

Evaluation of Secondary Acid and Enzymatic Hydrolysis of Hemicellulose in Hot Water Pre-Pulping Extract of Mixed Hardwoods*¹

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ABSTRACT

Pre-pulping extracts were found to contain a dilute amount of xylo-oligosaccharides and acetic acid as the major components, and many minor components including other organic acids, lignin-derived phenolics, and sugar degradation products. Once separated from the pulp, a secondary hydrolysis step was required to hydrolyze oligomeric hemicellulose sugars into monomeric sugars before fermentation. The following study detailed the extent of hemicellulose recovery by pre-pulping using hot water extraction and characterized the hydrolysis of the extract with respect to comparing acid and enzymatic hydrolysis. The secondary hydrolysis of hot water extracts made at an H-Factor of 800 was tested for a variety of acid and enzyme loading levels using the sulfuric acid and xylanases. The maximum fermentable sugar yield from acid and enzyme hydrolysis of the extract was 18.7 g/ℓ and 17.7 g/ℓ representing 84.6% and 80.1% of the maximum possible yield, respectively.

Keywords : acid hydrolysis, acetic acid, enzyme hydrolysis, hot water extraction, mixed hardwood, tubular reactor

1. INTRODUCTION

One promising development in conversion of lignocellulosic biomass to renewable fuels and chemicals is the process of pre-pulping hemicellulose extraction. Extracting hemicellulose prior to pulping creates a new feedstock within the existing pulp and paper industry while preserving cellulose for production of the more valuable pulp (van Heiningen, 2006). In present-day kraft pulp mills, hemicellulose is burned

during chemical recovery along with lignin to generate power and steam (Smook, 2002). Because hemicellulose does not have a high heating value, conversion by biological fermentation processes offers a potential way to increase the value derived from lignocellulosic feedstocks within an integrated bio-refinery.

Fig. 1 presents the integrated pulp and bio-refinery. To the chemical processing steps for producing value-added chemicals, it incorporates components from existing biorefinery type

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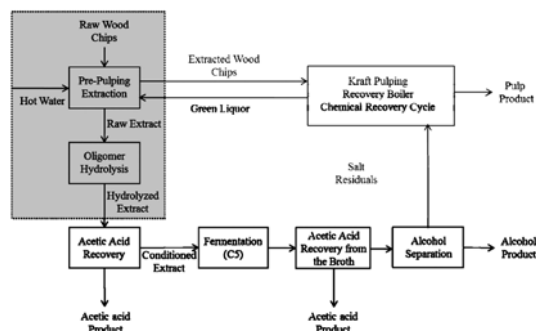


Fig 1. Simplified block diagram representing the process flow of a pulp and biorefinery. The pre-pulping extraction and oligomer hydrolysis steps are the focus of this study.

operations of wood pulp mill processes (Um *et al.*, 2011; Huang *et al.*, 2007). Carbohydrate separation and recovery involve both the five- and six-carbon compounds, these compounds respond to separations by selective hydrolysis because they have different activities in either chemical or enzymatic hydrolyses. The lignin component, the least developed of the biomass fraction, is often viewed as a fuel to drive the processing systems, but it is also a potential source of aromatic chemical products (van Heiningen, 2006). In order to achieve a true biorefinery status, each biomass component would be used in the appropriate process to yield the highest value product. Therefore, a combination of processing steps, tailored to fit the particular operation, will be used to produce a slate of chemical products, which will be developed in response to the market drivers to optimize feedstock utilization and overall plant income. In this manner, the return on investment for converting low-cost feed stock to high-value chemical products can be maximized.

On an annual basis, the US pulp and paper industry sustainably collects and processes approximately 108 million tons of wood for the production of pulp and paper (Ragauskas *et al.*,

