JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fractionation of Flax Shives by Water and Aqueous Ammonia Treatment in a Pressurized Low-Polarity Water Extractor

ANVAR U. BURANOV AND G. MAZZA*

National Bioproducts and Bioprocesses Program, Pacific Agri-Food Research Center, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC V0H 1Z0, Canada

Fractionation of flax shives into cellulose, hemicellulose, and lignin with a two-stage extraction process using water and aqueous ammonia was carried out in a pressurized low-polarity water extractor operated at different temperatures, flow rates, and ammonia concentrations. During the first stage with water, 84% of hemicellulose and 32% of lignin were removed at 190 °C at a flow rate of 1.5 mL/min for 30 min. During the second stage with aqueous ammonia, more than 77% of the lignin was removed, and hemicellulose removal reached 95% at 200 °C at a flow rate of 0.5 mL/min and with a solvent/feed ratio of 40 mL/g. The temperature and flow rate had a significant effect on lignin removal. The impact of additives (anthraquinone and hydrogen peroxide) and modifications (overnight soaking, reduced particle size, and elevated temperature) on lignin extraction was also studied. The combination of higher temperatures and reduced particle sizes resulted in enhanced lignin extraction. The extraction profiles of free phenolics (vanillin, acetovanillone, and vanillic acid) during the two-stage processing were monitored and compared with those of lignin.

KEYWORDS: Flax; *Linum usitatissimum*; biomass; biopolymers; biochemicals; hemicellulose; cellulose; lignin; phenolics; fractionation; extraction

INTRODUCTION

Flax (*Linum usitatissimum* L.) is grown worldwide for fiber and seed oil production. In Canada, flax is grown on 0.67 million hectares of land, and flax shive, the nonfiber fraction of the straw, is considered as one of the most abundant low-value biomass sources available for the production of bioethanol and biochemicals (*1*). Efficient fractionation of biomass into its major polymers (namely, hemicellulose, lignin, and cellulose) is the most important challenge to the efficient utilization of biomass from herbaceous crops for the production of biofuels and biochemicals. In lignocellulosic biomass, the lignin–carbohydrate complex (LCC) shields cellulose and limits the accessibility to enzymes through several physical factors such as crystallinity, surface area, and the degree of polymerization (*2–5*). Therefore, the cleavage of covalent bonds (ether, ester, glycosidic, and acetal) in LCC determines the efficiency of fractionation (*6, 7*). Lignin is rarely isolated as pure material and is always associated with carbohydrate linkages such as cellulose and hemicellulose to varying extents, depending on the isolation procedure. Commercial lignins always contain small amounts of carbohydrates as complexes (2–8% by weight), and this plays a crucial part in the final reactivity and properties of the polymer (*8, 9*). A variety of solvents was used for the extraction of LCC from ryegrass cell walls by Morrison (*10*), and the highest yields were achieved with dimethyl sulphoxide and N alkali extractions. Upon dilute acid hydrolysis, it was found that the carbohydrate fraction of the N alkali-extracted complex contained mainly xylose (70%) and arabinose, whereas the dimethyl sulphoxide extracted complex contained glucose (50%), xylose (30%), arabinose (12%), and galactose (5%). N alkali extractions were found to be the most efficient for the fractionation of biomass into hemicellulose, lignin, and cellulose (*10*). Hemicellulose and lignin fractionated from the biomass may have broader use in bioproducts, energy, and food applications (*3, 11*). The remaining cellulose can be used for the production of bioethanol after enzymatic hydrolysis with cellulases.

Recently designed fractionation processes employ dilute sulfuric acid (0.7%) extraction for high hemicellulose recovery and enhanced cellulose digestibility (*12, 13*). Sulfuric acid addition into batch processes increases the hemicellulose yield from 65% to 90%. However, this process is regarded as a costly pretreatment for the conversion of cellulose into ethanol due to the use of sulfuric acid, which is corrosive to the extraction equipment and requires neutralization for product recovery (*2*). Another promising fractionation process is the use of flowthrough technology, which can recover almost all of the hemicellulose sugars and digestible cellulose without any additives. In flowthrough technology, the extracting solvent is passed through the stationary biomass at varying temperatures without (or with low) pressure. Aqueous ammonia was used in the flowthrough

* Author to whom correspondence should be addressed. Tel.: +1 250 494 6376. Fax: +1 250 494-0755. E-mail: mazzag@agr.gc.ca.

10.1021/jf0714564 CCC: $37.00 Published 2007 by the American Chemical Society
Published on Web 09/27/2007

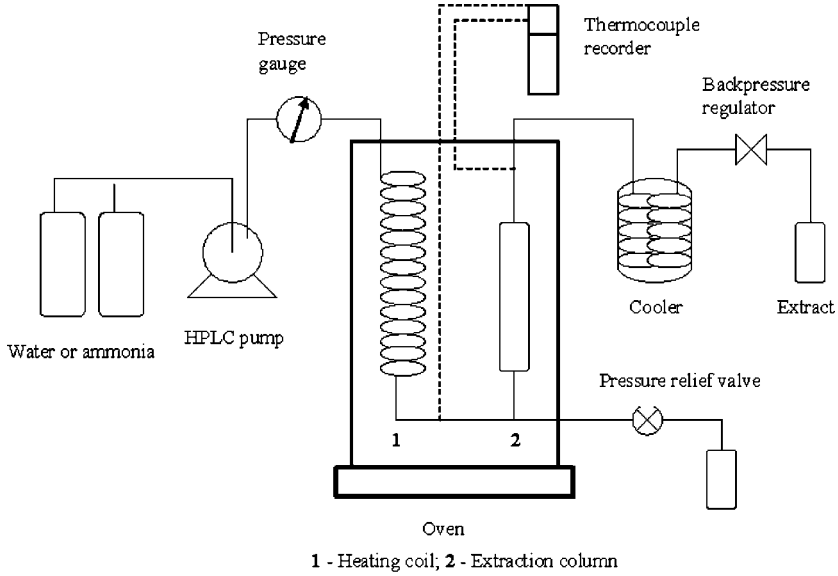

Figure 1. Pressurized low-polarity water (PLPW) extractor.

process for lignin extraction from corn stover for its unique properties, especially biomass swelling and recycling (*14*, *15*). The two-stage fractionation of corn stover by the flowthrough (percolation) process using hot water and aqueous ammonia has been performed successfully (*16*). Despite these advantages, flowthrough and batch operations use large amounts of water, resulting in excessive energy costs for both pretreatment and product recovery, and flowthrough equipment configurations are challenging to implement commercially (*2*).

