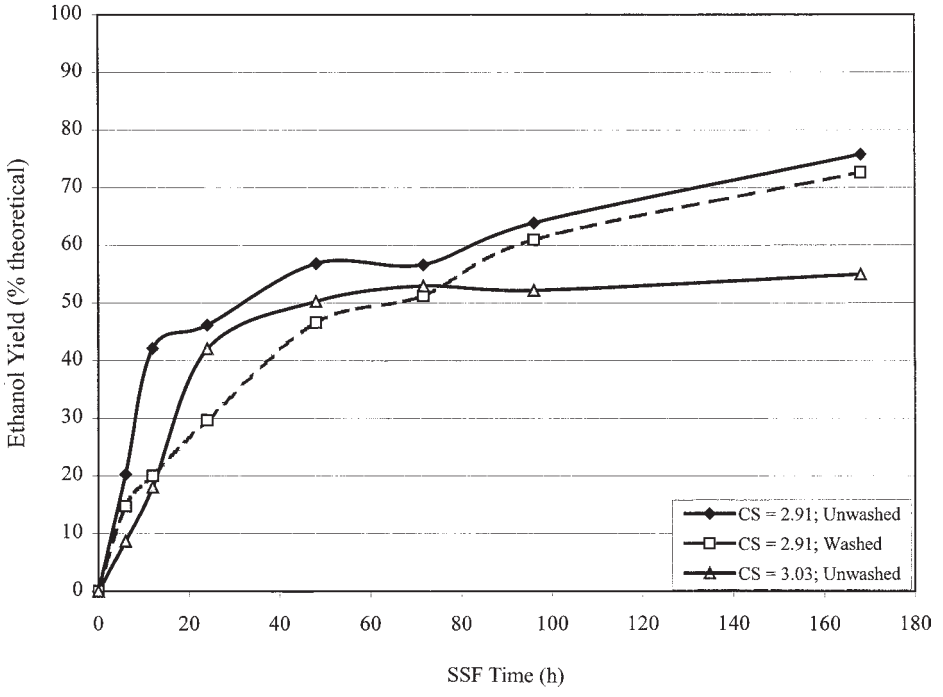

Fig. 7. SSF of second-stage hydrolysate (25 FPU/g cellulose; 10% insoluble solids).

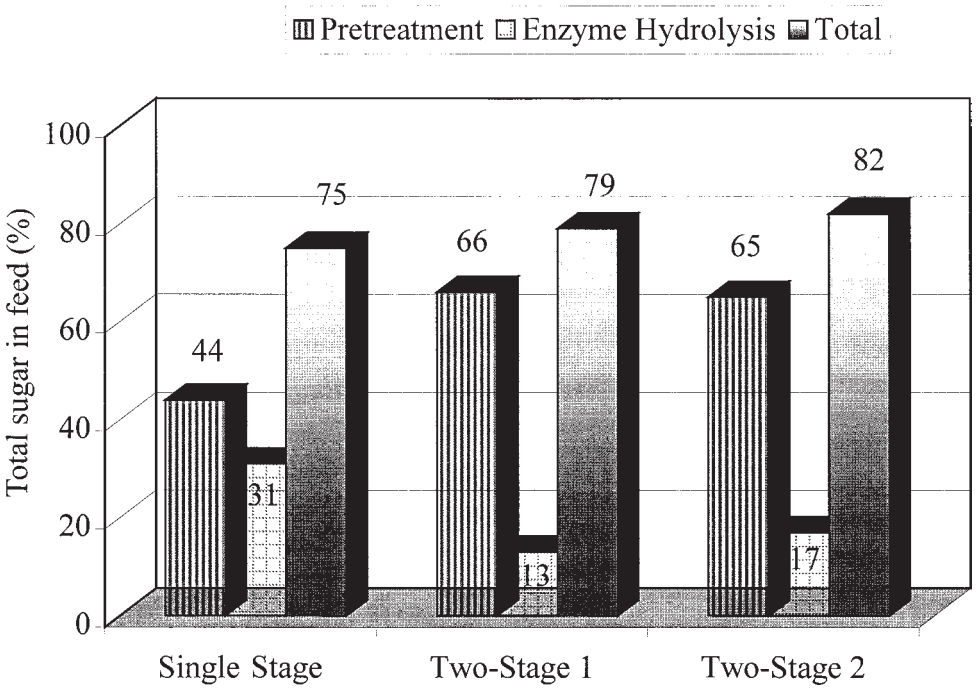

Fig. 8. Summary of total sugar yields for single- and two-stage dilute-acid pretreatment followed by enzymatic hydrolysis. Single-stage: CS = 2.56; two-stage 1: first CS = 2.41, second CS = 3.03; two-stage 2: first CS = 2.41, second CS = 2.91.

Conclusion

We have demonstrated that the overall sugar yield from softwood can be improved by using a two-stage pretreatment process. The concept of CS can be conveniently and effectively applied for preliminary pretreatment optimization. SSF of combined first- and second-stage hydrolysate was successfully demonstrated at high solids loading without any detoxification requirement. The experimental results suggest that by selecting a relatively low severity in the second-stage pretreatment, the enzyme digestibility, and thus ethanol yield, can be improved while significantly reducing the net enzyme requirement. In comparison to a single-stage pretreatment, a two-stage pretreatment process promises higher ethanol yield and substantially lower enzyme requirements. Whether these improvements can offset the increase in cost of adding the extraction and second-stage pretreatment steps remains to be resolved.

Acknowledgments

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