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# Optimization of hot-compressed water pretreatment of bagasse and characterization of extracted hemicelluloses

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ABSTRACT

Developing optimum treatment and separation procedures for hemicellulose components of lignocellulosic biomass could be useful in ethanol fermentation processes and obtaining pure hemicelluloses as biopolymers. Sugarcane bagasse analyses indicate that xylose is the major hemicellulose component constituting 17.7% of dry bagasse weight. In this study the effects of treatment conditions such as time, temperature and pressure on the yields of extracted hemicelluloses were studied. The optimum conditions were achieved at 180 °C for 30 min and 1 MPa pressure, with the yield of xylose reaching to 85% and the concentrations of sugar degradation products such as HMF and furfural remaining minimal at 0.95 and 0.07 g/L, respectively. Further, isolation of hemicelluloses from extracted hemicelluloses solutions was performed using Alfa Laval M20 membrane filtration system in two steps: (1) concentration of high molar mass hemicelluloses by ultrafiltration; and (2) separation of low molar mass hemicelluloses and oligomeric sugars by nanofiltration. The isolated hemicelluloses with the optimum pretreatment conditions were characterized by FT-IR and $^{13}C$ NMR techniques, resulting in agreement with typical spectra of xylan-type hemicelluloses.



## 1. Introduction

The demand for using renewable resources for energy and chemicals has been increasing rapidly because of population growth. Lignocellulosic biomass is abundant, renewable, and cost effective sources of energy and chemicals (Demirbas, 2001; Zhu & Pan, 2010). Ethanol produced from biomass provides many advantages over fossil fuels, most of ethanol currently produced in the world comes from corn as a major feedstock and the extra demand created a tight supply of corn for food production and higher food prices. Therefore, research on using lignocellulosic biomass for production of ethanol has been intensely pursued (Sun & Cheng, 2002; Wyman, 1994).

Production of ethanol from biomass requires releasing of cellulose and hemicelluloses sugars through hydrolysis and carrying out of a fermentation process (Mielenz, 2001). Hydrolysis and fermentation of biomass are much more complex than just fermentation of sugars and, currently, biomass conversion to ethanol needs further technical developments to make it competitive, such as increasing the hydrolysis yields of sugars and improving the fermentability of mixed sugars (Zhu, Wang, Pan, & Gleisner, 2008). Pretreatment of biomass is the most common technical approach used to hydrolyzing recalcitrance lignocellulosic biomass (Galbe & Zacchi, 2007; Zhu & Pan, 2010), wherein the lignin seal is broken down and structures of cellulose and hemicelluloses are disrupted to allow their separation and, most often, followed by further hydrolysis to mixtures of monomeric sugars and lignin components. The monomeric sugars mixture can be separated from the lignin fraction and subjected to a fermentation process for conversion to ethanol (Mosier et al., 2005; Taherzadeh & Karimi, 2007). However, the presence of different sugars requires different enzymes for fermentation, making the process complicated, costly, and inefficient due to differences in enzyme selectivity and durability. Also, certain by-products derived from hemicellulose degradation such as furfural, formic and acetic acids suppress the enzyme activities and inhibit the fermentation process (Kumar, Kothari, Lee, & Gupta, Ram, 2011; Sun, 2008). Therefore, in addition to separation of lignin components, separation of hemicellulosic components can be a useful approach for increasing the efficiency of the fermentation process of main glucose components. Membrane filtration methods have been used successfully to separate hemicelluloses and inhibitor compounds such as furans and carboxylic acids from pretreatment hydrolyzate solutions (Mousavi & Moghdam, 2009; Sjöman, Mänttäri, Nyström, Koivikko, & Heikkilä, 2008; Weng et al., 2010). Membrane filtration techniques have the advantages of ease and high efficiency; low energy consumption, adjustable separation capability, and applicability under various conditions.

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Pretreatment of lignocellulosic biomass materials has been investigated by using several methods such as dilute acid hydrolysis, liquid hot-water extraction, steam explosion-based extraction, dilute acid steam explosion, alkaline extraction, ammonia fiber explosion, hydrothermal pretreatment, and many other methods (Mosier et al., 2005). Among these methods, the hydrothermal pretreatment method using hot compressed water showed promising separation of hemicelluloses polymers from biomass because of a low capital cost and no use of major chemicals. Also, this method allows recovering of hemicelluloses as oligomers rather than monomeric sugars (Saha, 2003). Hardwoods and woody biomass can be hydrolyzed with yields of more than 90% oligomeric sugars using the hydrothermal pretreatment method (Mosier et al., 2005). Agricultural residues such as corn stover, cornhusk, wheat straw and sugarcane bagasse has very similar chemical compositions to woody biomass and hardwood and represent an abundant, inexpensive, and readily available source of lignocellulosic biomass (Gould, 1984). Sugarcane bagasse is produced in large quantities from sugar industry in many countries including the United States. Approximately, 280 kg of bagasse are generated from 1 ton of sugarcane and 54 million dry tons of bagasse is produced annually throughout the world (Sun, Sun, Sun, & Su, 2004).