Pressurized low-polarity water (PLPW) extraction, also known as subcritical water extraction, is a technology that modifies the properties of water by increasing the temperature up to 374 °C and keeping the pressure high enough to maintain water in the liquid state to improve its extraction ability. Thus, an increase in water temperature from 25 to 200 °C, for instance, decreases its dielectric constant from 79 to 35, reaching values similar to those for ethanol (24) or methanol (33). PLPW extraction has been reported to be superior to conventional extraction techniques including hydrodistillation, solid–liquid extraction, and supercritical CO_2 extraction. Some of the benefits include higher selectivity, increased cleanliness and speed, and cost savings on both raw material and energy (*17–21*).

PLPW extraction has been employed for the extraction of lignans from flaxseed (*22*) and free phenolics from flax shives (*23*). In this study, the PLPW process was evaluated using flax shives for hemicelluose removal with water and lignin removal with aqueous ammonia. A PLPW extractor was operated first with water and then with aqueous ammonia. The main advantage of this process is that almost all of the hemicellulose xylan is removed during the first stage with water, and the lignin is removed during the second stage, leaving solid residue containing mostly cellulose.

The purpose of this work was to fractionate flax shives into hemicellulose, lignin, and cellulose with a two-stage water and aqueous ammonia treatment in a PLPW extractor through variation of the process parameters for efficient separation.

MATERIALS AND METHODS

Substrate. Flax shives were obtained from Biolin Research Inc. (Saskatoon, Canada) and stored in a cold room at –35 °C until used. The shives were ground to a particle size of 1.0 mm with a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), using a 1 mm mesh screen. The ground flax shives were screened on #18 mesh screen for fiber and cleaned from the residual fiber. The ground flax shives were stored in the freezer at –15 °C. Flax shives with a particle size of 0.5 mm were prepared by milling clean 1.0 mm flax shives with a rotor mill (Retsch Company, Germany) then stored in a freezer at –15 °C. The composition of the sample was 34.2 ± 0.2% glucan, 21.3 ± 1.5% xylan, and 30.2 ± 0.2% Klason lignin as determined by the standard methods published by the National Renewable Energy Laboratory (*24*).

PLPW Extractor and Experiments. The PLPW extractor consisted of a high-performance liquid chromatography (HPLC) pump (Waters 515 model, Milford, MA), a temperature-controlled oven (Model 851F, Fisher Scientific, Pittsburg, PA), a 2.0 m (stainless steel tubing with 3 mm o.d.) preheating coil, an extraction column, a 1.0 m cooling coil (stainless steel tubing with 3 mm o.d.), and a back pressure regulator with a cartridge of 5.2 MPa (750 psi; Upchurch Scientific, Oak Harbor, WA). The columns used for the extractions were (1) 200 mm in length × 10 mm o.d. and (2) 200 mm in length × 20 mm o.d. Stainless steel tubing (3 mm o.d.) and connectors were used to connect the equipment pieces (HPLC pump, extraction cell, and back pressure regulator). A schematic diagram of the PLPW extractor setup is shown in **Figure 1**.

Milled flax shives (2.5 g) were packed in the extraction columns with frits and glass wool (0.1–0.2 g) at both ends. Previously, we reported the predicted extraction conditions for hemicellulose removal for different pH media, and our calculated values for water at pH = 7 were found to be 190 °C at a flow rate of 1.5 mL/min for 30 min. These predicted optimal extraction conditions for hemicellulose removal were used for the first stage extraction throughout the two-stage processing (*25*). The first stage of extraction was started by pumping deionized water to bring the pressure up in the system to the value fixed by the back pressure regulator [~5.2 MPa (750 psi)] at 190 °C and 1.5 mL/min for 30 min, and then the deionized water was changed to aqueous ammonia. Aqueous ammonia (15%) was passed through the system until the solvent/feed ratio reached 40 mL/g. The effects of three variables (temperature, flow rate, and ammonia concentration) and additives (anthraquinone and hydrogen peroxide) or process modifications (overnight soaking, reduced flax shives, and elevated temperature) on the extraction of lignin from flax shives were determined. The temperature experiments were performed at 120, 140, 150, 160, 180, and 200 °C at a constant flow rate of 1.0 mL/min. All other experiments were carried out at 160 °C, which was selected on the basis of the results from the temperature evaluations. Then, experiments were performed at four different flow rates and four different concentrations of ammonia. When the data from these trials was used, a flow rate of 0.5 mL/min and a concentration of 15% aqueous ammonia were chosen for the rest of the experiments. Finally, experiments were performed using additives and modifications in order to increase the lignin yield. After extractions, the column was washed

Table 1. Effect of Temperature on the Fractionation of Flax Shives by PLPW[a]

	solid (%)				liquid (%)			total (%)			yield in liquid (%)		
temp (°C)	SR[b]	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
untreated	100	30.2	34.2	21.3				30.2	34.2	21.3			
130	78.6	27.2	33.2	19.1	1.2	0.7	1.6	28.4	33.9	20.7	4.1	2.1	7.7
150	85.1	26.4	33.2	15.5	1.5	0.7	2.8	28.0	33.9	18.5	5.1	2.0	13.1
190	55.2	20.5	33.1	3.6	4.8	0.9	15.8	25.3	34.0	19.3	15.8	2.6	73.7

[a] Data are mean values of duplicate analyses. Standard error = 0.7–1%. Extraction conditions: T = 190 °C; flow rate = 1.5 mL/min; time = 30 min; extract volume = 45 mL; water/feed = 18 mL/g. [b] SR, solid remaining after extraction.

by pumping in deionized water at room temperature, and then it was removed. The residual biomass was dried at 45 °C overnight. The liquid extracts were neutralized with HCl (6 M) and kept at –35 °C prior to analysis.

Sample Analysis. Solid samples left after two-stage extractions were analyzed for sugars (glucose and xylose) and lignin according to the NREL Chemical Analysis and Testing Standard Procedures No. 001-004 (*24*). The solid samples were treated with 72% sulfuric acid for 2 h in a 30 °C water bath, diluted to 4%, and autoclaved at 121 °C for 1 h. The lignin content was measured gravimetrically. Sugar monomers were determined quantitatively by HPLC (Agilent model 1100) using a system equipped with a Rezex RCM-Monosaccharide column, a refractive index detector, and a G1329A autosampler using Agilent Chemstation Plus Software (Agilent Technologies, Palo Alto, CA).

