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Steam Pretreatment of Douglas-Fir Wood Chips

Can Conditions for Optimum Hemicellulose Recovery Still Provide Adequate Access for Efficient Enzymatic Hydrolysis?

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Abstract

Douglas-fir sapwood and heartwood were impregnated with SO_2 and steam exploded at three severity levels, and the cellulose-rich, water-insoluble component was enzymatically hydrolyzed. The high-severity conditions resulted in near complete solubilization and some degradation of hemicelluloses and a significant improvement in the efficiency of enzymatic digestibility of the cellulose component. At lower severity, some of the hemicellulose remained unhydrolyzed, and the cellulose present in the pretreated solids was not readily hydrolyzed. The medium-severity pretreatment conditions proved to be a good compromise because they improved the enzymatic hydrolyzability of the solids and resulted in the recovery of the majority of hemicellulose in a monomeric form within the water-soluble stream. Sapwood-derived wood chips exhibited a higher susceptibility to both pretreatment and hydrolysis and, on steam explosion, formed smaller particles as compared to heartwood-derived wood chips.

Index Entries: Douglas fir; sapwood; heartwood; steam explosion; enzymatic hydrolysis; fiber coarseness; particle size distribution.

Introduction

Bioconversion of lignocellulosic feedstocks to ethanol fuel holds promise in providing a cleaner and more environmentally benign alternative to gasoline for the transportation sector. Various types of hardwoods and agricultural residues have been studied as potential feedstocks for ethanol fuel production *(1–6)*, and extensive research has been conducted to

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optimize the different unit operations (e.g., pretreatment, enzymatic hydrolysis, and fermentation) of the overall wood-to-ethanol process *(7–10)*.

During the past decade, there has been an increasing interest in production of ethanol fuel from softwoods in Canada and Scandinavian countries. Bioconversion of softwood wastes has major environmental and socioeconomic benefits because it can both reduce the adverse impacts of the current wood-waste management practices, such as landfilling and incineration, and aid in the growth and sustainability of forestry-based industries.

Our past technoeconomic assessment *(11)* has shown that complete utilization of all the main wood components (cellulose, hemicellulose, lignin) as well as increasing the efficiency of the cellulose hydrolysis step are key factors in making the wood-to-ethanol process economically viable. Therefore, it is important to identify pretreatment conditions that not only allow for maximum recovery of hemicellulose-derived sugars in the liquid stream for subsequent fermentation, but also produce sufficiently accessible cellulose for efficient enzymatic digestion. The present study was undertaken to identify optimum pretreatment conditions for acid-catalyzed steam pretreatment of two distinct wood fractions (sapwood and heartwood) of a representative softwood species, Douglas fir, found in the Pacific Northwest. We, as well as other investigators *(12,13)*, have shown that impregnation of woody feedstocks with sulfur dioxide prior to steam explosion decreases the carbohydrate decomposition and increases the enzymatic hydrolysis of the substrates.

Although similar in many respects to hardwoods and herbaceous materials, softwoods show a greater resistance to pretreatment and enzymatic digestion primarily owing to their chemical and structural substrate characteristic *(14)*. It is also recognized that there are anatomical and chemical variations in different parts of a tree (e.g., sapwood vs heartwood) that may influence their response to pretreatment and subsequent enzymatic digestion. Heartwood, e.g., consists of more-mature wood cells and, in comparison with sapwood, contains more organic deposits such as resin, phenolic substances, and pigments *(15)*.

In the present study, Douglas-fir sapwood and heartwood fractions were impregnated overnight with SO_2, then steam exploded at various severity conditions (low, medium, and high), and subsequently hydrolyzed using commercial enzymes. The chemical composition of solid residues and liquid prehydrolysates was determined to monitor the material balance and the extent of hemicellulose solubilization and recovery. The enzymatic hydrolysis profiles of the water-insoluble, cellulosic component were also studied to determine the impact of pretreatment severity on the hydrolysis rate and yield. In an attempt to increase the digestibility of solids from the low-severity pretreatment without promoting the degradation of water-soluble sugars, the effect of a second-stage steam explosion under medium-severity conditions (195°C for 4.5 min with no SO_2 added) and mechanical refining were compared. The particle size distribution of each pretreated sample was also determined.

