

# Dilute Acid Hydrolysis of Softwoods

## Scientific Note

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### Abstract

Whole tree chips obtained from softwood forest thinnings were converted to ethanol via a two-stage dilute acid hydrolysis followed by yeast fermentation. The chips were first impregnated with dilute sulfuric acid, then pretreated in a steam explosion reactor to hydrolyze more than 90% of the hemicellulose and approx 10% of the cellulose. The hydrolysate was filtered and washed with water to recover the sugars. The washed fibers were then subjected to a second acid impregnation and hydrolysis to hydrolyze as much as 45% of the remaining cellulose. The liquors from both hydrolysates were combined and fermented to ethanol by a *Saccharomyces cerevisiae* yeast that had been adapted to the inhibitors. Based on available hexose sugars, ethanol yields varied from 74 to 89% of theoretical. Oligosaccharide contents higher than about 10% of the total available sugar appear to have a negative impact on ethanol yield.

**Index Entries:** Biomass; softwood; ethanol; pretreatment; bioconversion; acid hydrolysis.

### Introduction

Small-diameter trees and underbrush have overcrowded forests in the western United States, creating a severe fuel-loading problem. The resulting forest fires are often catastrophic. In an effort to reduce this fuel loading, the US and State Forest Services have started selective thinning operations in national and state forests. Forest thinnings have low economic value to the forest products industry. Furthermore, the volume of wood generated from forest thinning operations would be too large to be absorbed by traditional uses, such as producing wood composite products, firewood,

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compost, and mulch. A potential use of forest thinnings is to convert them into fuel ethanol and cogenerated electricity. This study is part of an effort to determine the feasibility of converting softwood forest thinnings to ethanol.

In a previous study (1), we obtained 90% glucose yield from enzymatic hydrolysis of pretreated softwoods. However, high enzyme loadings are required to achieve high glucose yields (2). Given the current high cost of commercial cellulase, an enzyme-based conversion process may not be attractive for near-term deployment. In this study we determined the potential ethanol yield from mixed softwood forest thinnings using dilute sulfuric acid hydrolysis. Dilute acid hydrolysis of lignocellulosic material for ethanol production has been extensively investigated (3–10). In general, two-stage or countercurrent hydrolysis processes, in which optimal conditions for sugar recovery from hemicellulose and cellulose are applied in sequence, give higher sugar yields than single-stage processes. Research results reported to date have been obtained for single-species feedstocks. The objectives of this study were to determine the sugar recovery yields from the whole-tree mixed wood feedstock and the feasibility of fermenting the hydrolysate to ethanol.

## Materials and Methods

### *Feedstock Preparation*

An 8-ton (approx 50% moisture content) load of softwood forest thinnings was harvested in early May 1997 from the Quincy, CA area by Pacific Wood Fuels Company (Redding, CA). The feedstock contained approx 70% White fir (*Abies concolor*) and 30% Ponderosa pine (*Pinus ponderosa*). The whole trees (including branches, bark, and needles) were chipped on site, placed in 700-lb totes, then trucked to the National Renewable Energy Laboratory (NREL). Immediately after receiving the shipment, the chips in all totes were mixed together by coning and quartering on polyethylene tarps at least six times with a front loader tractor equipped with a cleaned 1-yd<sup>3</sup> bucket. The chips were then milled using a Mitts and Merrill rotary knife mill (Model 10×12, Reduction Technology, Inc., Leeds, AL) equipped with a 1/2-in. (12.7-mm) rejection screen. The milled chips were repackaged in polyethylene lined drums and stored at –20°C. Milling of the coarse chips was necessary to improve acid impregnation and processing using the small hydrolysis reactor.

### *Dilute Acid Hydrolysis*

#### Acid Impregnation

The wood was impregnated with dilute acid before being subjected to high-temperature hydrolysis to ensure that uniform treatment was obtained and diffusion of acid into the chips was not a rate-limiting factor. Acid impregnation was carried out by soaking the chips in dilute sulfuric

acid (0.4–0.7%) solution at 60°C for 4 h, draining the acid-soaked chips overnight, then air-drying them to approx 40% solids. The acid-impregnated chips were then weighed into individual plastic bags. Each bag was used to charge the hydrolysis reactor. Samples of the sulfuric acid soaking medium were taken before and after soaking and analyzed for acid content. For second-stage hydrolysis, the wood fibers (obtained from washed first-stage hydrolysate) were impregnated with 0.4% sulfuric acid.