Liquid extracts collected sequentially were neutralized with HCl (6 M) and analyzed for sugars according to the NREL Chemical Analysis and Testing Procedure No. 001-014 (*24*). Total glucose and xylose in the liquid fraction were determined after hydrolysis with 4 wt % sulfuric acid at 121 °C for 1 h. Sugar monomers in the liquid samples were analyzed quantitatively by HPLC (Agilent model 1100) as described above. The lignin content of liquid extracts was determined by two methods: the acid hydrolysis method, by collecting precipitated lignin after a 4% sulfuric acid hydrolysis on LAP-014 procedure (*24*), and the nitrosation spectrophotometric method (*26*). The absorbance of nitrosophenol compounds formed in the reaction of dissolved lignin with sodium nitrate were read in an alkali medium at 430 nm.

Free Phenolics Analysis. The neutralized liquid extracts from both stages were analyzed for free phenolics (vanillin, vanillic acid, and acetovanillone) by a HPLC (Agilent model 1100) equipped with a Zorbax SB-C18 (5 μm, 3 × 250 mm) column, a Guard column (4.6 × 12.5 mm, 5 μm), an autosampler (G1329), and a pump (G1312A) using Agilent Chemstation Plus software (Agilent Technologies, Palo Alto, CA). The mobile phases were 50 μM phosphoric acid (solvent A) and methanol (solvent B). The methanol gradient was 5–55% B from 0 to 51 min, 55–100% B from 51 to 61 min, 100% B from 61 to 68 min, 100–5% B from 68 to 73 min, and 5% B from 73 to 83 min. Data were collected with a diode array detector between 210 and 400 nm, and absorbance was monitored at 280 nm. Concentrations of vanillin, vanillic acid, and acetovanillone were calculated using standards (0.1–10 mg/mL). Authentic phenolics were obtained from Sigma-Aldrich Canada Ltd. (Ontario, Canada).

RESULTS AND DISCUSSION

One-Stage PLPW Extraction. *Xylan Extraction.* The efficient hydrolysis of hemicelluloses with hot water is generally explained by the catalysis of acetyl group liberated during the breakdown of hemicellulose and hydronium ions of water. However, the latest experiments with acetic acid did not indicate that the acetyl group is the cause of hemicellulose hydrolysis (*2*). Previously, we reported the extraction conditions for xylan removal by PLPW and predicted conditions for different pH media. Our calculated data indicate that water (pH = 7) at 190 °C at a flow rate of 1.5 mL/min for 30–60 min to be optimal conditions for xylan removal (*25*). Test extractions were carried out to validate these optimal conditions with 2.5 g of flax shives, and the lignin content was determined in each fraction. The removal values of xylan, lignin, and glucan were 84, 32, and 2.8%, respectively, at the optimal conditions used for the first stage (**Table 1**). The improved removal of xylan by PLPW (pressure: 5.2 MPa) was significant compared to 9 and 40% for corn stover under batch conditions (0 MPa) and flowthrough systems (2.1–2.4 MPa) at 180 °C, respectively (*2*). The total yield of xylan recovered in the liquid phase was 73.7%, and the degradation level was 10% of the original xylan [degradation level = original xylan – (xylan in liquid + xylan in solid)]. A slight degradation of xylan to furfural and other undesirable products is unavoidable with any extraction, including under batch conditions and with flowthrough systems. However, it can be reduced to 3% at high flow rates (*2*). The lignin removal was approximately 32% and consistent with the values achieved by hot-water batch pretreatment (30%) and lower than those of hot-water flowthrough (35–65%) pretreatment of corn stover at 180–220 °C (*2, 16*). Enhanced lignin removal was also observed by others for flowthrough reactors (*28–30*). Glucan was very stable under PLPW conditions (0.22% degradation) with a removal rate from the feed of about 2.5% at 190 °C, consistent with those reported for hot-water batch and flowthrough pretreatment of corn stover at 180–220 °C (0–5.5%) and hot-water-only batch treatment of sugar cane bagasse at 200–220 °C (6%) (*2, 16, 27*). However, higher-temperature enhanced glucan dissolution and cellulose inertness had a limited impact (0.54%).

Overall, results with our PLPW extractor have shown improved xylan removal and consistent lignin and glucan removal compared with those of corn stover obtained using batch and flowthrough systems.

Two-Stage Extractions—Water and Aqueous Ammonia. *Lignin Extraction.* The two-stage PLPW process was used for the fractionation of flax shives. During the first stage, deionized water was used for hemicellulose extraction, and in the second stage, aqueous ammonia was used for lignin extraction. Both stages were performed successively in the same PLPW extractor and under varying conditions without intermittent sample taking. First-stage extraction with water was always performed under predetermined optimal conditions (190 °C, 5.2 MPa, 1.5 mL/min, 30 min, and 2.5 g of flax shives), and the second stage with aqueous ammonia was optimized for lignin extraction. The effects of temperature, flow rate and ammonia concentration on lignin removal were examined in order to optimize the second stage extraction process with aqueous ammonia. In the first series of experiments, the effects of six different temperatures (120 °C to 200 °C) on lignin removal were studied at constant flow rate of 1.0 mL/min (solvent/feed ratio, 40 mL/g). In the second series of experiments, the effect of five different flow rates (0.5, 1, 2, 3, and 5 mL/min) on lignin extraction at 160 °C was studied while keeping the solvent/feed ratio constant at 40 mL/g. For both series of experiments, 15% aqueous ammonia was used as a solvent. In the third series of experiments, four different concentrations of aqueous ammonia (15, 10, 5, and 2%) were used for lignin extraction, keeping the temperature,

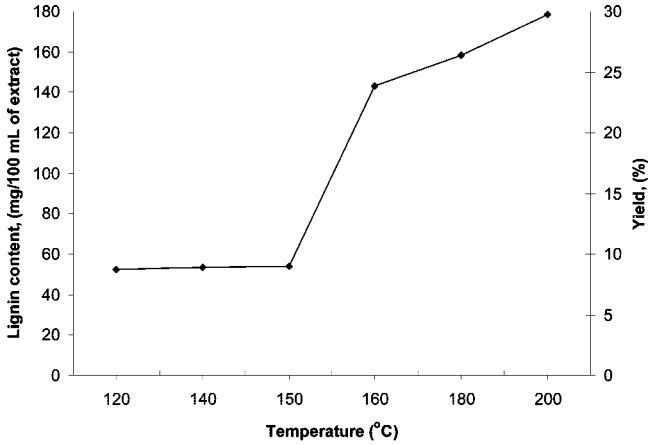

Figure 2. Effect of temperature on lignin extraction with 15% aqueous ammonia.

