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Pretreatment of Softwood by Acid-Catalyzed Steam Explosion Followed by Alkali Extraction

DANIEL SCHELL,* QUANG NGUYEN, MELVIN TUCKER, AND BRIAN BOYNTON

National Renewable Energy Laboratory, Golden, CO 80401

ABSTRACT

A process for converting lignocellulosic biomass to ethanol hydrolyzes the hemicellulosic fraction to soluble sugars (i.e., pretreatment), followed by acid- or enzyme-catalyzed hydrolysis of the cellulosic fraction. Enzymatic hydrolysis may be improved by using an alkali to extract a fraction of the lignin from the pretreated material. The removal of the lignin may increase the accessibility of the cellulose to enzymatic attack, and thus improve overall economics of the process, if the alkali-treated material can still be effectively converted to ethanol.

Pretreated Douglas fir produced by a sulfuric-acid-catalyzed steam explosion was treated with NaOH, NH_4OH, and lime to extract some of the lignin. The treated material, along with an untreated control sample, was tested by an enzymatic-digestion procedure, and converted to ethanol by simultaneous saccharification and fermentation using a glucose-fermenting yeast. NaOH was most effective at removing lignin (removed 29%), followed by NH_4OH and lime. However, the susceptibility of the treated material to enzymatic digestion was lower than the control and decreased with increasing lignin removal. Ethanol production was similar for the control and NaOH-treated material, and lower for NH_4OH- and lime-treated material.

Index Entries: Ethanol; lignocellulosic biomass; lignin extraction; cellulose hydrolysis; alkali.

INTRODUCTION

The production of ethanol from lignocellulosic biomass has received considerable attention, because of the potential of producing large quantities of ethanol for use as a transportation fuel *(1)*. The process involves

* Author to whom all correspondence and reprint requests should be addressed.

hydrolyzing the hemicellulosic and cellulosic fractions of biomass to their component sugars for subsequent conversion to ethanol by a fermentative process. Hemicellulose is typically hydrolyzed using a chemical process (e.g., by acid or caustic treatment, commonly referred to as "pretreatment"); cellulose is hydrolyzed by chemical (acid) or biological (enzyme) attack. The economic success of these processes will depend on their ability to obtain good sugar conversion, and to successfully convert the sugars to ethanol at high yields.

The enzymatic approach to hydrolyzing cellulose to glucose is receiving attention because enzymes can achieve high yields, since they do not catalyze glucose degradation reactions common for the acid process. However, the cellulose must be accessible to the enzyme. The accessibility depends on the severity of the pretreatment process. A greater degree of hemicellulose and lignin removal during pretreatment increases the accessibility of the cellulose, and thus the efficacy of cellulose hydrolysis.

One potential method of converting wood to ethanol involves removing the hemicellulosic sugars by an acid process, then, after washing the sugars from the solids, the solids are subjected to a lignin-extraction step. After separating the lignin-containing liquor from the solids, the soluble lignin is precipitated, and this liquor is combined with the hemicellulosic liquor (rich in six- and five-carbon sugars) and the solids in a simultaneous saccharification and fermentation (SSF) process. Combining the liquor streams reduces the amount of dilution water needed in the process, and thus increases ethanol concentrations. This should improve glucose conversion from enzymatic hydrolysis of cellulose, when compared to single treatment.

Heitz et al. *(2)* steam treated aspen wood *(Populus tremloides)* in a Stake II reactor (Stake Technology, Norval, Ontario, Canada). The treated material was delignified with NaOH at 5% by weight consistency, pH 13.0, and at 100°C for 30 min. Lignin in the caustic solution was recovered (precipitated) from solution by adjusting to pH 1.5–2.0, and holding at 80°C for 10 min. The amount of lignin recovered in the solution was strongly dependent on steam-treatment conditions ranging from 5 to 90% as treatment severity increased (residence time and steam temperature). Although, enzymatic glucose yields increased with steam treatment severity, delignification may or may not have increased yields.

Parajo et al. *(3)* delignified eucalpytus wood with a 95% acetic acid–0.2% HCl solution (boiling temperature for 1 h), and then treated the residue with varying concentrations (4, 12, and 20 wt%) of NH_4OH at 60°C for 3 h. NH_4OH caused little change in treated material composition, but more than doubled enzymatic hydrolysis yields. Varying NH_4OH concentration had no effect on yields.