Materials and Methods

Substrate Pretreatment

Bolts of a 129-yr-old Douglas fir (*Pseudotsuga menziesii*) obtained from the University of British Columbia research forest were debarked, sawn, and separated into sapwood and heartwood fractions. Each fraction was then chipped into smaller pieces with an average size of 30 × 30 × 2 mm. Samples of 100 g (dry) of wood chips were impregnated overnight with anhydrous SO_2 in plastic bags. The total weight of SO_2 was monitored. The wood samples were then steam exploded in a 2-L Stake Tech II (Stake Tech-Norvall, Ontario, Canada) reactor at three different severity conditions: low, medium, and high. These conditions were selected from the 13 sets of experimental conditions tested in our previous work *(16)*. Pretreatment at low severity (175°C, 4.5% of SO_2, 7.5 min) provided maximum hemicellulose recovery (in solids and liquid), whereas at high severity (215°C, 2.38% of SO_2, 2.38 min) produced solids with the highest enzymatic digestibility. Pretreatment at medium severity (195°C, 4.5% of SO_2, 4.5 min) was a compromise between the low and the high pretreatment conditions. The water-insoluble solid fraction, after being adjusted to 20% (w/w) dry matter content, was washed with deionized water and filtered through a Whatman microglass-fiber filter paper in a Buchner funnel. The water-insoluble residues were characterized as described in Table 3 and stored at 4°C until used in hydrolysis experiments. The water-insoluble residue obtained from the low-severity pretreatment was further treated using a second-stage steam explosion at 175°C for 7.5 min and 195°C for 4.5 min (no SO_2 was added). The same solid residue was also submitted to mechanical refining in a Sprout Waldron (Mundy, PA) refiner.

Substrate Characterization

The chemical composition of raw and steam-exploded solids was determined by performing a high-performance liquid chromatography (HPLC)–based sugar analysis assay after sulfuric acid hydrolysis according to the TAPPI Standard Method T222 om-88. The HPLC system (Dionex DX-300, Dionex, Sunnyvale, CA) was equipped with an ion-exchange PA1 (Dionex) column, a pulsed amperometric detector with a gold electrode, and a Spectra AS3500 autoinjector (Spectra-Physics, San Diego, CA). Prior to injection, samples were filtered through 0.45-μm HV filters (Millipore, Bedford, MA) and a volume of 20 μL was loaded. The column was equilibrated with 250 m*M* NaOH and eluted with deionized water at a flow rate of 0.8 mL/min.

Particle Fractionation

A Bauer-McNett fiber classifier (TMI) was used to determine the particle size distribution of sapwood and heartwood fractions after pretreatment at various severity conditions. Five consecutive screens of 14, 28, 50,

100, and 200 mesh were used. Fines were classified as particles that passed through the 200-mesh screen.

Enzymes

A complete *Trichoderma reesei* cellulase system (Celluclast®, Novo-Nordisk, Bagsvaerd, Denmark) in combination with a commercial β-glucosidase (Novozym 188®, Novo-Nordisk) was used in hydrolysis experiments. Celluclast contained 49 mg of protein/mL as measured by the Bio-Rad protein assay (Bio-Rad, Hercules, CA) and had the following activities: 80 FPU/mL of filter paper activity, 52 IU/mL of carboxymethylcellulase (CMCase), 226 IU/mL of xylanase, and 50 IU/mL of β-glucosidase. The protein content and activities of the Novozym 188 were as follows: 44 mg/mL; 5 FPU/mL; 34 IU/mL of CMCase; 94 IU/mL of xylanase; and 500 IU/mL of β-glucosidase.

Hydrolysis Experiments

Hydrolysis experiments were carried out at 2% (w/v) solid concentration in 50 m*M* sodium acetate buffer solution (pH 4.8) containing 0.5% sodium azide. Each flask was loaded with the desired enzyme loading of FPU of Celluclast/g of cellulose and Novozym 188/g of cellulose so as to have a CBU:FPU ratio of 2:1. These flasks were incubated in a shaking (200 rpm) water bath at 45°C. Samples of 500 μL were taken at various time intervals. The sugar concentrations were determined by HPLC as described in Substrate Characterization.