Table 2. Effect of Temperature on the Lignin Extraction with Aqueous Ammonia[a]

temp	solid (%)				liquid (%)			total (%)		
(°C)	SR[b]	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
untreated	100	30.2	34.2	21.3				30.2	34.2	21.3
120	52.9	18.3	31.7	3.0	7.0	0.9	15.8	25.3	32.6	18.8
140	50.5	17.9	29.4	2.2	7.1	0.9	15.8	24.3	30.2	18.1
150	49.9	17.9	29.6	2.3	7.1	0.9	15.8	25.0	30.4	18.2
160	47.7	16.5	28.9	2.4	10.9	0.9	15.8	27.4	29.8	18.2
180	46.1	16.6	28.6	2.5	11.5	1.0	16.1	28.1	29.6	18.6
200	40.5	12.2	26.1	1.4	12.4	1.0	16.3	24.6	27.1	17.7

[a] Note: Data are the mean value of duplicate analyses. Standard error = 0.7–1%. Extraction conditions are as follows. First stage: T = 190 °C; pressure = 5.2 MPa; flow rate = 1.5 mL/min; time = 30 min; extract volume = 45 mL; water/feed= 18 mL/g. Second stage: T = 120–200 °C; pressure = 5.2 MPa; flow rate = 1.0 mL/min; time = 100 min; extract volume = 100 mL; ammonia/feed = 40 mL/g. [b] SR, solid remaining after extraction.

flow rate, and solvent/feed ratio constant at 160 °C, 0.5 mL/min, and 40 mL/g, respectively. For these experiments, flax shives with a particle size of under 1.0 mm were used as the feed.

Effect of Temperature. The effect of temperature on lignin extraction (**Figure 2**) indicated that lignin yield increased with the temperature, especially between 150 and 200 °C. The difference in the amount of lignin extracted was significant at 150 °C versus 160 °C. The maximum lignin yield (32%) was achieved at 200 °C. The lignin extraction at lower temperatures (120–150 °C) was low, and the effect of changing temperature was negligible. The results indicate that the swelling of biomass with ammonia and lignin extraction is enhanced with increasing temperature starting at about 150 °C for flax shives.

The composition of solid samples presented in **Table 2** indicates that the two-stage process with water and aqueous ammonia was effective for hemicellulose xylan and total lignin with increasing temperature. The xylan content of the solid residue decreased to 1.4% after aqueous ammonia extraction at 200 °C. The residual xylan was further removed with aqueous ammonia reaching levels of 86–94%, and the xylan accountability content was 74–77%. An increase in the degradation of xylan was also observed with increasing temperature, but the impact was limited (12–17%; **Table 3**). Lignin extraction with aqueous ammonia increased with the temperature. The lowest lignin content of the remaining solid residue was 12.2% at 200 °C. More than 12% of the original lignin was detected in the liquid extract (**Table 2**). Glucan extraction and degradation also increased with the temperature during aqueous ammonia extraction. At 200 °C, the glucan content of solid residue decreased to 26.1% and the degradation level was 20.6% (**Tables 2** and **3**). The glucan content of the liquid extract was very low (1%), signifying that nearly all of the extracted glucan degrades. Glucan removal in corn stover at higher temperatures with water (*2*) and with aqueous ammonia at 200 °C with a flowthrough system was reported to be insignificant (*16*).

Table 3. Removal, Accounted, and Unaccounted Levels of Biopolymers with Aqueous Ammonia at Different Temperatures

temp	removal (%)			yield in liquid (accounted, %)			degradation (unaccounted, %)		
(°C)	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
120	39.3	7.1	85.8	23.2	2.5	74.2	16.1	4.6	11.6
140	40.7	14.0	89.5	23.3	2.5	74.3	17.4	11.5	15.3
150	40.6	13.4	89.1	23.4	2.5	74.4	17.2	10.9	14.7
160	45.3	15.4	88.9	36.1	2.5	74.4	9.2	12.9	14.5
180	45.0	16.3	88.3	38.1	3.0	75.7	6.9	13.3	12.6
200	59.5	23.7	93.5	40.9	3.1	76.5	18.6	20.7	17.0

Table 4. Fractionation of Flax Shives at Different Flow Rates at 160 °C[a]

flow rate	solid (%)				liquid (%)			total (%)		
(mL/min)	SR[b]	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
untreated	100	30.2	34.2	21.3				30.2	34.2	21.3
0.5	47.8	16.4	29.1	1.7	11.9	0.9	16.0	28.3	30.0	17.6
1	47.7	16.5	28.9	2.4	10.9	0.9	15.9	27.4	29.8	18.2
2	48.1	18.8	28.7	2.3	9.7	0.9	15.9	28.5	29.6	18.2
3	49.3	19.8	28.7	2.4	9.4	0.9	15.8	29.2	29.5	18.2
5	49.4	19.1	28.5	2.7	9.2	0.9	15.8	28.3	29.4	18.5

[a] Note: Data are the mean value of duplicate analyses. Standard error = 0.7–1%. Extraction conditions are as follows. First stage: T = 190 °C; pressure = 5.2 MPa; flow rate = 1.5 mL/min; time = 30 min; extract volume = 45 mL; water/feed = 18 mL/g. Second stage: T = 160 °C; pressure = 5.2 MPa; flow rate = 0.5, 1, 2, 3, and 5 mL/min; time = 100 min; extract volume = 100 mL; ammonia/feed = 40 mL/g. [b] SR, solid remaining after extraction.

