HYDROLYSIS OF *TILIA JAPONICA* WOOD FOR PRODUCTION OF A FERMENTABLE SUBSTRATE

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To prepare a substrate for microbial conversion of xylose into xylitol, *Tilia japonica* wood was hydrolyzed with dilute sulfuric acid. When the reaction temperature was fixed at 121 °C, an optimum yield of xylose was obtained by treatment with 3% sulfuric acid, for 60 min. Both an increase in the sulfuric acid concentration and a prolonged residence time resulted in a decrease in the xylose yield. A fermentable substrate with a relatively high xylose concentration (57.0 g L^{-1}) was obtained by hydrolysis with 3% sulfuric acid, at a liquid-to-solid ratio of 3 g g^{-1}. During hydrolysis at elevated temperatures, certain undesired by-products were also generated, such as degradation products of solubilized sugars and lignin, which are potential inhibitors of microbial metabolism. These compounds were, however, successfully removed from the hydrolyzate by treatment with activated char.

Keywords: *Tilia japonica*, hydrolysis with dilute sulfuric acid, xylose

INTRODUCTION

Xylitol, a five-carbon sugar alcohol with high sweetening power, has been used in the food and pharmaceutical industries due to its low caloric value and anticariogenic property. It is currently produced by catalytic reduction of xylose in the hemicellulose hydrolyzates of hardwoods or corn cobs. Since the hemicelluloses of hardwoods and agricultural residues contain other sugar units, such as arabinose, galactose and glucose, extensive separation and purification steps are necessary to remove these contaminants from the hydrolyzates, before chemical reduction. The yield of xylitol corresponds to only about 50-60% of the xylan present in the raw materials, and the chemical reduction process is, therefore, costly.[1]

An alternative method for xylitol production is microbial conversion of xylose in the hemicellulose hydrolyzates.[2]

It has been reported that the hemicellulose hydrolyzates of *Eucalyptus* wood,[3-5] sugar cane bagasse,[6-8] corn cob,[9,10] sorghum straw[11] and rice straw[12,13] prepared by hydrolysis with dilute sulfuric acid were successively converted into xylitol, in good yields, by yeasts. Among these, hardwoods are the most abundant and promising sources of xylose. The objective of this work is to prepare a fermentable substrate from *Tilia japonica* (Japanese linden) wood with a relatively high xylose concentration that can be converted into xylitol by yeasts. In addition to solubilized sugars, a range of toxic

compounds is generated during acid hydrolysis at elevated temperatures. Weak organic acids, furan derivatives and low molecular weight phenolics are potential inhibitors of microbial metabolism.[14] The effect of the treatment with activated char on the removal of such furan derivatives and phenolics was also investigated.

MATERIALS AND METHOD

Material

The *T. japonica* wood was ground in a Wiley mill to pass through a 1 mm screen. The P42-R80 mesh fraction of the ground wood was extracted in a Soxhlet apparatus with a benzene-ethanol (2:1, v/v) mixture, for 48 h. The defatted sample was further refluxed with water for 3 h. The homogenized extracted residue lot was composed of 14.7% pentosan (including 14.5% xylan), 39.6% hexosan (including 38.5% glucan), 21.6% lignin (including 3.4% acid soluble lignin) and 0.2% ash.

Acid hydrolysis and determination of neutral sugars in the hydrolyzates

The homogenized extracted residue was hydrolyzed with dilute sulfuric acid (1-4%) with a liquid-to-solid-ratio of 290 (g g^{-1}), at 121 °C. The resulting hydrolyzates were filtered to remove the insoluble residue. A known amount of *meso*-erythritol as an internal standard was added to a suitable aliquot of the filtrate and the solution was neutralized with a 0.2 M barium hydroxide solution, followed by centrifugation. Neutral sugars in the hydrolyzates were determined by high performance liquid chromatography (HPLC) with a refractive index detector. The Tosoh CO-8020 HPLC system (Tosoh Corp., Tokyo, Japan) was equipped with an Aminex HPX-87P column (300 × 7.8 mm, Bio-Rad, Richmond, VA), in combination with a Carbo-P micro-guard cartridge (4.6 × 30 mm, Bio-Rad, Richmond, VA). Ion exchanged, degassed and filtered water was used as a mobile phase, at a flow rate of 0.6 mL min^{-1} and 85 °C.

