

COMPARISON OF HEMICELLULOSES ISOLATED FROM SODA COOKING BLACK LIQUOR WITH COMMERCIAL AND BACTERIAL XYLAN

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In this study, hemicelluloses were isolated from soda cooking black liquor. The structural features of different raw materials (wheat straw, rice straw, reed) were characterized and compared with those of commercial 4-*O*-methyl-D-glucurono-D-xylan and bacterial xylan, using Fourier Transform Infrared spectroscopy (FT-IR), Thermogravimetry (TG) and Scanning Electron Microscopy (SEM). Furthermore, hemicelluloses isolated from black liquor and two kinds of xylan were converted to carboxymethyl hemicelluloses (CMH), using carboxymethylation with sodium monochloroacetate and sodium hydroxide in 2-propanol medium. The reactions were performed at 55 °C for 4 hours, with 33 mL 2-propanol and 1 mL 30% NaOH per 0.6 g hemicelluloses. The product had maximum degrees of substitution (DS) of 0.54 for wheat straw, 0.55 for rice straw, 0.67 for reed, 0.65 for commercial xylan and 0.43 for bacterial xylan (which can be completely soluble in water). The structures of the resulting polymers were characterized using FT-IR, TG, SEM and Gel Permeation Chromatography (GPC). The objectives of this study were to investigate the extractability of hemicellulosic components from cooking black liquors, to synthesize CMH and evaluate their performance.

Keywords: hemicellulose, carboxymethylation, bacterial xylan, 4-*O*-methyl-D-glucurono-D-xylan, comparison

INTRODUCTION

A large quantity of black liquor is collected from pulping mills to be used in alkali recovery processes. However, after alkali is concentrated and removed from the solution, significant amounts of organic matters other than lignins still remain in the residues.

In previous reports,¹⁻² three kinds of phenolic compounds and several plant fatty acids were extracted and converted to useful byproducts. In addition to these, a much larger amount of organic matters is considered as hemicelluloses and their degeneration products. With the research development on biomass resources, hemicelluloses gain increasing importance as a basis for biofuels and also for new biopolymeric materials and functional polymers accessible to chemical modification.³ Hemicelluloses are abundant biopolymers in wood and other plants,

such as grasses, sugarcane bagasse, and cereal straws. The amount of hemicellulose varies according to the particular plant species. For example, sugarcane bagasse has 25-34% hemicelluloses, poplar has 18-25%, wheat straw – 32-38%, sunflower husk – 23-26% and corncobs – 36-38%.⁴ Hemicelluloses are found in plant cell walls along with celluloses and lignin, and are heteropolymers. The principal sugars in hemicelluloses are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars.⁵ Hemicelluloses are branched polymers of low molecular weight with a degree of polymerization of 80-200.⁶ In most current literature, hemicelluloses are separated from

herbaceous lignocellulosics directly by acidic, alkaline or organic solvent extraction in one or multiple steps.⁷⁻¹⁰

There is almost no literature pertaining to the separation of hemicelluloses from black liquor using simple methods. This study explores a method for separating hemicelluloses from pulping black liquor, including wheat straw, rice straw and reed pulping.

Considering the application, many polysaccharide derivatives have been prepared by carboxymethylation reactions using substances such as cellulose,¹¹ starch,¹² and cashew tree gum.¹³ Carboxymethylation is one of the most versatile functionalization procedures as it provides access to bio-based materials with valuable properties, such as inspissations, filming, suspension, water maintaining and binding. Carboxymethylated polymers have been used for many applications, such as printing and dyeing, medicine, paper making, food, textiles, oil drilling etc. Carboxymethyl hemicelluloses (CMH), the derivatives of hemicelluloses, have better solubility in water than hemicelluloses, and have hydroxyl and carboxyl functional groups as well; it is believed that they can be used as replacement for carboxymethyl cellulose. Up to now, only a few papers described carboxymethyl hemicelluloses isolated from oats, rice, bagasse and beech wood.⁵ In this study, the aim was to investigate the extractability of hemicellulosic components from cooking black liquors, to produce CMH, using black liquor hemicelluloses, commercial xylan and bacterial xylan, and to

evaluate their features using FT-IR, GPC, SEM, and TGA analyses.