Table 5. Removal, Accounted, and Unaccounted Levels of Biopolymers with Aqueous Ammonia at Different Flow Rates

flow rate	removal (%)			yield in liquid (%) (accounted)			degradation (%) (unaccounted)		
(mL/min)	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
0.5	45.9	14.8	92.2	39.6	2.5	74.9	6.3	12.2	17.3
1	45.3	15.5	88.9	36.0	2.5	74.4	9.3	12.9	14.5
2	38.9	15.9	89.0	32.2	2.5	74.6	6.7	13.4	14.5
3	34.4	16.2	88.6	31.0	2.5	74.2	3.5	13.6	14.5
5	36.7	16.6	87.2	30.5	2.5	73.9	6.2	14.1	13.3

The difference in the removal of lignin, glucan, and xylan from flax shives at different temperatures of the two-stage process is particularly noteworthy (**Table 3**). Lignin removal increased significantly at 200 °C with undesirable high glucan degradation (20.7%). The lignin removal was about 40% between 120 and 150 °C, 45% between 160 and 180 °C, and further increased to 59.5% at 200 °C. Thus, lignin and xylan removal reached their maximum values of 59.5% and 93.5%, respectively, at 200 °C. For our further studies, we chose 160 °C as the optimal temperature, because of high lignin extraction (45%) and low degradation of glucan (12.9%).

Effect of Flow Rate. The extractions at different flow rates were carried out at a fixed extract volume. The results (**Tables 4** and **5**) show that the extraction of lignin was higher at 0.5 and 1.0 mL/min than at 2, 3, and 5 mL/min. The highest lignin removal was achieved at lower flow rates, particularly at 0.5 mL/min. This can be attributed to swelling of the lignin polymer

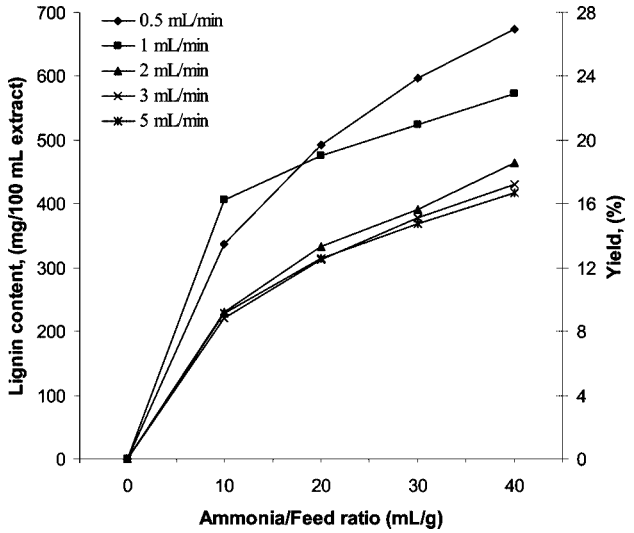

Figure 3. Sequential extraction of lignin with aqueous ammonia at 160 °C.

Table 6. Fractionation of Flax Shives at Different Concentrations of Ammonia at 160 °C[a]

ammonia	solid (%)				liquid (%)			total (%)		
concn (%)	SR[b]	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
untreated	100	30.2	34.2	21.3				30.2	34.2	21.3
15	47.8	16.4	29.1	1.7	11.9	0.9	16.0	28.3	30.0	17.6
10	47.0	18.6	27.4	1.5	11.2	1.3	15.9	29.9	28.7	17.4
5	47.0	18.6	27.4	1.8	10.2	1.3	15.9	28.8	28.7	17.7
2	47.6	18.5	26.6	1.5	10.0	1.4	15.9	28.5	28.1	17.4

[a] Note: Data are the mean value of duplicate analyses. Standard error = 0.7–1%. Extraction conditions are as follows. First stage: T = 190 °C; pressure = 5.2 MPa; flow rate = 1.5 mL/min; time = 30 min; extract volume = 45 mL; water/feed = 18 mL/g. Second stage: T = 160 °C; pressure = 5.2 MPa; flow rate = 0.5, 1, 2, 3, and 5 mL/min; time = 100 min; extract volume = 100 mL; ammonia/feed = 40 mL/g. [b] SR, solid remaining after extraction.

in aqueous ammonia at lower flow rates and its gradual removal with time (*15*). The solubilization of residual xylan increased significantly at lower flow rates. Decreasing flow rate increased the removal of lignin (45.8%) and xylan (92.2%) but increased xylan degradation from 13.3 to 17.3% (**Table 5**). Also, glucan dissolution in aqueous ammonia increased with flow rate insignificantly from 14.8 to 16.6%.

Figure 3 illustrates sequential lignin extraction at different flow rates in aqueous ammonia. Small differences were observed at the higher flow rates of 2, 3, and 5 mL/min. The major amount of lignin was removed during the first half of the extraction period (20 mL/g). As shown, the maximum lignin yield was achieved at a flow rate of 0.5 mL/min, and this flow rate was therefore chosen for further studies.

Effect of Aqueous Ammonia Concentration. The effect of ammonia concentration (2, 5, 10, and 15%) on the fractionation of flax shives was tested (**Tables 6** and **7**). Maximum lignin removal (46%) was achieved with 15% aqueous ammonia. Glucan dissolution increased with decreasing concentrations of ammonia. Maximum glucan removal (22%) was observed with 2% ammonia. Ammonia concentration had no apparent effect on xylan removal, since xylan removal and yield were almost constant at 93% and 74.5%, respectively.

Figure 4 illustrates the sequential extraction of lignin with aqueous ammonia. Yield was based on the liquid extracts. Lignin extraction with 10 and 15% aqueous ammonia was very similar. Lignin yield decreased with decreasing ammonia concentrations, but no difference was observed between 2 and 5% ammonia. Efficient lignin extraction can only be achieved with a minimum ammonia concentration of 10%.

A value of 15% aqueous ammonia was chosen for further studies since lignin is removed efficiently with higher concentrations of aqueous ammonia and glucan dissolution was low (14.8%).

Extraction of Free Phenolics during the Two-Stage Extractions. The extraction profiles of selected free phenolics including vanillin, vanillic acid, and acetovanillone were monitored during the two-stage extractions. During the first stage, 4.21 mg of vanillin, 2.26 mg of vanillic acid, and 0.16 mg of acetovanillone/100 mL were extracted from 2.5 g of flax shives. The amounts of free phenolics extracted during the second stage are presented in **Table 8**. Vanillin and acetovanillone extraction showed the same trend as with lignin extraction (increased with increasing temperature and ammonia concentration and decreasing flow rate). The content of vanillic acid increased from 0.22 to 0.37 mg/100 mL with decreasing ammonia concentration.

