Degradation of Saccharides with Raw Wood (Larch, Bamboo and Cherry) Particles and Its Application to Separation of Arbutin and Glucose

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Note
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In this paper, we examined the availability of raw woods as glycolic materials.

It was found that some wood materials such as bamboo, larch and cherry could degrade glucose. Glucose is degraded to carbon dioxide via an unknown acidic compound. We then examined whether these wood materials could degrade various sugars. Larch, bamboo and cherry show high degradation rates for glucose, galactose, mannose and sucrose. Disaccharide, sucrose is hydrolyzed to glucose and fructose following glucose consumption. The degradation of saccharides by these raw wood materials may mainly be a result of microorganisms on the woods.

Bamboo particles applied to the separation of glycoside, arbutin, and glucose are found to selectively degrade glucose.

Key words:
Saccharides, degradation, wood particle, arbutin

Introduction

The process of turning felled timber into usable materials produces huge quantities of waste materials such as bark and wood chips, which stretch the limits of waste processing facilities and are a source of growing environmental problems when disposed of inappropriately. This material has been reused in a growing number of ways. These include the use of bamboo in applications that exploit its antioxidant, antibacterial and deodorizing properties, the use of woody waste material in the production of particle board and fiber board, and the use of wood ash as a water purifying agent. On the other hand, research is also being conducted on methods for utilizing the chemical functions of these materials. For example, studies are being conducted into the use of fresh woody material and pretreated woody material in the adsorption of metals[1–5] or the adsorption of metals with modified lignin prepared from woody waste material,[6] and into the use of woody materials with adsorbed metals as catalysts.[7]

Recently, inexpensive natural resources such as sugar cane and sugar beet have been adopted as sources of polysaccharides, and large amounts of sugars are discharged from places such as food and drink factories. To meet legal restrictions, these discharges are treated by methods such as dilution or highly expensive microbial treatment.

In this study, with the aim of establishing new ways of using woody material, we report on a new inexpensive glycolytic material made from the abundant and environmentally responsible resource of woody waste material. Also, as an example of application of our technique, we attempted to separate arbutin (a new inexpensive skin whitening agent) from its raw material glucose using bamboo sawdust.

Experimental

Degradation of saccharides

Aqueous solutions were prepared by dissolving saccharides shown in Tables 1 and 2 of G.R. grade in a phosphate buffer and sodium hydroxide solution. The experiments were performed using a 1.0 mol dm^{-3} phosphate buffer at pH 6. The effect of pH on the degradation of glucose and galactose in the presence of larch were examined. Both saccharides were completely degraded at pH 6. Raw

Table 1 – *Fraction of monosaccharide degraded by wood after 300 h*

Monosaccharide	$1-c/c_0$ [–]				
	larch	bamboo	cherry	sugarcane	wood chips
glucose	0.715	0.647	0.705	0.525	0.346
galactose	0.790	0.685	0.548	0.471	0.181
mannose	1.000	0.696	0.396	0.469	0.123
fructose	0.396	0.135	0.068	0.091	0.027
xylose	0.409	0.326	0.053	0.189	0.021

Initial saccharide concentration was 50 mmol dm^{-3} and pH was adjusted to 6 with a phosphate buffer.

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Table 2 – *Fraction of disaccharide degraded by wood after 300 h*

Di-saccharide	$1-c/c_0$ [−]				
	larch	bamboo	cherry	sugarcane	wood chips
sucrose	1.000	0.943	0.998	0.887	0.095
maltose	0.413	0.216	0.111	0.229	0.029
lactose	0.196	0.058	0.075	0.108	0.020

Initial saccharide concentration was 50 mmol dm^{-3} and pH was adjusted to 6 with a phosphate buffer.

wood particles used were epidermis of bamboo (*Phyllostachys pubescens*, Ban Co., Anan-shi, Tokushima), dried leaf of larch (*Larix leptolepis*, Anan-shi, Tokushima), cherry bark (*Prunus jamasakura,* Anan-shi, Tokushima) and stem of sugarcane (*Saccharum officinarum*, Okinawa). These wood samples were selected because they are considered to be traditionally physiologically active.[8] For comparison, we used chips of mixed wood from a timber mill. The raw wood samples were milled and sieved with 50–70 mesh (d = 200–300 μm). The concentration value of wood particles used in the experiment was γ = 0.01 kg dm^{-3} and the experiments were performed at 303 K for 300 h in a vial tube. After centrifugation, the composition of supernatants was measured by HPLC. After the contact, the apparent aspect of particles remained unchanged. HPLC analysis was performed with a Sugar Column SH1011 column (Showa Denko, Japan) using a c = 5 mmol dm^{-3} sulfuric acid solution as the mobile phase and an RI detector (Shimadzu, Japan, RID10A). The glucose concentration was also determined by UV/VIS spectrum (Shimadzu UV2500-PC) at λ = 550 nm using a colorimetric enzyme assay. The pH of the aqueous solution was determined using a Horiba F-12 pH meter. Contents of CO_2 in the headspace were analyzed by GC (Shimadzu Corp., GC14B equipped with a FID detector; WG100 column [d_i = 3.0 mm, length l = 2 m, GL Science], carrier gas He).

