DETOXIFICATION OF JAPANESE WHITE BIRCH WOOD HEMICELLULOSE HYDROLYSATE WITH A CARBONACEOUS SORBENT PREPARED FROM BIRCH WOOD HYDROLYSIS RESIDUE

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During hydrothermal treatment of lignocelluloses, a wide range of compounds that inhibit microbial metabolism are formed. To eliminate inhibition, an acid hydrolysate of Japanese white birch (*Betula platyphylla* var. *japonica*) wood was detoxified with different carbonaceous sorbents, a dehydration product prepared from the birch wood hydrolysis residue, steam- and $ZnCl_2$-activated charcoals. Furfural and low molecular weight phenols in the hydrolysate were successfully removed by these sorbents, whereas the concentrations of neutral sugars and acetic acid stayed almost constant. Sorption capacities of the steam-activated charcoal were superior to those of the other sorbents. However, when the dose of the dehydration product prepared from the birch wood hydrolysis residue was increased up to 125 g L^{-1}, the concentrations of furfural and phenols in the hydrolysate decreased by their harmless levels to the yeast, *Candida magnoliae*.

Keywords: *Betula platyphylla* var. *japonica*, acid hydrolysis residue, carbonaceous sorbents, removal of fermentation inhibitors

INTRODUCTION

Hemicelluloses found in hardwoods and agricultural wastes are a renewable source of xylose, which can serve as substrates for the production of biofuel, food additives and chemical feedstuffs. Hydrolysis with dilute mineral acids under relatively mild conditions is the most conventional method employed for preparing xylose from these raw materials. The resulting sugars can be converted into ethanol or xylitol by fermentation. However, acid hydrolysates prepared from lignocelluloses usually contain not only fermentable sugars, but also some undesirable byproducts, such as acetic acid, furan derivatives and low molecular weight phenols originated from lignin and extractives. They act as an inhibitor of the microbial metabolism.[1] Direct use of the neutralised hydrolysates reduces the efficiency of the growth of microorganisms and product formation.[2] Detoxification of the hydrolysates before fermentation is necessary for successful bioconversion of the solubilised sugars. Various detoxification methods including biological, physical and chemical ones have been proposed to transform inhibitors to inactive compounds or to reduce their concentration.[3,4] Among these, charcoal sorption is considered to be a suitable method for removing furan derivatives and low molecular weight phenols.[5,6] It has been reported that charcoal treatment improved the conversion of sugars in the wood hydrolysates into xylitol[7] or into ethanol[6,8] by yeasts. However, 20 g L^{-1} of steam-activated charcoal was necessary for detoxifying bamboo hydrolysate containing 19 g $L^{-1}$of xylose.[9] Xylose in the detoxified hydrolysate was converted into xylitol with a yield of about 60% by the yeast *Candida magnoliae*. However, the circulating price of commercially available steam-activated charcoal corresponds to more over half of the product price. Activated charcoals are generally too expensive to employ as a sorbent for

detoxifying lignocellulose hydrolysates. On the other hand, mild hydrolysis of lignocelluloses produces waste fibre in large quantities. In this study, we prepared a hemicellulose hydrolysate containing 42.9 g L^{-1} of xylose from Japanese white birch (*Betula platyphylla* var. *japonica*) wood. An alternative carbonaceous sorbent was also prepared from the birch wood hydrolysis residue by dehydration with concentrated sulphuric acid, in order to detoxify the birch wood hemicellulose hydrolysate. Its sorption capacities towards the inhibitors were examined. The use of the hydrolysis residue fits into the biorefinery concept.