2006; Hekkert *et al.*, 2001). Extractives from the pulping process provide approximately 700 million liters of turpentine and tall oil annually that could be employed for biodiesel applications (Ragauskas *et al.*, 2006; Mabee, 2006). Hemicelluloses occur in relatively large amounts in hardwoods (12~20%) and lesser amounts in softwoods (3~8%) (Blanchette, 2000). They normally have monomeric substituents or, in some cases, branched oligomer chains, and vary in composition and structure. In the case of hardwoods, the hemicelluloses consist of linear chains of β -1-4 glycosidic bonded xylose monomers with an average degree of polymerization of 100~200 (Bailey and Ollis, 1986). Hemicellulose is a byproduct which is largely being wasted at pulp mills today. It is often burned in boilers with the lignin after the cellulose has been sorted out for pulp production. Recovery of hemicellulose sugars prior to the pulping operation reduces the mass of dissolved wood components and provides new product opportunities enhancing production of Kraft pulps. Extraction of hemicellulosic sugars by a hot water prehydrolysis produces sugars that are mostly oligosaccharides and small monosaccharides. Acetyl esters normally present on hemicellulose are partly cleaved, leaving some esters still attached to the sugars (Um, 2008; Kenealy *et al.*, 2007). This complicates a detailed analysis of the sugars present and necessitates a secondary hydrolysis in the analysis of the total carbohydrates present. A second hydrolysis is even more important for the identification of the carbohydrate composition when the hemicellulose is extracted by an alkaline system. Here the hemicellulose comes out as a polymeric material and the acetyl esters as acetate. Polymeric oligosaccharides are easier to separate and concentrate from aqueous solutions by ultrafiltration and evaporation. Acetate could be recovered as a salt, but

if acetic acid is the desired product then acid addition will be required. The separation of acetic acid and sugars can be accomplished with membrane filtration and has been proposed as a part of ethanol production from wood (Kenealy *et al.*, 2007; Pu *et al.*, 2007).

Advances are being made in the conversion and processing of lignocellulosic biomass to useful products. When the cellulose is converted to ethanol or other products the hemicellulose must also be used since it is a significant fraction of lignocellulosic materials. If cellulose is to be a significant portion of the product, as in paper, the hemicellulose and lignin are the only materials that can provide new products. In both cases, the processing and ultimate fate of the hemicellulose is important for the economics and design of the process. In this study, we investigated the optimal conditions of secondary acid and enzyme hydrolysis for maximum fermentable sugar recovery from hemicellulose extracts of mixed pulping wood. Furthermore, we characterize hydrolysis of the extract with respect to variable parameters and conclude with a comparison of acidic and enzymatic hydrolysis results.

2. MATERIALS and METHODS

2.1. Raw Material

Chips of mixed hardwood were supplied by the Red-Shield Kraft Pulp Mill in Old Town, ME, USA. The hardwood chips comprised primarily of maple (~50%) with lesser amounts of beech, birch and poplar. The chips were screened to an average 7/8 ~ 5/8 in (22.6 ~ 16.0 mm) using a mechanically vibrated horizontal screen. The screened wood chips were used directly in hot water extraction studies. Some of the screened chips were ground to an average size of 30 ~ 40 mesh (0.595 ~ 0.420 mm) using

a laboratory knife mill. The milled wood chips were used directly for determination of total solids/moisture and carbohydrate content in biomass. The composition of this material was analyzed according to NREL laboratory analytical procedures: NREL/TP-510-42618 for Structural carbohydrates, TP-510-42623 for Sugars (Sluiter *et al.*, 2008; Sluiter *et al.*, 2006). The sugar composition of raw wood chips was 43.3% glucan, 2.6% arabinan, 19.4% XMG, and 23.1% Klason lignin. Here, XMG (capitalized) represents the sum total of the oligomeric sugar (xylan + mannan + galactan) and xmg (lowercase) represents monomeric sugar.

2.2. Pre-Pulping Extraction

The mixed northeast hardwood chips were used throughout this study. The hemicellulose extractions were performed using a 20 l rocking digester. The digester was loaded with 2 kg of chips of between 16 and 22.6 mm and cooked. An effective hot water on chips was used for all cases with liquor to wood ratio of 4 : 1. This system was agitated (2 rpm) at 160°C for 110 min yielding an H-factor 800. The H-factor is a kinetic model applicable to alkaline pulping that expresses cooking time and temperature as a single variable. The digester heat-up period lasted 50 min before steady state was reached, and cooled down after 110 min. at operating temperature required an additional 50 min. Reactor pressure reached 130 psig during extraction. The reactor was then cooled below 100°C. Samples were withdrawn and the total sugar and reducing sugar contents were quantified in the supernatant from the xylan suspension. Under extraction conditions approximately total 13.5% of the mass of the wood was extracted.