In a recent study (Boussarsar, Roge, & Mathlouthi, 2009), the hydrothermal pretreatment of bagasse was optimized in a temperature ranging from 170 to 190 °C, resulting in an optimum reaction time of 2 h at 170 °C. This pretreatment time appears to be long and, therefore, the major aim in this study was to investigate the pretreatment conditions for sugarcane bagasse at the same temperature range but using shorter reaction time of less than 1 h. The second aim was isolating of hemicellulose components from the pretreatment solutions using ultra and nanoflitration techniques, characterizing and investigating them as biopolymers.

## 2. Experimental

### 2.1. Chemicals

All chemicals used in this study were purchased from commercial resources and used without further purification. Calcium carbonate ≥99%, sulfuric acid 98%, certified ethanol, methanol and HPLC water were purchased from Fisher Scientific. Sugars used for HPLC analysis, D-glucose, D-mannose, D-galactose, D-arabinose and D-xylose, and dextran (1000, 5000, 12,000, 25,000, 50,000 and 80,000 Da) standards used for average molecular weight determinations were purchased from Sigma–Aldrich.

### 2.2. Raw material

De-pithed sugarcane bagasse used was provided by Sustainable Fuels LLC, New Iberia, LA. First it was air dried, then ground in a Wiley mill, and sieved through 40–80 mesh size sieve. Bagasse (15 g) was extracted with ethanol/benzene (1:1) mixture in a Soxhlet apparatus for 6 h to remove extractives and wax. Then bagasse was dried off from the solvent under the hood at room temperature for 24 h, and stored in plastic bag for moisture conditioning.

The chemical composition of the extracted bagasse sample was determined according to a known procedure (Sluiter et al., 2008b). Summary of this method is as follows: 0.3 g of sample was hydrolyzed by treating with 3 mL of 72% sulfuric acid for 1 h at room temperature by agitating and crushing constantly with glass rod. Then, 84 g deionized water (DI) was added to obtain 4% sulfuric acid solution and the mixture autoclaved at 121 °C for 1 h in a pressure tube to completely hydrolyze all oligomeric sugars into monomers. Then, the sugar solution was neutralized with calcium carbonate to pH 5.5 and filtered with 0.2 μm syringe nylon membrane (Millex-GN) for removal of fine particles. The solution was then analyzed by high performance liquid chromatography (HPLC) using an Agilent 1200 instrument equipped with a refractive index detector and Biorad column HPX 87P (7.8 mm × 300 mm) at 80 °C. Deionized water was used as an eluent at flow rate of 0.6 mL/min and the run last 50 min. Quantification of the sugars was done by comparing the peak integration values with those of chromatograms made with known sugar standards and presented in percentages based on dry bagasse weight. The acid soluble lignin content of the hydrolyzed sample was determined by Cary 100 Bio UV–vis Spectrophotometer (Varian Australia, Australia). The Klason lignin content of the hydrolyzed sample was determined by NREL/TP-510-42623 method. Ash content of bagasse was determined by NREL/TP-510-42622 technique by burning at 600 °C for 4 h.

### 2.3. Pretreatment procedures

The hot compressed water (HCW) pretreatment of bagasse was carried out in a high pressure Parr Reactor, series 4550 (Parr Instrument Company, Moline, IL) with a total volume of 450 mL. The reactor was loaded with bagasse (10 g) and water (100 g) in a 1:10 solid:liquid ratio and sealed. Then, the reactor was flushed with nitrogen for 10 min to remove the air and then filled with nitrogen until targeted pressure reached. Subsequently, heating and stirring at 200 rpm was applied until the desired reaction condition was reached. The pretreatment condition was varied: temperature, 170, 180, 190 °C; time, 10, 20, 30, 40, and 50 min, and pressure, 1, 2, 3, 4 and 5 MPa, resulting in 20 pretreatment samples in total. The pretreatment time began when the selected temperature was reached (approximately 20–30 min) and when the targeted time attained, the reaction was stopped by quenching the reactor in cold water. The liquid and solid portions were separated by vacuum pump filtration.