EXPERIMENTAL

Acid hydrolysis

A fraction of the ground wood of Japanese white birch (P42-R80 mesh, P355-R 180 μm) was hydrolysed with 3% sulphuric acid with a solid-to-liquor ratio of 0.25 (g g^{-1}) at 120 °C for 1 h using an autoclave (LSX-300, TOMY SEIKO CO., LTD., Tokyo, Japan). The reaction mixture was filtered to separate the hydrolysate and solid residue. The yield of hydrolysis solid residue was 66.4 ± 0.2% (mean ± SD, n = 5, dry material basis). Neutral sugars in the hydrolysate were determined by HPLC using an Aminex HPX-87P column (300 × 7.8 mm, Bio-Rad) eluted with water at a flow rate of 0.5 mL min^{-1} and 70 °C.[10]

Preparation of sorbent

The hydrolysis solid residue was neutralised with diluted ammonia solution, washed thoroughly with hot water and dried in an electric oven at 105 °C. The residue was treated with concentrated sulphuric acid according to the method of Namasivayam *et al*.[11] The sorbent was prepared by mixing 1 part of the hydrolysis solid residue, 1.8 parts of concentrated sulphuric acid and 0.1 part of ammonium persulfate and keeping the mixture in an electric oven at 80 °C for 12 h. The reaction mixture was soaked in water, and the supernatant was neutralised with diluted ammonia solution to remove any residual acid. The solid residue was washed thoroughly with hot water, dried in an electric oven at 105 °C, and pulverised to pass a 100 mesh screen. The yield of the sorbent was 68.9 ± 0.6% (mean ± SD, n = 5, based on dry hydrolysis solid residue).

Detoxification experiments

Sorption experiments were conducted by agitating 20 mL of the hydrolysate with desired amounts of sorbents in a reciprocal shaker (160 strokes min^{-1}) at 30 °C for 1 h. The suspensions were filtered off, and furfural and acetic acid in the filtrates were determined by HPLC using a Shodex SH column (300 × 8 mm, Showa Denko) eluted with 0.01 M sulphuric acid at a flow rate of 0.7 mL min^{-1} and at 50 °C.[12] For determiing the lignin degradation products, the pH of the samples was adjusted to 12.0 before dilution at 1:1000 with distilled water[13] and was analysed by a Hitachi U-2810 spectrophotometer.

RESULTS AND DISCUSSION

When Japanese white birch wood was hydrolysed with 3% sulphuric acid with a solid-to-liquor ratio of 0.25 (g g^{-1}) at 120 ºC for 1 h, the resulting hydrolysate contained 42.9 g L^{-1} xylose. However, the hydrolysate also contained significant amounts of undesirable compounds, such as acetic acid, furfural and low molecular weight phenols. Watson *et al*.[14] reported that the maximum specific growth rate of *Pachysolen tannophilus* was reduced by 63% at a furfural concentration of 0.35 g L^{-1}. Delgenes *et al*.[15] found that the cell growth of *C. shehatae* was reduced by 38% at furfural concentration of 1 g L^{-1}. Detoxification before fermentation is, therefore, necessary for the successful bioconversion of the birch wood hydrolysate.

Tables 1 and 2 show the chemical composition of the birch wood hydrolysate treated with three different carbonaceous sorbents. The sorption properties of carbonaceous sorbents are due to factors such as surface area, porous structure and degree of surface reactivity.[16] The chemical composition and the BET surface area of the sorbents are listed in Table 3.

When the hydrolysate was treated with these sorbents, the amounts of furfural and low molecular weight phenols decreased with increasing the amount of sorbents added, whereas the concentrations of neutral sugars and acetic acid stayed almost constant (Tables 1 and 2). Furfural and low molecular weight phenols are selectively removed from the hydrolysate by sorption onto the sorbents. Among these inhibitors, low molecular weight phenols, such as Hibbert's ketones, vanillin and syringaldehyde, are more toxic than furan derivatives.[5,7,15,17] In this study, we focused our interest on the removal of low molecular weight phenols monitored by the absorbance at 280 nm (A_{280}). Sorption capacities of the steam-activated charcoal were superior to those of the other sorbents. Although $ZnCl_2$-activated charcoal has the largest surface volume (Table 3), its sorption capacities to the inhibitors was inferior to those of the steam-activated charcoal. $ZnCl_2$-activated charcoal is probably quite suitable for the relatively

large molecular size of the sorbate due to its well-developed mesoporous structure.[16] As mentioned before, commercially available activated charcoals are too expensive to employ for detoxifying the hydrolysate..