2.3. Secondary Acid Hydrolysis

All batch acid hydrolysis experiments were performed using sealed tubular reactors. The vessel (12 cm³ of internal volume) was constructed out of stainless steel tubing because of its strength at elevated temperatures, and corrosion resistance. Both ends of the vessel were sealed with Swagelok end-caps into a size of 0.39 in. (1 cm) diameter × 5.91 in. (15 cm) length. The acid hydrolysis was conducted with 10 mL hemicellulose extract under conditions ranging from 110 to 130°C, 0 to 4% H₂SO₄, and 20- to 120-min residence time. Temperatures of the vessel were adjusted with oil (Heat Transfer Fluid 550, Fisher, Pittsburgh, PA), which temperatures were controlled with external heating/cooling baths. The vessel was initially submerged into oil bath set at 50°C above the desired reaction temperature for rapid preheating. The vessel was then quickly transferred into next oil bath set at the precise desired reaction temperature. The vessel temperature was monitored by a thermocouple (KQXL-18G-12, Omega Eng. Inc., Stamford, CT) inserted into the reactor. After finishing the reaction, the vessel was quenched in an ice bath for 10 min.

2.4. Secondary Enzyme Hydrolysis

The xylo-oligosaccharide extracts were prepared for enzyme reaction by the previously described extraction process; the resulting solid concentration in the extract solution was 3.91%. The experiments were conducted according to the NREL Laboratory Analytical Procedures (LAP): NREL/TP-510-42629 (Selig *et al.*, 2008). The enzymatic hydrolyses were conducted with 3,000 U/g xylanase from *Trichoderma viride* (Xylanase 1, Fluka, distributed by Sigma-Aldrich, St. Louis, MO, USA) and 2,500 U/g xylanase from *Thermo- myces lanuginosus* (Xylanase 2, Sigma-Aldrich, St. Louis, MO, USA)

under agitation (68 RPM) for 96 h at the temperatures of 40°C. One unit (U) of each enzyme activity is defined as the amount of enzyme, which produces 1 μmol reducing sugar as xylose in the reaction mixture per minute. Before adding the enzymes, the samples were placed in the shaker bath for 10 min to ensure thorough mixing and to warm the samples partially to the hydrolysis temperature. The enzymatic hydrolysis was conducted with 100 ml working volume under various conditions ranging from pH 1.5 to 6.0 in increments of one-half pH unit, and 0.5 to 4% enzyme. The pH was adjusted with 1 M NaOH or HCl. These samples were then left to equilibrate for 30 min, and then the pH was checked. In order to monitor the hydrolysis, samples were withdrawn and the reducing sugar contents and acetic acid were quantified by HPLC, after removal of the insoluble materials by centrifugation and syringe filtration.

2.5. High Performance Liquid Chromatography

The sugar composition of reaction products was quantitatively analyzed by HPLC. The Shimadzu model (LC-10AT Liquid Chromatogram, Shimadzu Corp., Kyoto, Japan) HPLC used for carbohydrates measurement had Bio-Rad Aminex HPX-87H (300 mm × 7.8 mm, Bio-Rad Laboratories Inc., Hercules, CA) and Cation H micro-guard cartridge (30 mm × 4.6 mm, Bio-Rad Laboratories Inc., Hercules, CA). The column was maintained at 60°C, and 5 mM H₂SO₄ was used for the Aminex 87H-column as eluent at a flow rate of 0.6 ml/min. All of the sugar peaks were detected by a RI detector and UV absorption (215 nm) and were identified and quantified by comparison to retention times of authentic standards. The Bio-Rad Aminex HPX-87H analytical column allows the concur-

Table 1. Analysis of raw extract composition used for experiments

	Total Solid ^a [%]	pH	Glucan [g/ℓ]	XMG ^b [g/ℓ]	Arabinan [g/ℓ]	Acetyl [g/ℓ]	Furfural [g/ℓ]	Total Sugars ^c [g/ℓ]
Theoretical Maximum Yield ^d			1.5	22.1	1.1	5.5	1.2	11.8
Untreated Blind Control ^e	3.91	3.55	0.3	3.4	0.8	3.0	0.8	0.7