EXPERIMENTAL

Cooking raw material

Air dried wheat straw, rice straw, and reed collected from JiangSu Province in China were used. The compositions of all raw materials are shown in Table 1. All methods were based on Tappi Standards.

Separation of hemicellulose from black liquors

Wheat straw, rice straw and reed (moisture content 10%) were chopped to 2-3 cm in length and cooked with a 2% sodium hydroxide solution (straw to liquid ratio 1:7 w/v) at 121 °C, for 2 hours, using electric steam autoclave (H-factor: 10.6). The amount of straw used in each run was 300 g on an oven-dried (OD) basis. After cooling the cooked slurry, the pulp (solid part) was filtrated and washed with hot water three times. The filtrate and washing liquor were adjusted to pH 3.5-4 and allowed to stand overnight (15 hours). Then the mixture was centrifuged to remove the precipitates (PPT-1). PPT-1 may have been contaminated with some hemicelluloses, but it was not discussed in this paper. The remaining liquor was concentrated to a volume between 500 and 1000 mL, then acetone (1:1 v/v) was added while stirring. It was allowed to stand to produce a precipitate (PPT-2) that was recovered by filtration using a Buchner funnel and filter paper. PPT-2 was believed to be crude hemicelluloses. It was washed three times using acetone-water (1:1 v/v), and the products were used for analysis and as reaction raw materials for carboxymethylation treatment.

Table 1
Composition of raw materials

Raw material	Alcohol-benzene extractives (%)	α -cellulose (%)	Holocellulose (%)	KL-Lignin (%)
Wheat straw	4.2	39.6	75.9	17.7
Rice straw	2.5	37.0	72.8	20.4
Reed	1.9	40.5	76.4	24.0

Synthesis of bacterial xylan

Xylose was incubated with *Acetobacter xylinum*, using a medium similar to the one reported by Hestrin and Schramm.¹⁴ The detailed incubation process was as follows: peptone (5 g), yeast extract (5 g), sodium phosphate dibasic anhydrous (Na_2HPO_4) (2.7 g) and citric acid monohydrate (1.15 g) were dissolved in 1 L de-ionized water. The solution was divided into four 500 mL Erlenmeyer flasks (each of them contained around 250 mL) and 2.5 g of xylose was added to each

flask. They were then sterilized by autoclave at 121 °C for a minute. (It is important not to hold the maximum temperature for long, because it will cause the production of furfural during sterilization. This works as an inhibitor to bacterial activities, causing the incubation to fail). After cooling, *Acetobacter xylinum* was planted and maintained at room temperature (optimum temperature 30 °C). It was grown up for 3-4 weeks and harvested after 6 weeks. The yield of bacterial xylan was 2.8%.

The commercial xylan was 4-*O*-methyl-D-glucurono-D-xylan, which was provided by Sigma Aldrich Trading Co., Ltd (Catalog No. M5144-1G).

Carboxymethylation of hemicellulose

Samples of hemicelluloses (600 mg) were mixed with 33 mL of 2-propanol in an Erlenmeyer flask (125 mL). Under magnetic stirring, 1 mL of 30% NaOH was added to the mixture, followed by 450 mg of monochloroacetic acid (MCA), and then it was heated on an oil bath at 55 °C for 4 h. The cooled mixture was separated using a centrifuge (at 3000 rpm) into a solvent and a solid phase. The insoluble material was washed several times with a water/methanol solution (1:9 by volume), and then with methanol. After drying over P₂O₅ in a vacuum oven, the resulting carboxymethylated hemicellulose (CMH) yield was determined.

Fourier Transform Infrared Spectroscopy (FT-IR)

The chemical structure of carboxymethyl hemicelluloses was evaluated by FT-IR spectroscopy. FT-IR spectra were obtained on an FT-IR spectrophotometer (Tensor 27), using a KBr disc containing 1% finely ground samples.

Thermal Gravimetric Analysis (TGA)

The thermal stability of the isolated hemicelluloses was measured using a simultaneous thermal analyzer (TGA/SDTA851e, Mettler-Toledo). The sample weights were between 5 and 10 mg. The scans were run from room temperature to 600 °C at a rate of 10 °C/min under nitrogen flow. Prior to thermal analysis, the samples were dried in a vacuum oven at 40 °C for 24 h.

