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Optimization of Steam Explosion to Enhance Hemicellulose Recovery and Enzymatic Hydrolysis of Cellulose in Softwoods

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Abstract

A combination of Douglas fir heartwood and sapwood chips were steam pretreated under three conditions as measured by the Severity Factor (log R_o), which incorporated the time, temperature/pressure of pretreatment. By adjusting the steam pretreatment conditions, it was hoped to recover the majority of the hemicellulose component as monomers in the water-soluble stream, while providing a cellulosic-rich, water-insoluble fraction that could be readily hydrolyzed by cellulases. These three conditions were chosen to represent either high hemicellulose sugar recovery (low severity [L], log R_o = 3.08), high-enzyme hydrolyzability of the cellulosic component (high severity [H], log R_o = 4.21), and a compromise between the two conditions (medium severity [M], log R_o = 3.45). The medium-severity pretreatment conditions (195°C, 4.5 min, 4.5% SO_2, logR_o = 3.45) gave the best compromise in terms of relatively high hemicellulose recovery after stream pretreatment and the subsequent efficiency of enzymatic hydrolysis of the water-insoluble cellulosic fraction. The percent recovery of the original hemicellulose in the water-soluble fraction dropped significantly when the severity was increased (L-76.8%, M-64.7%, and H-37.5%). However, the ease of enzymatic hydrolysis of the cellulose-rich, water-insoluble fraction increased with increasing severity (L-24%, M-86.6%, and H-97.9%). Although more severe pretreatment conditions provided optimum hydrolysis of the cellulosic component, less severe conditions resulted in better recovery of the combined hemicellulose and cellulosic components.

Index Entries: Steam explosion; hemicellulose; enzymatic hydrolysis; softwood.

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Introduction

In British Columbia, about 9 million tons of wood residue are produced annually from sawmills with over one-third (3.3 million tons) of this material currently incinerated or landfilled *(1)*. The production of fuel ethanol from this residual material could alleviate the associated environmental and economic problems while providing an alternative transportation fuel from a renewable resource (no net greenhouse gases are released to the environment).

There are a number of ways to produce ethanol from wood. Our group has defined a generic wood-to-ethanol process (Fig. 1) comprised of the following steps: pretreatment, fractionation, enzymatic hydrolysis of cellulose, fermentation, product recovery (ethanol and lignin) and waste treatment *(2)*. Pretreatment using steam explosion of SO_2-impregnated softwood has been shown to be an effective way to enhance the fractionation of wood into its constituents of hemicellulose, cellulose, and lignin for further product recovery, as well as improving the ease of enzymatic hydrolysis of the cellulosic fraction *(3)*. Most of the past research in this area has been directed towards optimization for conversion of cellulose to monomeric glucose with subsequent fermentation to ethanol. However, under the severe pretreatment conditions required for optimum enzymatic hydrolysis, to a significant extent the hemicellulose sugars are degraded and lost *(4)*. At the same time, carbohydrate- and lignin-derived degradation products can also contribute to the inhibition of sugar fermentation to ethanol while the lignin becomes progressively more condensed and less reactive *(5)*.

Previously we have shown that, in order for the wood-to-ethanol process to be cost-effective, all of the carbohydrate components present in the wood substrate need to be recovered *(6)*. It was also found that the feedstock and enzyme costs were the primary barriers to maximizing the return on investment *(7)*. We have shown that less severe pretreatment conditions provided high release of hemicellulose sugars in a monomeric form while conserving the glucose in the cellulosic fraction *(8)*. However, pretreatment at low-severity conditions results in poor glucose recoveries, because the water-insoluble, cellulosic-rich fraction is not readily hydrolyzed by cellulase enzymes. Although extensive post-treatment after steam explosion to reduce the amount of lignin can be effective in improving the extent of enzymatic hydrolysis for some softwoods *(3)*, these additional process steps add to the overall production costs.