Results and Discussion

We initially determined the chemical composition of the sapwood- and heartwood-derived wood chips (Table 1), and it was apparent that the sugar and lignin content of the two fractions were quite similar. The total mass recovery for the sapwood- and heartwood-derived chips was slightly lower and higher than 100%, respectively. While the >100% mass recovery could be justified as the result of polysaccharide hydration (cleavage of glycosidic bonds) during hydrolysis, a <100% recovery could have been attributed to the presence/formation of water-soluble components (e.g., extractives and sugar degradation products) that were not quantified. Moreover, it is probable that sampling and analytical errors are the main reasons for the observed, slight variations.

The pretreatment severity had a strong effect on total recovery of solids after steam explosion (Table 2). It was evident that increasing the reaction severity increased the extent of feedstock solubilization (decreasing percentage of solid) and reduced the recovery of the original materials. This was not unexpected, because it is known that harsh pretreatment conditions can dissolve not only the hemicellulose fraction of the substrate but also portions of the cellulose that will result in a reduction in total mass of solids. On the other hand, degradation of water-soluble, hemicellulose-

Table 1
Sugar (hydrated form) and Lignin Content
of Original Douglas-Fir Sapwood and Heartwood

Sugars (g/100 g dry substrate)	Sapwood	Heartwood
Glucose	48.3	49.4
Mannose	13.2	14.0
Galactose	3.6	4.2
Xylose	2.3	3.0
Arabinose	1.2	1.2
Acid-insoluble lignin	29.1	30.9
Total	97.7	102.7

Table 2
Recovery of Original Feedstock (% dry wt) in Solid and Liquid Streams
After Steam Explosion of Douglas-Fir Sapwood and Heartwood
at Low, Medium, and High Steam Pretreatment Severity Conditions[a]

Feedstock and conditions	Solids (%)	Liquid (%)	Total recovery (%)
Sap-L	77	13	90
Hrt-L	89	8	97
Sap-M	76	12	88
Hrt-M	69	13	82
Sap-H	58	16	74
Hrt-H	61	16	77

[a]Sap, sapwood; Hrt, heartwood; L, low; M, medium; H, high. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

derived sugars can result in the increased loss of total mass recovery (solid + liquid) observed at higher severity conditions (Table 2).

It is known that hydrolysis of glycosidic bonds in wood polysaccharides is catalyzed by the hydronium ion (H^+) generated from the reaction between SO_2 and water during the steam-explosion operation. Owing to their extremely small size (4 Å), hydronium ions can easily penetrate into the tight structure of cellulose and hydrolyze the polymer into its monomeric constituents, glucose. Glucose, as well as other hemicellulose-derived monomeric sugars, will undergo degradation reactions that produce byproducts such as hydroxymethylfurfural and furfural. These compounds are known to have toxic/inhibitory effects on ethanologenic microorganisms during the fermentation of sugars to ethanol *(7,8)*.

The effect of increasing pretreatment severity on the degradation of the hemicellulose-derived sugars was apparent (Fig. 1) because, in general, the sugar loss increased with an increase in pretreatment severity, with the exception of glucose. The increase in glucose concentration in the liquid prehydrolysate was the result of cellulose depolymerization and corre-