Semiquantitative determination of potential inhibitors

The acid hydrolysis of lignocellulosic materials gives rise to solubilized sugar degradation products, such as furfural and hydroxymethylfurfural, which absorb strongly at 280 nm.[15] The overall amounts of furan derivatives and soluble lignin fragments in the hydrolyzates were estimated as furfural, by absorbance at 280 nm (A_{280}).[10]

Treatment with activated char

The homogenized extracted residue was hydrolyzed with 3% sulfuric acid with a liquid-to-solid ratio ranging between 10 and 3 (g g^{-1}), at 121 °C, for 60 min. Sorption experiments were conducted by agitating 15 mL of the hydrolyzate with desired amounts of an activated char (Shirasagi M, Japan EnviroChemicals, Ltd., Osaka, Japan), in a reciprocal shaker (160 strokes min^{-1}) at 30 °C, for a certain period of time.

RESULTS AND DISCUSSION

The pre-extracted wood meal was hydrolyzed with dilute sulfuric acid with a liquid-to-solid ratio of 290 (g g^{-1}), at 121 °C, for 60 min. The effect of sulfuric acid concentration on the yield of each neutral sugar in the hydrolyzates is listed in Table 1. The yield of xylose increased with increasing sulfuric acid concentration, reaching a maximum (17.2%, DM basis) at 3% sulfuric acid. An additional increase in the sulfuric acid concentration resulted in the thermal decomposition of xylose. In contrast, the

yield of arabinose was constant (0.2%) at a sulfuric acid concentration ranging between 1-4%. Parajó *et al*.[16] have reported that the arabinose units from the hemicellulose of *Eucalyptus* wood were susceptible to hydrolysis with dilute sulfuric acid and that most arabinose (69.6-84.3% of the potential amount) was generated in the first hour of reaction. Small amounts of glucose and mannose were also generated by hydrolysis with dilute sulfuric acid.

The glucose in the hydrolyzate would be, however, preferentially used as a carbon source by yeast in the early stage of cultivation.

In this study, the generation of degradation products from solubilized sugars and lignin were evaluated by absorbance of the hydrolyzates at 280 nm (A_{280}).[10] These compounds inhibit the microbial conversion of xylose. The A_{280} value increased proportionally with sulfuric acid concentration (Table 1).

To evaluate the effect of the residence time on the yield of each neutral sugar, the wood meal was hydrolyzed with 3% sulfuric acid, at 121 °C, for a period of time between 30-180 min. The yield of xylose increased with increasing the residence time up to 60 min (Table 2). A prolonged residence time (90 min and more) resulted in a decrease in the xylose yield. On the other hand, the yield of glucose increased steadily with increasing the residence time. These results indicate that, when the reaction temperature was fixed at 121 °C, 3% sulfuric acid and a 60 min residence time were considered as optimum conditions for the production of xylose from *T. japonica* wood. As the objective of this study was to prepare a fermentable substrate with a relatively high xylose concentration, the wood meal was hydrolyzed with 3% sulfuric acid at a liquid-to-solid ratio from 3 to 10 (g g^{-1}). As shown in Table 3, xylose concentration in the hydrolyzate (g L^{-1}) increased with decreasing the liquid-to-solid ratio.

The values of xylose concentration in the hydrolyzates compare[3] favorably with those of *Eucalyptus* wood [17 g L^{-1}, liquid-to-wood ratio = 8 (g g^{-1})]. Although the highest xylose concentration (57.0 g L^{-1}) was obtained with a liquid-to-solid ratio of 3, the A_{280} value of the hydrolyzate (248.0) was too high to perform the microbial conversion of xylose. Tada *et al*.[10] have reported that successful xylitol production from corn cob hydrolyzates using *Candida* yeast required an A_{280} value below 20 (corresponding to 0.13 g L^{-1} as furfural).

To minimize the inhibition of microbial metabolism, the hydrolyzate was treated with a commercially available activated char at 30 °C, for 60 min (Table 4). The dose of activated char increased with decreasing the A_{280} value, whereas the xylose concentration remained almost unchanged (55.3-54.2 g L^{-1}). When the hydrolyzate (15 mL) was treated with 0.6 g activated char, the A_{280} value was reduced from 248.0 (corresponding to 1.59 g L^{-1} as furfural) to 9.5 (corresponding to 0.06 g L^{-1} as furfural). On the other hand, 95% of the xylose originally present in the hydrolyzate could be recovered after treatment with activated char. The results indicate that the inhibitors are selectively removed from the hydrolyzate by sorption onto the activated char. When the hydrolyzate (15 mL) was treated with activated char (0.6 g) for a contact time between 30-90 min (Table 5), no significant difference was observed in the

recovery yield of each solubilized sugar. The inhibitors could be, therefore, removed from the hydrolyzate within 30 min.

