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Separation and Features of Carbohydrates in Bamboo Lignocellulosics

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Bamboo is a kind of natural lignocellulosic composites, in which cellulose fibers are embedded in a lignin matrix. Cellulose is not only useful as natural fibers, but has the potential to produce valuable chemicals such as ethanol, lactic acid, acetone and butyl alcohol. However, the molecular level utilization of cellulose can be achieved only after separating cellulose and lignin. In this work, bamboo lignocellulosics were converted into carbohydrates and lignophenols under normal pressure and at room temperature using the phase-separation system. The resulting carbohydrates were characterized in comparison with those from woody materials. The cell wall Interpenetrating Polymer Network (IPN) structure of bamboo was looser than woody materials, leading to quick hydrolysis of carbohydrates to monomers, dimmers, trimers and soluble polymer fractions. The sugar compositions from bamboo were similar to those from hardwood. The proportion of low molecular weight sugars was higher than that of woody materials.
Key words: bamboo, carbohydrates, the phase-separation system, ligno-*p*-cresols

1. INTRODUCTION

Global environmental protection and depletion of fossil fuel have highlighted the importance of establishing an efficient methodology for producing bioethanol from renewable biomass sources [1, 2]. To promote the use of bioethanol, production costs must be reduced to the point where ethanol prices are competitive with those of fossil energy resources. It is extremely important, therefore, to find simple and effective methods for hydrolyzing cellulosic biomass to sugars.

Lignocellulosic materials such as agricultural residues, hardwood, and softwood species are sugar sources for ethanol production [3]. Nevertheless, the conversion of lignocellulosic biomass, and particularly wood, to ethanol is difficult owing to the presence of rigid lignin which protects carbohydrates from acid or enzymatic attack [4, 5].

The utilization of carbohydrates until now is limited in natural fibers such as pulps. Usually, in pulping industry, through high temperature, high pressure and drastic reagent treatment, lignin is modified into structural complicated materials with many condensation structures. It is very difficult to use it as raw materials for functional materials. In conventional methods for utilization of carbohydrates, lignins are wasted. Actually, the biotechnology process is only approved when the ecosystem is not disturbed, so both of carbohydrate and lignin should be used effectively.

A new technique for separating wood into lignin and carbohydrates with concentrated acid-phenol was developed successfully in our laboratory. The cellulose and hemicellulose within the cell wall were converted into aqueous sugars and lignin was converted into lignophenol quantitatively [6, 7].

Bamboo is a wide-spread plant family in all continents except Europe. It has been accumulated in a large amount especially in Asia. Its utilization has been limited to building materials or articles of handicraft and a large amount of bamboo remains unutilized. In the present work, three species bamboo (*Phyllostachys bambusoide, Phyllostachys heterocycla*, *Phyllostachys nigra*) were treated through the phase-separation system. Sugar

compositions and molecular weight distributions of aqueous phase were characterized.

2. EXPERIMENTAL

2.1 Materials

Internodal pieces of bamboo were mechanically liberated from the epidermis, cut into short rings (about 1 cm in length) and reduced to small pieces. Bamboo chips were ground using a Wiley mill and a vibrational mill to pass an 80 mesh screen, and extracted with ethanol-benzene (1:2, v/v) for 48 hr.

2.2 Synthesis and isolation of ligno-p-cresols and sugars

Synthesis and isolation of ligno-*p*-cresols were carried out by the method in literature [8]. The flow charts are shown in Fig. 1 and Fig. 2. As the yields of bamboo ligno-*p*-cresols reached their maximum at reaction time 20 min., in this study, the reaction time was set at 20 min. to prepare sugar analysis samples.

2.3 Sugar compositions of aqueous phase

The sugars in the aqueous phase were hydrolyzed at 100°C for 4 hr by heating, after the aliquot 10 mL was diluted to 3% H_2SO_4 concentration. During the boiling time, distillated water was added gradually to keep up the solution volume, and then 20 mg of ribose was added as internal standard. The solution were neutralized by Ba $(OH)_2$ saturated solution, and the sugars compositions were determined by High Performance Liquid Chromatography (HPLC). Measurement conditions are as follows: Column; Shim-pack ISA-07/S2504 (4 mm ID.×25 cm L.), Eluent; 0.1 M potassium borate buffer (A) and 0.4 M potassium borate buffer (pH 9.0) (B), Flow rate; 0.6 ml/min, Gradient; A 100% to B 100% (2%/min), Temp.; 65°C, Detector; RI, Reaction reagent; 1% *L*-arginine and 3% Boric acid, Reaction temp.; 150°C, Detection wave length; Ex-320 nm, Em-430 nm. The molecular weight distributions of carbohydrates were determined by Gel Permeation Chromatography (GPC). Measurement conditions are as follows: Column; Asahipak GS-520 HQ, GS-320 HQ, GS-220 HQ, Eluent; H_2O, Flow rate; 0.6 ml/min, Temp.; 50°C, Detector; RI.

