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Fermentability of Water-Soluble Portion to Ethanol Obtained by Supercritical Water Treatment of Lignocellulosics

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Abstract

The water-soluble (WS) portion obtained by supercritical water treatment of lignocellulosics was studied for its fermentability to ethanol. A fermentation test of the WS portion showed it was not fermented to ethanol. Therefore, a wood charcoal treatment was applied to the WS portion to remove furan and phenolic compounds, which are thought to be the inhibitors to sugar fermentability. It was found that treatment with wood charcoal can be effective at removing these inhibitors and improving the fermentability of the WS portion without reducing the levels of fermentable sugars.

Index Entries: Lignocellulosics; supercritical water; inhibitor; wood charcoal; ethanol fermentation.

Introduction

Energy and environmental issues such as the exhaustion of fossil resources and global warming are of major concern. Increased attention, therefore, has been focused on ethanol from biomass as an alternative to fossil fuels owing to its environmental friendliness. Various studies have been performed such as hydrolysis of lignocellulosics by acid catalysis *(1, 2)* and enzymatic saccharification *(3)* to obtain the fermentable sugars for ethanol production.

In our laboratory, on the other hand, supercritical fluid technology has been applied for the conversion of lignocellulosics to fuels and chemicals *(4–12)*. Supercritical water treatment of lignocellulosics is thought to be a promising method because of it very short reaction time, within several seconds, without any catalysts. It is reported that the fermentable sugars such as glucose and mannose can be recovered as cellulose and hemicellulose-derived products in the water-soluble (WS) portion by the supercritical water treatment of woody biomass *(5,11).* Furthermore, the lignin-derived

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products are also reported to be recovered simultaneously as the methanol-soluble portion *(12)*. For these separated portions, however, the fermentability of the obtained WS portion has not been evaluated yet. Possibly, the WS portion would be contaminated with lignin-derived products, such as furfural and 5-hydroxymethyl furfural, which are known to be inhibitors for ethanol fermentation.

In this study, therefore, the fermentability of the WS portion was studied by fermentation test with *Saccharomyces cerevisiae*, and evaluated the potential of the supercritical water treatment for ethanol production.

Materials and Methods

Preparation of the Water-Soluble Portion

Japanese cedar (*Cryptomeria japonica* D. Don) was treated using batch-type supercritical fluid biomass conversion system contained a 5 mL volume reaction vessel made of Inconel-625 (*6,7,11,12)*. Water was fed with 150 mg of wood flour (below 80 μm) to this reaction vessel, and then it was quickly heated by immersing it for 8 s in the molten tin bath preheated at 400°C. The reaction vessel was then immersed in a water bath to stop the reaction. After the treatments, the WS portion was retrieved by filtration. The pH of the obtained WS portion was 3.8.

Preparation of the Wood Charcoals

Western red cedar (*Thuja plicata* D. Don) flours were treated under nitrogen at a heating rate of 4°C/min and maintained at the designated temperature for 1 h in a range between 400 and 900°C to prepare various types of wood charcoals.

Wood Charcoal Treatment for the WS Portion

The wood charcoal was added to the WS portion at a loading of 7 wt% the WS portion. The WS portion with the wood charcoal was then stirred for 10 min at room temperature. Subsequently, the wood charcoal was separated from the WS portion by filtration.

Fermentation

Prior to the fermentation, the pH of the WS portions untreated and treated with the wood charcoals was adjusted to be 5.5 in pH with solid $Ca(OH)_2$. The WS portion was then centrifuged to remove the precipitates and the obtained supernatant was used for the fermentation.

For inoculum preparation, a yeast, *Saccharomyces cerevisiae* (IFO 233), was grown in a medium containing 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone and 10 g/L glucose for 24 h at 28°C with shaking. The nutrient broth was prepared at concentrations of 120 g/L yeast extract, 120 g/L malt extract, and 200 g/L peptone.

The fermentation test was then carried out in a 7 mL glass bottle containing 3.7 mL of the WS portion after pH adjustment at 5.5, 0.1 mL of the nutrient broth, and 0.2 mL of the inoculum, equipped with the cannula for exhaust of carbon dioxide with 0.2 μm filter. The fermentation medium was then incubated at 28°C with magnetic stirring at 180 rpm.

