



Development of efficient system for ethanol production from paper sludge pretreated by ball milling and phosphoric acid

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ABSTRACT

High-solid paper sludge was investigated for its potential to produce renewable biofuel. As a pretreatment method without consuming a large amount of energy, mechanical grinding for a comparatively short time followed by chemical swelling was used for optimal enzyme saccharification and ethanol production from paper sludge. Chemical swelling by phosphoric acid was more favorable than ball mill grinding. However, sequential pretreatment system using ball milling for 2 min and then phosphoric acid swelling for 1 h enhanced the enzyme saccharification rate and the reducing sugar productivity, 84.1% and 28.1 mg/g/h, respectively. The simultaneous saccharification and fermentation (SSF) from the sequential pretreatment system resulted to 81.5% ethanol conversion rate, the productivity being 1.27 g/L/h compared to untreated raw paper sludge which gave 54.3% ethanol conversion rate and 0.424 g/L/h productivity. Our work shows that consecutive mechanical grinding and chemical swelling are effective pretreatment methods for the enhancement of enzyme saccharification and efficient ethanol production from paper sludge.

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1. Introduction

Fossil fuels are implicated in the emission of greenhouse gases that pollute the environment. The utilization of bioethanol as an alternative fuel has attracted a lot of attention because it results to zero net carbon dioxide output into the atmosphere since it is recycled through photosynthesis (Araque et al., 2008; Ohgren, Rudolf, Galbe, & Zacchi, 2006). Commercial bioethanol production mostly relies on fermentation of sucrose from sugarcane and molasses or glucose derived from starch-based feedstocks such as corn that are easy to degrade to fermentable sugars. Though cellulosic substrates provide cheaper raw materials for bioethanol production, they are more difficult to biodegrade compared to starch-based materials (Davis, Rogers, Pearce, & Peiris, 2006; Farrell et al., 2006). Therefore, pretreatments of cellulosic materials prior to their utilization have attracted enormous research interest for efficient and cost effective production of bioethanol.

Paper sludge is a solid residue from paper-making industries. It contains large quantities of short cellulose fibers. These residues are usually disposed of in landfills or subjected to incineration after dewatering, which has a significant cost-increasing factor on paper production (Kadar, Szengyel, & Reczey, 2004; Lark, Xia, Qin, Gong, & Tsao, 1997). However, since paper sludge constitutes large amounts of polysaccharides, they are feasible raw materials for

production of fermentation products such as ethanol and lactic acid (Fan et al., 2003; Marques, Alves, Roseiro, & Girio, 2008; Schmidt & Padukone, 1997).

Two steps are required for the conversion of paper sludge to ethanol. The first step is the hydrolysis of the polysaccharide component to fermentable sugars while the second step is the fermentation of sugars to ethanol. Enzymatic saccharification is often more preferable than acid hydrolysis, since it makes it possible to combine cellulose hydrolysis with ethanol fermentation (Xu, Wang, Jiang, Yang, & Ji, 2007). In addition, the simultaneous saccharification and fermentation (SSF) provides the possibility to overcome the disadvantage of carrying out separate saccharification and fermentation processes because SSF decreases the amount of enzyme used and therefore the production cost (Kadar et al., 2004). However, since cellulose has a crystalline structure in which molecules are subjected to extensive hydrogen bonding (Nishiyama, Sugiyama, Chanzy, & Langan, 2003) and van der Waals forces (Notley, Pattersson, & Wagberg, 2004), this structure provides low accessibility to chemicals and enzymes. Therefore, it is important to facilitate the conversion of cellulose to glucose in order to develop an efficient production of bio-based products from cellulosic materials (Zhang, Cui, Lynd, & Kuang, 2006).

In this work, the utilization of high solid-content paper sludge as an alternative substrate for the ethanol production using SSF process was studied. In addition, for the process enhancement, the effect of mechanical and chemical pretreatment of paper sludge on the enzyme saccharification and the ethanol production was investigated.