2.4 Elemental analysis of ligno-p-cresols

Carbon, hydrogen and nitrogen contents of bamboo (Madake), softwood and hardwood ligno-*p*-cresols were determined by Gas Chromatography (GC) method in Elemental analysis center of Kyoto University.

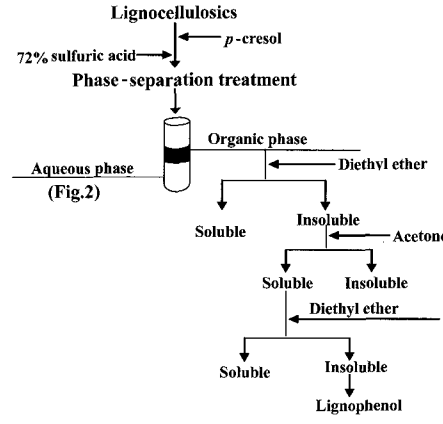

Fig.1 Synthesis of ligno-*p*-cresols through the phase-separation system (1 step process)

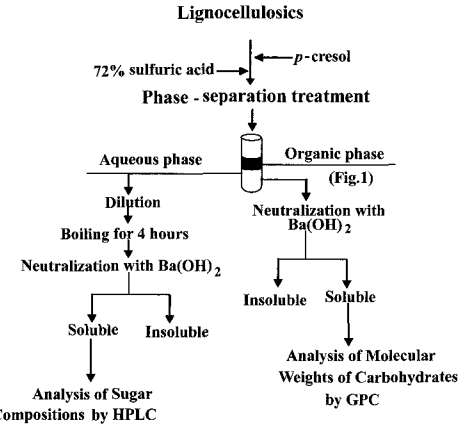

Fig.2 Analysis of sugars received from the phase-separation system (1 step process)

2.5 Methoxyl group content

The methoxyl group was determined by modified Zeisel's method [9, 10, 11]. The method based on the formation of methyl iodide from methoxyl groups. When lignin is heated with hydriodic acid, methyl iodide was removed from the reaction flask with a current of air into absorption vessels filled with molecular sieve 13X. The amount of methyl iodide was weighed and the methoxyl

content was calculated by the equation (2),

$$R\text{-}O\text{-}R' + HI \rightarrow R\text{-}OH + R'I \quad (1),$$

$$OCH_3\ (\%) = W \times F \times 100/S \quad (2),$$

Where W is weights of absorbed methyl iodide (mg), F is OCH_3 (31.04)/CH_3I (141.95) =0.2186 and S is weight of sample (mg).

3. RESULTS AND DISCUSSION

3.1 Sugar compositions of aqueous phase

When the phase-separation treatment was stopped at 20 min., the aqueous phase included mannose, arabinose, galactose, xylose and glucose. The proportions of them to dry bamboo meal were showed in Table 1.

Table 1 Sugar proportions (%) in aqueous phase

	Bamboo	Poplar	Western hemlock
Mannose	2.0	4.0	13.0
Arabinose	4.3	1.9	1.9
Galactose	2.0	1.1	2.6
Xylose	8.9	7.7	1.8
Glucose	56.5	49.3	48.7

(% to dry bamboo meal)

In case of bamboo and poplar, the proportions of sugars from hemicellulose were 23.3% and 22.9%, respectively. In case of western hemlock, it was 28.5%, and the proportion of mannose to total sugars was 19.1% and to hemicellulose was 67.1%. Hemicellulose was readily hydrolyzed to get soluble in the early stage of phase-separation treatment. Cellulose with crystalline region was difficult to be hydrolyzed, and the yield of glucose slowly increased with the reaction time. The total sugar yields to dry wood meals were 73.7%, 64.0% and 68.1%, for bamboo, hardwood and softwood, respectively. The proportion of bamboo sugars to its holocellulose was 95.2%. The hydrolysis pattern of carbohydrates was totally different between bamboo and wood with the treatment time of 20 min. The sugar yield from bamboo was the highest among the three samples, with the maximum amount of lignophenols. It indicated the rapid releasing of IPN structures of the cell wall for bamboo.

3.2 Molecular weights distribution of carbohydrates

GPC profiles of carbohydrates in the aqueous layer are shown in Fig. 3. Through the phase separation treatment time of 20 min., cellulose and hemicellulose were hydrolyzed into monomers, dimmers, trimers and polymer fractions. In the case of bamboo [Fig. 3 (1)], the fractions with more than 20000 of average molecular weight ($\overline{M}w$) were observed in the range of retention time 26.5-47.5 min. The fractions with 380-2000 of $\overline{M}w$ were observed in the range of retention time 47.5-66.5 min., and the monomer was observed in a softwood (S), the fractions with over 100 thousands of Mw were observed in the range of retention time 27-49 min. and 27.5-47 min., respectively, and their proportions were 49.2% and 51.9%, respectively. The fractions with several thousands of $\overline{M}w$ were observed in the range of retention time 49-66 min. and 47-66 min. Their proportions are 26.6% and 37.9%, respectively. Monomers were observed in the range of retention time 66-70 min. (H) and 66.5-70 min. (S) and their proportions were 24.3% and 10.2%, respectively.