Analytical Methods

To evaluate the adsorption capacity of the wood charcoals prepared, about 50 mg of wood charcoals, dried and degassed, were subjected to a nitrogen-adsorption measurement. The drying and degassing were carried out with a heating device (Shimadzu, Flow Prep 060) in nitrogen at 120°C for 3 h. During the subsequent adsorption of nitrogen, isotherms at −196°C were measured to determine the Brunauer–Emmett–Teller (BET) surface area by the *t*-plot method using micromeritics (Shimadzu, Gemini 2375).

For the quantification of furan and phenolic compounds in the WS portions untreated and treated with the wood charcoals, the WS portion was analyzed by high-performance liquid chromatography (HPLC) (Shimadzu, LC-10A) equipped with a STR ODS-II column (Shinwa Chem. Ind. Co.) and an ultraviolet detector (Shimadzu, SPD-10A) set at 280 nm. CH_3OH/H_2O (20/80–100/0, 0–60 min) was used as mobile phase at a flow rate of 1.0 mL/min. The column oven temperature was set at 40°C.

For determining the concentrations of sugars in the WS portions untreated and treated with the wood charcoals, the analysis of the WS portion was done by HPLC equipped with an Aminex HPX-87H column (Bio-Rad Lab, Inc.) and a refractive index detector (Shimadzu, RID-10A); 0.005 mol/L H_2SO_4 was used as mobile phase at a flow rate of 0.6 mL/min. The column oven temperature was set at 45°C. The sugars—fructose, mannose, galactose, and xylose—could not be separated on this column. Therefore, these were analyzed as a single peak.

For determining produced ethanol and consumed sugars during the fermentation, the fermentation medium was filtered through a 0.45 μm filter to separate the yeast. The obtained filtrate was then analyzed by HPLC with the same conditions as in the analysis method for sugars mentioned above.

Results and Discussion

Fermentability of the WS Portion

Figure 1 shows the concentration changes in the sugars (the total of glucose, fructose, mannose, galactose, and xylose) and ethanol during the fermentation of the WS portion. The sugars and ethanol were found to be almost constant in their concentrations, indicating that no fermentation of sugars was occurring.

In a previous report on the lignin-derived products in the methanol-soluble portion obtained by the supercritical water treatment of Japanese

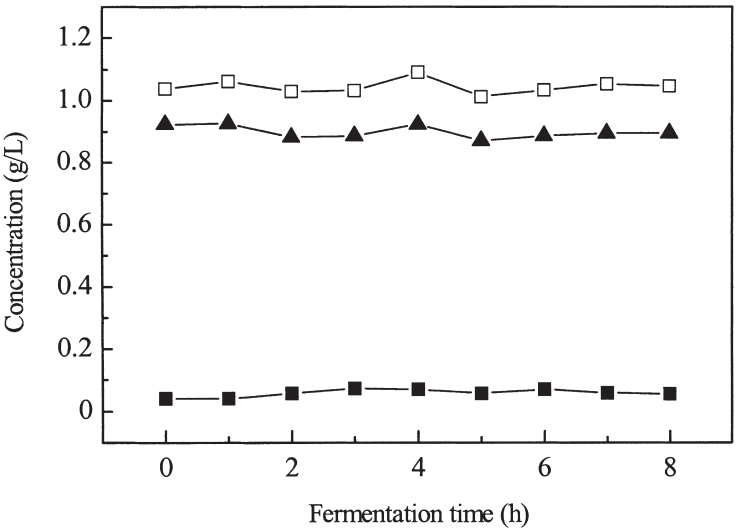

Fig. 1. Concentration changes of sugars and ethanol during the fermentation of the WS portion. —□— glucose; —▲— fructose + mannose + galactose + xylose; —■— ethanol.