Separation of arbutin and glucose

Aqueous solutions were prepared by dissolving the glucose and/or arbutin shown in Fig. 1 in a phosphate buffer and sodium hydroxide solution. The experiments were performed using a c = 1.0 mol dm^{-3} phosphate buffer at pH 6. The concentration value of bamboo particles used in the experiment was γ = 0.01 kg dm^{-3} and the experiments were performed at 303 K for 170 h in a vial tube. The concentrations of glucose, hydroquinone and arbutin were determined by HPLC (Shimadzu Corp., LC10AD with a RID detector (RID-10A); Sugar SH1011 column; elutant, 0.5 mmol dm^{-3} H_2SO_4 solution).

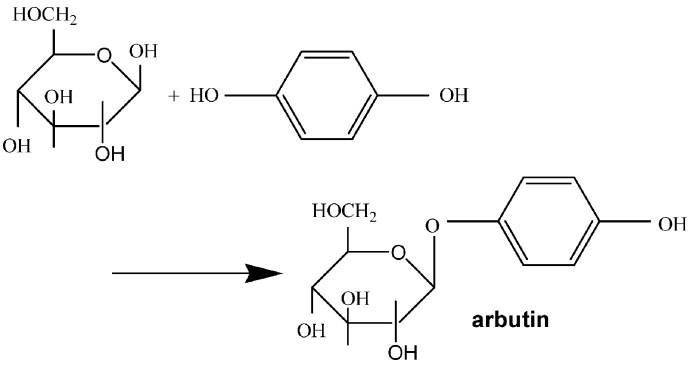


Fig. 1 – *Arbutin synthesis from glucose and hydroquinone*

Results and discussion

Effect of raw wood on degradation of saccharides

Fig. 2 shows the time courses of glucose concentration and peak area of unknown product in glucose degradation in the presence of larch and that of glucose concentration in the absence of larch. A new peak appeared in the HPLC chromatogram and the pH value decreased after the addition of wood particles, and this peak area increased with decreasing glucose concentration as shown in Fig. 2, suggesting that glucose was degraded by larch. We tried to indentify the unknown product by using LC-Mass and comparing retention time of the unknown product with that of the possible acidic candidates (pyruvic, lactic, succinic, and formic acids). Unfortunately, we could not identify the compound giving the new peak.

Fig. 3 shows the concentration profiles of glucose degradation by bamboo. From Figs. 2 and 3, although the difference in rates of glucose degradation

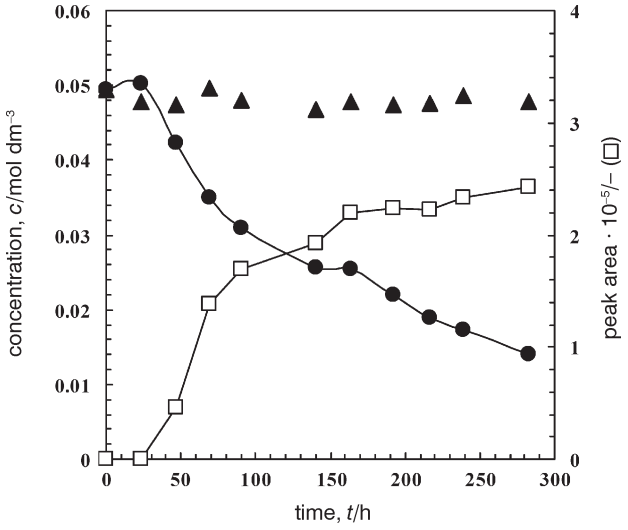
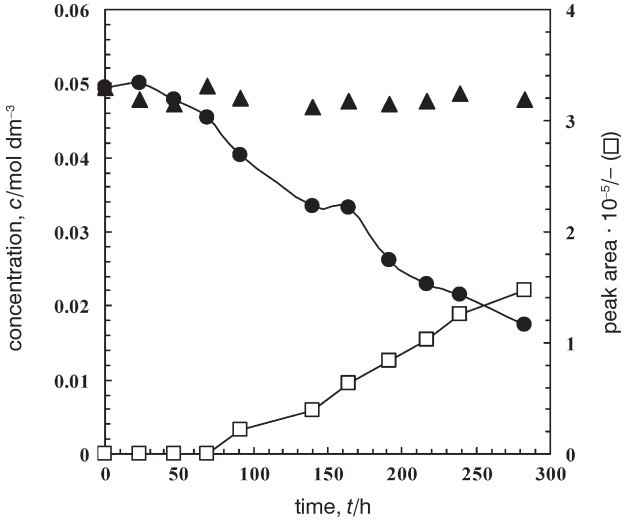