### 2.4. Acid hydrolysis and sugar analyses of hemicelluoses in pretreatment filtrates

Pretreatment filtrates were weighed and aliquots of them were hydrolyzed. The sugar contents were detected to determine the pretreatment efficiencies according to the standard NREL method (Sluiter et al., 2008a). Summary of this method is as follows: 20 mL of aliquots was placed in a pressure tube and certain amount of 72% of sulfuric acid was added to obtain a 4% sulfuric acid solution using the pre-calculated value reported in the appendix of the method. The, the sample mixture was autoclaved for 60 min at 121 °C, neutralized, filtered, and the sugar contents were determined by the same HPLC method described in Section 2.2. The sugar content weight values were then converted to those based on dry bagasse weights and then to percentage values based on each sugar types.

### 2.5. Determination of lignin components in pretreatment filtrates

The content of dissolved lignin in the extract was determined by scanning the aliquot on the UV–vis spectrophotometer as described in Section 2.2.

### 2.6. Molecular weights determination for extracted hemicelluloses

Average molecular weights of extracted hemicelluloses were determined by gel permeation chromatography (GPC). Aliquotes

(1 mL) were filtered through a 0.45 μm Millex® syringe driven filter unit into a 4 mL vial. GPC analyses were performed on a Waters HPLC system, consisting of a Waters 600E System Controller, Waters 717 plus autosampler, and waters 410 differential refractometer. Six dextran standards with peak molecular weights of 80,000, 50,000, 25,000, 12,000, 5000, and 1000 Da were used for building molecular weight calibration curve. Twenty micro litres aliquots were injected and separated through Shodex OHpak SB805 HQ and SB804 HQ columns connected in series. The mobile phase was water with a flow rate of 1 mL/min and the total sample run time was 25 min. Data from the differential refractometer was acquired and processed using PC-based Viscotek GPC Software.

### 2.7. Membrane filtration procedures for concentration of hemicelluloses

Concentration of hemicelluloses in the pretreatment filtrate obtained at optimum conditions was carried out on an Alfa-Laval M-20 (Alfa Laval Corporate AB, Lund, Sweden) membrane filtration system. Ultrafiltration was chosen to separate hemicellulose molecules with high molecular weight followed by nanofiltartion to separate low molecular weight and monomeric hemicelluloses components. Four litres of filtrate was required for this membrane equipment and, therefore, 12 batches of pretreatment (360 g of bagasse in total) were performed in the Parr reactor under the optimum pretreatment condition (180 °C, 30 min, and 1 Mpa), the solid particles in the slurry were separated by filtration on a nylon filter cloth using vacuum. The filtrates were combined and filtered again on 3–5 μm filter paper (Whitman international, England) to remove a small amount of particles that can plug the feed channels of the membrane modules. The pH of the filtrate solution was then adjusted to 5.5 from the original value of 3.6, with 5 N sodium hydroxide to neutralize some carboxylic acids to salts. Three different membranes (ETNA10PP, ETNA01PP and Alfa Laval-NF) with different molecular cutoff (10 kDa, 1 kDa and 400 Da), respectively, were used in sequence. For each membrane, four double-sided membrane discs were used to give a total membrane area of 0.14 $m^2$. The flow mode was cross flow. In the beginning, the feed solution was pumped from the feed tank to the membrane module by Hydra-Cell pump and the pressure over the membrane was varied by using control valve. During filtration, the retentate was kept flowing back to the feed tank while the permeate was continuously collected, giving the total amounts of permeate removed at any time. This also allowed calculating the flux values of the filtration.

Further, the pure water flux value was measured before and after experiments to identify the changes in the pure water flux due to the fouling or damage of the membranes. Fouling was determined based on the difference between the pure water flux of 500 mL water before (PWFb) and after (PWFa) filtration of the experimental solution as follows:

$$\text{Fouling}(\%) = \frac{\text{PWFb} - \text{PWFa}}{\text{PWFb}} \times 100$$

All variables such as pressure and temperatures were kept the same during determination of fouling. Also, performance of the membranes was determined by solid contents in retentates, filtrates and permeates. Hemicelluloses contents in retentate, permeate, and feed solution were verified by acid hydrolyses of aliquots and analyses of the sugar contents on HPLC. Further concentration for hemicelluloses in retentate was performed on a freeze dryer 4.5 (Labconco Corporation, Kansas, Missouri). The obtained crude hemicelluloses sample was further dried in a desiccator over calcium chloride and characterized using FTIR and $^{13}C$ NMR.

## 3. Result and discussion

### 3.1. Bagasse chemical composition

The chemical compositions of sugarcane bagasse are listed in Table 1. The results indicate that 44% of sugarcane bagasse composition is cellulose, 24% is lignin and 27% is hemicelluloses. The xylose content value represents almost 66% of total hemicelluloses. This result allowed determining the efficiency of pretreatment of extraction of hemicelluloses based on xylose content after converting xylan to xylose by acid hydrolysis.