cedar, which is one of the portions after the fractionation of the treated sample, various compounds were identified such as guaiacol, methylguaiacol, ethylguaiacol, vinylguaiacol, propylguaiacol, eugenol, propylguaiacol, vanillin, *cis*-isoeugenol, homovanillin, *trans*-isoeugenol, acetoguaiacone, propioguaiacone, guaiacylacetone, 2-methoxy-4-(1-hydroxypropyl)phenol, homovanillic acid, 2-methoxy-4-(prop-1-en-3-one)phenol, and *trans*-coniferylaldehyde *(12)*. These compounds are mainly recovered in the methanol-soluble portion; however, they can be contaminated in the WS portion. Therefore, HPLC analysis on the WS portion was carried out to study these contaminants. The obtained result is shown in Fig. 2 as designated by "Untreated." Among the compounds mentioned above, vanillin, acetoguaiacone, guaiacol, and coniferylaldehyde could be identified, and quantified as in Table 1 although some peaks in the HPLC chromatograms were unidentified. In addition to these compounds, furan compounds such as furfural and 5-hydroxymethyl furfural were also found in the WS portion. It is known that various furan and phenolic compounds derived from sugars and lignin during the hydrolysis treatment, respectively, can inhibit the fermentation of sugars to ethanol *(13)*. Therefore, the poor fermentability of the WS portion observed in Fig. 1 would be due to the presence of these compounds.

Wood Charcoal Treatment for Removing the Furan and Phenolic Compounds

Various wood charcoals were prepared at different temperatures and their effect on the removal of inhibitors in the WS portion was studied.

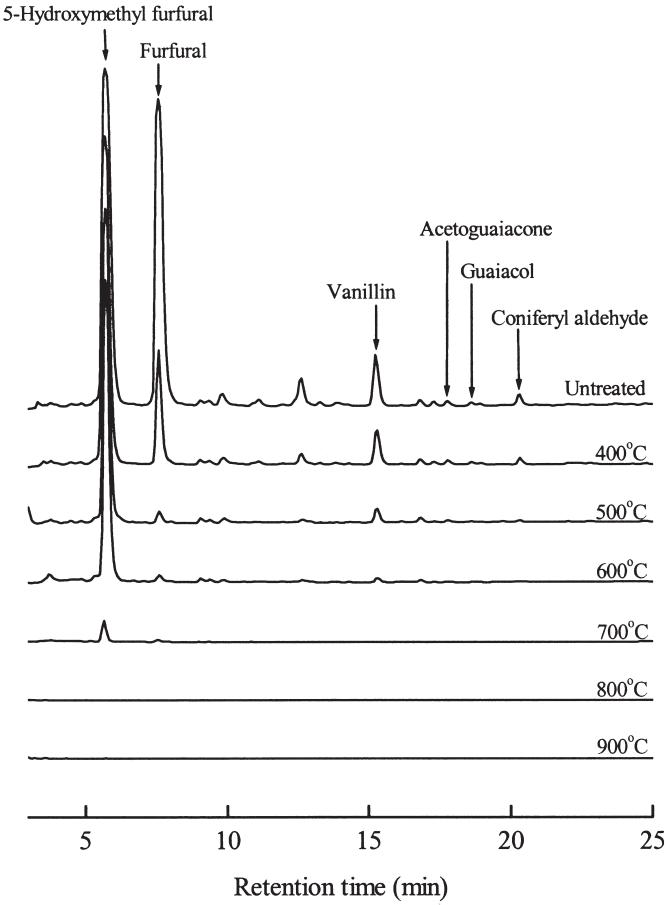

Fig. 2. Compariosons of the HPLC chromatograms for the WS portions before and after its treatment with the wood charcoals prepared at variours temperatures.

Table 1
Concentrations of Furan and Phenolic Compounds in the WS Portion Before and After its Treatment with the Wood Charcoals Prepared at Various Temperatures

Preparation temperature (°C)	BET surface area (m^2/g)	Concentrations in the WS portion (mg/L)					
		5-Hydroxymethyl furfural	Furfural	Vanillin	Aceto-guaiacone	Guaiacol	Coniferyl aldehyde
Untreated	—	378	240	818	3	33	94
400	148	334	71	241	3	21	42
500	361	303	5	18	2	22	31
600	450	276	3	10	N.D.	4	26
700	450	15	N.D.	3	N.D.	N.D.	N.D.
800	415	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
900	475	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D., not detectable.