Schwald et al. *(4)* investigated steam explosion (240°C, 60–140 s) of aspen wood chips and steam explosion of SO_2-impregnated chips (1.6% SO_2 dry basis, 220°C, 100 s). The treated chips were washed with water,

and then twice washed with 0.4% NaOH. There were no significant differences in enzymatic hydrolysis yields and rates between untreated and alkali-washed material.

Steam-exploded aspen wood with no catalyst, and with impregnation by SO_2 and sulfuric acid, was investigated by Mackie et al. *(5)*. The treated chips were washed with water, and then washed with 0.4% NaOH at room temperature. Extracted material (as percent of original wood weight) was approx 15% for no acid and SO_2-treated wood, and approx 10% for sulfuric-acid-treated wood. The effectiveness of the various treatments on the enzymatic susceptibility of the treated wood was tested by performing an SSF procedure. However, no comparison was made between alkali-washed and unwashed material.

The goal of this work was to test the enzymatic digestibility and SSF performance of a softwood feedstock (Douglas fir) subjected to sulfuric-acid-catalyzed steam explosion, followed by alkali extraction. Various alkalis (NaOH, NH_4OH, and lime) were tested for their ability to remove lignin, increase enzymatic cellulose hydrolysis yields, and not significantly affect fermentation performance. Although NaOH is a stronger caustic and should be a more effective delignification agent, NH_4OH may have some nutritive effect, and lime is known to detoxify hydrolysates *(6)*.

METHODS

Pretreatment

Debarked Douglas fir logs were chipped and milled in a knife mill equipped with a 9.5-mm screen. Particles smaller than 2 mm were discarded. Screened particles were soaked in 0.35% sulfuric acid at 60°C for 6 h. They were then placed in a steam explosion apparatus previously described *(7)*, and heated to 215°C by direct steam injections. After heating for 140 s, the particles were explosively decompressed into a collection tank. The solids were collected and frozen for later use.

Solids concentrations were determined by oven-drying a sample of solids or liquid at 105°C. The composition of the pretreated biomass was determined by methods previously described *(8)*.

Alkali Extraction

Figure 1 shows a flow diagram of the process used to produce the alkali-treated material used in this study. Pretreated wood (at 31% solids) was diluted to 20% solids with 60°C water (~100 g of water) and filtered, then washed with another 100 g of water to extract most of the soluble sugars. This low-pH sugar solution was saved, and the solids were extensively washed with fresh 60°C water. The washed solids were diluted to a 15% solids concentration with an alkali solution and held at 60°C in either 0.4% NaOH (0.1 *M*), 10% NH_4OH (2.8 *M*), or 0.06% lime (0.008 *M*).

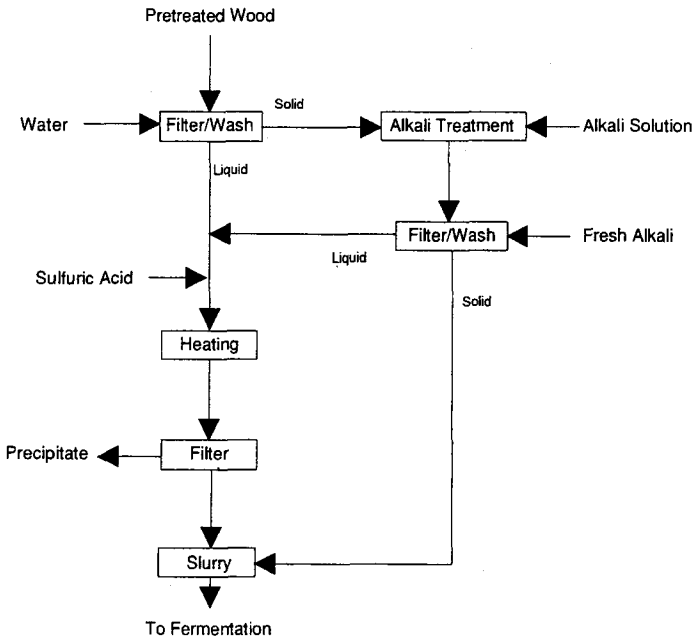

Fig. 1. Alkali treatment experimental procedure.