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2. Experimental

2.1. Material

Paper sludge was used as same as described previously (Yamashita, Kurosumi, Sasaki, & Nakamura, 2008), which the particle size of raw paper sludge was 2–4 mm. The estimated holocellulose and cellulose contents of raw paper sludge were 47.6% and 33.4%, respectively. Before use as the material for ethanol production, paper sludge was stored in container at ambient temperature.

Saccharomyces cerevisiae AM 12 was obtained from Bio Academia Co. Ltd, Japan, and used for ethanol production. It was incubated on potato dextrose agar plates at 30 °C and then stored in the refrigerator at 4 °C.

$$\text{Ethanol conversion rate (\%)} = \frac{\text{Amount of ethanol produced}}{\text{Amount of theoretical maximum ethanol produced (0.51 g ethanol/g glucose) from paper sludge}} \times 100$$

2.2. Pretreatments methods

The effects of three different pretreatments (mechanical grinding by ball mill, chemical swelling by phosphoric acid and sequential pretreatment system with a ball mill and phosphoric acid) were investigated for the effective saccharification of paper sludge and fermentation of the formed sugars to ethanol.

The mechanical grinding by ball milling is as follows: 20 g of raw paper sludge was ground by a vibrating ball mill (Vibrating sample mill CMT-TI-300, C. M. T. Co., Ltd.) at 60 cycles/s for 2 min. The particle size of the sample after ball mill treatment was approximately 0.1 mm.

The chemical swelling by phosphoric acid is as follows: 0.5 g of raw paper sludge was added to a 100 mL vial container and 0.6 mL of distilled water was added. 10 mL of ice-cold phosphoric acid was slowly added to the vial containing wet paper sludge and the mixture was vigorously stirred. After the paper sludge-phosphoric acid mixture was left for about an hour on ice with occasional stirring, 40 mL of ice-cold water was added at a rate of 10 mL per addition with vigorous stirring between additions. The paper sludge mixture was centrifuged at 3500 rpm for 30 min at 4 °C. The paper sludge solid portion was resuspended in ice-cold water, followed by centrifugation at 3500 rpm to remove the supernatant containing phosphoric acid. 0.5 mL of 2 M Na₂CO₃ was added to neutralize the residual phosphoric acid and the ice-cold water was used to suspend the paper sludge. After centrifugation, the treated paper sludge was stored at 4 °C (Zhang et al., 2006).

The pretreatment with mechanical grinding by a ball mill followed by chemical swelling by phosphoric acid was carried out sequentially as described above in an attempt to enhance the enzyme saccharification rate and the ethanol productivity.

2.3. Enzymatic hydrolysis

The hydrolysis was performed in 100 mL Falcon tubes at an initial sample concentration of 5% (w/w) in 10 mL of 100 mM sodium acetate buffer at pH 5.0 using commercial enzyme (Meicelase) purchased from Meiji Seika Co., Ltd, Japan, at a loading rate of 20 FPU/g sample. The enzymatic hydrolysis reaction was carried out in a reciprocating water bath shaker at 140 strokes/min for 48 h at 45 °C. The supernatant was centrifuged and removed for sugar con-

tent determination. The saccharification rate is calculated using the following equation (Sharma, Kalra, & Kocher, 2004):

$$\begin{aligned} \text{Cellulose hydrolysis rate (\%)} \\ = \frac{\text{The amount of glucose produced} \times 0.9}{\text{The amount of cellulose included of raw paper sludge}} \times 100 \end{aligned}$$

2.4. Simultaneous saccharification and fermentation (SSF)

Pure yeast culture from an agar plate was added to 30 mL L-tubes containing 10 mL of sterile medium. The medium compositions were as follows: 10 g/L glucose, 1 g/L yeast extract, 0.1 g/L KH₂PO₄, 0.1 g/L MgSO₄·7H₂O and 0.1 g/L (NH₄)₂SO₄. This precul-

ture was incubated at 30 °C for 24 h using a seesaw incubator at 60 rpm (Itoh, Wada, Honda, Kuwahara, & Watanabe, 2003). The ethanol conversion rate is calculated using the following equation:

200 g/L of the raw and the pretreated paper sludge were put into separate 500 mL Erlenmeyer flasks, and then autoclaved for 20 min at 121 °C. Then, the sterilized nutrient solution, the enzyme and the sodium acetate buffer were added. The composition of the nutrient solution and enzyme loading in the fermentation medium were adjusted as follows: 2 g/L yeast extract, 0.05 g/L MgSO₄·7H₂O, 1 g/L (NH₄)₂HPO₄, 20 FPU/g sample of Meicelase, and 100 mM of sodium acetate buffer at pH 5.0 (Sharma et al., 2004). The previously precultured yeast suspension was used for inoculation and the mixture was incubated in a rotary shaker at 40 °C with gentle agitation at 100 rpm.

