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Pretreatment of Bagasse by UCT-Solvent for the Enzymatic Hydrolysis

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ABSTRACT

A thermochemical pretreatment of bagasse for the enzymatic hydrolysis has been carried out, in which pretreatment bagasse was autoclaved with binary solvent, composed of water and organic solvent having upper critical temperature (UCT) on the mutual solubility curve. The pretreatment was named "UCT-solvent pretreatment." The hydrophobic decomposition products from lignin and hemicellulose, that dissolved in organic phase at room temperature, could be easily separated from the solid and sugars in the aqueous phase. By using UCT-solvent instead of only water, the sugar recoveries from bagasse through the pretreatment and the enzymatic hydrolysis were much improved. There exists an optimal mixing ratio between organic solvent and water to maximize the effect of the pretreatment for enzymatic hydrolysis. The optimal ratio can be explained by the competitive effect between the ability of water as a reagent for the hydrolysis and the ability of solvent for the extraction of the decomposition product, and furthermore by the competitive effect between affinities of the solvent to hydrophilic hemicellulose and hydrophobic lignin. Decomposition of hemicellulose at lower temperature than 190°C was decreased, and hence the degradation of xylose during the pretreatment decreased. These favorable effects of UCT-solvent pretreatment are significantly attributed to the formation of the homogeneous single phase of organic solvent and water at high temperature and the phase separation at room temperature.

Index Entries: Pretreatment; enzymatic hydrolysis of cellulose; bagasse; lignocellulose; organic solvent; upper critical temperature; biomass.

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INTRODUCTION

Various kinds of pretreatments of cellulosic materials for the enzymatic hydrolysis have been developed (*1*). The enzymatic hydrolysis rate is strongly related to the surface area accessible to enzyme cellulase (*2,3*). The larger the accessible surface area of cellulose, the faster the hydrolysis. The hydrolysis rate also depends on the amount of enzyme adsorbed on cellulose. Cellulase adsorbs on not only cellulose, but also lignin and hemicellulose (*4,5*). The enzyme adsorbed on lignin and hemicellulose is wasted for the hydrolysis of cellulose. In other words, even if the accessible surface area would be large enough to be quickly hydrolyzed, the hydrolysis would be signficantly reduced by coexisting lignin and hemicellulose. The purpose of the pretreatment, therefore, is to increase the accessible surface area of cellulose and to reduce surface area of lignin and hemicellulose. These purposes can be attained by effective removal of lignin and hemicellulose from the lignocellulosic materials.

For the effective removal of lignin and hemicellulose, two things must go well: degradation of lignin and hemicellulose and removal of the degradation products from the inside of cellulose fiber.

In this work, we propose the thermochemical pretreatment with binary solvent composed of water and organic solvent having upper critical temperature (UCT) in the mutual solubility curve. We call such mixed solvent "UCT-solvent." We assumed that organic solvent mixed with water should satisfy the following:

1. High ability in extraction of degradation product from lignin;
2. Low solubility in water at room temperature;
3. Less toxic and explosive;
4. Specific gravity of less than 1.0 (it would make the solvent separation from solids easier.); and
5. The upper critical temperature of the mixed solvent is less than 200°C.

In this work, we chose cyclohexanol and *n*-pentanol as organic solvent mixed with water. The upper critical temperatures in the mutual solubility curves are 184°C in cyclohexanol–water (UCT-solvent A) and 187.5°C in *n*-pentanol–water (UCT-solvent B).

MATERIALS AND METHODS

Materials

Cellulase from *Trichoderma viride*, Meicelase CEPB-5029 (Filter paper activity: 8000 U/mg), was supplied from Meiji Seika Kaisha Ltd., Japan and used without any further purification. Bagasse was obtained from Prof. Toyama's Laboratory in University of the Ryukyus. All other chemicals were of reagent grade.

Pretreatment

Four g of dry bagasse ground to less than 35 mesh and 36 mL of UCT-solvent were autoclaved, where the temperature was raised from room temperature to a given high temperature of 170 to 220°C at the gradient of 1.5°C/min and unless otherwise noted, the ratio of organic solvent in UCT-solvent was 41.3 vol %. As soon as the high temperature was reached, the mixture was cooled down to room temperature leaving the autoclave sealed. Through a series of the process, the phase of UCT-solvent changes as following: initially two phases at room temperature, then single phase at high temperature, and again two phases at room temperature. The mixture thus pretreated was centrifuged. The upper organic phase was replaced and used for the determination of furfural concentration. One mL of the lower aqueous phase was used for the determination of total reducing sugar, glucose, xylose, and furfural. The pretreated bagasse suspended in the lower aqueous phase was enzymatically hydrolyzed with the aqueous phase.