^aTotal solid fraction in the extracts after extraction

^bXMG = xylan + mannan + galactan

^cTotal sugars = glucan + XMG + arabinan

^dDetermination of initial sugar and acetyl concentrations were performed by acid hydrolysis, using NREL standard methods No.002, under conditions of 120°C, 4% H₂SO₄, and 60 min residence time

^eNo secondary acid hydrolysis

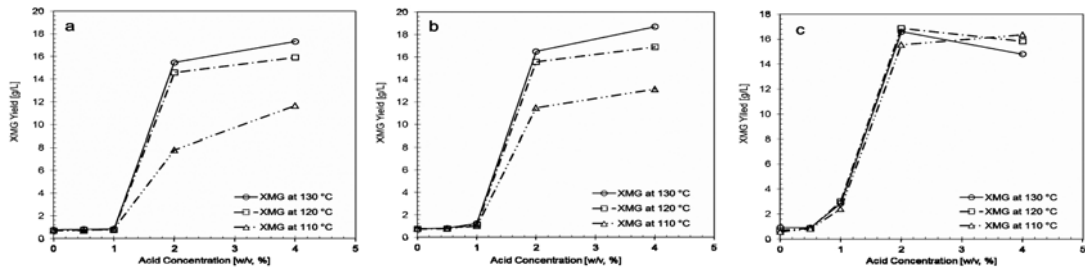


Fig 2. XMG yield from hot water pre-pulping extract of mixed northeast hardwood followed by secondary acid hydrolysis as function of temperature and acid concentration: reaction time (a) 20 min (b) 40 min, and (c) 120 min.

rent analysis of liquid sample for the presences of acetic acids and sugar degradation products.

3. RESULTS and DISCUSSION

3.1. Pre-Pulping Extract

The concentration of carbohydrates released from mixed hardwood chips following hot water extraction treatment is listed in Table 1. Total carbohydrates extracted represented 1.5, 22.1, and 1.1 g/ℓ of glucan, XMG, and arabinan respectively. The ratio of glucan/XMG/arabinan mannose liberated from hemicellulose extract liquid was 0.07 : 1 : 0.05. Analysis of these ratios implies that the XMG component is pre-

dominantly derived from arabinoglucuronoxylan. Small amounts of glucan are likely the result of hydrolysis of galactoglucomannan. All process sugar yields from acid and enzymatic secondary hydrolysis were calculated from the basis of the sugar composition of extracts.

3.2. Dilute-Acid Hydrolysis Results

The main purpose of this part of the work was to investigate the influence of the known important reaction variables, temperature, time, and acid concentration. The effects of temperature (ranging from 110 to 130°C) on dilute H₂SO₄ (0.5, 2, and 4 w/v) hydrolysis of pre-pulping extract for various reaction time (20,

40, and 129 min) were evaluated. The data in Fig. 2 indicates that the XMG yields are dependent upon the reaction temperature as well as H_2SO_4 dose of secondary acid hydrolysis. The maximum yield of XMG was 18.7 g/l after dilute acid hydrolysis of 4% H_2SO_4 (w/v) extracted hemicellulose liquor for 40 min at 130°C . (Fig. 2(b)). The acetate formation appears to peak at a temperature of 130°C . Furfural, the decomposition of xylose, increased with the temperature (data not shown). This clearly indicates that pentose sugars (xylose and arabinose) derived from hemicellulose were further degraded. But, no HMF was produced per liter of extract. One reason is that small amounts of glucose are relatively extracted from hot water extraction process in the pre-pulping extract. The data on these minor constituents are, however, subject to little errors because their quantities are so small.

Pre-pulping extracts used in this study theoretically contained 1.5 g/l glucan, 22.1 g/l XMG and 1.1 g/l arabinan, which make up the total carbohydrate content of 24.7 g/l (Table 1). To reduce further degradation of monosaccharide formed, we have evaluated the effects of various acid doses on dilute H_2SO_4 hydrolysis. With 3.91% (w/v, consistency) extracts, an acid dose of 4% (w/v) gave maximum yield (18.7 g/l) of fermentable sugars at 130°C (Fig. 2(b)). About 85% of hemicellulose was converted to sugars and only 50% glucan (data not shown) was converted to glucose. This trend in the results was similar to that reported for rapeseed straw (Jeong *et al.*, 2010). Although the trends in the results were similar, the actual pentose sugar yields from the experiments were much higher than those collected by Jeong using rapeseed straw. The effect of the 0.5, 1, and 2% of H_2SO_4 dose on the hydrolysis was also studied. The maximum xylose sugars concentration present in the hydrolyzates were