The lime solution was at its solubility limit at 60°C. After 1 h, the solids were filtered and washed with the fresh 60°C alkali solution. This alkali solution was saved, and the solids were again extensively washed with fresh alkali solution. The treated washed solids were analyzed for enzymatic digestibility. The total solids concentrations were measured on all saved and discarded solutions for tracking solids.

The low-pH sugar solution was combined with the alkali solution, and was adjusted to pH 2.0–2.5 with sulfuric acid. The solution was heated to 80–90°C and held for 15 min, to precipitate the lignin solubilized during the alkali treatment. The solution was filtered to remove precipitated lignin, and later combined with the treated solids to test performance during SSF. When NH_4OH was used, the alkali solution was heated to 80–90°C and held until the pH stabilized as the ammonia vapor was driven off. This additional step simulates an ammonia recovery step. A control sample (nonalkali-treated) was generated by following only the first filter-and-wash step, and saving the produced solids and liquid.

Enzyme Digestibility

Enzymatic digestibilities (defined as glucose produced divided by potential glucose) were performed on the extensively washed alkali-treated and control samples, using the following procedure. The amount of washed solids required to give 0.1 g cellulose in 10 mL was added to a vial. The buffer for the digestion was 50 m*M* citrate, pH 4.8, containing

40 μg/mL tetracycline and 30 μg/mL cycloheximide. The Iogen (Ottawa, Ontario, Canada) cellulase enzyme (Iogen Super Clean cellulase, lot #BRC 191095) loading was adjusted to 60 FPU/g of cellulose in the vials. The contents of the vials were prewarmed to 50°C before enzyme was added. Digestibility assays were carried out in duplicate at 50°C on a Roto-Torque rotaton (Cole Parmer, Niles, IL), with rotation at a 45-degree angle from the horizontal and 120 rpm, and compared to an identical no-enzyme blank. Duplicate Solka Floc (grade NF-FCC, lot #1016, Fiber Sales and Development, Urbana, OH 43078) digestions were used as controls. One-half mL samples were removed and analyzed for glucose, using a YSI Model 2700 Select Biochemistry Analyzer equipped with immobilized glucose oxidase membranes (Yellow Springs Instruments, Yellow Springs, OH). Samples were centrifuged at 12,000*g* for 5 min and diluted to keep the glucose readings below the 2.5 g/L level used for calibrating the instrument.

Simultaneous Saccharification and Fermentation

Alkali-treated solids and liquor were thawed and recombined. The pH levels of the NaOH and lime-treated slurries were near the required 5.0, after the acidified liquor was combined with the high-pH solids. The ammonia-treated sample was near pH 10.0 and was adjusted to 5.0 with sulfuric acid. The control sample was raised to pH 5.0 with lime. Because of dilution caused by adding liquid during the treatment steps described above, the total solids concentration of the combined slurries was 6–7%. The solids concentration of the control sample was adjusted to be in this range. The concentration of Difco (Detroit, MI) yeast extract and peptone in each SSF flask was 2.5 g/L and 5.0 g/L, respectively. The enzyme loadings were 70–75 FPU/g cellulose, so that adequate glucose would be available from cellulose hydrolysis. Each flask was inoculated with a 10% (w/w) *Saccharomyces cerevisiae* D_5A *(9)*, a glucose-fermenting yeast, grown on 50 g/L glucose at 30°C. Inoculated flasks were placed on a rotary shaker that operated at 30°C and 150 rpm.

Flasks were analyzed for sugars with a Hewlett-Packard (Palo Alto, CA) 1090L high-pressure liquid chromatograph (HPLC) equipped with a Bio-Rad (Hercules, CA) HPX-87P carbohydrate-analysis column that operated at 85°C. The mobile phase was deionized water at a flow rate of 0.6 mL/min. Fermentation products were quantified using the same samples on another HPLC equipped with a Bio-Rad HPX-87H organic-acid analysis column operating at 65°C. The mobile phase was 0.01 *N* sulfuric acid at a flow rate of 0.6 mL/min.