The maximum amount of vanillin (2.75 mg) was extracted at 160 °C at a 0.5 mL/min flow rate with 15% ammonia, which corresponds with the optimal conditions observed for lignin extraction. The amount of extracted acetovanillone varied widely with the highest amount (0.36 mg/100 mL) obtained at 160 °C, 0.5 mL/min, and 5% ammonia. The highest extraction levels of vanillic acid (0.48 and 0.37 mg/100 mL) were observed at 200 °C with 15% ammonia and at 160 °C with 2% ammonia, respectively. Therefore, a high temperature only enhanced the extraction of vanillic acid probably due to the fragmentation of vanillic acid from lignin at high temperatures.

Effect of Additives and Modifications. In order to increase lignin yield during extraction with aqueous ammonia, the effects of variables such as oxidants, soaking, particle size of the flax shives, solvent/feed ratio, and combined effect of these variables with the temperature were studied to achieve high cellulose purity. For comparative purposes, the extraction was carried out solely with aqueous ammonia. The experimental conditions and results are presented in **Table 9**. Additions and processing modifications were made to the observed optimal conditions for lignin extraction: 160 °C, 0.5 mL/min flow rate, 1.0 mm flax shives, 40 mL/g solvent/feed ratio unless noted otherwise. The results of the sequentially extracted lignin contents are presented in **Tables 9** and **10** and are illustrated in **Figure 5**. The catalytic amount of anthraquinone (0.2%) and the combined effect of higher temperatures (180 and 200 °C) and reduced particle size of flax shives (0.5 mm) had a noticeable effect on lignin extraction. The reduction of particle size from 1.0 to 0.5 mm improved the extraction of lignin and resulted in higher cellulose purity at elevated temperatures of 180 and 200 °C.

Therefore, an increase in lignin yield can be achieved by increasing the temperature, reducing the particle size, or adding efficient oxidants (**Tables 9** and **10**).

Small differences were observed in the lignin content of solids remaining after modified extractions except with 200 °C (**Table 9**). Overnight soaking, the addition of oxidants (0.2% anthraquinone or 15% hydrogen peroxide), and reducing the particle size from 1.0 to 0.5 mm at 160 °C did not affect the lignin content of the solids (~19%) and lignin removal (~39%), as indicated in **Tables 9** and **10**. However, improvement in sequential lignin extraction was observed with catalytical amounts (0.2%) of anthraquinone (**Figure 5**). No significant difference in the glucan and xylan content of solid samples was observed.

The use of aqueous ammonia in both stages of extraction also showed results similar to those with two-stage extractions

Table 7. Removal, Accounted, and Unaccounted Levels of Biopolymers at Different Ammonia Concentrations

	removal (%)			yield in liquid (%) (accountability)			degradation (%) (unaccountability)		
ammonia concn (%)	lignin	glucan	xylan	lignin	glucan	xylan	lignin	glucan	xylan
15	45.9	14.8	92.2	39.6	2.5	74.9	6.3	12.2	17.3
10	38.3	19.7	93.1	37.2	3.8	74.5	1.1	15.9	18.5
5	38.3	19.8	91.5	33.7	3.9	74.6	4.7	15.9	16.9
2	38.9	22.0	92.8	33.2	4.2	74.5	5.6	17.8	18.3

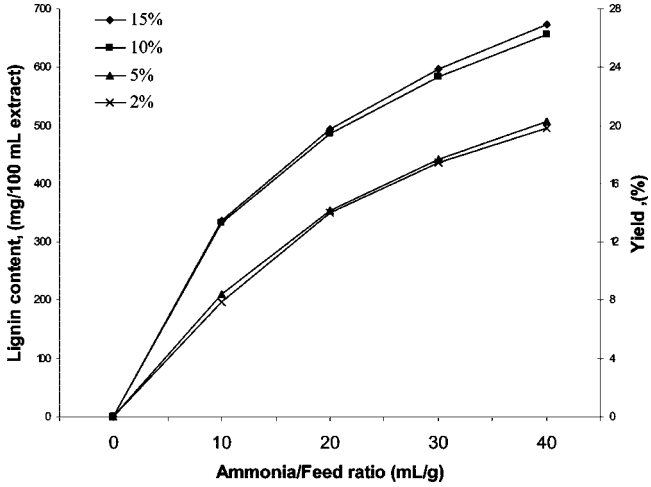

Figure 4. Sequential extraction of lignin at different concentrations of ammonia.

Table 8. Free Phenolics Extraction during Aqueous Ammonia Treatment

	free phenolics (mg/100 mL)		
experimental	vanillin	vanillic acid	acetovanillone
	Temperatures (°C)		
120	1.01		
140	1.10	0.12	
150	1.05	0.14	0.12
160	1.14	0.22	0.18
180	1.42	0.40	0.49
200	1.62	0.48	0.80
	Flow Rates (mL/min)		
0.5	2.75	0.26	0.16
1	1.14	0.22	0.18
2	1.32	0.22	0.10
3	1.12		
5	1.05		
	Ammonia Concn (%)		
2	1.31	0.37	0.14
5	1.57	0.21	0.36
10	0.97	0.26	0.30
15	1.14	0.22	0.18

using water and aqueous ammonia, except for the xylan removal (**Table 9**). More than 20% of the xylan remained in the solid sample, and the xylan yield in the liquid sample accounted for 55% of the total, indicating that aqueous ammonia alone was not as effective as water for xylan extraction. This is consistent with xylan extraction by aqueous ammonia treatment of corn stover (*15*). Lignin removal (48%) was higher than other two-stage extractions at 160 °C but was low compared to those of corn stover at 70–85% (*15*). Glucan removal and degradation rate were both 13.8%, confirming glucan degradation with aqueous ammonia under PLPW conditions used in this study. Glucan degradation in the range of 10–12% with aqueous ammonia has been previously reported (*15, 16*). The percentage of remaining solids decreased with increasing temperature and reduced particle size, reaching 28% at 200 °C (**Table 9**). Flax

