Carbohydrate Polymers 79 (2010) 250–254

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

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

Yuya Yamashita, Chizuru Sasaki, Yoshitoshi Nakamura *

Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

ARTICLE INFO

Article history:
Received 21 July 2009
Received in revised form 28 July 2009
Accepted 29 July 2009
Available online 3 August 2009

Keywords:
Paper sludge
Mechanical grinding
Chemical swelling
Saccharification
Ethanol production

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.

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.

* Corresponding author. Tel.: +81 88 656 7518; fax: +81 88 656 9071.
E-mail address: ynakamu@bio.tokushima-u.ac.jp (Y. Nakamura).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.07.054

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.

2.2. Pretreatments methods

The effects of three different pretreaments (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 Na_2CO_3 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 content determination. The saccharification rate is calculated using the following equation (Sharma, Kalra, & Kocher, 2004):

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

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 KH_2PO_4, 0.1 g/L $MgSO_4{\cdot}7H_2O$ and 0.1 g/L $(NH_4)_2SO_4$. This preculture 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:

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

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 $MgSO_4{\cdot}7H_2O$, 1 g/L $(NH_4)_2HPO_4$, 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,