RESULTS

Alkali Extraction

After pretreatment, the wood slurry is 31.1% total solids and 21.6% insoluble solids, and approx one-third of the wood was solubilized by the treatment. The composition is 46% cellulose and 45% lignin. One goal

Table 1
Amount of Lignin Dissolved from Pretreated Douglas Fir

	Lignin dissolved during alkali treatment step[a]	
Alkali	% original pretreated wood	% lignin in pretreated wood
NaOH	13.3	29.5
NH_4OH	4.7	10.5
Lime	0.2	0.5

[a] Assumes only lignin is dissolved during the alkali extraction step, and includes lignin in the discarded alkali-washed liquor.

of this work was to determine the ability of each alkali to solubilize lignin that remained in the pretreated wood. The results are presented in Table 1.

As expected, NaOH was the most effective delignification agent. It solubilized 13.3% of the pretreated wood (after water washing), or 29.5% of the lignin. This is comparable to the results of Mackie et al. *(5)*, who achieve 15% solubilization of the pretreated wood. Ten percent NH_4OH solubilized only 10.5% of the lignin, about one-third of the solubilization achieved by NaOH, and was inferior to NaOH as a delignification agent. Lime was clearly not effective as a delignification agent, particularly with its limited solubility.

Enzymatic Digestibility

Enzymatic digestibilities as functions of time for each alkali are shown in Figure 2. For calculation purposes, the authors assumed that the cellulose content of all the samples was the same as the control; this is not true, because some lignin was dissolved during the alkali treatment (cellulose content was not measured for the treated material). This would increase the potential glucose, and bias the reported digestibilities higher than they should be, which makes it even more clear that the control was superior to any of the alkali-treated material. The results indicate that, as the extent of delignification increased, digestibilities decreased, which was counter to the expected trend. This phenomena has been reported before *(10)*, and researchers have suggested that either redistribution of the lignin or alteration in the crystalline cellulose structure is responsible for loss of hydrolysis performance.

Simultaneous Saccharification and Fermentation

Figure 3 shows ethanol production during SSF for the control and each of the three alkali-treated samples. The initial glucose concentration for each sample was 7, 5, 3, and 1 g/L for the control, NaOH-, lime-, and

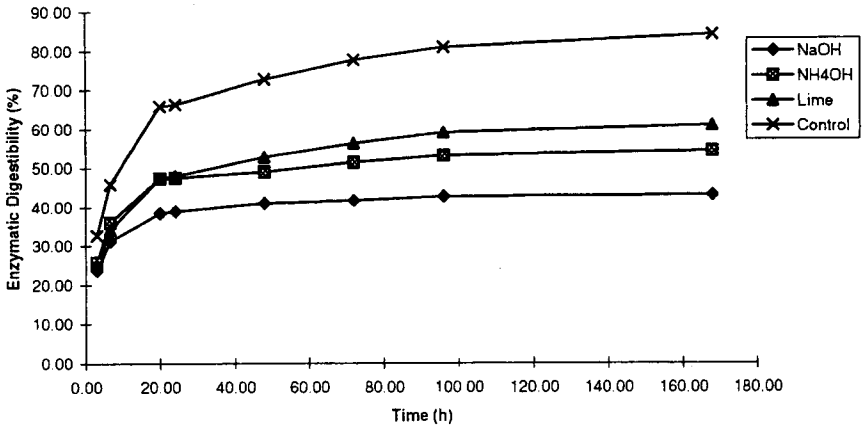

Fig. 2. Enzymatic digestibility as a function of time for each of the alkali-treated samples and the untreated control sample.