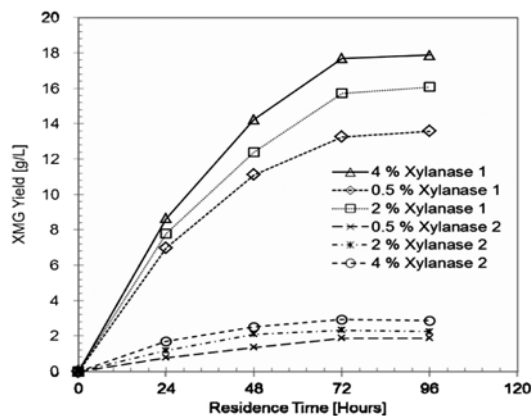


Fig. 3. Effect of enzyme loading on XMG yield at constant temperature (40°C).

0.8, 1.2, and 16.5 g/l with the acid doses of 0.5, 1, and 2% (w/v) at the temperature of 130°C , respectively.

The effect of residence time on various dilute H_2SO_4 hydrolysis of hemicellulose extract at the temperature ranged from 110 to 130°C was also studied (Fig. 2). Fig. 3 shows the XMG yield for 120 min reaction time as function of reaction temperature and acid concentration. The overall xylose yield did not increase with the increase of treatment time as a result of decrease in hemicellulose conversion (Fig. 3). Going to higher severity might somewhat reduce both the XMG remaining in the extract and the loss. Tested reaction times were selected such that the longest reaction time was sufficiently long to result in a decreased XMG yield, ensuring that a maximum yield had been at least bracketed by each experimental condition of temperature and acid concentration.

3.3. Enzymatic Hydrolysis Results

To explore various options for enzymatic conversion of the hemicellulose extract after hot water extraction process, we performed a series

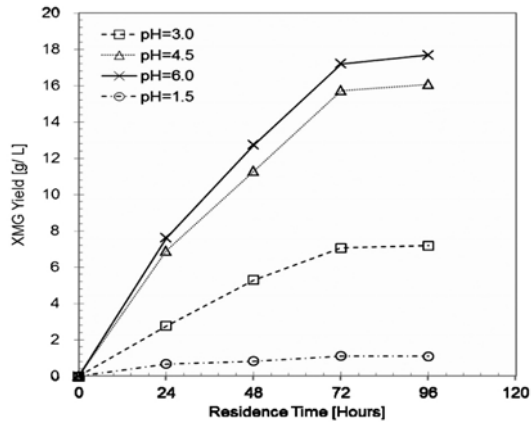


Fig 4. Enzymatic hydrolysis of pre-pulping extract for XMG yield as function of enzyme loading amount at constant temperature (40°C).

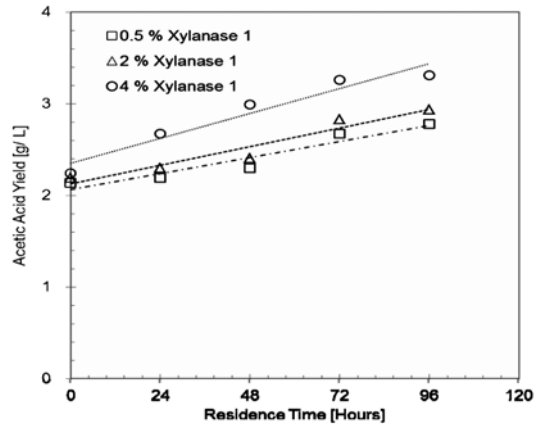


Fig 5. Acetic acid yield for different enzyme loading at constant temperature (40°C).