Scanning Electron Microscopy (SEM)

Particle surface features of hemicelluloses and CM-hemicelluloses were studied with a JEOL (JSM-6490A) scanning electron microscope (SEM), with an acceleration voltage of 30 kV. The sample surfaces were sputter coated with gold to avoid charging.

Gel Permeation Chromatography (GPC)

The number and weight average molecular weights of carboxymethylated hemicelluloses were determined using gel permeation chromatography on a Waters

GPC system with Waters Ultrahydrogel 120 columns and a refractive index detector. Water (flow rate: 0.8 mL/min) was used as mobile phase, the analysis was done at 25 °C. A universal calibration was used for the calculation of the molecular weights, using polyethyleneglycol standard.

Determination of substitution degree

The average values of degree of substitution (DS) were determined by acidometric titration. Exactly 0.2 g of carboxymethyl hemicelluloses was weighed in a 250 mL flask, and then 80 mL distilled water was added and stirred for 10 min. The pH value of the solution was adjusted up to 8 by acid (0.5 mol/L HCl) or alkali (0.5 mol/L NaOH). Then the solution was titrated with 0.05 M H₂SO₄ until the pH value of the solution decreased to 3.74 by acidometer. The degree of substitution was calculated based on the equations shown below:

$$DS = 0.162B/(1-0.08B) \quad B = 2MV/m$$

where M = normality of H₂SO₄ used, V = ml of H₂SO₄ used to titrate sample, B = mmol/g of H₂SO₄ consumed per gram of carboxymethylated products.⁵ All the titrations were carried out in triplicate and standard deviations were less than 4.0%.

RESULTS AND DISCUSSION

Yield of hemicelluloses

The aim of this part of the work was to investigate the extractability of hemicellulosic components from cooking black liquors. The yields of the isolated hemicelluloses and lignin precipitate are given in Table 2. As can be seen, hemicellulosic components are roughly 15-18% of the black liquor solid. If the acetone-water mixture ratio is optimized, the components will be able to reach 25-30%. The separation of hemicelluloses can reduce black liquor viscosity, reducing the loss of useful organic matter in the alkali recovery. At the same time, the formation of foams during the solvent extraction is reduced tremendously and improves the effect of the extraction for the next step. (In Table 2, lignin percentage is to the dry matter of dried Filtrate 1).

Table 2
Characterization of the residue lignins, hemicellulosic components and fatty acids extracted from black liquor

Raw material	Hemicellulose (%)	Residue lignin (%)	Fatty acid (%)
Wheat straw	15.7	20.7	1.5
Rice straw	16.5	21.7	1.7
Reed	17.3	23.0	2.0

#: Weight percent based on dry matter of Filtrate-1

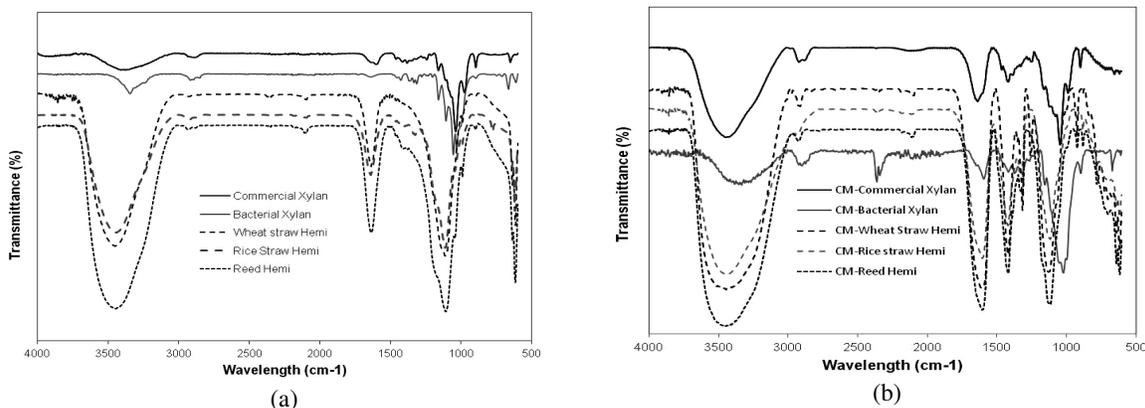


Figure 1: FT-IR spectra of hemicelluloses (a) and CM-hemicelluloses (b)