### 3.2. Optimization of pretreatment conditions

In this study, the effects of pretreatment time and temperature were first determined, followed by the effects of pressure. Fig. 1a shows the yields of extracted hemicelluloses, in the form of hydrolyzed monomeric sugars, for pretreatments at 170 °C and 1 MPa pressure for 10–50 min. The highest yield of xylose (82% of xylose, based on total xylose content in sugarcane bagasse

**Table 1**
Chemical composition analysis results of bagasse (%, dry basis).

| | (%) |
|---|---|
| Glucose | 43.8 |
| Xylose | 17.9 |
| Galactose | 3.1 |
| Arabinose | 3.6 |
| Mannose | 2.1 |
| Lignin | 24.1 |
| Ash | 2.3 |
| Extractives | 2.2 |
| Total | 99.1 |

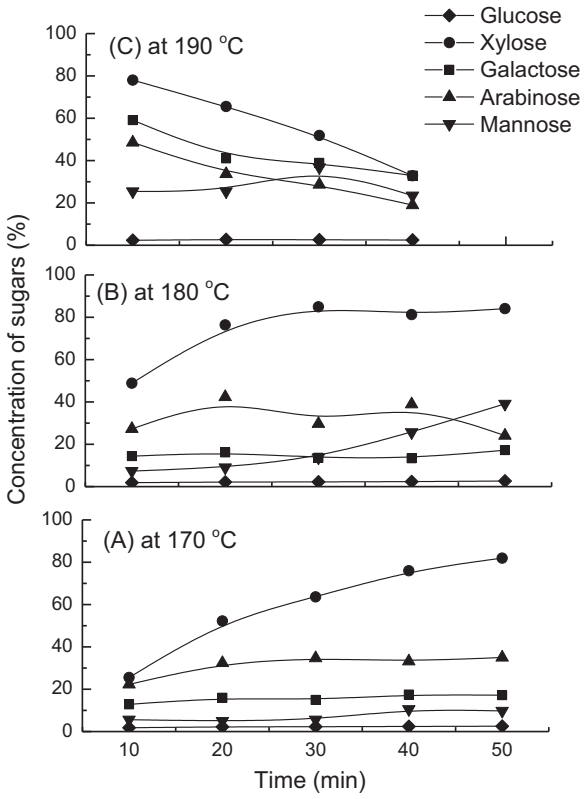


**Fig. 1.** Effects of treatment time and temperature on the yields of extracted hemicelluloses in terms of the sugar contents (%) based on the sugar contents of bagasse. (Pressure = 1 MPa; bagasse to liquor ratio = 1:10.)

hemicelluloses) was obtained after 50 min pretreatment compared to 49% xylose yield at 170 °C for 2 h treatment (Boussarsar, Roge, & Mathlouthi, 2009). Extraction of 82% xylose at 170 °C, 50 min and 1 MPa initial pressure indicates that pressure assists the extractability of xylose. According to Garrote et al. (2007), severe extraction condition solubilized higher amount of hemicelluloses. Since the aim of this work was to reduce the cost of the hot-compressed water pretreatment technique of sugarcane bagasse, prolonged extraction times were not studied. Instead, the pretreatment process was performed at slightly high temperature (180 °C) and keeping other parameters without change. At this temperature, the yield of xylose reached its maximum (85%) after 30 min as shown in Fig. 1b. As the extraction continued from 30 to 50 min, the yield of xylose was relatively unchanged. In case of mannose, as the extraction yield was increased and reached 39% after 50 min. The yields of galactose and glucose were increased by small increment. The yields of arabinose varied because arabinose is unstable at the severe condition (Boussarsar, Roge, & Mathlouthi, 2009; Garrote et al., 2007).

In Fig. 1c, the pretreatment results at 190 °C indicate that the amounts of extracted hemicelluloses were decreasing with the time. This indicates that at severe extraction condition hemicelluloses was unstable. The highest amount of extracted sugars, except mannose, was obtained after 10 min extraction. The yields of xylose, galactose and arabionose were 78%, 59% and 48%, respectively. However, those sugar yields were lower than yields obtained at 30 min and 180 °C. The yields of extracted glucose were relatively constant and they were less than 5% at all reaction times and temperatures, indicating that this pretreatment condition had small effect on the cellulose component of biomass even at prolonged extraction times.