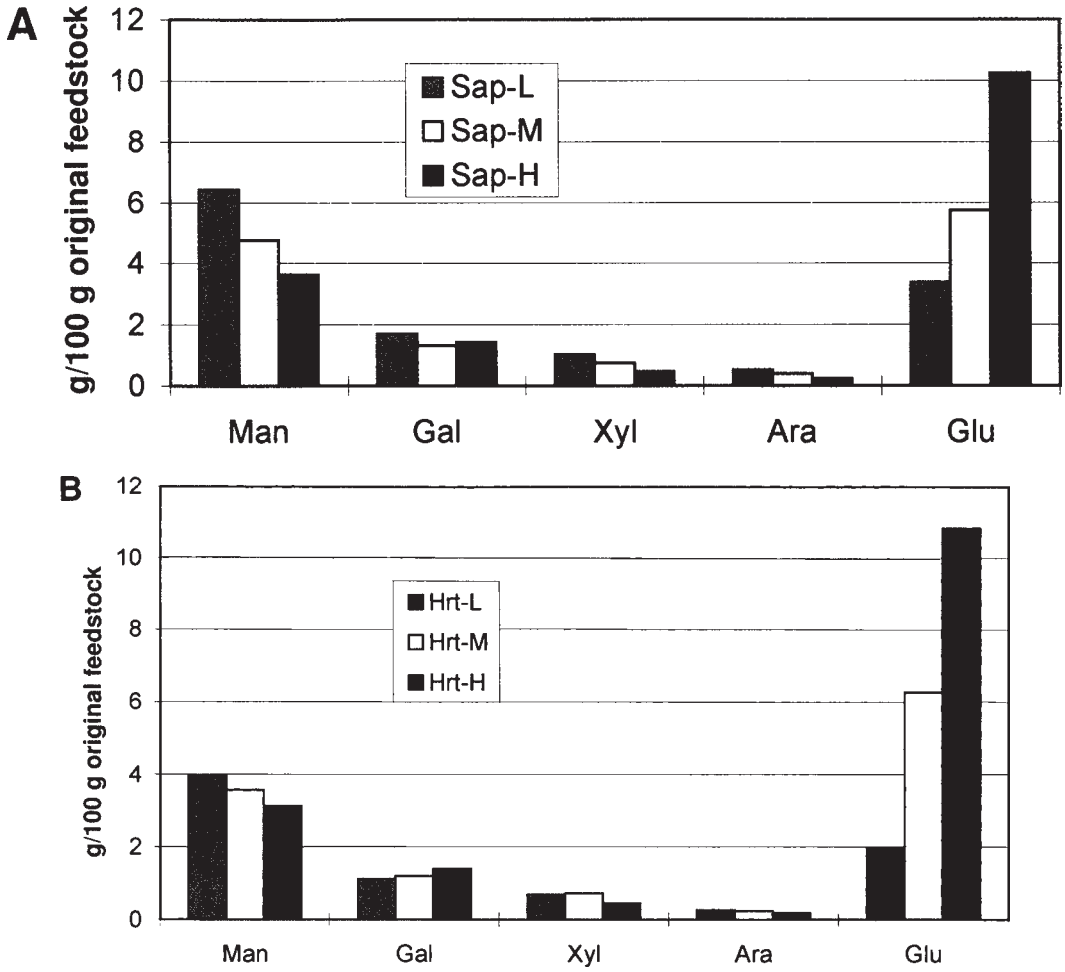

Fig. 1. **(A)** Concentration of various sugars in the water-soluble fraction of steam-exploded Douglas-fir heartwood (Hrt) at low (L), medium (M), and high (H) severities. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min. **(B)** Concentration of various sugars in the water-soluble fraction of steam-exploded Douglas-fir sapwood (Sap) at low (L), medium (M), and high (H) severities. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

sponded closely to the decrease in solids' glucose content. The extent of cellulose depolymerization was great enough to override the effect of the hemicellulose-derived glucose degradation, whose ultimate effect is the accumulation of glucose in the liquid. The minor variations in concentration of galactose seem to be the result of experimental errors rather than any other significant reason.

At each of the three pretreatment conditions, the majority of hemicellulose-derived sugars (except glucose) were recovered as monomers in the water-soluble component (Table 3), although the effect was more pro-

Table 3
Sugar (monomers) and Lignin Composition (g/100 g dry substrate) of Pretreated Solids and Liquid Prehydrolysate Resulting from Steam Explosion of Douglas-Fir Sapwood and Heartwood at Low, Medium, and High Pretreatment Severity Conditions[a]