### Two-Stage Dilute Acid Hydrolysis

All dilute acid hydrolysis experiments (first- and second-stage) were performed in a 4-L steam explosion reactor made of Hastelloy C-22™ to resist corrosion. The reactor was equipped with a steam jacket, electrical heat bands, and two direct steam injection ports near the top and bottom to quickly and uniformly heat the biomass during hydrolysis. Two thermocouples, inserted near the top and bottom of the reactor, measured the reaction temperature. A 4-in. (10-cm) ball valve at the top of the reactor allowed the chips to be loaded quickly. Before the wood chips were pretreated, the reactor was preheated to near the desired operating temperature by setting the electrical heaters to the desired temperature and admitting steam into the jacket and cycling steam repeatedly through the reactor. A quick-opening 2-in. (5-cm) ball valve at the bottom of the reactor allowed the hydrolyzed chips to be discharged completely.

In first-stage hydrolysis experiments, a batch of preweighed acid-impregnated chips (approx 1 kg wet weight) was loaded into the reactor and saturated steam was admitted (defined as time zero). The hydrolysis temperature for first-stage hydrolysis is normally 180–200°C. After a predetermined hydrolysis time the steam was shut off, then the contents of the reactor (cooked wood chips, condensate, and steam) were discharged into a cooled flash tank with the flash vapor condensed in a separate condenser. Three replicate “shots” were made for each processing condition. The contents of the flash tank were emptied and blended, then the flash tank surfaces were thoroughly rinsed with water before processing the next batch. The pretreated materials (or hydrolysates) were processed into liquor samples (obtained by pressing the liquid from the wet samples) and water-insoluble solid samples (obtained by extensively washing the samples) for chemical analyses.

The feedstock for the second-stage pretreatment was generated from first-stage hydrolysate. A large batch of first-stage hydrolysate was collected following multiple shots from the 4-L reactor. The hydrolysate (about 70% moisture content) was mixed by coning/quarterming at least three times. The liquor was then pressed from the hydrolysate using a 24-in. (61-cm) diameter vacuum Buchner funnel (VWR, Denver, CO) lined with muslin. The dewatered solid residue was then washed by gently resuspending the pressed solids in a 55-gallon plastic drum filled with hot (about 60°C) water, and transferring the suspended slurry into the Buchner funnel fitted with muslin cloth to collect the washed solids for another

round of washing. The washing was repeated five times or until the pH of the filtrate was approx 5.5. The washed fibers were resuspended in 60°C dilute sulfuric acid solution (0.4%) in a 33-gallon plastic barrel, allowed to settle overnight, and filtered with an 18-in. (45.7-cm) Buchner funnel. The filter cake was then pressed to approx 30% solids and air-dried to approx 50% solids before second-stage hydrolysis. The procedure for second-stage hydrolysis is similar to that for the first-stage except for a higher reaction temperature (210–220°C).

### *Analysis of Wood, Water-Insoluble Solids, and Liquor*

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

### *Ethanol Fermentation*

#### Combined Hydrolysate Liquor Preparation

Combined liquors were prepared by combining first-stage (80% v/v) and second-stage (20% v/v) liquors (filtrates obtained from acid hydrolysate slurries) at 17% equivalent total solids. The 80%/20% ratio chosen was based on the estimated volume of liquors generated from each stage. The 17% total solid concentration was based on the estimated amount of water required to recover the sugars in the hydrolysate slurries. Calcium hydroxide was added to raise the pH of the liquor to  $\text{pH } 5.0 \pm 0.2$ .