Effects of temperature and time on the pH value of the reaction mixture at the end of pretreatment, concentrations of degradation products, and the amount of soluble lignin were also investigated. Fig. 2a exhibits the effects of extraction time and temperature to the pH value. Identical trends were observed at 170, 180 and 190 °C. The pH values decreased as the extraction time increased. Similarly, the pH values decreased as the temperature of extraction increased from 170 to 190 °C. These indicate that severe pretreatment condition (high temperature and prolonged time) promote disintegration of acetyl groups from xylan backbone structures, producing acetic acid and other acidic compounds (Klinke, Thomson, & Ahring, 2004).

Fig. 2b and c shows the effects of temperature and time on the amounts of furfural and HMF produced from degradation of hemicelluloses. The concentrations of furfural and HMF increased by raising the temperature or extending the reaction time, indicating that the production of acetic and other acids increases and promote hydrolysis and degradation reactions to form furfural and HMF. The effect of temperature on the yields of degradation products was much more pronounced than the effect of time. The concentrations of HMF and furfural at 190 °C and 10 min were higher than those observed at 180 °C for 30 min and 170 °C for 50 min. Since the formation of furfural and HMF is undesirable, the pretreatment of bagasse at 190 °C was considered incompatible even though the reaction time was short. The concentrations of HMF and furfural at 180 °C and 30 min were comparable with those at 170 °C for 50 min.

Fig. 2c shows the effects of time and temperature on the amount of soluble lignin in the pretreatment filtrate samples, measured by UV spectroscopy. The major absorption for lignin components measured at 275–278 nm range (Fukushima & Hatfield, 2001). Similarly, the amount of soluble lignin was increasing as the temperature and time of pretreatment increasing. However, the effect of temperature on the amounts of dissolved lignin was much higher than the effects of time. The amount of dissolved lignin at 190 °C and 10 min was higher than that at 180 °C for 30 min and 170 °C for 50 min. Based on the above results, it can be summarized that the optimum

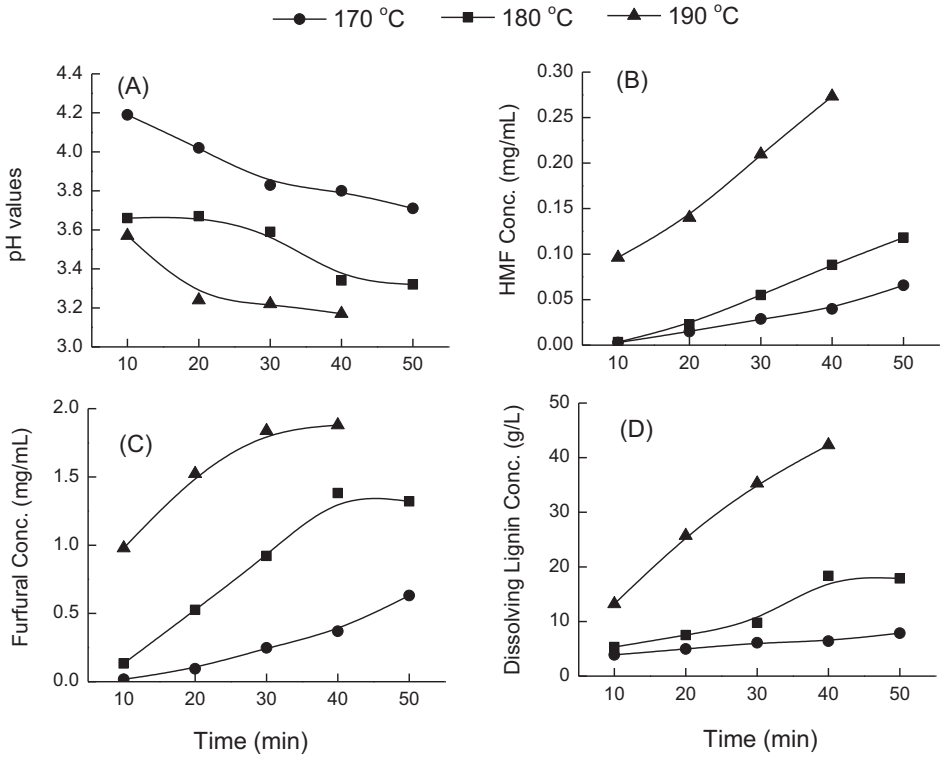


**Fig. 2.** Effects of treatment time and temperature on the pH values, concentration of degradation products and the yields of dissolved lignin in the extract. (Pressure = 1 MPa; bagasse to liquor ratio = 1:10.)