2.5. Analytical methods

The amount of ethanol and glucose from the fermentation broth were determined by HPLC using an Aminex column HPX-87H (Bio-rad, Richmond, CA) (Davis et al., 2006) and total sugar concentration was measured by phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). In case of the enzymatic hydrolysis, glucose concentration was measured by the mutarotase GOD method (Glucose C-Test; Wako Pure Chemicals, Osaka, Japan) and the reducing sugar concentration was determined according to the Somogyi-Nelson method (Somogyi, 1952).

3. Results and discussion

3.1. Enzyme saccharification of raw and pretreated paper sludge

The effects of the mechanical grinding by a ball mill, the chemical swelling by phosphoric acid, and the consecutive treatments (by mechanical grinding and chemical swelling) were examined for the enhancement of both enzyme saccharification and ethanol production. Fig. 1 shows the time courses of glucose and reducing sugar productions on enzyme saccharification of raw and pretreated paper sludge. The amounts of glucose and reducing sugar produced from raw paper sludge gradually increased through the reaction time of 24 h and reached their maximum values of 111 and 146 mg/g (dry sample), respectively. Though Marques, Santos,

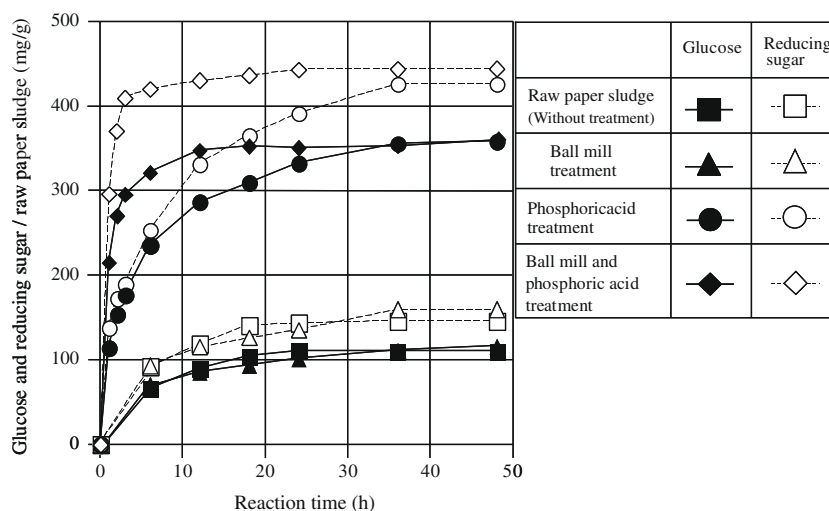


Fig. 1. Time courses of glucose and reducing sugar production on enzyme saccharification from raw and pretreated paper sludge.