#### Yeast Adaptation

Wild-type *S. cerevisiae* yeast does not perform well in softwood hydrolysate liquors at equivalent total solid concentrations greater than about 10% (1). We have successfully adapted a *S. cerevisiae* strain to Douglas fir hydrolysate (11) by repeatedly transferring cultures from weak liquors to higher-solids liquors. We then adapted the same adapted strain to hydrolysates obtained from pretreated mixed softwoods. We plated the adapted strain on hydrolysate plates: 15% (w/v) filter-sterilized hydrolysate liquor, 1.5% ammonium sulfate, 0.25% clarified corn steep liquor (CSL), and 1.5% (w/v) agar. This agar may be supplemented with glucose, or other hexose, if needed. The clarified CSL is the clear supernatant resulting from permitting 50% (w/v) fermentation-grade CSL (CPC International, Summit, IL) to settle, or the whole CSL may be centrifuged at 9000g for 20 min and 0.2  $\mu\text{m}$  filtered to render it aseptic. The adapted cultures can be preserved frozen  $\leq -40^\circ\text{C}$  in  $\geq 20\%$  (v/v) glycerol or  $\geq 20\%$  sucrose.

#### Ethanol Fermentation

Ethanol fermentation of the combined hydrolysate liquor was performed using a *S. cerevisiae* yeast strain that had been adapted to softwood hydrolysates. Fermentation was carried out at 30°C in 50-mL baffled DeLong flasks (Bellco, Vineland, NJ) with Morton closures placed in

Table 1  
Composition of Mixed Softwood Forest Thinnings (in wt%)

Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin	Ash	Extractives	Unidentified
33.9	6.5	3.4	3.0	8.9	27.2	0.9	7.5	8.7

150-rpm orbital shaker-incubators. Clarified CSL (0.25% w/w) and ammonium sulfate (1.5% w/v) were added to the pH-adjusted combined liquor as a nutrient supplement. Yeast pellets obtained from a 20% inoculum were added to minimize dilution of the liquor. No further pH adjustment was made during the fermentation. Samples were withdrawn every 4 h initially, then every 8 h, and analyzed for cell growth, residual glucose, and ethanol concentration.

Cell growth was monitored by measuring absorbance at 600 nm using a Genesys 5 Spectronic spectrophotometer (Milton Roy, Rochester, NY). Viability of yeast cells was determined by adding Wolford viable stain (12) and counted with a Petroff-Hauser hemocytometer (VWR Scientific Products, Denver, CO). Glucose concentration was determined by YSI (Yellow Springs Instruments, Yellow Springs, OH) and monomeric sugars by high-performance liquid chromatography (HPLC) using a Hewlett Packard liquid chromatograph HP #1090, equipped with a refractive index detector and a Bio-Rad (Hercules, CA) HPX-87P column. Ethanol was determined by gas chromatography (GC) using a Hewlett Packard Series II gas chromatograph fitted with a Porapak Q column.

## Results and Discussion

### *Dilute Acid Hydrolysis*

The chemical composition of the milled feedstock is presented in Table 1. Because the feedstock contained bark and needles, the extractives content (7.5%) was higher than the value (3–4%) normally found in debarked softwood chips. Earlier work on single-stage prehydrolysis (1) indicates that high hydrolysis temperatures (>200°C) resulted in a higher degree of degradation of hemicellulose. Therefore, in our first attempt at two-stage dilute acid hydrolysis we carried out the first-stage hydrolysis at 200°C to improve the hemicellulosic sugar yield. The soluble sugar recovery yields for the two-stage dilute acid hydrolysis are summarized in Table 2. Based on the large amounts of furfural and hydroxymethyl furfural (HMF) formed, the first-stage hydrolysis conditions appear to be too severe. Lignin recovery, based on Klason lignin content of the water-insoluble solids after second-stage hydrolysis, was 135% of the Klason lignin content of the wood fed into first-stage hydrolysis. This indicates that condensation products (possibly from the degradation of carbohydrates and extractives) were analyzed as pseudo-lignin in the solid residues after dilute acid hydrolysis. A set of hydrolysis experiments was then carried out to determine the

Table 2  
Soluble Sugar Yields<sup>a</sup> from Mixed Softwood Forest Thinnings  
After a Two-Stage Dilute Acid Hydrolysis

	Glucose	Xylose	Galactose	Arabinose	Mannose
After first stage <sup>b</sup>					
% Theoretical value in original feedstock	19.8	63.5	81.3	47.6	62.0
% Monomeric sugar	92	98	93	100	92
After second stage <sup>c</sup>					
% Theoretical value in original feedstock	30.1	0	0	2.2	1.5
% Monomeric sugar	87	N/A	N/A	100	88
Combined two-stage					
% Theoretical value in original feedstock	49.9	63.5	78.1	49.8	63.5
% Monomeric sugar	89	98	93	100	91
% Converted to furfural	N/A	27%	N/A	N/A	N/A
% Converted to HMF	3	N/A	N/A	N/A	24
Unaccounted	12%	18%	-5%	-5%	-5%

<sup>a</sup>Monomers + oligomer; N/A: not applicable.  
<sup>b</sup>First stage at 200°C, 0.4% sulfuric acid for 5 min.  
<sup>c</sup>Second stage at 215°C, 0.4% sulfuric acid for 3 min.