As a control, another pretreatment was carried out, that was the same except that water was used instead of UCT-solvent.

Enzymatic Hydrolysis

Four g of untreated bagasse or all residue after the pretreatment of 4 g bagasse was used as the substrate. Unless otherwise noted, the aqueous phase separated from the organic phase in the pretreatment was included in the residue as substrate. The substrate was hydrolyzed in a jacketed batch reactor with 36 mL of working vol of the suspension at 40°C with gentle magnetic stirring and buffered to pH 4.8 with 0.1 *M* acetate. The concentration of cellulase was 6.7 mg/mL. One mL of the mixture was withdrawn and centrifuged. The supernatant was used for the determination of sugar concentration.

Determination of the Concentration of Sugars and Furfural

The concentration of the total reducing sugar (TRS) was determined by DNS method, and the concentration of glucose and xylose were determined separately by an enzymatic method using the glucose oxidase/peroxidase reagent and modified orcinol-Fe^{3+}-hydrochloric method (*6*), respectively. TRS as cellobiose was determined from calibration curves for cellobiose, glucose, and xylose. The concentration of furfural was determined by gas chromatography.

Definition of Sugar Yield After the Pretreatment and the Hydrolysis

Sugar yield was defined as following: Yield (TRS) was based on the total amount of cellulose (0.47 g glucose/g bagasse) and hemicellulose

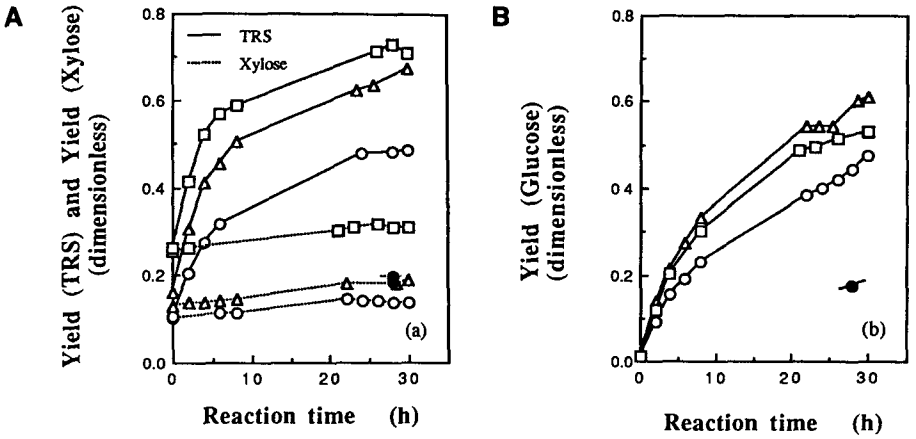

Fig. 1. Hydrolysis of untreated and pretreated bagasse at 40°C and pH 4.8. (a) Yield of total redusing sugar (TRS) based on hollocellulose contained in untreated bagasse and Yield of xylose from xylane, (b) Yield of glucose from cellulose. Pretreatment: (●) untreated, (○) water (control), (□) UCT-solvent A, (△) UCT-solvent B. Pretreatment temperature: 200°C. Enzyme concentration: 6.7 mg/mL.

(0.32 g xylose/g bagasse) contained in untreated bagasse, where unknown reducing sugar in the sample was evaluated as cellobiose. Yield (glucose) and yield (xylose) were based on the amount of cellulose and hemicellulose contained in untreated bagasse, respectively.