Table 9. Fractionation of Flax Shives in the Presence of Additives and Process Modifications[a]

		solid (%)			liquid (%)		total (%)	
additives/modifications	SR[b]	lignin	glucan	xylan	glucan	xylan	glucan	xylan
untreated	100	30.2	34.2	21.3				
anthraquinone (0.2%)	47.0	18.4	29.8	2.2	0.9	15.9	30.7	18.0
hydrogen peroxide (15%)	45.5	18.3	28.3	1.7	0.9	15.9	29.2	17.5
overnight soaking	47.6	18.5	29.5	2.3	0.9	15.8	30.4	18.1
0.5 mm flax shives	47.1	19.5	28.9	2.1	0.9	15.8	29.7	17.9
ammonia (15%); 190 °C	48.3	15.9	29.5	4.2	0.9	11.8	30.3	16.0
180 °C; 0.5 mm flax	43.6	16.8	27.8	1.6	1.2	15.9	29.0	17.5
200 °C	33.5	10.2	23.3	1.1	0.9	15.9	24.1	17.0
200 °C; 0.5 mm flax shives; 120 mL/g	28.0	7.1	20.2	1.1	0.9	15.9	21.1	17.0

[a] Note: Data are the mean value of duplicate analyses. Standard error = 0.7–1%. Extraction conditions are as follows. First stage: T = 190 °C; pressure = 5.2 MPa; flow rate = 1.5 mL/min; time = 30 min; extract volume = 45 mL; water/feed = 18 mL/g. Second stage: T = 160 °C or 180 and 200 °C as noted; pressure = 5.2 MPa; flow rate = 0.5 mL/min; time = 100 min; extract volume = 100 mL; ammonia/feed = 40 or 120 mL/g as noted. Particle size = 1.00 or 0.5 mm as noted. [b] SR, solid remaining after extraction.

Table 10. Removal, Accounted, and Unaccounted Levels of Biopolymers with Aqueous Ammonia in the Presence of Additives and Process Modifications

additives or modifications	removal (%)			yield in liquid (%) (accounted)		degradation (%) (unaccounted)	
	lignin	glucan	xylan	glucan	xylan	glucan	xylan
anthraquinone (0.2%)	39.2	12.7	89.9	2.5	74.4	10.2	15.5
hydrogen peroxide	39.5	17.1	92.2	2.5	74.5	14.5	17.7
overnight soaking	38.9	13.7	89.1	2.5	74.2	11.2	14.9
0.5 mm flax shives	35.5	15.5	90.3	2.5	74.2	13.0	16.1
ammonia (15%), 190 °C	47.4	13.8	80.4	0.0	55.5	13.8	24.8
180 °C; 0.5 mm flax	44.4	18.5	92.7	3.4	74.8	15.1	17.8
200 °C	66.2	31.9	95.0	2.5	74.7	29.4	20.3
200 °C; 0.5 mm flax shives; 120 mL/g	76.6	40.8	94.7	2.5	74.6	38.3	20.0

shives milled to a particle size of 0.5 mm were used in this case. A total of 40% of solids remained at 200 °C (**Table 2**) with a particle size of 1.00 mm. The combined effect of reduced particle size (0.5 mm) and higher temperature (200 °C) enhanced lignin extraction. The lignin removal reached 66% at 200 °C at the solvent/feed ratio of 40 mL/g and increased further up to 77% at an increased solvent/feed ratio of 120 mL/g (**Table 10**). Glucan content of the sample decreased to 20%, and removal and degradation reached 41% and 38.3 %, respectively. Thus, while achieving 10% additional lignin extraction with 120 mL/g rather than 40 mL/g, more than 9% of the glucan was lost.

The lignin/glucan removal ratio was apparently useful in estimating the efficiency of the fractionation process. In these experiments, the highest lignin/glucan removal ratio (3.1) was achieved with the anthraquinone-added extractions. This ratio was 1.9:2.5 with other extractions with additives and modifications.

In summary, the fractionation of flax shives into hemicellulose, lignin, and cellulose with a two-stage treatment process

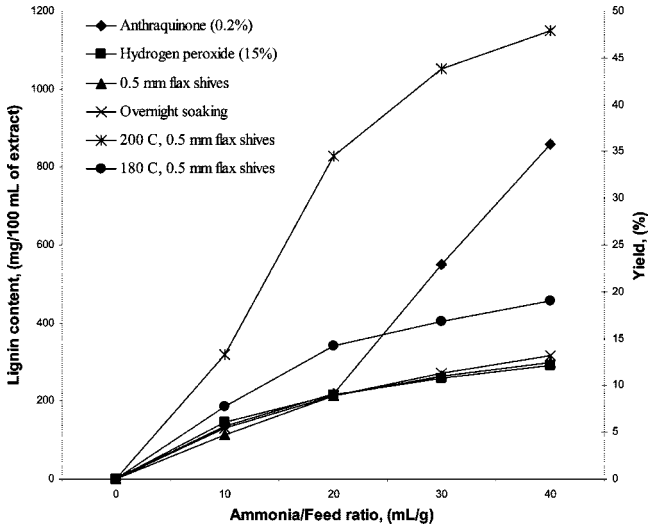

Figure 5. Sequential lignin extractions at different conditions.

using water and aqueous ammonia in a PLPW extractor was efficient for hemicellulose removal, reaching 85% after the first stage with water and 95% after the second stage with 15% aqueous ammonia. The hemicellulose extraction with PLPW required only 20 mL/g for 2.5 g of flax shives and has a promising future due to the use of a small amount of water as the solvent.

Efficient lignin extraction from flax shives with aqueous ammonia required a temperature of 200 °C, a flow rate of 0.5 mL/min, and a particle size of 0.5 mm to achieve 77% lignin removal and 67% cellulose purity. This can be attributed to the higher lignin content of flax shives (30.2%). Significant glucan dissolution (10–41%) occurred in aqueous ammonia with increasing temperature between 160 and 200 °C. Lignin extraction in aqueous ammonia was very efficient in terms of concentrated extracts and required only 40 mL/g of solvent/feed ratio. However, glucan degradation hinders the increase of temperature for greater lignin extraction. Fractionation of flax shives in a PLPW extractor using a two-stage process—first with water, then with ammonia for improved efficiency—is promising for the separation of hemicellulose and lignin. However, further work is required for the removal of the residual lignin.

ACKNOWLEDGMENT

We thank Tony Cottrell and John Drover for their help with the HPLC analysis.

LITERATURE CITED

(1) Wood, S.; Layzell, D. B. A Canadian Biomass Inventory: Feedstocks for a Bio-based Economy. *BIOCAP Can. Foundation* **2003**, 18–24.

(2) Liu, C.; Wyman, C. E. The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover. *Ind. Eng. Chem. Res.* **2003**, *42*, 5409–5416.