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of the three extracted hemicellulosic components are shown in Fig. 1. The hemicelluloses from three kinds of herbaceous cooking black liquor showed the typical signal pattern expected for a hemicellulosic moiety, and showed no significant differences from commercial xylan. Signals seen in the 3600-2800 cm⁻¹ region are due to stretching vibrations of CH and OH. The C-O stretching region (1200-950 cm⁻¹) is also visible. A sharp band at about 1110 cm⁻¹ in the spectra is typical of the absorption of xylans, indicating a dominant xylan in the isolated hemicelluloses. Another sharp band was observed at 1639 cm⁻¹, which indicated uronic acid existing on the hemicelluloses. In the anomeric region (950-700 cm⁻¹), a small sharp band at 903 cm⁻¹ is typical of β -anomers, indicating the presence of dominant β -glycosidic linkages between the sugar units in all the hemicellulosic fractions. The sharp band at 618 cm⁻¹ is the characteristic absorption peak of carbon five ring. When compared with ordinary hemicelluloses, the IR spectra of CM-hemicelluloses show an additional strong absorption band at 1601 cm⁻¹, confirming the presence of COO⁻ group.⁵ Strong absorption peaks were observed at 1314 and 1417 cm⁻¹, which were assigned to -CH₂ scissoring and -OH bending vibration, respectively. These results indicated that the carboxymethyl groups had successfully been introduced into the product. FT-IR spectra of commercial xylan, bacterial xylan, CM-commercial xylan and CM-bacterial xylan are also shown in Fig. 1. Bacterial xylan and commercial xylan have similar FT-IR absorptions. Similar to hemicelluloses, both CM-samples of

bacterial xylan and commercial xylan have strengthened absorptions peaks at about 1600-1700 cm⁻¹, which indicates that carboxymethyl groups were also successfully introduced into the xylans. The peak at 2361 cm⁻¹ in the spectrum of CM-bacterial xylan was a carbon dioxide peak from KBr contamination.

Degree of substitution (DS) of CM-hemicelluloses

The DS of hemicelluloses are shown in Table 3, the DS are 0.54 (CM-wheat straw), 0.55 (CM-rice straw), 0.53 (CM-reed), 0.65 (CM-commercial xylan), 0.23 (CM-bacterial xylan) respectively. The low DS of CM-bacterial xylan is caused by the dense network structure of bacterial xylan (Fig. 2), which makes it difficult for reagents to penetrate. This is supported by the fact that while the other four kinds of CM-hemicelluloses are soluble in water, CM-bacterial xylan is not completely soluble. The bacterial xylan is a homopolymer comprising a single unit of xylose. As a result, it is tightly attached like a nanocellulose and is difficult to dissolve in solvents. On the other hand, the other four hemicelluloses are composed of multiple units, they are attached as branches on the main xylose chain and are easy to dissolve in solvents. This difference in the structure caused different solubility.

Molecular weight of CM-hemicelluloses

Table 4 shows the molecular weights of the different products, indicating that the molecular weights of CM-hemicelluloses from black liquor were smaller than those of CM-commercial xylan and CM-bacterial xylan. The CM-bacterial xylan showed a lower molecular weight than CM-commercial xylan. Because the CM-bacterial

xylan was not completely soluble in water, only the soluble fraction could be measured by GPC.

In other words, only a part of the fraction can be carboxymethylated under these conditions.