RESULTS AND DISCUSSION

Figures 1 (a) and (b) present the hydrolysis of untreated and pretreated bagasse pretreated with water and UCT-solvents A and B at 200°C. The sugar presented at zero reaction time in Fig. 1 was obtained by the pretreatment, and hence the increment from the value was coming from the enzymatic hydrolysis. As can be seen in Fig. 1, the pretreatment improved recovery of sugar from bagasse, and particularly we can see the favorable effect in the UCT-solvent pretreatment. Figure 1 also shows that a significant amount of sugar is produced by the pretreatment. UCT-solvent is also useful in the recovery of sugar produced by the pretreatment. The sugar produced by the pretreatment is composed of xylose, a very small amount of glucose, and unknown reducing sugar. The total sugar recoveries are represented in Table 1. It shows that, by using UCT-solvent A instead of only water, 1.5 times of sugar can be recovered. In all cases shown in Table 1, the recovery of xylose was small, compared with the value obtained through the microwave irradiation pretreatment (26.7 mg/mL) (7). This is because the pretreatment time is much longer than 5 min of the time (7) in the microwave irradiation pretreatment, and hence

Table 1
Comparison of UCT-Solvent Pretreatment of Bagasse with the Control Pretreatment that Uses Water as Solvent (Pretreatment Temperature: 200°C)

Pretreatment (solvent*1)	Sugar recoverd after pretreatment and 28h-enzymatic hydrolysis [mg/ml]			Yield [dimensionless]	Klason lignin [%]	Initial rate of enzymatic hydrolysis [mg/(ml•h)]
	Glucose	Xylose	TRS			
Water	23.2	5.3	41 6	0.50	22.4	4.4
UCT-solvent A	27 5	11.3	60.6	0.73	5.0	11.1
UCT-solvent B	28.9	6.5	54.7	0 66	11.8	9.6
Untreated	9.2	7.1	16.3	0.19	20.9	

*1 UCT-solvent A: cyclohexanol-water, UCT-solvent B: *n*-pentanol-water.

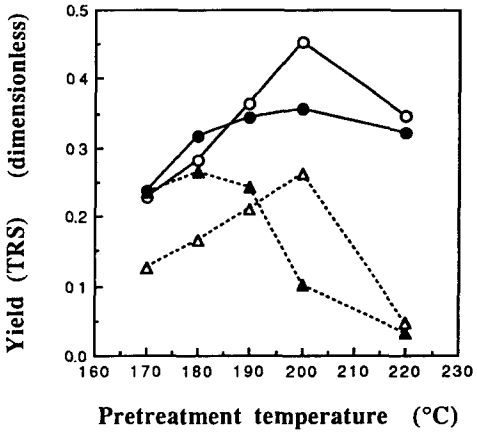

Fig. 2. Sugar production by the pretreatment with UCT-solvent A (cyclohexanol and water) and the enzymatic hydrolysis as a function of the pretreatment and temperature. Pretreatment with (○,△) UCT-solvent, and (●,▲) water (as the control pretreatment). Sugar production by (----) the pretreatment, and (——) the enzymatic hydrolysis.

as described later, xylose produced is further decomposed to other chemicals such as furfural. Table 1 also present the initial hydrolysis rate, v_0. The initial hydrolysis rates for the bagasses pretreated with UCT-solvent, in particular with UCT-solvent A, are larger than that for the bagasse pretreated with only water. This result can be explained in terms of lignin removal from bagasse shown in Table 1. The removal of lignin makes the surface area of the lignocellulosics increase (2,3), resulting in the larger reaction rate.

In Fig. 2, the sugar production by the pretreatment with UCT-solvent A and by the enzymatic hydrolysis was separately evaluated as a function of the pretreatment temperature, comparing with the result by the control pretreatment. Figure 3 was obtained for UCT-solvent B. Between the pre-

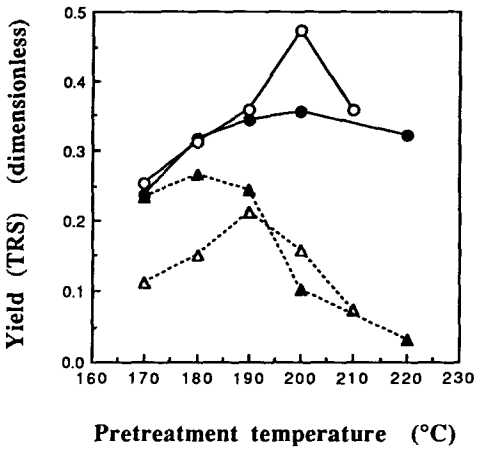

Fig. 3. Sugar production by the pretreatment with UCT-solvent B (*n*-pentanol and water) and the enzymatic hydrolysis as a function of the pretreatment and temperature. Pretreatment with (○,△) UCT-solvent, and (●,▲) water (as the control pretreatment). Sugar production through (----) the pretreatment, and (——) the enzymatic hydrolysis.