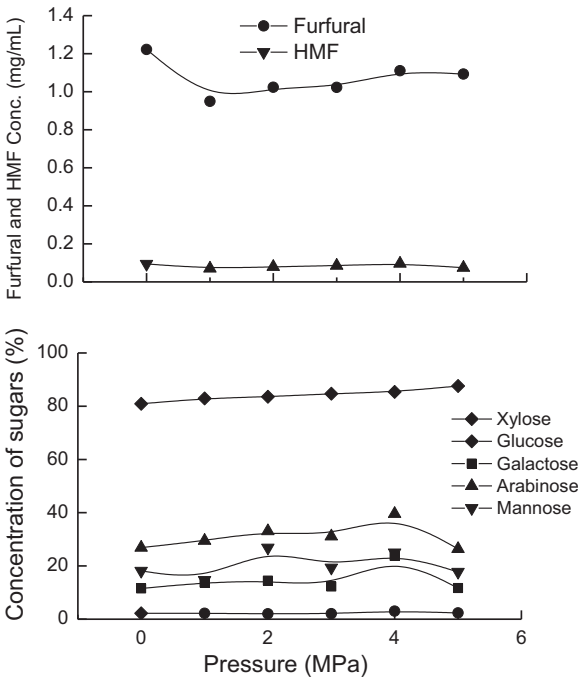


**Fig. 3.** Effects of the applied pressure on the yield of extracted hemicelluloses in terms of the sugar contents based on the sugar contents of bagasse and degradation products. (Temperature = 170 °C; Time = 30 min; bagasse to liquor ratio = 1:10.)

condition for extraction of hemicelluloses with highest xylose content, lowest degradation products and dissolved lignin was 180 °C and 30 min.

The pressure is another factor in hydrothermal pretreatments of biomass because it keeps water in the liquid state and this assists to alter lignocellulosic material structures (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010). In this study, the application of 1 MPa pressure resulted in the highest amount of xylose with moderate amount of sugar degradation compounds at 180 °C and 30 min. Thus, the pressure effect was investigated under this condition, by applying different pressures of 0–5 MPa. The effect of pressure on the yield of extracted hemicelluloses and degradation products were studied and the results are illustrated in Fig. 3. It is obvious that changing of pressure (at chosen range) has smaller effects than reaction time and temperature. The yield of xylose at pressure 0, 1, 2, 3, 4 and 5 MPa was 81%, 85%, 83%, 84%, 85% and 87%, respectively, based on the xylose value in the sugarcane bagasse. At 1 MPa the sugar degradation products were lower at 0.95 mg/mL HMF and 0.07 mg/mL furfural compared with the values observed at 0, 1, 2, 3, 4 and 5 MPa. Therefore, 1 MPa applied pressure was chosen as optimum pressure in this system.

### 3.3. Concentration of hemicelluloses using membrane filtration

Concentration of hemicelluloses in the bagasse extraction aliquot was carried out by sequential filtration on membranes in following order ETNA10PP, ETNA01PP, and NF in the manner

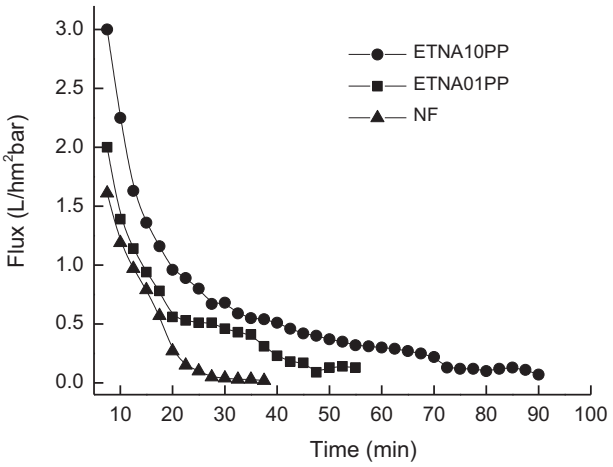


**Fig. 4.** Flux values of three different membranes during the filtration of sugarcane bagasse hemicelluloses extract.

that permeate of bigger size membrane would be the feed of next lower size membranes. The main feed solution prepared under the optimum pretreatment condition was containing 53.57 g hemicelluloses with average molecular weight 27,700 Da. First ETNA10PP membrane was fed with feed solution (Table 2) and it was observed about 57% of hemicelluloses with average MW 20,540 Da and lower permeated through the membrane. Twenty eight percent of hemicelluloses with average MW 34,100 Da and higher were retained in the retentate. Then, the permeate from ETNA10PP used for feeding the second membrane, ETNA01PP, wherein about 29% hemicelluloses were retained in the retentate. Similarly, the permeate of second membrane was used to feed the third membrane, NF, wherein more than 68% of hemicelluloses were retained in the retentate solution. Totally 42 g hemicelluloses were obtained from freeze drying of all retentate solution. The actual obtained weight of hemicellulose was slightly higher than the calculated weight (Table 2). It can be explained due to the incorporation of some lignin compounds.