Fig. 2 – *Time courses of glucose concentration (●) and peak area of unknown product (□) in glucose degradation in presence of larch and that of glucose concentration in absence of larch (▲)*



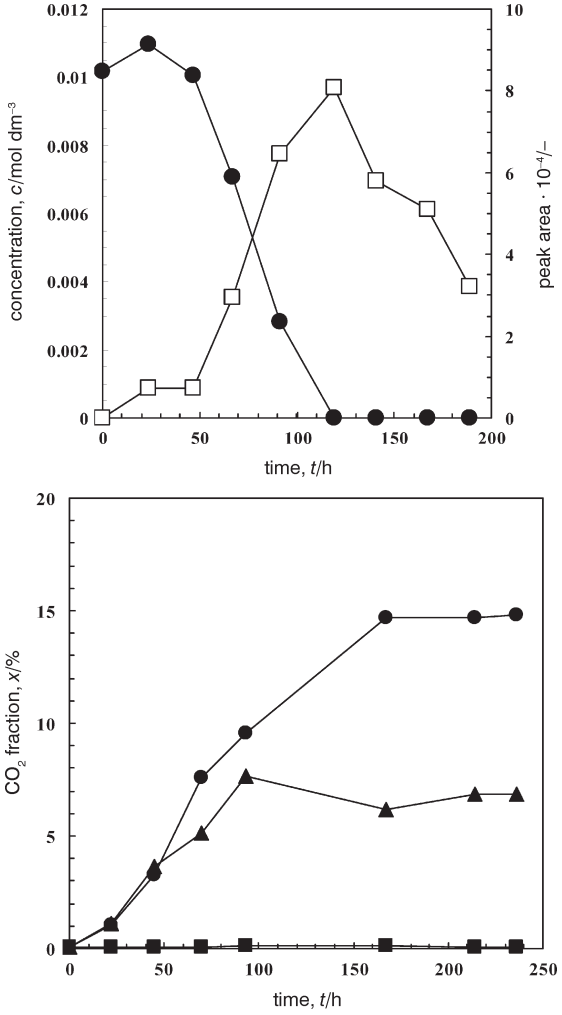
F i g . 3 – *Time courses of glucose concentration (●) and peak area of unknown product (□) in glucose degradation in presence of bamboo and that of glucose concentration in absence of bamboo (▲) (initial saccharide concentration was c = 5 mmol dm⁻³)*

for both cases is relatively small, the production rates of the unknown product are different, suggesting that microorganisms on larch and bamboo are different.

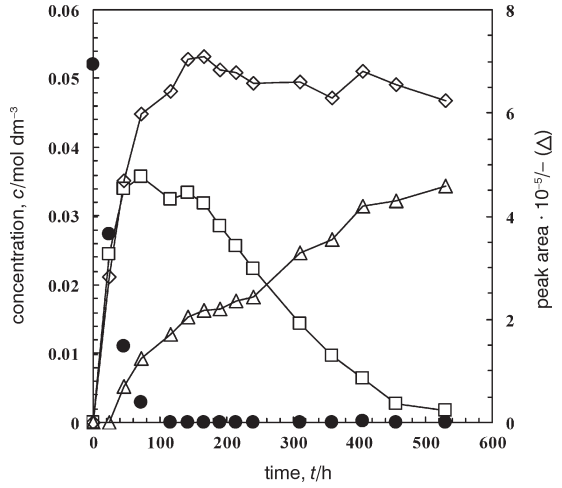
Table 1 lists the fraction of monosaccharides degraded by raw woods. From Table 1, larch has the highest capacity of degradation among the woods investigated, while wood chips did not have degradation ability. We guess that chemical modification of woods may reduce the degradation ability. For all wood materials, the degradation ratio of fructose and xylose is smaller than other monosaccharides, suggesting that degradation of monosaccharides is caused by microorganisms because it is difficult for most bacteria and some fungi to utilize fructose and xylose as a carbon source.