Table 1

Effect of sulfuric acid concentration on chemical composition of hydrolyzates[1]

Sulfuric acid concentration (%)	A_{280}[2]	Yield (%, dry material basis)			
		Xylose	Arabinose	Glucose	Mannose
4.0	9.0	16.7	0.2	1.6	1.0
3.0	6.0	17.2	0.2	1.3	0.9
2.0	4.5	16.5	0.2	1.0	0.7
1.0	2.5	14.1	0.2	0.5	0.5
0	0.3	nd	nd	nd	nd

[1] The pre-extracted wood of *T. japonica* (0.3 g) was hydrolyzed with 87 g of dilute sulfuric acid at 121 °C for 60 min; [2] Absorbance of the hydrolyzate at 280 nm ; [3] Not detected

Table 2

Effect of residence time on chemical composition of hydrolyzates[1]

Residence time (min)	A_{280}[2]	Yield (%, dry material basis)			
		Xylose	Arabinose	Glucose	Mannose
30	3.9	15.3	0.2	1.1	0.5
60	6.0	17.2	0.2	1.3	0.9
90	8.0	16.5	0.1	1.7	0.7
120	11.4	16.2	0.2	2.1	0.7
180	15.1	15.6	0.1	2.6	0.9

[1] The pre-extracted wood meal of *T. japonica* (0.3 g) was hydrolyzed with 87 g of 3% sulfuric acid at 121 °C; [2] Absorbance of the hydrolyzate at 280 nm

Table 3

Effect of liquid-to-solid ratio on chemical composition of hydrolyzates[1]

Liquid-to-solid ratio (g g^{-1})	A_{280}[2]	Concentration (g L^{-1})			
		Xylose	Arabinose	Glucose	Mannose
290	6.2	0.6	-[3]	-	-
10	122.7	15.8	0.1	1.6	0.6
5	195.6	31.9	0.2	2.0	1.1
3	248.0	57.0	0.5	5.7	2.0

[1] The pre-extracted wood meal of *T. japonica* was hydrolyzed with 3% sulfuric acid at 121 °C for 60 min; [2] Absorbance of the hydrolyzate at 280 nm; [3] Less than 0.1 g L^{-1}

Table 4

Effect of activated char on chemical composition of hydrolyzate[1]

Dose of activated char (g L^{-1})	A_{280}[2]	Yield (g L^{-1})			
		Xylose	Arabinose	Glucose	Mannose
0	248.0	57.0	0.5	5.7	2.0
20	37.4	55.3	0.3	4.7	2.1
30	16.6	54.8	0.3	4.9	2.1
40	9.5	54.2	0.4	5.1	2.0

[1] The pre-extracted wood meal of *T. japonica* (10 g) was hydrolyzed with 30 g of 3% sulfuric acid at 121 °C for 60 min. The resulting hydrolyzate was treated with activated char at 30 °C for 60 min; [2] Absorbance of the hydrolyzate at 280 nm

Table 5

Effect of contact period of time with activated char on chemical composition of hydrolyzate[1]

Contact period of time (min)	A_{280}[2]	Yield (g L^{-1})			
		Xylose	Arabinose	Glucose	Mannose
0	248.0	57.0	0.5	5.7	2.0
30	9.4	55.0	0.4	5.4	2.0
60	9.5	54.2	0.4	5.1	2.0
90	9.6	55.6	0.3	5.3	2.1

[1] The pre-extracted wood meal of *T. japonica* (10 g) was hydrolyzed with 30 g of 3% sulfuric acid at 121 °C for 60 min. The resulting hydrolyzate (15 mL) was treated with activated char (0.6 g) at 30 °C; [2] Absorbance of the hydrolyzate at 280 nm

Acetic acid, also generated during wood hydrolysis, is a potential inhibitor for the microbial conversion of xylose. However, in this study, no attempt was made at determining the acetic acid present in the hydrolyzates. The hemicellulose hydrolyzates are generally concentrated *in vacuum*, then neutralized with lime, followed by subsequent fermentation. During the concentration stage, the acetic acid can be partially removed from the substrate.[17]

In conclusion, a fermentable substrate with a relatively high xylose concentration can be prepared from *T. japonica* wood by hydrolysis with 3% sulfuric acid, under mild operation conditions. The inhibitors, such as the dehydration products of solubilized sugars and the phenolics originated from lignin, are successfully removed from the hydrolyzates by treatment with activated char. The hemicellulose hydrolyzate from *T. japonica* wood is a potential source of the substrate that can be converted into xylitol by yeasts.

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