Table 1
Effect of treatment with various sorbents on the concentrations of neutral sugars in the hydrolysate of Japanese white birch wood[1]

Sorbent	Dose ($g\ L^{-1}$)	Concentration ($g\ L^{-1}$)				
		Xylose	Arabinose	Glucose	Mannose	Galactose
Dehydration product from Japanese white birch wood[2]	0	42.9	1.8	3.1	1.7	4.8
	100	39.1	2.6	2.4	1.8	4.6
	125	38.7	2.7	2.4	1.8	4.5
	150	37.6	2.5	2.3	1.8	4.4
Steam-activated charcoal[3]	10	41.5	1.7	2.8	1.6	4.8
	15	41.3	2.7	2.7	1.9	4.8
	20	41.3	1.7	2.7	1.6	5.0
	30	42.3	1.6	2.8	1.6	3.8
$ZnCl_2$-activated charcoal[4]	10	38.9	2.5	2.4	1.8	4.6
	20	38.0	2.2	2.4	1.7	4.5
	30	37.5	1.7	2.4	1.6	4.9

[1] Sorption experiments were conducted by agitating 20 mL of the hydrolysate with desired amounts of sorbents in a reciprocal shaker (160 strokes min^{-1}) at 30°C for 1 h; [2]The ground wood of Japanese white birch (*Betula platyphylla* var. *japonica*) was hydrolysed with 3% sulphuric acid with a solid-to-liquor ratio of 0.25 ($g\ g^{-1}$) at 120°C for 60 min. The resulting hydrolysis residue was treated with concentrated sulphuric acid according to the method of Namasivayam *et al.*[11] ; [3] Steam-activated charcoal (Shirasagi M, Japan EnviroChemicals, Ltd., Osaka, Japan)
[4] $ZnCl_2$-activated charcoal (Wako Pure Chemicals Industries, Ltd., Osaka, Japan)

Table 2
Effect of treatment with various sorbents on the concentrations of inhibitors in the hydrolysate[1]

Sorbent	Dose ($g\ L^{-1}$)	A_{280}[5]	Concentration ($g\ L^{-1}$)	
			Acetic acid	Furfural
Dehydration product from Japanese white birch wood[2]	0	0.19	12.6	1.2
	100	0.05	10.5	0.1
	125	0.03	10.4	0.1
	150	0.03	10.4	0.1
Steam-activated charcoal[3]	10	0.06	11.6	0.2
	15	0.03	11.5	0.2
	20	0.02	11.8	0.1
	30	0.01	11.7	-[6]
$ZnCl_2$-activated charcoal[4]	10	0.06	11.6	0.5
	20	0.05	11.5	0.3
	30	0.03	11.4	0.2

[1-4] See Table 1; [5] Absorbance at 280 nm; [6] Detection limit 0.01 $g\ L^{-1}$

Table 3
Chemical composition and BET surface area of various sorbents

Sorbent	C (%)	H (%)	N (%)	Ash (%)	Surface area ($m^2\ g^{-1}$)
Dehydration product from Japanese white birch wood[1]	44.3	3.7	0.9	0.1	5
Steam-activated charcoal[2]	93.3	0.5	0.3	2.5	959
$ZnCl_2$-activated charcoal[3]	85.6	2.2	0.4	0.6	988

[1-3] See Table 1

Although the pore structure of the dehydration product prepared from the acid hydrolysis residue of the birch wood was not sufficiently developed, when the dose increased up to 125 g L^{-1}, the concentrations of low molecular weight phenols and furfural reduced significantly. Undesirable effects caused by these inhibitors on the microbial metabolism can be eliminated. Acetic acid, which was released from the birch wood xylan, could not be removed after the treatment with the carbonaceous sorbents used (Table 2). The sensitivity to acetic acid is, however, yeast species dependent. Pessoa *et al*.[18] reported that 3.7 g L^{-1} of acetic acid in sugar cane hemicellulose hydrolysate was completely consumed by *C. tropicalis*. *C. guilliermondii* and *P. stipitis* were also able to assimilate significant amounts of acetic acid in hemicellulose hydrolysates of eucalyptus wood[19,20] and sugar cane bagasse.[21] Our recent study showed that *C. magnoliae* consumed large parts of acetic acid present in the fermentation media (7.44-7.81 g L^{-1}) as a carbon source under microaerobic conditions.[22]