Table 2
Concentration of Sugars in the WS Portion Before and After its Treatment with the Wood Charcoals Prepared at Various Temparatures

Preparaion temperature (°C)	Concentration in the WS portion (g/L)	
	Glucose	Other sugars*
Untreated	1.04	0.92
400	1.06	0.95
500	1.04	0.92
600	1.03	0.93
700	1.03	0.93
800	1.04	0.92
900	1.04	0.94

*The total of fructose, mannose, galactose, and xylose.

To evaluate the adsorption ability of the prepared wood charcoals, BET surface areas were measured (Table 1). It seems apparent that it is increased up to 500°C and remained rather constant above 600°C in preparation temperature. This result indicates that the wood charcoals prepared at higher temperature have higher adsorption capacity. Additionally, the degree in hydrophobicity is reported to be increased as the preparation temperature of the wood charcoal is raised *(14)*. Therefore, the wood charcoal prepared at higher temperatures can be expected to adsorb hydrophobic compounds. In fact, as in Table 1 and Fig. 2, furan such as furfural and 5-hydroxymethyl furfural and phenolic compounds such as guaiacol, vanillin, acetoguaiacone, and coniferylaldehyde were decreased with wood charcoals prepared at higher temperatures. Especially in the wood charcoals prepared above 700°C, these compounds were adsorbed completely and not detected any more. These results revealed that the wood charcoal treatments are effective in removing these compounds above from the WS portion.

Table 2 shows the concentrations of sugar in the WS portion before and after its treatment with the wood charcoals prepared at various temperatures. The untreated WS portion is on the data before its treatment. It is apparent from these data that for all the wood charcoal treatments, the concentrations of sugars remained same as in the untreated WS portion. From these results in Tables 1 and 2, the wood charcoals can selectively remove the furan and phenolic compounds without removing the fermentable sugars. This absorption behavior characteristic of the wood charcoals is preferable to achieve the high fermentability of sugars in the WS portion to ethanol.

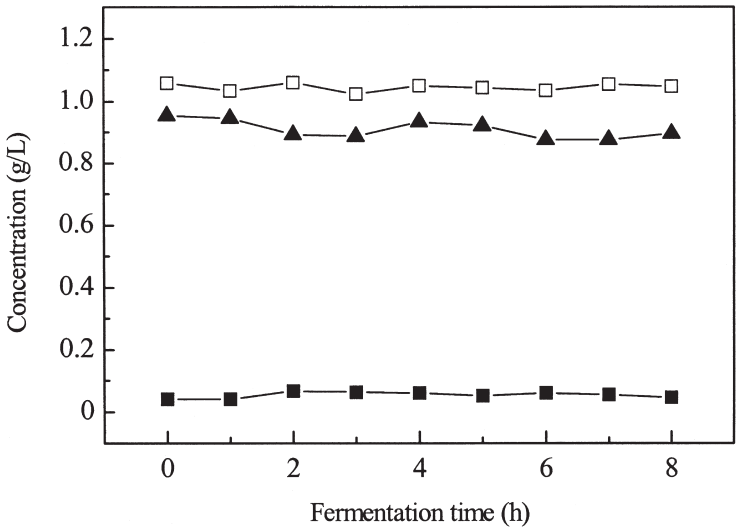

Fig. 3. Concentration changes of sugars and ethanol during the fermentation of the WS portion after the treatment of wood charcoal prepared at 400°C. —□— glucose; —▲— fructose + mannose + galactose + xylose; —■— ethanol.

Fermentability of the WS Portion after its Treatment with Various Wood Charcoals

To evaluate the fermentability of the WS portion after its treatment with wood charcoal, the fermentations of its portion were carried out to produce ethanol. Figure 3 shows the obtained concentration changes of sugars and ethanol during its fermentation after the wood charcoal treatment prepared at 400°C. It is evident that sugars and ethanol concentrations remained almost constant as in the untreated WS portion in Fig. 1. This indicates that the treatment with wood charcoal prepared at 400°C cannot be effective to enhance fermentability. A similar result was obtained in the wood charcoals prepared at 500°C. However, in the wood charcoal prepared at 600°C in Fig. 4, the sugar concentrations were found to be decreased somewhat. Instead, ethanol could be produced. Such a trend was even more enhanced as in Fig. 5 for the wood charcoal prepared above 700°C.