Table 3  
Soluble Sugar Yields<sup>a</sup> from Hydrolysate Liquor  
of Pretreated Mixed Softwood Forest Thinnings (% Theoretical Value)

Run #	T (°C)	t (min)	% H <sub>2</sub> SO <sub>4</sub>	G <sup>b</sup>	X	GA	A	M
1	180	7	0.7	14.0	78.0	74.6	50.9	79.8
2	180	9	0.7	19.8	75.8	72.3	52.3	73.0
3	190	3	0.7	16.1	70.3	81.3	98.0	87.3
4	190	4	0.7	15.7	62.5	74.0	>100.0	77.2

<sup>a</sup>Monomers + oligomers.  
<sup>b</sup>G, glucose; X, xylose; GA, galactose; A, arabinose; M, mannose.

hydrolysis conditions that yield higher hemicellulosic sugar recovery and fewer degradation products. The results are shown in Table 3.

Based on the results shown in Table 3, a large batch of first-stage hydrolysate was generated at 0.7% sulfuric acid, 190°C, and 3-min reaction time. The pretreated material was washed with water, then hydrolyzed in a second-stage pretreatment at 0.4% acid, 215°C, for 3 min. The soluble-sugar recovery yields for the two-stage dilute acid hydrolysis are summarized in Table 4. The concentrations of sugars and degradation products in the hydrolysate liquors are shown in Table 5. The gravimetric recoveries for both stages were 97–100%. Although hemicellulosic sugar recoveries were improved over the high-temperature first-stage hydrolysis, the fractions of oligosaccharides also increased. Lignin recovery was 109%. This indicates that a portion of the carbohydrate or its degradation products (likely from



Table 4  
Soluble Sugar Yields<sup>a</sup> from Mixed Softwood Forest Thinnings  
After a Two-Stage Dilute Acid Hydrolysis

	Glucose	Xylose	Galactose	Arabinose	Mannose
After first stage <sup>b</sup>					
% Theoretical value in original feedstock	16.1	70.3	81.3	98.0	87.3
% Monomeric sugar	88	100	87	100	79
After second stage <sup>c</sup>					
% Theoretical value in original feedstock	34.0	0.5	1	0	2
% Monomeric sugar	84	100	100	N/A	100
Combined two-stage					
% Theoretical value in original feedstock	<b>50.1</b>	<b>70.8</b>	<b>82.3</b>	<b>98.0</b>	<b>89.3</b>
% Monomeric sugar	85	100	87	100	79
% Converted to furfural	N/A	10	N/A	N/A	N/A
% Converted to HMF	2	N/A	N/A	N/A	14.5
Unaccounted	12%	18%	-5%	-5%	-5%

<sup>a</sup>Monomers + oligomers; N/A: not applicable.

<sup>b</sup>First stage at 190°C, 0.7% sulfuric acid for 3 min.

<sup>c</sup>Second stage at 215°C, 0.4% sulfuric acid for 3 min.

Table 5  
Concentration of Sugars and Degradation Products in Hydrolysates (g/L)

Stage	G	X	GA	A	M	AC <sup>a</sup>	HMF	FL
First	27.8 (31.4) <sup>b</sup>	25.4 (27.5)	13.4 (15.5)	9.8 (9.2)	33.9 (42.7)	7.0	2.4	2.5
Second	74.0 (88.4)	0.6 (0.6)	1.7 (0.5)	0 (0)	3.0 (2.9)	0	5.8	0

<sup>a</sup>AC, acetic acid; HMF, hydroxymethyl furfural; FL, furfural.