	Ara		Gal		Glu		Xyl		Man		Lignin
Feedstock	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Sap-L	0.23	0.51	0.62	1.69	44.66	3.38	0.92	1.01	3.62	6.42	28.49
Hrt-L	0.27	0.26	0.89	1.12	51.62	1.98	0.98	0.69	4.09	3.95	32.93
Sap-M	0.21	0.40	0.56	1.32	35.70	5.75	0.91	0.75	3.15	4.77	28.70
Hrt-M	0.23	0.24	0.83	1.20	34.50	6.27	0.98	0.72	3.38	3.57	33.00
Sap-H	0.06	0.24	0.64	1.42	23.20	10.25	0.23	0.47	1.86	3.63	26.10
Hrt-H	0.06	0.19	0.79	1.40	20.74	10.84	0.18	0.45	1.77	3.12	32.94

[a]Sap, sapwood; Hrt, heartwood; L, low; M, medium; H, high. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

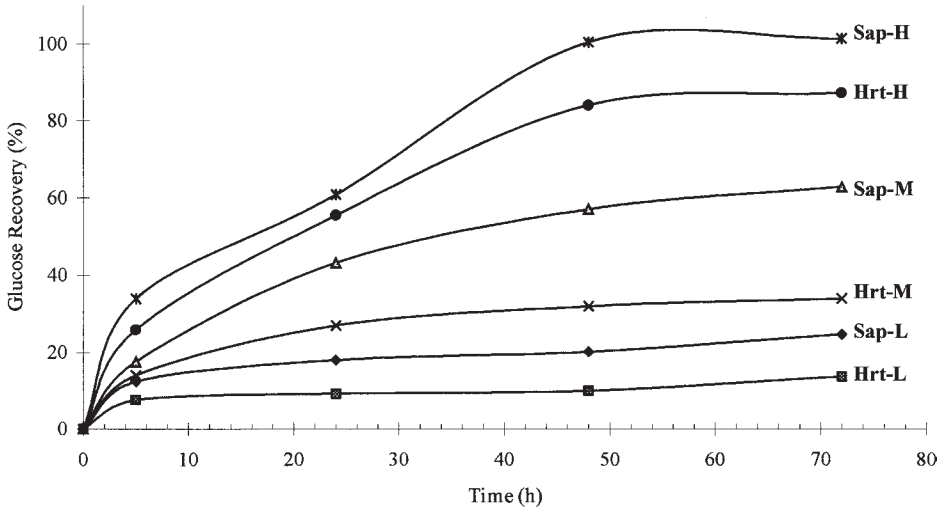

Fig. 2. Enzymatic hydrolysis of Douglas-fir sapwood (Sap) and heartwood (Hrt) pretreated at low (L), medium (M), and high (H) severities. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

nounced at higher severities. The lignin content remained relatively constant at all conditions. The total recovery (solid + liquid) of each sugar, including glucose, decreased as the pretreatment severity increased, clearly indicating the drawback of excessively harsh pretreatments, which reduce the potential to obtain ethanol from the hemicellulose-derived sugars.

At low- and medium-severity conditions, the heartwood-derived hemicellulose (Ara, Xyl, Gal, and Man) was not as readily solubilized (Table 3). These solids contained a higher percentage of hemicellulose as compared to the sapwood pretreated at the same sets of conditions. This trend was not observed at the higher severity conditions perhaps owing to extreme severity of the treatment and extensive solubilization of all components.

We next followed the enzymatic hydrolysis profile when the pretreated solids were subjected to enzymatic hydrolysis for 72 h (Fig. 2). The hydrolyzability of the solids improved as the pretreatment conditions became more severe. This was probably owing to extensive hemicellulose solubilization since the lignin content remained fairly constant, even at the highest severity (Table 3). Previously, it has been suggested *(18,19)* that hemicellulose hydrolysis increases the pore volume of the substrates, hence increasing the cellulose accessibility by cellulolytic enzymes. These pores can be the anatomical features of the wood, such as lumen and pit aperture, or the externally induced cavities and slits formed during dissolution of hemicellulose and lignin. The total volume of these pores, which could also be interpreted as the surface area available for protein interaction, has been shown to be closely related to the enzymatic digestibility of a variety of substrates *(17–19)*.