treatments with UCT-solvents A and B, similar relationships were obtained. The enzymatic hydrolysis data shows the UCT-solvent is more effective when the pretreatment is carried out at higher than upper critical temperature of the solvent. The best pretreatment temperature for the enzymatic hydrolysis is 200°C. It seems that at over 200°C, decomposition of cellulose occurs. Almost all of sugar produced by the pretreatment is from hemicellulose. There is a maximum in the sugar production by pretreatment against the temperature, and the temperature to give the maximum is higher in the UCT-solvent pretreatment rather than the control pretreatment with water. In order to understand the shift, the decomposition of xylose was separately examined, that is, 47 mg/mL xylose was treated in the same way as carried out for bagasse, using water and UCT-solvents A and B at 200°C. Xylose was decomposed to the other chemicals such as furfural, but the extent of the decomposition was not different among the three pretreatments: 84±1% of xylose was decomposed. This result means that the shift in the temperature to give the maximum in sugar production by the pretreatment is caused by the difference in the extent of the hydrolysis of hemicellulose. The concentration of water in UCT-solvents A and B is low (58.7 vol %), and furthermore the organic solvent would make the water activity low. These negative factors for the hydrolysis probably diminished the hydrolysis of hemicellulose.

Table 2 presents the amount of furfural produced by the pretreatment. In the pretreatment with UCT-solvent, furfural is partitioned between the organic phase and aqueous phase after the pretreatment. The partition coefficient (organic/aqueous) was 3.4 for cyclohexanol and 3.1

Table 2
Formation of Furfural During the Pretreatment

Pretreatment temperature (°C)	Amounts of furfural produced by the pretreatment of bagasse (mg/g-bagasse)		
	Water*1	UCT-solvent A*2	UCT-solvent B*3
180			1.3
190	17.3	6.8	8.3
200	35.5	15.5	18.3
210			26.5
220	30.5	36.0	

*1 Pretreatment carried out as a control, using water instead of UCT-solvent.
*2 cyclohexanol-water
*3 *n*-pentanol-water

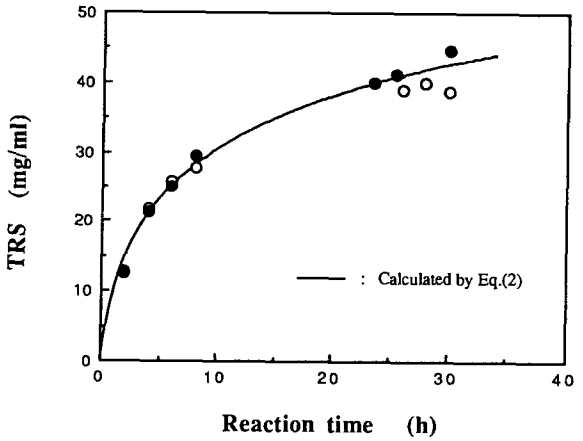

Fig. 4. Data-fitting by the empirical rate equation (Eq. (2)) for the enzymatic hydrolysis of bagasse pretreated with UCT-solvent A and UCT-solvent B at 200°C. (○) UCT-solvent A, (●) UCT-solvent B. Parameter values used for the estimation: k=4.1 (dimensionless), V_0=15.0 (mg/(mL·h)).

for *n*-pentanol. The large coefficient is favorable to remove furfural from the pretreated bagasse. The amount presented in Table 2 is the total of furfural contained in the two phases. In all cases, the higher the pretreatment temperature, the more furfural is produced. At given temperature, the pretreatment with water produces the larger amount of furfural than the other two pretreatments. That is because, as mentioned above, hemicellulose is more hydrolyzed to xylose in the control pretreatment with water compared with in the UCT-solvent pretreatment. Little difference between the two UCT-solvent pretreatments was observed.