By controlling the severity of the pretreatment conditions, it may be possible to achieve a compromise between high enzymatic hydrolysis rates for the cellulose while providing good recovery of the hemicellulose components in the form of monomeric sugars. The purpose of the present study is to obtain some initial data that can test this hypothesis and to obtain an estimate of the range of pretreatment conditions that give good total sugar yields. Further work by others in the group is being carried out to fine-tune the pretreatment process. In the work described here, we

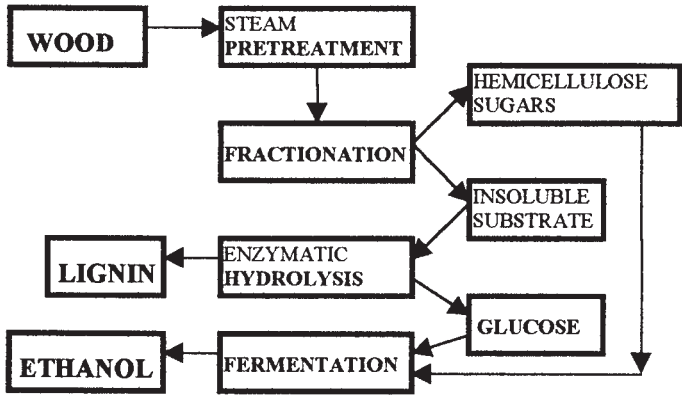

Fig. 1. Generic wood-to-ethanol process.

report the preliminary work on the effects of pretreatment conditions on 1. the release and recovery of sugars in the soluble fraction, and 2. the recovery of glucose after enzymatic hydrolysis of the cellulose-rich, water-insoluble fraction.

Materials and Methods

Severity of Pretreatment

The important variables in steam-explosion pretreatment are time, temperature, chip size, and moisture content *(9)*. The effects of time and temperature/pressure of the treatment process can be represented by the Severity Parameter as defined by Overend and Chornet *(10)*. The parameter combines time, t (min), and temperature, T (°C) in the form of:

$$R_o = te^{[(T-100)/14.75]} \quad (1)$$

In this work, we looked at three sets of pretreatment conditions: one for maximum hemicellulose recovery (low severity [L], log R_o = 3.08, 175°C, 7.5 min., 4.5% SO_2), one for maximum cellulose conversion via enzymatic hydrolysis (high severity [H], logR_o = 4.21, 215°C, 6.6 min, 2.38% SO_2), and a compromise between the other two conditions (medium severity [M], logR_o = 3.45, 195°C, 4.5 min, 4.5% SO_2).

Preparation of Water-Soluble (Hemicellulose-Rich) and Water Insoluble (Lignin-Cellulose Rich) Fractions

Mixed wood chips (sapwood: heartwood, 2:1) of Douglas-fir (*Pseudotsuga menziesii*) were steam-exploded, as described previously *(8)* at the three conditions described above. The ratio of sapwood to heartwood was used to reflect the likely composition of wood residue found in local sawmills in British Columbia. The pretreated materials, adjusted to a dry-matter

content of 5% (w/w) with de-ionized water, were filtered through a Whatman microglass filter paper under suction. The resultant filtrates were characterized and quantified for hemicellulose sugar content. The equivalent, cellulose-rich, water-insoluble retentates were used for characterization and subsequent enzymatic hydrolysis.

Sugar Analysis

The Klason lignin in the original wood chip samples were calculated using the TAPPI Standard method T222 om-88. The filtrates were then analyzed for reducing sugars content by the Somogyi-Nelson Method *(11)*. Similarly, the water-insoluble fractions of the steam-pretreated wood chips were analyzed for reducing sugar content after the removal of the acid-insoluble lignin. The Somogyi-Nelson Method was also used to measure the sugar concentrations of hydrolysate removed at different stages of enzymatic hydrolysis. The sugar content of the soluble fractions of the pretreated wood samples were measured as monomers. Samples were post-hydrolyzed by autoclaving in 4% sulfuric acid for 1 h at 120°C. A batch of sugar standards was also autoclaved at the same time to estimate hydrolysis loss factors. All sugar measurements were performed using high-performance liquid chromatography (HPLC) *(8)*.

Enzymes

Hydrolysis experiments were performed using a commercial *Trichoderm reesei* cellulase preparation (Celluclast from Novo-Nordisk, Denmark) that contained 49 mg/mL protein with the following activities: 80 FPU/mL, carboxymethyl-cellulase (CMCase) 52.3 IU/mL, xylanase 225.7 IU/mL, and β-glucosidase 50.2 IU/mL. Celluclast was supplemented with β-glucosidase (Novozym 188 from Novo-Nordisk, Denmark) with the following characteristics: protein 44 mg/mL, 5.1 FPU/mL, CMCase 33.8 IU/mL, xylanase 93.57 IU/mL and β-glucosidase 500.3 IU/mL. The enzyme mixture had a final filter paper to β-glucosidase activity ratio of 1:3.5 to avoid product inhibition owing to cellobiose accumulation *(12)*.