The improvement in substrate digestibility resulting from an increase in pretreatment severity has also been suggested to be owing to such factors

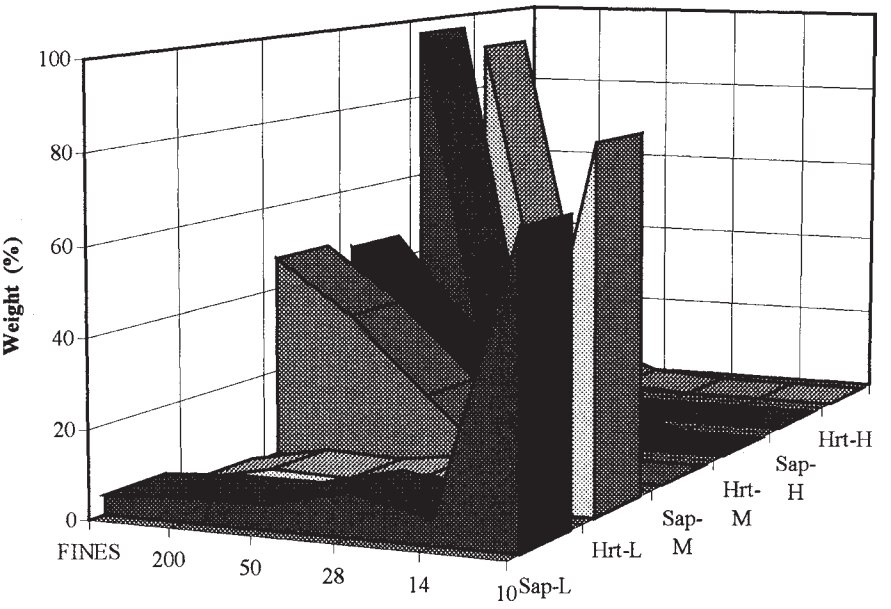

Fig. 3. Particle size distribution of Douglas-fir sapwood (Sap) and heartwood (Hrt) pretreated at low (L), medium (M), and high (H) severities. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

as enhancing amorphogenesis within the cellulose structure and formation of smaller particles *(20,21)*.

It was probable that the type and degree of lignification within the pretreated substrate had an effect because at each of the three severity conditions, the sapwood was hydrolyzed more efficiently than heartwood (Fig. 2). This effect could be attributed to the consistently lower lignin content (~4 to 7%) of the sapwood fractions (Table 3). The difference in hydrolyzability of sapwood and heartwood was most pronounced at medium severity (Sap-M and Hrt-M), although it did not coincide with the largest difference in lignin content (Table 3).

It was also noted that steam pretreatment reduced the feedstock particles' size, and that the final size distribution depended on the pretreatment severity. By increasing the reaction severity, the percentage of smaller particles and fines in each sample increased (Fig. 3). It is known that the finer particles have higher surface area and adsorb more enzyme and, as a result, are hydrolyzed faster and more efficiently *(22)*; this trend was observed in our data as well. The Sap-H and Hrt-H samples that underwent the most severe pretreatment exhibited the highest hydrolyzability followed by those treated at medium- and low-severity conditions, respectively (Fig. 2). Also, at every severity level (low, medium, or high), the sapwood fraction contained more fines than the heartwood, which, in part, can explain the better hydrolyzability of the pretreated sapwoods. The fines content alone, however, cannot explain the significant difference between the digestibility of Sap-M and Hrt-M (Fig. 2). The mildly pre-

Table 4
Fiber Coarseness of Sapwood- and Heartwood-Derived P200 Fraction Pretreated at Low, Medium, and High Pretreatment Severity Conditions[a]

Pretreatment condition	Coarseness (mg/mm)	
	Sapwood	Heartwood
Low	0.54	0.57
Medium	0.59	0.66
High	0.82	1.44

[a]Low: 175°C, 4.5% of SO_2, 7.5 min; medium: 195°C, 4.5% of SO_2, 4.5 min; high: 215°C, 2.38% of SO_2, 2.38 min.

treated materials (low severity) contained very little fines and the majority of particles were found in the longer fiber length fractions.