Girio, and Roseiro (2008) reported that almost complete saccharification of raw paper sludge was accomplished by cellulolytic enzyme, the lower amount of glucose production was observed in this work. This could be attributed to the fact that the paper sludge used in this work formed aggregates, which inhibited the enzyme accessibility and/or cellulose digestibility. Therefore, the pretreatments by mechanical grinding and/or chemical swelling were attempted for the efficient enzymatic saccharification. When the paper sludge was pretreated by a ball mill, the amount of glucose and reducing sugar productions were higher (116.4 and 159.7 mg/g dry sample, respectively) compared to untreated samples. Though the pretreatment by a ball mill could reduce the particle size of the paper sludge, the significant increases of the amounts of glucose and reducing sugar were not appeared. In contrast, the amounts of glucose and reducing sugar production from paper sludge pretreated by phosphoric acid increased and reached 359.0 and 426.6 mg/g dry sample, respectively, after 36 h of reaction. This observation suggests that the chemical swelling is more efficient in the pretreatment of paper sludge compared to mechanical grinding for the enhancement of enzymatic saccharification. Moreover, the sequential pretreatment system with mechanical grinding by a ball mill and chemical swelling by phosphoric acid resulted in the significant increases of the amounts of glucose and reducing sugar production by 6 h of reaction. Although the enzymatic saccharification was accomplished after 12 h of reaction completely, the produced glucose and reducing sugar were 360.2 and 445.0 mg/g dry sample, respectively, which obtained as same as those from phosphoric acid pretreated paper sludge after 36 h of reaction. Table 1 shows the kinematic parameters of the enzymatic saccharification of raw and pretreated paper sludge. Similar results in cellulose hydrolysis rate and glucose productivity were obtained when the raw and the ball mill treated paper sludge were used. However, when phosphoric acid pretreated paper sludge was

used, the cellulose hydrolysis rate increased from 29.9% to 96.7% while the glucose productivity increased from 4.6 to 10.0 mg/g/h. In addition, the sequential pretreatment system with mechanical grinding by a ball mill and chemical swelling by phosphoric acid introduced the significant increases of not only the cellulose hydrolysis rate but also the glucose productivity, which obtained the maximum values of 97.1% and 30.0 mg/g/h, respectively. The improvements of both the cellulose hydrolysis rate and the kinematic parameters of enzymatic saccharification could be attributed to the sequential effects of the reduction of particle size of paper sludge aggregation and the enhancement of the enzyme accessibility and cellulose digestibility by the chemical changes in supramolecular structure of cellulose contained in the paper sludge (Sharma et al., 2004). These results indicate that the sequential pretreatment through mechanical grinding by ball mill and chemical swelling by phosphoric acid is effective pretreatment method for the enhancement of enzyme saccharification of paper sludge.

3.2. Ethanol production from raw and pretreated paper sludge in using SSF process

Fig. 2 shows the time courses of glucose, ethanol and total sugar concentration in using SSF process for the raw and the pretreated paper sludge obtained from the sequential pretreatment system with mechanical grinding by a ball mill and chemical swelling by phosphoric acid. After inoculation, the glucose and the total sugar concentration in each fermentation broth started to increase and reached maximum values of 6.2 and 18.6 g/L in using raw paper sludge, and 40.0 and 66.9 g/L in using pretreated paper sludge, respectively, after 6 h of incubation. Thereafter, the glucose concentration in each fermentation broth dropped sharply and then remained at low levels during fermentation. This phenomenon indicates that during this time, the yeast cells were fermenting

Table 1
Kinematic parameters of enzyme saccharification after 48 h reaction.

	Raw paper sludge	Pretreatment		
		Ball mill	Phosphoric acid	Ball mill and phosphoric acid
Glucose/dry sample (mg/g) ^a	110.8	116.4	359.0	360.2
Reducing sugar/dry sample (mg/g) ^a	146.4	59.7	426.6	445.0
Cellulose hydrolysis rate (%)	29.9	31.3	96.7	97.1
Glucose productivity (mg/g/h)	4.6 (24 h)	2.4 (48 h)	10.0 (36 h)	30.0 (12 h)

^a The amount of production calculated from values on a dry-weight basis before pretreatment.

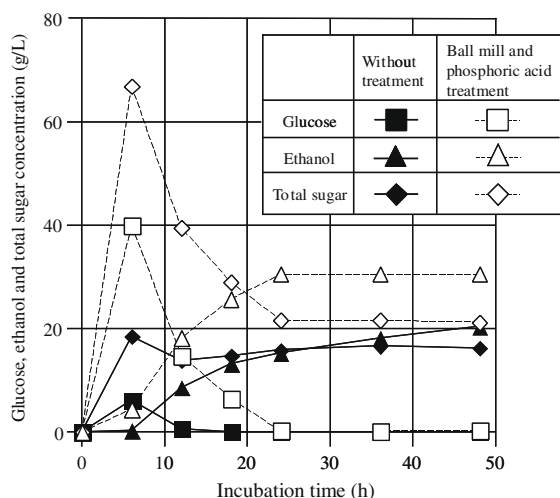


Fig. 2. Time courses of glucose, ethanol and total sugar concentrations in SSF.