The performance of the three membranes used for concentration of hemicelluloses extract was determined by pure water flux (PWF) testing, Fig. 4. Pure water permeability flux of membranes ETNA10PP, ETNA01PP and NF was tested at 4, 6 and 8 bar pressures, respectively. For all membranes, PWF values before filtration was higher than PWF values after the filtration, meaning the performance of membranes diminished due to the fouling. The fouling factor of membranes was in the following order ETNA10PP < ETNA01PP < NF, reflecting that the bigger the pore size of membrane the smaller the fouling of the membranes.

### 3.4. FT-IR analysis for hemicelluloses

The FT-IR spectrum of the freeze-dried fraction (Fig. 5) showed signals in the 1200–1000 $cm^{-1}$ region typical of isolated hemicelluloses previously reported by (Sun & Tomkinson, 2002). The band at 1033 $cm^{-1}$ represents dominant stretching and bending

**Table 2**
The average molecular weight, concentration, and yield of isolated hemicelluloses (HC) in the pretreatment solution after each membrane step.

| | Feed | | | Permeate | | | Retentate | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mw | HC Conc. (mg/mL) | HC weight (g) | Mw | HC Conc. (mg/mL) | HC weight (g) | Mw | HC Conc. (mg/mL) | Weight (g) |
| ETNA 10PP | 27,700 | 14.8 | 53.5 | 20,540 | 10.2 | 30.5 | 34,100 | 22.5 | 15.0 |
| ETNA 1PP | 20,540 | 10.2 | 30.5 | 8350 | 5.5 | 13.5 | 29,100 | 16.3 | 9.0 |
| NF | 8350 | 5.5 | 13.5 | 550 | 1.1 | 2.1 | 4430 | 14.4 | 9.2 |

Hemicelluloses contents were determined by hydrolyses to monomeric sugars followed analyses by HPLC.