It has also been suggested that gross fiber characteristics, such as cell wall thickness (or coarseness), can be as influential as particle size and surface area in affecting hydrolysis. Cell wall thickness varies both within and between species, and within the one tree, compression and tension woods have thicker-walled fibers, whereas juvenile wood has thinner cell walls, as does the sapwood portion of any species *(15)*. Coarseness, an important parameter in papermaking, is defined as the weight-to-length ratio of a population of fibers. Therefore, lengths being equal, a coarse fiber weighs more than a fiber with a thinner cell wall. Earlier work has shown that coarser fibers were hydrolyzed less efficiently, perhaps owing to having a lower "specific" surface area, in which specific surface area was defined as the available surface area per unit weight of a fiber *(20)*. It is obvious that in two fibers with equal lengths, the one that has a thicker wall weighs more, and therefore has a smaller specific surface area. It has also been observed that the hydrolysis of coarse and fine fibers proceeds through two distinct mechanisms. The coarser fibers are first "peeled" into smaller fibers, whereas the finer particles become "eroded" *(18)*. When the coarseness of the P200 fraction (very small size particles of about 0.5 mm retained by the no. 200 screen of Bauer McNett) of each substrate was measured (Table 4), the sapwood particles always had a lower coarseness, which should make them more susceptible to enzyme adsorption and subsequent hydrolysis.

The low-severity pretreatment did not enhance the hydrolyzability of either of the wood fractions to a substantial level, and the high-severity conditions resulted in significant loss of hemicellulose-derived sugars and partial solubilization of cellulose. The medium-severity set of conditions appeared as a good compromise between the two extremes because it resulted in a considerable improvement in hydrolyzability of solids without significant polysaccharide degradation.

Previously, our and other groups have suggested that a sequential, cascade or "sequencing batch" mode of pretreatment/acid hydrolysis

Table 5
Chemical Composition of Sugar (monomers) and Lignin Content (g/100 g dry substrate) of Sapwood Pretreated at Low Severity Conditions (Sap-L) and After Secondary Treatment[a]

Feedstock	Arabinose	Galactose	Glucose	Xylose	Mannose	Lignin
Sap-L	0.3	0.8	56.9	1.2	4.6	36.3
Sap-L/L	Neg	Neg	66.1	Neg	Neg	37.7
Sap-L/M	Neg	Neg	52.2	1.0	3.7	50.2
Sap-L/R	Neg	Neg	66.0	0.7	0.7	38.2

[a]Sap-L/L, low severity; Sap-L/M, medium severity; Sap-L/R, mechanical refining; neg, negligible. L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

might be the best way to recover most of the hemicellulose-derived sugars while providing a readily hydrolyzable cellulose component. To achieve enhanced feedstock hydrolyzability without excessive sugar degradation, a series of sequential pretreatments was carried out in which the sapwood chips that were previously pretreated at low severity (Sap-L) were then subjected to three different secondary treatment schemes: additional steam explosion (no SO_2 added) at low severity (Sap-L/L), additional steam explosion (no SO_2 added) at medium severity (Sap-L/M), and mechanical refining (Sap-L/R). It was hoped that the first two treatments would increase the availability of the cellulose for enzymatic digestion while minimizing the degradation of water-soluble, hemicellulose-derived sugars as the liquid prehydrolysates were separated prior to secondary treatment. The last treatment was chosen to elucidate the effect of particle size reduction on the rate and extent of hydrolysis in the absence of further chemical alteration, since refining was solely a mechanical treatment.

When the chemical composition of the Sap-M before and after secondary pretreatment was determined (Table 5), it was found that the secondary, low-severity treatment dissolved the small amounts of hemicellulose sugars left in the water-insoluble fraction after primary treatment, consequently increasing the percentage of cellulose (glucose) and lignin detected. Increasing the severity of the secondary treatment from low to medium resulted in the acid hydrolysis of a significant amount of cellulose, thereby lowering the amount of glucose present in the water-insoluble fraction, and consequently increasing the lignin content. The effect of refining was similar to that of secondary low-severity pretreatment, i.e., a slight increase in lignin content and a significant increase in glucose content.