(3) Saddler, J. N. *Introduction, Biotechnology in Agriculture*; CABI: U.K., 1993; Vol. 9, pp 1–11.

(4) Schwald, W.; Brownell, H. H.; Saddler, J. Enzymatic hydrolysis of steam treated aspen wood: Influence of partial hemicellulose and lignin removal prior to pretreatment. *J. Wood Chem. Technol.* **1988**, *8* (4), 543–560.

(5) Chang, V. S.; Holtzapple, M. T. Fundamental factors affecting biomass enzymatic reactivity. *Appl. Biochem. Biotechnol.* **2000**, *84* (86), 5–37.

(6) Watanabe, T. Analysis of native bonds between between lignin and carbohydrate by specific chemical reactions. In *Association between Lignin and Carbohydrates in Wood and Other Plant Tissues*; Koshijima, T. Watanabe, T., Eds.; Springer-Verlag: Heidelberg, Germany, 2003; pp 91–130.

(7) Converse, A. O. Substrate factors limiting enzymatic hydrolysis. *Biotechnology in Agriculture*; CABI, U.K., 1993; Vol. 9, pp 93–106.

(8) Singh, R.; Singh, S.; Trimukhe, K. D.; Pandare, K. V.; Bastawade, K. B.; Gokhale, D. V.; Varma, A. J. Lignin-carbohydrate complexes from sugarcane bagasse: Preparation, purification, and characterization. *Carbohydr. Polym.* **2005**, *62* (1), 57–66.

(9) Wallace, G.; Russell, W. R.; Lomax, J. A.; Jarvis, M. C.; Lapierre, C.; Chesson, A. Extraction of phenolic-carbohydrate complexes from graminaceous cell walls. *Carbohydr. Res.* **1995**, *272*, 41–53.

(10) Morrision, I. M. Isolation and analysis of lignin-carbohydrate complexes from Lolium multiflorum. *Phytochemistry* **1973**, *12*, 2979–2984.

(11) Adler, E. Lignin chemistry—Past, present and future. *Wood Sci. Technol.* **1977**, *11*, 169–218.

(12) Grohmann, K.; Torget, R. W.; Himmel, M. E. Optimization of dilute acid pre-treatment of biomass. *Biotechnol. Bioeng.* **1985**, *15*, 59–80.

(13) Torget, R.; Walter, P.; Himmel, M.; Grohmann, K. Dilute-acid hydrolysis pre-treatment of corn residues and short-rotation woody crops. *Appl. Biochem. Biotechnol.* **1991**, *28* (29), 75–86.

(14) Kim, S. B.; Lee, Y. Y. Fractionation of herbaceous biomass by ammonia-hydrogen peroxide percolation treatment. *Appl. Biochem. Biotechnol.* **1996**, *57* (58), 147–156.

(15) Kim, T. H.; Kim, J. S.; Sunwoo, C.; Lee, Y. Y. Pretreatment of corn stover by aqueous ammonia. *Bioresour. Technol.* **2003**, *90*, 39–47.

(16) Kim, T. H.; Lee, Y. Y. Fractionation of corn stover by hot water and aqueous ammonia treatment. *Bioresour. Technol.* **2006**, *97*, 224–232.

(17) Fernandez Perez, V.; Jimenez Carmona, M. M.; Luque de Castro, M. D. An approach to the static-dynamic subcritical water extraction of laurel essential oil: comparison with conventional techniques. *Analyst* **2000**, *125*, 481–485.

(18) Gamiz Gracia, L.; Luque de Castro, M. D. Continuous subcritical water extraction of medicinal plant essential oil: comparison with conventional techniques. *Talanta* **2000**, *51*, 1179–1185.

(19) Hawthorne, S. B.; Grabanski, C. B.; Martin, E.; Miller, D. J. Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix. *J. Chromatogr., A* **2000**, *892*, 421–433.

(20) Jimenez Carmona, M. M.; Ubera, J. L.; Luque de Castro, M. D. Comparison of continuous subcritical water extraction and hydrodistillation of marjoram essential oil. *J. Chromatogr., A* **1999**, *855*, 625–632.

(21) Kubátová, A.; Lagadec, A. J. M.; Miller, D. J.; Hawthorne, S. B. Selective extraction of oxygenates from savory and peppermint using subcritical water. *Flavour Fragrance J.* **2001**, *16*, 64–73.

(22) Cacace, J. E.; Mazza, G. Pressurized low polarity water extraction of lignans from whole flaxseed. *J. Food Eng.* **2006**, *77*, 1087–1095.

(23) Kim, J. W.; Mazza, G. Optimization of extraction of phenolic compounds from flax shives by pressurized low polarity water. *J. Agric. Food Chem.* **2006**, *54*, 7575–7584.

(24) *Chemical Analysis and Testing (CAT) Standard Procedure Nos. 001-004 and -014*; National Renewable Energy Laboratory, NREL: Golden, CO, 1996.

(25) Kim, J.; Mazza, G. Extraction and separation of carbohydrates and phenolic compounds in flax shives with pH-controlled pressurized low polarity water. *Bioresour. Technol.* Submitted.

(26) Dence, C. W. Determination of Dissolved Lignin by the Modified Pearl-Benson (Nitrosation) Method. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C.W., Eds.; Springer-Verlag: Heidelberg, Germany, 1992; pp 55–57.

(27) Laser, M.; Schulman, D.; Allen, S. G.; Lichwa, J.; Antal, M. J.; Lynd, L. R. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. *Bioresour. Technol.* **2002**, *81*, 33–41.

(28) Bobleter, O. Hydrothermal degradation of polymers derived from plants. *Prog. Polym. Sci.* **1994**, *19*, 797–841.

(29) Mok, W.; Antal, M. J. Hot water only solvolysis of whole biomass hemicellulose by hot water compressed liquid water. *Ind. Eng. Chem. Res.* **1992**, *31*, 1157–1161.

(30) Allen, S. G.; Kam, L. C.; Zemann, A. J.; Antal, M. J. Fractionation of sugar cane with hot compressed liquid water. *Ind. Eng. Chem. Res.* **1996**, *35*, 2709–2715.

Received for review May 16, 2007. Revised manuscript received July 30, 2007. Accepted August 2, 2007. We are grateful to the CBIN program of Natural Resources Canada for partial funding of this project.

JF0714564