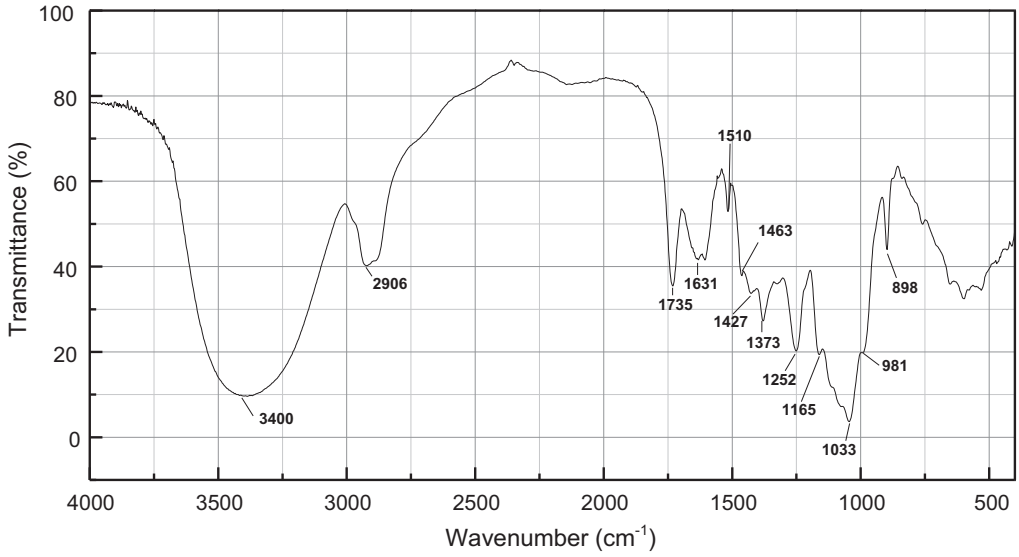


**Fig. 5.** FT-IR spectrum of the isolated sugarcane bagasse hemicelluloses sample in KBr pellet.

vibrations from C—O, C—C, C—OH and C—O—C bonds. Bands between 1165 and 1000 $cm^{-1}$ represent typical xylan structures. The two low-intensity shoulders at 1165 $cm^{-1}$ and represent arabinosyl side chains (Ma et al., 2012). This is due to the vibration of xylan units at the branched positions of C-2 and C-3. According to a report (Sun & Tomkinson, 2002), the arabinosyl side chain is attached only to the xylopyranosyl backbone and these two peaks identify the arabinoxylan structure. Also, it was reported (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000) that the intensity of these two peaks decrease as the number of branching increases. The intense band at 1735 $cm^{-1}$ represents the carbonyl stretching assigned to the acetyl, glucuronic acid and ferulic ester groups of polysaccharides. The sharp and intense peak at 898 $cm^{-1}$ represents the C-1 group ring frequency that characterizes β-glycosidic linkage between the sugar units (Fang, Sun, & Tomkinson, 2000; Gupta, Madan, & Bansal, 1987). Bands at 1463 and 1427 $cm^{-1}$ represent the $-CH_2-$ stretching vibration. Bands at 1373, 1323 and 1252 $cm^{-1}$ are originated from C—H, OH, or $-CH_2$ bending vibrations. The insensitive signal at 2906 $cm^{-1}$ is due to the C—H stretching vibration and the extensive band at 3400 $cm^{-1}$ is due to the —OH stretching vibrations of hemicelluloses and water. The sharp peak at 1510 $cm^{-1}$ represents the existence of lignin in the isolated hemicelluloses sample (Owen & Thomas, 1989; Sun et al., 2004).

### 3.5. $^{13}C$ NMR analysis for hemicelluloses

The isolated hemicellulosic fraction was analyzed by $^{13}C$ NMR spectroscopy in Fig. 6. Five strong signals at 101.49, 76.18, 73.47, 72.53 and 62.796 ppm, which are ascribed respectively to C-1, C-4, C-3, C-2 and C-5 of →4)-β-d-Xylp-(1→ residue substituted at 2 and 3 positions with —OAc and 4-O-Me-GlcA. Signals 102.49, 75.07, 75.07 and 65.05 ppm represent C-1, C-2, C-2, C-4 and C-5 carbons of →4)-β-d-Xylp- (1→ residue. The carbonyl carbon resonance signal at 173.01 ppm indicates C-6 in methyl uronic acids. Also anomeric carbon of α-D-GlcA gives the signal at 97.54 ppm. The carbonyl carbon resonance of acetyl group attached to the C-2 and C-3 of xylan backbone gives signal at 173.53 ppm. Signals at 20.22 and 20.45 ppm arise from $-CH_3$ group carbon of —OAc group at C-3 and C-2 carbons of xylan backbone (Ebringerova, Hromadkova, Alfoldi, & Berth, 1992; Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000; Vignon & Gey, 1998). This result implies that the isolated hemicellulosic fraction can be represented by (4-O-methyl-α-D-GlcA)

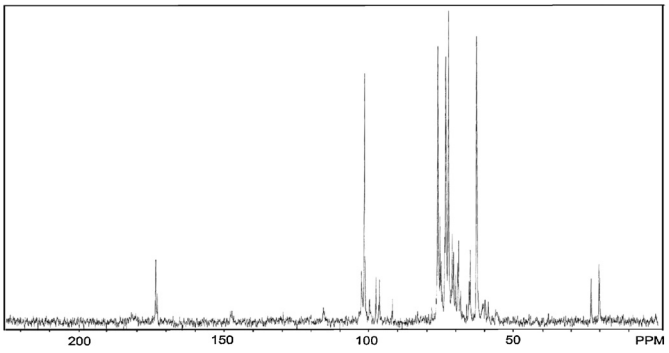


**Fig. 6.** $^{13}C$ NMR of the isolated sugarcane bagasse hemicelluloses in water.

→4)-β-d-Xylp- (1→ with a small amount of lignin impurities. In fact, the lignin impurity in the sample showed signals at $\delta$ 173.53 and 56 ppm from carbonyl and methoxyl group carbons of lignin (Capek et al., 2000).

## 4. Conclusion

Extraction of sugarcane bagasse hemicelluloses was conducted by a hot-compressed water extraction technique under a relatively mild condition regarding to time (10–50 min), temperature (170–190 °C) and pressure (1–5 MPa). It was observed that increasing the temperature by 10 °C from 170 to 180 °C resulted in extraction of a higher amount of hemicelluloses with 20 min less reaction time. The effect of temperature on the yield of degradation products and dissolved lignin was much more pronounced than the effect of time. For example, the concentration of HMF, furfural and dissolved lignin at 190 °C and 10 min was higher than those compounds at 180 °C min for 30 min and 170 °C min for 50 min. Changing of the applied pressure from 1 to 5 MPa slightly affected on the yield and properties of isolated hemicelluloses. The optimum condition for extraction of hemicelluloses with highest xylose content, lowest degradation products and dissolved lignin was 180 °C for 30 min at 1 MPa applied pressure. The extracted hemicelluloses solutions were concentrated by ultrafiltration with three different membranes in series. FT-IR and $^{13}C$ NMR spectra of isolated hemicelluloses showed typical spectrum of xylose based hemicelluloses which consistent with References.

## Acknowledgment

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