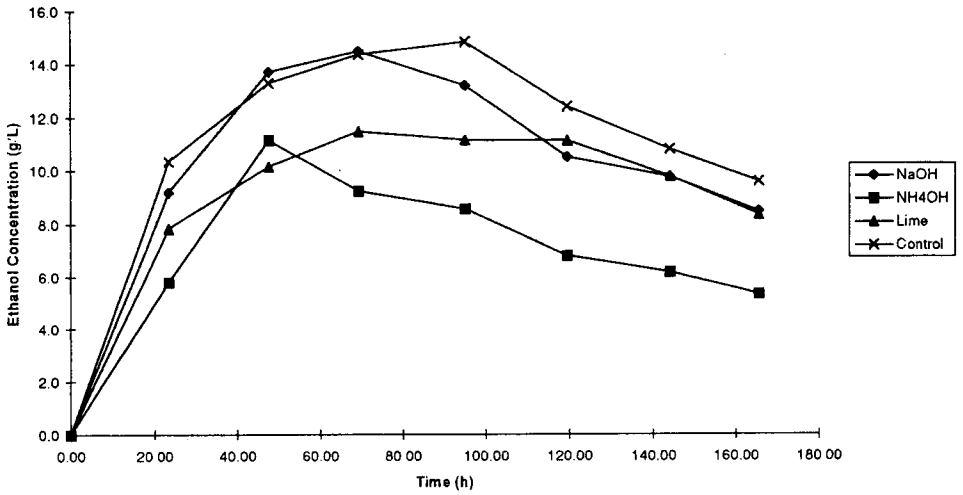

Fig. 3. Ethanol concentration as a function of time for each of the alkali-treated samples and the untreated control sample.

NH_4OH-treated samples, respectively. The alkali treatment is apparently responsible for some sugar loss, particularly with NH_4OH. However, the data in Fig. 3 includes the contributions from the initial glucose and glucose produced during enzymatic hydrolysis of the cellulose, since this reflects the overall performance of the system.

The SSF results show that the NaOH treatment had performance similar to the control (both peaked at about 80% ethanol yield); both NH_4OH and lime treatments produced significantly less ethanol. A small part of this can be attributed to the lower amount of initial glucose in the samples. But reduced enzymatic digestibility is probably the primary factor respon-

sible for the reduced yields. The enzymatic digestibility measured above for the NaOH sample was significantly lower than the control, but both samples had similar performance during SSF. The reason for this behavior is not known. But it does illustrate the importance of evaluating processes based on fermentation performance of treated material and not extrapolating fermentation performance based on digestibility measurements.

SUMMARY

This work has shown that NaOH is the most effective delignification agent, compared to NH_4OH and lime. Relatively low concentrations (0.1 *M*) of NaOH can dissolve 30% of the lignin in acid-pretreated Douglas fir. But this alkali treatment does not improve either enzymatic digestibility or SSF performance compared to an untreated control. The fractionation process would have to show a significant performance improvement to justify the increased cost and complexity that would be added to a biomass-to-ethanol process.

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REFERENCES

1. Schell, D. J., McMillan, J. D., Philippidis, G. P., Hinman, N. D., and Riley, C. (1992), in *Advances in Solar Energy*, vol. 7, Boer, K. W., ed., American Solar Energy Society, Boulder, CO, pp. 373–448.
2. Heitz, M., Capek-Menard, E., Koeberle, P. G., Gagne, E., Chornet, E., Overend, R. P., Taylor, J. D., and Yu, E. (1991), *Bioresource Technol.* **35,** 23–32.
3. Parajo, J. C., Alonso, J. L., and Santos, V. (1995), *Process Biochem.* **30,** 537–545.
4. Schwald, W., Breuil, C., Brownell, H. H., Chan, M., and Saddler, J. N. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 29–44.
5. Mackie, K. L., Brownell, H. H., West, K. L., and Saddler, J. N. (1985), *J. Wood Chem. Technol.* **5,** 405–425.
6. McMillan, J. D. (1994), in *Enzymatic Conversion of Biomass for Fuels Production*, Himmel, M. E., Baker, J. O., and Overend, R. P., eds, American Chemical Society, Washington, DC, pp. 411–437.
7. Nguyen, Q. A., Tucker, M. P., Boynton, B. L., Keller, F. A., and Schell, D. J. (1998), to be published in *Appl. Biochem. Biotechnol.*, accepted.
8. Vinzant, T. B., Ponfick, L., Nagle, N. J., Ehrman, C. I., Reynolds, J. B., and Himmel, M. E. (1994), *Appl. Biochem. Biotechnol.* **45/46,** 611–626.
9. Spindler, D. D., Wyman, C. E., Grohman, K., and Philippidis, C. P. (1992), *Biotechnol. Lett.* **14,** 403–407.
10. Ramos, L. P., Breuil, C., and Saddler, J. N. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 37–47.