Enzymatic Hydrolysis

In this work, the three substrates, representing different levels of pretreatment severity, were hydrolyzed using cellulase enzyme at a concentration of 60 FPU/g cellulose. Batch hydrolysis was carried out in 250-mL stoppered Erlenmeyer flasks containing 2% substrate (dry weight/volume) in 50 mL 50 m*M* acetate buffer at pH 4.8 supplemented with 0.5% sodium azide. The flasks were incubated in a shaker at 45°C and 200 rpm. They were pre-incubated for 10 min at the indicated temperature before the addition of the enzymes. Aliquots of 1 mL were taken after 1, 4, 24, 48, 72, and 96 h. The samples were immediately chilled on ice and centrifuged at 3000 rpm for 5 min. Supernatants from the samples were frozen and stored for sugar measurement.

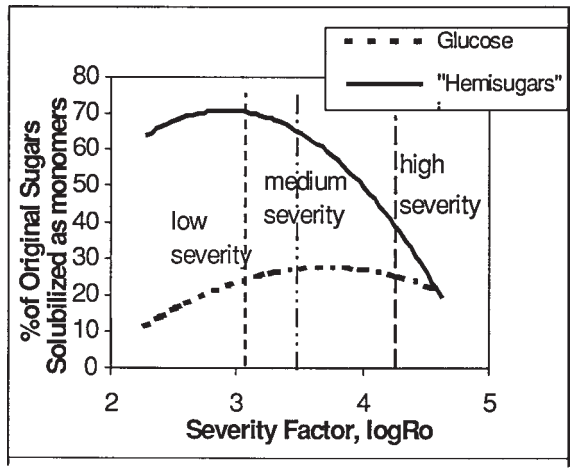

Fig. 2. Effect of pretreatment severity on solubilization of sugars after acid-catalyzed steam pretreatment of Douglas Fir wood chips.

Results and Discussion

Survival of Hemicellulose Sugars in Solution

Previously, it was shown that the severity of pretreatment affected both the solubilization and recovery of hemicellulose sugars *(8)*. For example, it was reported *(4)* that about 65% of the original pentosan in *Populus tremuloides* could be recovered at a severity factor of log R_o = 3.8, whereas at higher severities, there was a significant loss of pentosans to furfurals and other degradation products. Initially, with the Douglas Fir substrate, we wanted to find the pretreatment conditions that would provide maximum solubilization of the hemicellulose-derived sugars, with the majority of these sugars present in a monomeric form. Using the sum of monomeric arabinose, xylose, galactose, and mannose ("hemisugars") as representative of the trend in solubilization of hemicellulose (Fig. 2), maximum monomer recovery was found at a Severity Factor of approx 3 (175°C, 7.5 min, 4.5% SO_2). As the Severity Factor increased to 4.21, the amount of "hemisugars" in solution decreased sharply to 37% of that detected in the original wood (Table 1). This contrasted with a recovery of approx 77 and 65% of the original "hemisugars" in a monomeric form for the water-soluble fraction derived from steam treatment at low- and medium-severity conditions, respectively. As an increase in sugar breakdown products such as furfural and hydroxy methyl furfural was observed in the HPLC, it seems that reduction in "hemisugars" with increasing severity is owing mainly to sugar decomposition *(4)*.

Survival and Recovery of Sugars in the Water-Insoluble Cellulosic Fraction

Previously it has been shown that a higher degree of severity was usually required to obtain a water-insoluble, cellulosic-rich fraction that

Table 1
Effect of Severity of Pretreatment on Percent Recovery of Hemicellulose, Cellulose and Degree of Enzymatic Hydrolysis of the Cellulosic Component

Steam-explosion conditions	Low	Medium	High
Severity factor ($logR_o$)	3.08	3.45	4.21
Recovery of hemicellulose component			
% Original "hemisugars" recovered in monomeric form	76.8	64.7	37.5
Enzymatic hydrolysis of the cellulosic fraction			
Cellulose content (g/100 g of water insoluble component)	58.4	53.0	19.4
% of Cellulose hydrolyzed (60 FPU/g cellulose)	24.2	86.6	97.9
Sugar yield from enzymatic hydrolysis (g/100 g of water insoluble component)	14.1	45.9	19.0

could be readily enzymatically hydrolyzed *(9)*. However, it was important that as much of the original cellulose as possible was recovered in a utilizable form as either fermentable glucose released into the water-soluble stream as a result of "acid hydrolysis" occurring during steam pretreatment, or as readily enzymatically hydrolyzed cellulose available in the water-insoluble fraction.