The three sequentially pretreated substrates were then subjected to enzymatic hydrolysis. In comparison with the original substrate (Sap-L), the secondary medium-severity treatment had the highest impact on the extent of hydrolysis (Fig. 4). As a result, the amount of cellulose hydrolyzed after 72 h of enzyme treatment increased to 2.4 times that of the original material (from ~25 to 60%). Even at a shorter, and more realistic, residence

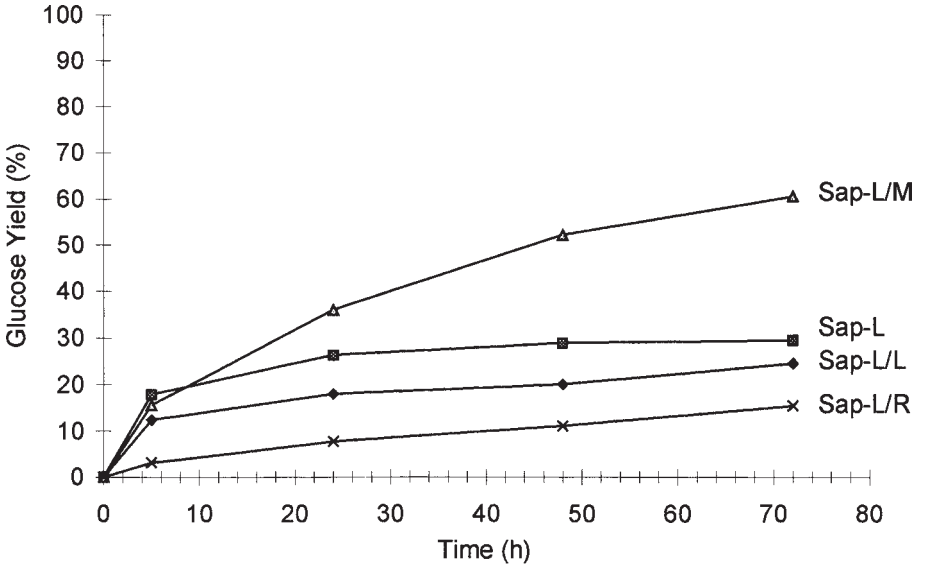

Fig. 4. Enzymatic hydrolysis of single-stage pretreated sapwood at low severity (Sap-L) and after secondary pretreatment at low severity (Sap-L/L), medium severity (Sap-L/M), and mechanical refining (Sap-L/R). L: 175°C, 4.5% of SO_2, 7.5 min; M: 195°C, 4.5% of SO_2, 4.5 min; H: 215°C, 2.38% of SO_2, 2.38 min.

time (e.g., 24 h), the glucose recovery doubled from 18 to 36%. However, after secondary treatment at low-severity conditions, the increase in cellulose hydrolysis was only between 5 and 9%, depending on the hydrolysis time. Mechanical refining decreased both the rate and extent of hydrolysis as compared to the original substrate (Sap-L) (Fig. 4). This was unexpected because it had been shown previously that a reduction in particle size can increase hydrolyzability *(20)*.

The data also indicated that both sequential treatments—low followed by low and low followed by medium severity—did not increase the glucose yield beyond those obtained from the single-stage pretreatment at medium severity (Sap-M). Therefore, considering the operational costs associated with the additional pretreatment operation, single-stage pretreatment at medium severity remains superior to a two-stage operation.

Conclusion

The severity of pretreatment conditions had a direct impact on the hydrolyzability of both sapwood and heartwood fractions. The improvement in rate and yield of hydrolysis resulting from the increase in pretreatment severity was caused by chemical as well as structural changes in both the sapwood and heartwood fractions. These changes include hemicellulose solubilization and a possible reduction in feedstock particle size. At a certain severity, the sapwood fraction was more susceptible to acid-catalyzed steam pretreatment, hence producing substrates with better enzy-

matic digestibility. While pretreatment at low-severity conditions did not render the feedstocks sufficiently amenable to enzymatic digestion, the high-severity treatment caused excessive loss of hemicellulose-derived sugars. The medium-severity conditions were a good compromise between the two extremes (i.e., high and low) because they improved the hydrolyzability of the solids and allowed for recovery of the majority of water-soluble, hemicellulose-derived sugars.

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