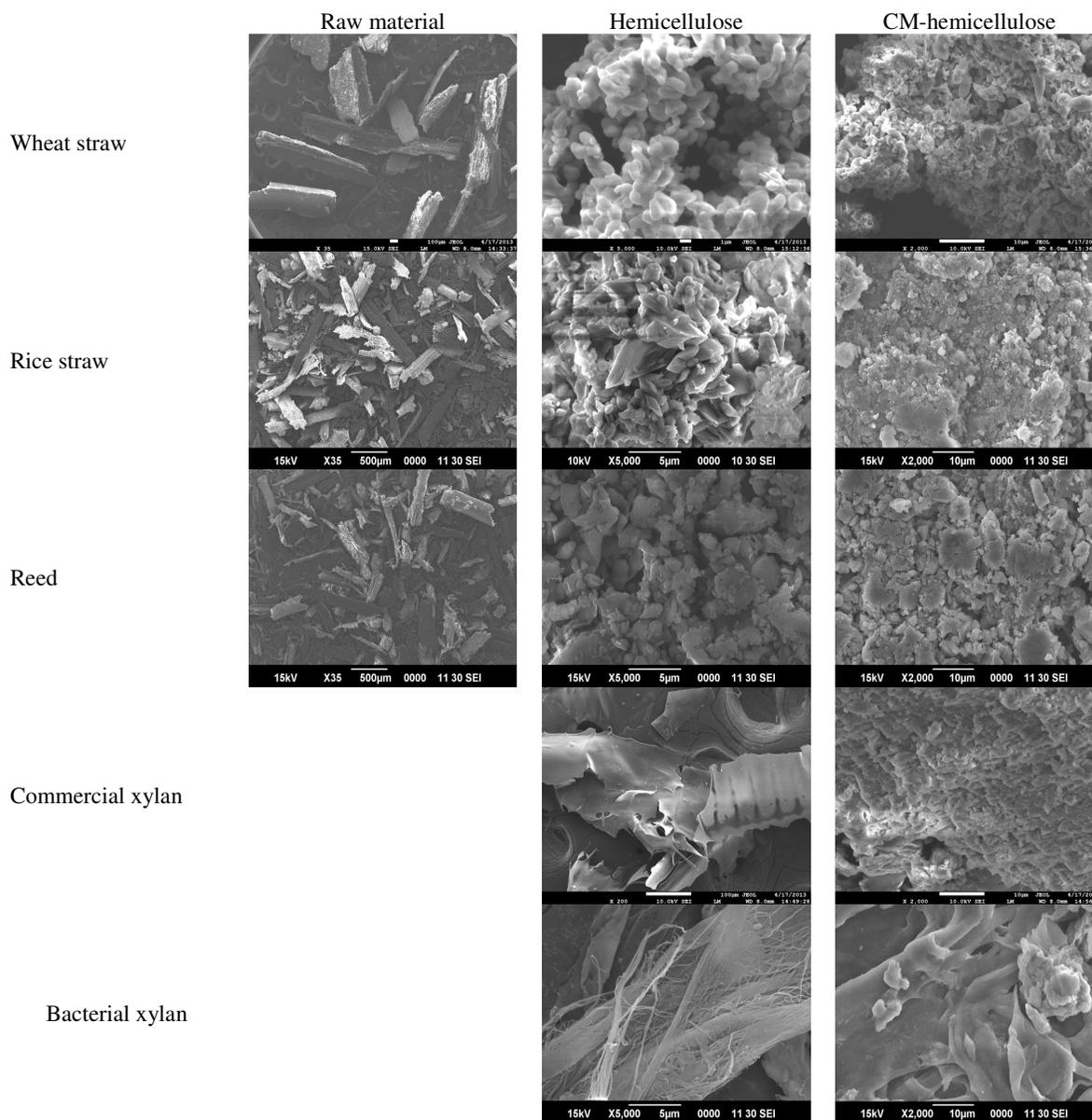


Figure 2: SEM photos of hemicelluloses and CM-hemicelluloses

Table 3
DS of carboxymethylated hemicelluloses

Sample	DS
CM-W.Straw H.	0.54
CM-R.Straw H.	0.55
CM-R.H.	0.53
CM-Com. Xylan	0.65
CM-Bac. Xylan	0.33

Table 4
Molecular weights of CM-hemicelluloses

Sample	Mw	Mn	Polydispersity
CM-W.Straw H.	1810 (608)	1565 (577)	1.16 (1.05)
CM-R.Straw H.	1683 (609)	1413 (590)	1.19 (1.03)
CM-R.H.	1803 (709)	1554 (692)	1.16 (1.02)
CM-Com.Xylan	3504	3414	1.03
CM-Bac.Xylan	2871	2698	1.06