In the water-soluble fraction, 6.34 g glucose/100 g original wood was detected at low-severity conditions. The concentration of mannose was 9.98 g/100 g. Assuming that the glucose in hemicellulose is one-third of the mannose concentration *(13)*, the amount of glucose solubilized from hemicellulose would be 3.3 g/100 g, or 52% of the glucose detected. At high severity, the amount of glucose in solution was 22.93 g/100 g, but the amount of mannose was only 5.19 g/100 g. Using the same assumption that the amount of glucose from the hemicellulose fraction is one-third of mannose *(13)*, the amount of glucose from the hemicellulose fraction makes up only 7.5% of the glucose detected in solution at high severity. Although the majority of the glucose detected in the water-soluble fraction after pretreatment at low-severity condition was derived from the hemicellulose component, at high severity, the low cellulose content of the water-insoluble fraction (Table 1) and the corresponding higher glucose concentration of the water-soluble fraction (Fig. 2) all indicated that extensive acid hydrolysis of the cellulose component occurred *(4)*. It was also apparent that at higher severity conditions, there was substantial degradation of the water-soluble sugars, which greatly reduced the recovery and yield of the original carbohydrates.

As well as recovering as much of the original carbohydrate in either the water-soluble or water-insoluble fraction as possible, it was also important to determine how readily the cellulosic fraction could be enzymatically hydrolyzed (Fig. 3, Table 1). As expected, the more severe the pretreatment, the more readily the water-insoluble cellulosic component could be hydro-

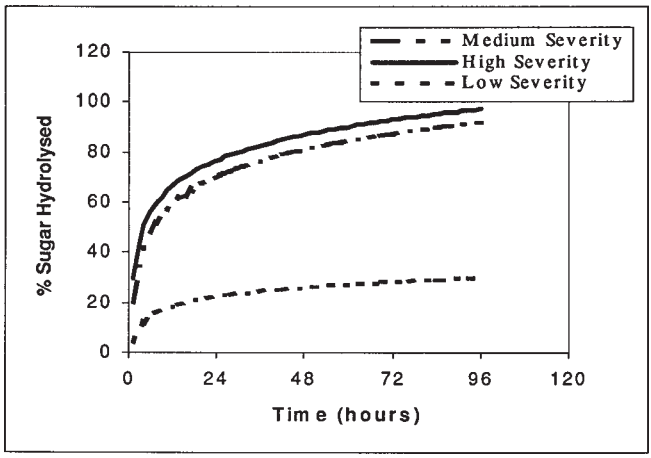

Fig. 3. Effects of severity on enzymatic hydrolysis of steam-pretreated Douglas Fir.

lyzed (Table 1). When the rate and extent of hydrolysis was followed over time (Fig. 3), the low-severity pretreatment conditions resulted in initially slow rates of hydrolysis and the release of only 24.2% of the available cellulose after 72 h (Table 1). Although almost complete enzymatic hydrolysis of the water-insoluble fraction (97.7%) could be achieved after severe pretreatment, the medium-severity treatment also resulted in a similar hydrolysis profile (Fig. 3) and a final yield of 86.6% (Table 1).

Because at high severity, a significant quantity of the sugar in the wood is already lost during pretreatment, despite near-complete enzymatic hydrolysis of the cellulosic residue, the net sugar yield from hydrolysis of the water-insoluble cellulosic fraction was 19.0 g/100 g of substrate after 72 h of hydrolysis (Table 1). On the other hand, at low severity, although most of the cellulose was conserved, the material was not readily hydrolyzed. As a result, the net sugar yield was only 14.1 g/100 g substrate. The medium-severity condition gave a significantly higher net sugar yield of 45.9 g/100 g after enzymatic hydrolysis of the substrate. Although the medium-severity pretreatment conditions resulted in a slightly greater loss of the cellulosic sugar than did the low-severity case (from 58.4% to 53%) (Table 1), the extent of enzymatic hydrolysis was greatly enhanced (Fig. 3).

Conclusions

Pretreatment of Douglas Fir wood chips at low-severity conditions provided best recovery of the original hemicellulose sugars in a monomeric form. After medium-severity pretreatment, the amount of "hemisugars" recovered dropped slightly, whereas at high severity, the amount dropped drastically. After pretreatment at low severity, although most of the cellulose in the wood was conserved, this material was not readily hydrolyzed. Although the samples pretreated at higher severity could be hydrolyzed completely, a significant amount of the original hemicellulose- and cellu-

lose-derived sugars were solubilized and degraded during the pretreatment stage. Medium-severity conditions provided the best results in terms of combined sugar yields from both enzymatic hydrolysis and through near-optimum recovery of sugars in the water soluble fraction.

Acknowledgments

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