Figure 4 presents the hydrolysis of bagasse pretreated with water and UCT-solvents A and B at 200°C. The solid curves were obtained from the following empirical rate expression (*8*),

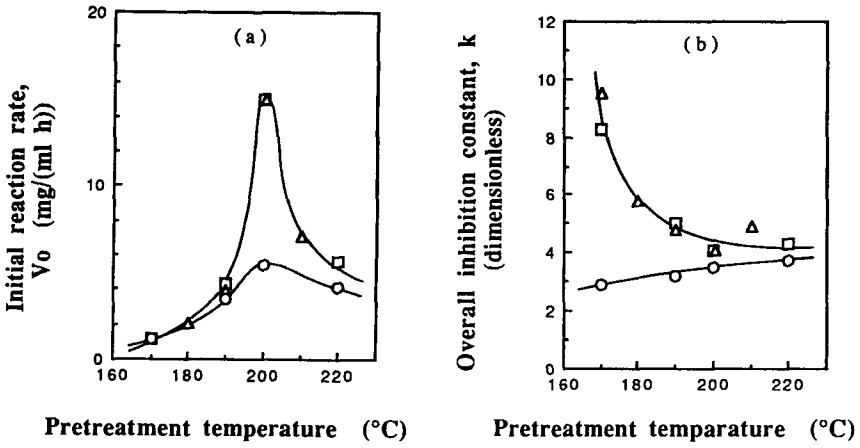

Fig. 5. Effects of the pretreatment on the kinetics of the enzymatic hydrolysis of the pretreated bagasse. (a) Initial hydrolysis rate, V_0. (b) Overall inhibition constant estimated by Eq. (1), k. Pretreated with (○) water as the control pretreatment, (□) UCT-solvent A, and (△) UCT-solvent B.

$$-dV/dX = kV \tag{1}$$

where V and X are the hydrolysis rate and the fractional conversion, respectively. The k is dimensionless overall inhibition constant. The k value means that the larger the value, the more quickly the hydrolysis rate decreases. By the integration of Eq. (1), we obtain,

$$P = (S_0/k)ln\{1+(V_0/S_0)kt\} \tag{2}$$

where P is the concentration of product, S_0 and V_0 are initial substrate concentration and initial reaction rate, respectively. The t is reaction time. As can be seen in Fig. 4, the experimental data can be well expressed by Eq. (2), and hence Eq. (1). Figures 5 (a) and (b) present the values of V_0 and k, respectively, obtained for the hydrolysis of bagasses pretreated at various temperatures, where the bagasse was used without any washing after the pretreatment. Figure 5 shows that, as partially described in Table 1, the UCT-solvent pretreatment increases the initial hydrolysis rate, probably because of the enlargement of the surface area of cellulose accessible to cellulase (3). In the range of pretreatment temperature of 190 to 220°C, k-values were almost same. The large k-value at 170 to 190°C, compared with the reference pretreatment, can be explained in terms of smaller removal of hemicellulose that hinder attack of enzyme to cellulose. In other words, rapid decrease in the surface area of cellulose accessible to cellulase by remaining hemicellulose reflects the large k-value.

Finally, the effect of phase change in UCT-solvent has been investigated. In the principle, the pretreatment with UCT-solvent could be expected to proceed by two steps. The first step, that proceeds at high temperature near or over UCT, is simultaneous decomposition of lignin and hemicellulose, and extraction of the decomposition products by UCT-

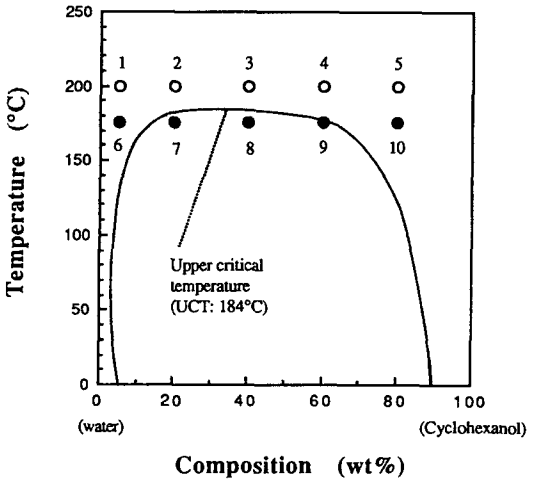

Fig. 6 (a). Experimental design on the mutual solubility curve for UCT-solvent A (cyclohexanol and water). Plots from no. 1 to no. 10 point conditions of the pretreatment with UCT-solvent A. Temperature in °C: (●) 176, and (○) 200.