<sup>b</sup>Values in parentheses were obtained after 4% sulfuric acid "posthydrolysis" at 121°C for 1 h to hydrolyze all soluble oligomers to monomers.

glucose and xylose) was analyzed as pseudolignin. Despite the heterogeneous composition of the whole-tree mixed species feedstock, the sugar yields are similar to those obtained for clean wood chips from a single species, as reported in the literature. A large fraction of the high-unaccounted values for glucose and xylose was presumably analyzed as pseudolignin (i.e., condensation products). We are currently improving the hydrolysis methods to increase the monomeric sugar yields. One method was to perform "posthydrolysis" of the liquors at 120–125°C and 2% H<sub>2</sub>SO<sub>4</sub> for 1 h. Hydrolysis at these conditions converted about 98% of the oligomers to monomers. Another method is to improve the monomeric sugar yields during the initial hydrolysis. Previous results (1) indicate that high temperatures generally lead to greater degradation of sugars, for a reaction time range of 2–5 min. Based on these results and the success in

Table 6  
Comparison of Ethanol Yields from Two Combined Hydrolysate Liquors

Liquor no.	Oligosaccharide, %	Time, h	AC <sup>a</sup>	FL	HMF	Ethanol, g/L	Ethanol yield, % <sup>b</sup>	Residual hexose, g/L	Productivity, g/Lh
1 <sup>c</sup>	10	48	2.8	2.1	2.6	10.0	81	N/M	
		118				10.9	89	0	0.28
2 <sup>d</sup>	24	16	2.4	0.9	1.5	13.4	74	5.2	0.83
		24				10.8	60	5.2	
		43				11.0	60	5.1	

<sup>a</sup>AC, acetic acid (g/L); FL, furfural (g/L); HMF, hydroxymethyl furfural (g/L); N/M, not measured.

<sup>b</sup>Ethanol yield was based on total available monomeric hexose.

<sup>c</sup>Liquor 1, 80% (v/v) from first-stage liquor (200°C, 0.4% H<sub>2</sub>SO<sub>4</sub>, 5 min) plus 20% (v/v) from second-stage liquor (215°C, 0.4% H<sub>2</sub>SO<sub>4</sub>, 3 min).

<sup>d</sup>Liquor 2, 80% (v/v) from first-stage liquor (190°C, 0.7% H<sub>2</sub>SO<sub>4</sub>, 3 min) plus 20% (v/v) from second-stage liquor (215°C, 0.4% H<sub>2</sub>SO<sub>4</sub>, 3 min).



hydrolyzing oligomers to monomers at low temperature and higher acid concentrations, one may expect improved monomeric sugar yields by lowering the hydrolysis temperature and at the same time raising the acid concentration. For example, the acid concentration in both hydrolysis stages can be raised to 1–2% while lowering the hydrolysis temperature to about 185°C for first stage and 210°C for second stage.

### *Ethanol Fermentation*

The ethanol yields (based on available monomeric hexose) from two combined hydrolysate liquors using the adapted *S. cerevisiae* yeast strain are presented in Table 6. The new combined hydrolysate liquor, which contains 24% oligosaccharides, exhibits more inhibitory effects than the previous combined hydrolysate liquor, which contains about 10% oligomers. Because both liquors contain fairly low levels of acetic acid, furfural, and HMF, we postulate that the reduction in ethanol yield is caused by the high concentration of oligosaccharides. The significant improvement in ethanol productivity from 0.28 to 0.83 g/L/h was caused mainly by continuing adaptation of the yeast over a period of 2 mo and possibly by the lower level of inhibitors (furfural and HMF). The viability of the yeast cells was 80–90%. The high content of extractives and phenolics of the whole-tree feedstock does not adversely affect the ethanol yield once the yeast is adapted to these and other inhibitors.

### **Conclusion**

Using a two-stage dilute sulfuric acid hydrolysis process, recovery yields of 50% for glucose and 70–98% for other sugars were obtained from whole-tree mixed softwood forest thinnings. A *S. cerevisiae* yeast strain was successfully adapted to the hydrolysates at 17% total solids. Ethanol yield in the 74–89% theoretical range was obtained. In order to improve the ethanol yield, the concentration of oligosaccharides in the hydrolysate must be reduced by optimizing first- and second-stage hydrolysis parameters and/or adding a post-hydrolysis step.

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