Table 2. Comparison of different hydrolysis conditions at the highest xylose yield

Conditions	Acid Hydrolysis	Enzyme Hydrolysis
Extracts	3.91% Total Solid	
Temperature	130°C	40°C
Residence Time	40 min.	72 hr.
Acid/Enzyme	4% (w/v)	4% (w/w)
Acid/Enzyme	Sulfuric Acid ^a	Commercial Xylanase ^b
pH	0.85	6.00
XMG ^c Yield	18.7 g/ℓ	17.7/ℓ
XMG Digestibility	84.6%	80.1%
Temperature Controller	Oil bath	Convection Incubator
Reactor	Tubular Reactor	Erlenmeyer Flask

^aSulfuric Acid: Pure 96.00% (+/- 0.15% solution in water).

^bXylanase: 3,000 U/g xylanase from *Trichoderma viride*.

^cXMG = xylan + mannan + galactan

of enzymatic hydrolysis experiments to determine the effect of enzyme loading, pH, and type of enzyme at a constant temperature. Fig. 3 presents the effect of different enzyme and enzyme loading on xylose yield from 3.91% con-

sistency of extract as function of hydrolysis time at 40°C. The range of xylanase loading, from 0.5 to 4%, was selected for comparison with the xylose yield of the extract. The XMG yield of from the extract hydrolyzed by xyla-

nase 1 (*Trichoderma viride*) was 17.7 g/l with a digestibility of 80.1% at 72 h. Based on the amount of xmg released in a hydrolysis experiment, the yield of the total potential XMG in the extract solution increased with increasing the amount of enzyme loading. In the case of other xylanase 2 (*Thermomyces lanuginosus*), lower xmg was produced per liter of extract for the any enzyme loading at the temperature. From the XMG yield, it is seen that the activity of xylanase 1 (*Trichoderma viride*) was more stable for 72 h at 40°C than that of xylanase 2 (*Thermomyces lanuginosus*). Fig. 4 compares the XMG yield of the extract (3.91% consistency) over a pH range of 1.5~6.0 in increments of one-half pH unit. The slurry extract was adjusted with NaOH and HCl followed by leaving to equilibrate for 30 min, and then the pH was checked. As a result, the enzymatic XMG yield is 17.2 g/l for the pH = 6.0 samples and 15.7 g/l for the pH = 4.5 sample. The digestibility of these pH was 80.4% (pH = 6.0) and 71.7% (pH = 4.5) respectively. Meanwhile, the acidic hydrolysis (pH = 3 and 1.5) did not give good yield of XMG from the extract. From the comparison, the optimum pH for the enzyme activity, as determined under these hydrolysis conditions was found to occur between a pH of 4.5 and 6.0 for xylanase 1 (*Trichoderma viride*). However, this trend is similar to results obtained from sugarcane bagasse (Adsul *et al.*, 2005) and hardwood (Umand vanWalsum, 2009; 2010). Both of these studies suggested that residual lignin and acetic acid presented in hydrolyzate may play an important part in attracting the hydrolysis enzymes to the surface of the biomass.

The time courses of acetic acid yield for the enzyme loading by the xylanase 1 from the extract is shown in Fig. 5. Unlike previous acid hydrolysis, the acetic acid concentration was linearly increased. The acetic acid is likely de-

riving from further hydrolysis of components in the extract. The mode of inhibition of acetic acid on enzyme hydrolysis may be via reduction of the pH below the optimal range, resulting in a decrease in XMG yield. Meanwhile, no furfural was detected per liter of extract in all the enzyme experiments. It is evident that most of the hemicellulose was solubilized and degraded to component monosaccharides (xmg) without further degradation of monosaccharides to HMF or furfural at the mild hydrolysis conditions.

4. CONCLUSION

The low production rates resulted from the low extraction yield, which was limited to approximately 13.5% of the debarked wood mass in the current design. This was done because of concerns over loss of pulp yield and the effect that the extraction process has on the wood pulping process. More ethanol and acetic acid could be produced provided more of the hemicelluloses in the wood were extracted while still maintaining the pulp yield. However, we are now concentrating our effort to hydrolyze and saccharify the extract in such a way as not to produce any decomposition products or minimize the formation of these fermentation inhibitors. The maximum fermentable sugar yield from acid and enzyme hydrolysis of the extract was 18.7 and 17.7 g/l representing 84.6% and 80.1% of the maximum possible yield, respectively. These results did prove to provide a useful means of trading off the hydrolysis effects of these two different reagents on total pentose sugar yields.

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