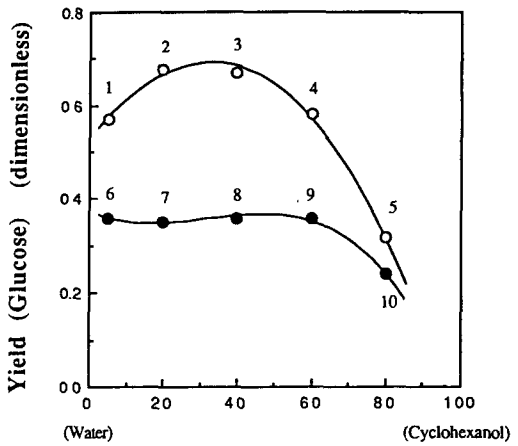

Fig. 6 (b). Enzymatic hydrolysis of the bagasses pretreated under the conditions shown in Fig. 6 (a). Pretreatment temperature in °C: (●) 176, and (○) 200. The numbers in the figure are corresponding to those presented in Fig. 6 (a).

solvent, where the organic solvent and water would form homogeneous solution. The second step is phase separation between the organic phase containing hydrophobic products from lignin and hemicellulose and the aqueous phase containing hydrophilic products such as sugar. In order to understand how UCT-solvent works in the pretreatment, the following experiment was carried out. Figure 6 (a) presents a design of the experiment, where the solid curve shows the mutual solubility for UCT-solvent A and each of ten black circles points the condition at which the pretreatment was carried out. Figure 6 (b) presents the results of hydrolysis of

bagasses pretreated under the conditions, where the pretreated bagasse was washed with acetone and water before the enzymatic hydrolysis. Figure 6 (b) shows that the pretreatment at 200°C is more effective than that at 176°C, and also that the effect of the pretreatment depends on the ratio of organic solvent (cyclohexanol) and water. Since such dependency can be seen only for the pretreatment with UCT-solvent A under the homogeneous single phase (Exps. 1 to 5), it can be concluded that a formation of the homogeneous single phase of the solvent is contributing to the positive effect of the pretreatment. However, it is also true that only the homogeneity in the solvent does not always produce better result (Exps. 5 and 10). The more the content of organic solvent in UCT-solvent, the higher the ability of UCT-solvent for the extraction of the degradation product from lignin and hemicellulose, but also the lower the activity of water as a reagent for hydrolysis becomes. The existence of the optimal ratio between organic solvent and water to maximize the extent of the hydrolysis can be explained by the competitive effect between the ability of water as a reagent for the hydrolysis and the ability of solvent for the extraction of the decomposition product. Furthermore, it can be true that the more the content of water in UCT-solvent, the stronger the affinity of the solvent to hemicellulose, and the more the content of organic solvent, the stronger the affinity of the solvent to lignin. For the effective pretreatment, it is needed that both hemicellulose and lignin are simultaneously decomposed. This situation must require the optimum in the solvent composition. Actually, both of the two explanations could be true.

CONCLUSIONS

UCT-solvent pretreatment of bagasse was proposed for the effective enzymatic hydrolysis, in which pretreatment the decomposition products from lignin and hemicellulose can be easily separated from the solid and sugars produced during the preteatment. The characteristics of UCT-solvent pretreatment can be summarized as follows:

1. UCT-solvent is useful for the extraction of the degradation products from lignin and hemicellulose compared with water, resulting in the enhancement of enzymatic hydrolysis.
2. There exists an optimal mixing ratio between organic solvent and water to maximize the effect of the pretreatment for enzymatic hydrolysis. It can be explained in terms of the competitive effect between the ability of water as reagent for the hydrolysis and the ability of solvent for the extraction of decomposition product. It can also be explained in terms of affinity of the solvent to hemicellulose and lignin. There must be the optimal

ratio between organic solvent and water at which the solvent well simultaneously attacks to hydrophilic hemicellulose and hydrophobic lignin.

3. Decomposition of hemicellulose is depressed, and hence the degradation of xylose during the pretreatment is diminished. This effect of UCT-solvent makes the xylose recovery higher even at high temperature around 200°C at which the largest effect of the pretreatment on the enzymatic hydrolysis of the lignocellulose is attained.
4. The favorable effect of the UCT-solvent pretreatment is significantly attributed to the formation of the homogeneous single phase of organic solvent and water and the phase separation at room temperature.

In this work, bagasse with UCT-solvent was batchwise autoclaved, and the process took a long time (about 2 h). Reduction in the pretreatment time, for example by using continuous pretreatment reactor, would increase the recovery of xylose. The pretreatment with UCT-solvent was useful for the other lignocellulosics rather than bagasse, although the data are not shown here. The data will be reported in another paper.

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