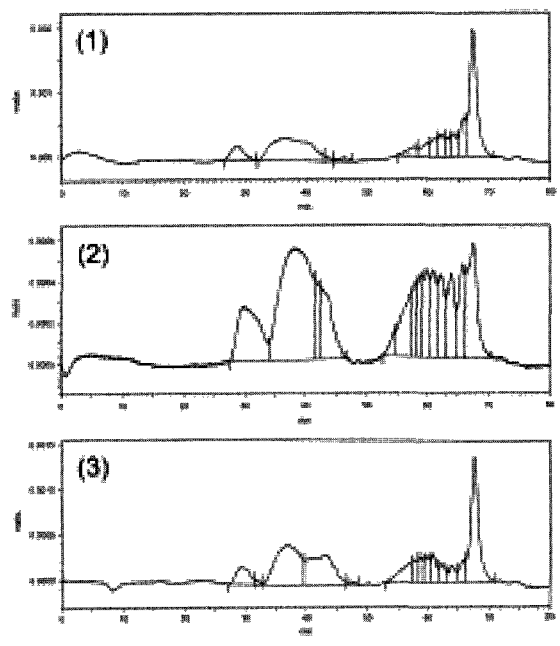

Fig. 3 GPC profiles of carbohydrates in the aqueous layer. (1) Bamboo (Madake), (2) Western hemlock, (3) Poplar.

These data show that the hydrolysis of bamboo carbohydrates proceeded more rapidly than that of woods. This is due to unique anatomical characteristics of bamboo compared to wood. The vascular bundles of bamboo are embedded in the parenchyma and the cell wall structures are looser than that of woods. As sugar sources for industrial utilization, the bamboo has more advantages for the structure and life cycle compared to woods.

3.3 Elemental analysis and methoxyl content

The elementary composition and empirical formula of obtained lignophenol are shown in Table 2. Both methoxyl group contents and the grafted cresol amounts of bamboo ligno-*p*-cresols are higher than those of softwood and are similar to those of hardwood. The methoxyl group contents of ligno-*p*-cresols in their C_9-unit formulae are similar to that of milled bamboo lignin. It indicated that the core units of ligno-*p*-cresols retain the structure of native bamboo lignin.

Table 2 Chemical Composition of Bamboo Ligno-*p*-cresols

	Analytical composition(%)					Empirical formula
	C	H	O	N	OCH_3	
Bamboo	66.39	5.84	27.77	-	14.21	$C_9H_{7.20}O_{2.91}(OCH_3)_{1.35}(C_7H_7O)_{0.85}$ [294.56]
Poplar	66.65	6.17	27.18	-	15.27	$C_9H_{7.88}O_{2.68}(OCH_3)_{1.43}(C_7H_7O)_{0.82}$ [290.83]
Western hemlock	66.46	5.82	27.72	-	10.88	$C_9H_{7.88}O_{2.97}(OCH_3)_{0.92}(C_7H_7O)_{0.66}$ [262.54]

4. CONCLUSION

In this work, bamboo lignocellulosics were converted into carbohydrates and lignophenols under normal pressure and at room temperature using the phase separation system. The resulting carbohydrates were characterized in comparison with those from bamboo was looser than woody materials, being hydrolyzed more quickly into monomers, dimmers, trimers and soluble polymer fractions. The sugar compositions from bamboo were similar to those from hardwood. The proportion of low molecular weight sugars was higher than that of woody materials.

All the analytical data indicated that bamboo has high potential to produce aromatic and aliphatic compounds as raw materials.

5. REFERENCE

[1] A. Petersson, MH. Thomsen, H. H.-Nielsen, A.B. A.B.Thomsen, *Biomass and Bioenergy*, **in press** (2007).

[2] S. Kim, B.E. Kim, *Biomass and Bioenergy*, **26**, 361-375 (2004).

[3] C. Munoz, R. Mendonca, J. Baeza et al., *Journal of Chemical Technology and Biotechnology*, **82**, 767-774 (2007).

[4] B. Fouad, J. Jover, E. Gonzalez, *Ingenieria Quimica (Madrid, Spain)*, **37**(425), 240-248 (2005).

[5] O.P. Ward, A. Singh, *Microbial Biotechnology in Agriculture and Aquaculture*, **1**, 449-479 (2005).

[6] M. Funaoka, I. Abe, *Tappi Journal*, **August**, 145-149 (1989).

[7] M. Funaoka, *Polymer International*, **47**, 277-290 (1998).

[8] M. Funaoka, S. Fukatsu, *Holzforschung*, **50**, 245-252 (1996).

[9] C.W. Dence, in "Methods in Lignin Chemistry," S.Y. Lin and C.W. Dence, Ed., Berlin Heidelberg: Springer-Verlag, 1992, p336-341.

[10] G. Gran, *Sven Papperstidn*, **56**, 179-180 (1953a).

[11] G. Gran, *Sven Papperstidn*, **56**, 202-203 (1953b).

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