Table 2

Kinematic parameters of ethanol production in SSF using raw and pretreated paper sludge.

	Raw paper sludge	Ball mill and phosphoric acid
Produced ethanol (g/L)	20.4	30.5
Ethanol yield (–)	0.277	0.416
Conversion rate (%)	54.3	81.5
Ethanol productivity (g/L/h)	0.424 (48 h)	1.27 (24 h)

the produced glucose (from enzymatic saccharification) to ethanol. Meanwhile, the total sugar concentration from raw paper sludge was also reduced to 13.8 g/L at 12 h incubation, then slightly increased again and stayed at around 16.5 g/L. In case of the pretreated paper sludge, the total sugar was reduced to 21.6 g/L at 24 h of incubation and then remained constant. The large differences between each fermentation in final total sugar concentration were not observed, which means that the large amount of non-fermentable sugars resulting from the enzyme saccharification of paper sludge are accumulated in both fermentation broths. In addition, the amount of ethanol production in each fermentation gradually increased with incubation time and peaked at 20.4 g/L after 48 h of incubation in using raw paper sludge. However, the fermentation in using pretreated paper sludge was finished at 24 h of incubation, corresponding to maximum ethanol production of 30.5 g/L. This result indicated that the enzyme saccharification is the rate-limiting step for ethanol production from raw paper sludge during the SSF process, in contrast, the ethanol fermentation is that in using the pretreated paper sludge since the enzyme saccharification and the ethanol fermentation were accomplished completely at the same time (within 24 h of reaction) in using pretreated paper sludge. Table 2 shows the kinematic parameters of ethanol production in SSF in using raw and pretreated paper sludge. The ethanol yield and the conversion rate of fermentation in using raw paper sludge were 0.277 and 54.3%, respectively. This conversion rate is consistent with the report by Kadar et al. (2004) using commercial baker's yeast, *Saccharomyces cerevisiae*, although there are some differences in the types of paper sludge used. These low values of the ethanol yield and the conversion rate obtained from raw paper sludge could be attributed to the poor enzyme saccharification following the low production of ethanol. On the other hand, the ethanol yield and the conversion rate in using pretreated paper sludge obtained higher values, corresponding to 0.416%

81.5%, respectively. This result confirms that almost all the cellulose contained in the paper sludge was fermented to ethanol because the similar values of the ethanol yield and the conversion rate were obtained in comparison with the batch fermentation of glucose medium by *S. cerevisiae* AM 12, which obtained the ethanol yield of 0.434 and the conversion rate of 85.4% (data not shown). In addition, the ethanol productivity in using pretreated paper sludge (1.27 g/L/h, 24 h) was about 3-fold compared to that obtained in using raw paper sludge (0.424 g/L/h, 48 h). Therefore, it has been shown that the sequential pretreatment system with mechanical grinding and chemical swelling is the effective pretreatment method prior to enzymatic saccharification of paper sludge and therefore it facilitates efficient ethanol production.

4. Conclusion

The effect of pretreatment of paper sludge on the enzyme saccharification and ethanol production was investigated for the utilization of high solid-content paper sludge as an alternative material for renewable energy. The cellulose hydrolysis rate and the glucose productivity in using paper sludge treated by phosphoric acid gave higher values compared to those in using untreated or ball mill pretreated paper sludge. This result suggested that chemical swelling is favorable as the pretreatment of paper sludge on enzyme saccharification compared to the mechanical milling. In addition, the sequential pretreatment system with mechanical grinding and chemical swelling resulted to significant increases not only of the amount of potential sugars production by enzyme saccharification but also of ethanol production and productivity in SSF process. Therefore, it was concluded that the sequential pretreatment system with mechanical grinding and chemical swelling is effective pretreatment method for the production of ethanol from paper sludge.

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