CONCLUSION

A hemicellulose hydrolysate with a relatively high xylose concentration (42.9 g L^{-1}) was prepared from Japanese white birch wood by hydrolysis with 3% sulfuric acid under mild hydrolysis conditions. Furfural and low molecular weight phenols in the hydrolysate were successfully removed by carbonaceous sorbents, whereas the concentrations of neutral sugars and acetic acid stayed almost constant. Sorption capacities of the dehydration product prepared from the birch wood hydrolysis residue were inferior to those of the steam-activated charcoal. However, when the dose of the dehydration product was increased up to 125 g L^{-1}, the concentrations of furfural and phenols in the hydrolysate decreased to their harmless levels to the yeast, *C. magnoliae*. The dehydration product prepared from the birch wood hydrolysis residue can be used as an inexpensive sorbent for detoxifying the birch wood hemicellulose hydrolysate.

REFERENCES

[1] E. Palmqvist and B. Hahn-Hägerdal, *Bioresour. Technol*., **74**, 25 (2000).
[2] F. R. Frazer and T. A. McCaskey, *Biomass*, **18**, 31 (1989).
[3] J. C. Parajó, H. Domínguez and J. M. Domínguez, *Bioresour. Technol*., **66**, 25 (1998).
[4] S. I. Mussatto and I. C. Roberto, *Bioresour. Technol*., **93**, 1 (2004).
[5] J. C. Parajó, H. Domínguez and J. M. Domínguez, *Bioprocess Eng*., **16**, 39 (1995).
[6] H. Miyafuji, H. Danner, M. Neureiter, C. Thomasser, J. Bvochora *et al*., *Enz. Microb. Technol*., **32**, 396 (2003).
[7] J. C. Parajó, H. Domínguez and J. M. Domínguez, *Bioresour. Technol*., **57**, 179 (1996).
[8] G. L. Woo, J. S. Lee, C. S. Shin, S. C. Park, H. M. Chang *et al*., *Appl. Biochem. Biotechnol*., **78**, 547 (1999).
[9] M. Miura, I. Watanabe, Y. Shimotori, M. Aoyama, Y. Kojima *et al*., *Wood Sci. Technol*., **47**, 515 (2013).
[10] M. Aoyama, K. Seki and N. Saito, *Holzforschung*, **49**, 193 (1995).
[11] C. Namasivayam and K. Kadirvelu, *Chemosphere*, **34**, 377 (1997).
[12] M. Miura, T. Seo, Y. Shimotori, M. Aoyama, H. Nakatani *et al*., *Holzforschung*, **67**, 881 (2013).
[13] S. I. Mussatto, J. C. Santos and I. C. Roberto, *J. Chem. Technol. Biotechnol*., **79**, 590 (2004).
[14] N. E. Watson, B. A. Prior, P. M. Lategan and M. Lussi, *Enzyme Microb. Technol*., **6**, 451 (1984).
[15] J. P. Delgenes, R. Moletta and J. M. Navarro, *Enzyme Microb. Technol*., **19**, 220 (1996).
[16] D. Mohan and C. U. Pittman, Jr., *J. Hazard. Mater*., **B137**, 762 (2006).
[17] T. A. Clark and K. L. Mackie, *J. Chem. Technol. Biotechnol*., **34B**, 101 (1984).
[18] A. Pessoa, I. M. Mancilha and S. Sato, *J. Biotechnol*., **51**, 83 (1996).
[19] M. D. Ferrari, E. Neirotti, C. Albornoz and E. Saucedo, *Biotechnol. Bioeng*., **40**, 753 (1992).
[20] M. G. A. Felipe, L. A. Alves, S. S. Silva, I. C. Roberto, I. M. Mancilha *et al*., *Bioresour. Technol*., **56**, 281 (1996).
[21] M. G. A. Felipe, M. Vitolo, I. M. Mancilha and S. S. Silva, *J. Ind. Microbiol. Biotechnol*., **18**, 251 (1997).
[22] M. Miura, N. Matsumoto, K. Seino, Y. Shimotori, H. Nakatani *et al*., *Cellulose Chem. Technol*., **49**, 807 (2015).