Since unknown products were appeared in the degradation of most saccharides, we measured the CO_2 content in the headspace, which is considered a final product. Degradation of mannose in the presence of larch was selected because of quantitative degradation of mannose as shown in Table 1. Figs. 4(a) and 4(b) show the time-courses of concentrations of mannose and unknown products, and CO_2 concentration in the headspace during 220 h, respectively. Evidently, the concentration of unknown products decreases after disappearing mannose and CO_2 content increases compared with the control experiment, suggesting that saccharides degrade through an unknown product to CO_2.

Table 2 lists the fraction of disaccharides degraded by raw woods. It is found that sucrose is easily degraded by all raw woods except wood chips. The concentration profile of sucrose degradation by larch is shown in Fig. 5. First, the sucrose



F i g . 4 – *(a) Time courses of mannose concentration (●) and peak area of unknown product (□) in mannose degradation in presence of larch; (b) Time courses of carbon dioxide concentration in headspace in the presence of mannnose (c = 10 mmol dm⁻³) and larch (●), in the presence of larch and absence of mannose (▲) and in the presence of mannnose and in the absence of larch (■).*

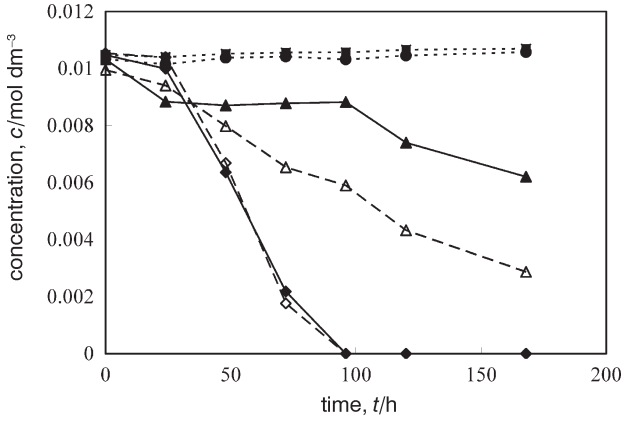


F i g . 5 – *Time courses of sucrose concentration (○), glucose (●) and fructose (◇) and peak area of unknown product (□) in sucrose degradation in presence of larch (initial saccharide concentration was c = 5 mmol dm⁻³)*

was hydrolyzed to glucose and fructose. Then the produced glucose was degraded to an unknown product and the produced fructose remained in the solution, as expected from the results of the monosaccharides. If this experiment was performed in a longer period, fructose would be consumed as well. It is possible that some fungi (white rot fungi) participate in the saccharides degradation as it is well known that they have higher affinity for glucose than fructose.[9,10]

Separation of arbutin and glucose

As shown in Fig. 1, arbutin was enzymatically synthesized from hydroquinone and a large excess of glucose. In such a case, arbutin must be economically separated. Fig. 6 shows the time course of glucose and arbutin concentration in the presence of bamboo wood. We used bamboo because degradation rates of arbutin by other wood materials were higher than that with bamboo in the preliminary experiment. In the absence of wood particles, glucose and arbutin were not degraded. In the case of a solution containing arbutin only, arbutin degraded slowly compared with glucose in the presence of bamboo. In experiments when arbutin and glucose were exposed to wood particles, 60 % of arbutin remained while all glucose was degraded after 100 h. In the case of the coexistence of glucose and arbutin, the degradation rate of arbutin was depressed compared with the above case, although the degradation rates of glucose in both cases were almost the same. In a coexistence system, glucose was first degraded easily and then arbutin was degraded. The degradation of glucose and arbutin is not a parallel but serial process. Therefore, separation of glucose and arbutin became better until glucose completely disappeared in the solution. Consequently, after 100 h, more than 85 % of the arbutin remained, while glucose degraded completely, suggesting that bamboo particles are promising for the degradation of sugar.



F i g . 6 – *Time courses of arbutin and glucose in cases of: a) only glucose (◇); b) only arbutin (△); c) coexistence of glucose (◆) and arbutin (▲) in presence of bamboo; d) coexistence of glucose (■) and arbutin (●) in absence of bamboo as a control (initial glucose and arbutin concentrations were c = 10 mmol dm^{-3})*

Conclusion

Saccharides including glucose by raw wood materials, such as larch, bamboo and cherry were degraded. Larch, bamboo and cherry showed high degradation capacities for glucose, galactose, mannose and sucrose at pH 6. Glucose was degraded to carbon dioxide via an unknown acidic compound. The degradation of saccharides by raw wood materials may mainly be a result of microorganisms on the woods.

These results were applied to the separation of arbutin and glucose, bamboo particles were found to selectively degrade glucose.

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List of symbols

c – concentration, mmol dm^{-3}, mol dm^{-3}
d – diameter, μm, mm
l – length, m
t – time, h
x – mole fraction, %
γ – mass concentration, kg dm^{-3}

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