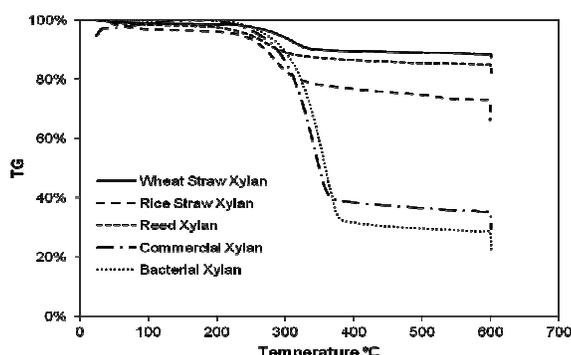


Figure 3: TG charts of five kinds of hemicelluloses

Thermal Gravimetric Analysis (TGA)

The thermal properties of the five hemicellulosic preparations were investigated by thermogravimetric analysis (TGA). TGA is one of the most convenient techniques used to determine general degradation characteristics and activation energies of materials under pyrolysis and combustion. Fig. 3 gives the TG charts of the five types of hemicelluloses. They began to decompose at 149 °C (wheat straw), 154 °C (rice straw), 159 °C (reed), 236 °C (commercial xylan), 210 °C (bacterial xylan), respectively. Their maximum rate of weight loss occurred between

340-400 °C. As the heating temperature approached the appropriate limit of maximum weight loss, the weight loss peaked at 89.86% (wheat straw), 75.17% (rice straw), 87.41% (reed), 63.63% (commercial xylan), 68.32% (bacterial xylan), respectively. The results suggested that the hemicellulosic components from black liquors had lower thermal stability than that of commercial xylan and bacterial xylan. It was also observed that their thermal stability correlated with the increasing molecular weight (Table 4). The TGA results reinforce the fact that bacterial xylan and commercial xylan have similar structures.

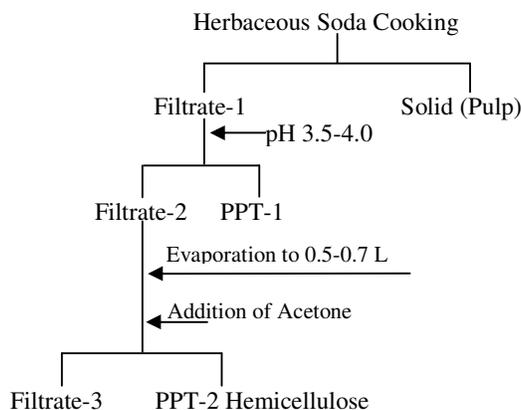


Figure 4: Flow sheet of solvent fractionation

Also, a part of the hemicelluloses were mixed in the crude lignin fraction (PPT-1), but it is not discussed in this paper (Fig. 4). The reason is that the purification of the hemicelluloses mixed in PPT-1 is very difficult and the amount is little. It is more realistic to think of the utilization of lignin.

Scanning Electron Microscopy (SEM)

In Fig. 2, SEM photos show that the hemicelluloses consisted of larger particles, while CM-hemicelluloses were observed to be much finer. The photos show the macromolecular structure of the carboxymethylated samples resulting from the $-\text{CH}_2\text{COOH}$ group. The SEM photo of bacterial xylan shows that its structure was different from the others. It was a dense network, which made it very difficult to grind. The carboxymethylation of bacterial xylan was also more difficult to grind than the other samples, the photo of CM-bacterial xylan confirmed limited changes to its structure. These data were consistent with the low DS of CM-bacterial xylan.

CONCLUSION

Hemicelluloses can be easily precipitated from black liquor using acetone, with a high yield. The removal of hemicelluloses is easy and significantly reduces the viscosity of their filtrate and improves the solvent extraction process by the dramatic decrement of total contents in the black liquor.

The structure of the hemicelluloses isolated from black liquor is different from that of commercial xylan, however, it has a similar main chain as that of commercial xylan.

In the carboxymethylation process, the hemicelluloses from black liquor and commercial xylan were easily converted into carboxymethylated hemicelluloses and had good solubility in water, while carboxymethylated bacterial xylan was difficult to dissolve in water due to its dense network structure.

Throughout this study, the precipitation using an organic solvent proved to be a useful method

for separating the hemicellulosic components from black liquor.

Carboxymethylation is a good method for converting hemicellulosic components into water soluble materials.

These results show that the methods employed in this study could be exploited in order to effectively recover and utilize black liquor on an industrial scale.

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