The glucose concentration became zero and maximum ethanol concentration was achieved after 6 h in fermentation time. However, the concentration of sugars except for glucose was found to decrease and become constant at around 0.2 g/L. This may be due to the presence of xylose in the WS portion, which cannot be fermented to ethanol by *S. cerevisiae*. Considering that the maximum ethanol concentration is around half as the consumed sugars concentration, all fermentable sugars in the WS portion were thought to be fermented to ethanol. This result indicates that the WS portion treated with the wood charcoal prepared at 700°C could be

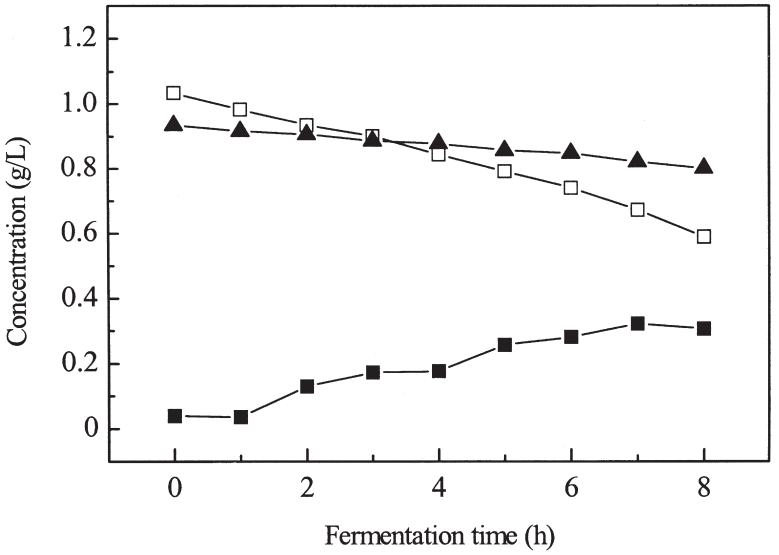

Fig. 4. Concentration changes of sugars and ethanol during the fermentation of the WS portion after the treatment of wood charcoal prepared at 600°C. —□— glucose; —▲— fructose + mannose + galactose + xylose; —■— ethanol.

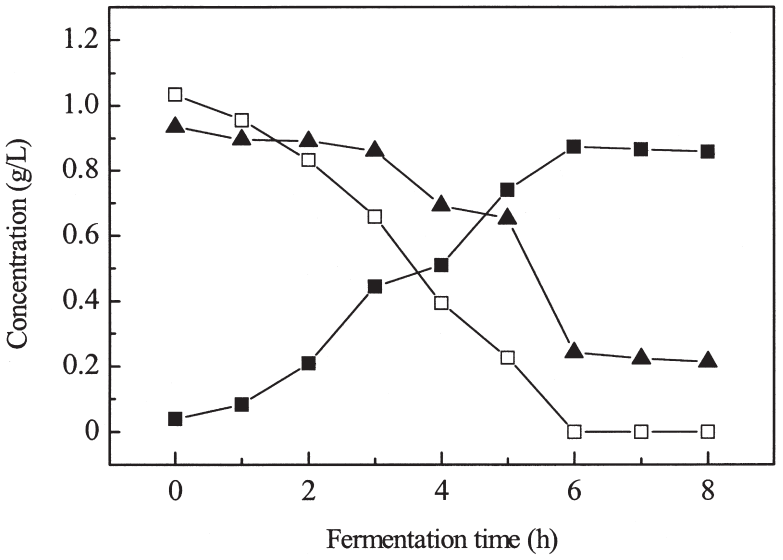

Fig. 5. Concentration changes of sugars and ethanol during the fermentation of the WS portion after the treatment of wood charcoal prepared at 700°C. —□— glucose; —▲— fructose + mannose + galactose + xylose; —■— ethanol.

fermented effectively. The similar enhanced fermentability could be achieved for the wood charcoals prepared at 800°C and 900°C.

Conclusion

The WS portion cannot be fermented due to various furan and phenolic compounds contained. To improve its fermentability, the wood charcoals prepared at various temperatures have been applied. The wood charcoals prepared at higher temperatures have the enhanced adsorption ability for the furan and phenolic compounds in the WS portion. Wood charcoal treatment for only 10 min can improve the fermentability of the WS portion without removing the fermentable sugars.

Acknowledgment

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