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Short Communication

Aqueous ammonia pretreatment of oil palm empty fruit bunches for ethanol production

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ABSTRACT

Oil palm empty fruit bunches (EFB) were pretreated by aqueous ammonia soaking for ethanol production. Pretreated EFB, which were pretreated at the optimal conditions of 60 °C, 12 h, and 21% (w/w) aqueous ammonia, showed 19.5% and 41.4% glucose yields during an enzymatic digestibility test for 96 h when using 15 and 60 FPU of cellulase, respectively. Using the pretreated EFB, simultaneous saccharification and fermentation for 168 h with 5% (w/v) glucan loading and 60 FPU of cellulase and 30 CBU of β-glucosidase per gram glucan resulted in ethanol production of 18.6 g/L titer, 65.6% of theoretical maximum yield, and 0.11 g/L/h of productivity.

1. Introduction

Oil palm trees, *Elaeis guineensis*, are cultivated extensively in humid tropical areas for edible oil production (Kelly-Yong et al., 2007). After the reddish fruits of the palm oil tree have grown in large bunches, the empty fruit bunches (EFB), which account for 20% of the total oil palm biomass, are removed during oil processing (Kelly-Yong et al., 2007). Every year 14.9 and 37.7 million tons of EFB are produced in Malaysia and worldwide, respectively (Akhtar et al., 2010). Since the bunches are rich in cellulose and hemicellulose that are not easily digested, these bunches are the primary material that must be subjected to waste treatment in the palm industry.

In this study, aqueous ammonia pretreatment, which is known to enhance saccharification and ethanol yields mainly by removing lignin from lignocellulose (Li and Kim, 2011; Jung et al., 2011), was applied to EFB containing lignin of over 30%, which is higher than that of other lignocellulosic biomass. The optimization of the aqueous ammonia pretreatment process for EFB was conducted by varying the pretreatment temperature, pretreatment time, solid:liquid (S/L) ratio, and ammonia concentration based on the glucose yield as determined by an enzymatic digestibility test. Protein binding experiments using a carbohydrate-binding module (CBM), *Ct*CBD3, were conducted to enzyme accessibility of the pretreated EFB. Also, simultaneous saccharification and fermentation (SSF) were conducted using pretreated biomass to evaluate the performance of the ammonia-pretreated EFB. This study provides the experimental results describing the feasibility of using EFB for ethanol production following alkaline pretreatment.

2. Methods

2.1. EFB and cellulose

EFB obtained from Tropical Chase (Kuala Lumpur, Malaysia) were ground using a high-speed rotary cutting mill (MF 10, IKA, Staufen, Germany) to give particle sizes ranging from 706 to 125 μm (25–120 mesh). The composition of the EFB based on its total dry weight was 35.6% (w/w) glucan, 14.7% xylan, 3.6% mannan, 35.4% acid-insoluble lignin, and 5.8% ash. The water-soluble solids content of the EFB biomass was 17.7% (w/w) of the total biomass, in which the monomeric sugar contents of glucose, xylose, galactose, arabinose, mannose, fructose, and sucrose were 2.4%, 2.3%, 1.4%, 3.1%, 3.3%, 1.2%, and 4.2% (w/w) of the water-soluble solids. Avicel (Sigma–Aldrich, St. Louis, MO) was used as a pure cellulose control.

2.2. Ammonia pretreatment of EFB

Ten grams of dried EFB were soaked in a certain concentration of aqueous ammonia solution (Junsei, Tokyo, Japan). The slurry was then incubated at a certain temperature for a certain length of time without agitation. The pretreated EFB slurry was then

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filtered using a filtration cloth (a pore size of 22–25 μm; Calbiochem, La Jolla, CA) to recover the insoluble solids. The recovered solids were washed with distilled water until pH 6.5–7.0, and were transferred to a clean dish and then dried in a vacuum-drying oven (SH-45S, BioFree, Seoul, Republic of Korea) at 45 °C for 3 days.

2.3. Enzymatic hydrolysis of pretreated EFB

Enzymatic hydrolysis to evaluate pretreatment effectiveness was conducted in a 20 mL vial with 1% (w/v) glucan loading and 15 FPU of Accellerase 1000/g glucan according to NREL LAP-009 (Brown and Torget, 1996). The enzyme reaction mixture containing pretreated and washed EFB, cellulase and 0.05 M sodium citrate buffer at pH 4.8 was incubated at 50 °C and 170 rpm in duplicate.

2.4. SSF of pretreated EFB

To investigate the fermentability of the pretreated EFB, SSF were conducted at 38 °C using NREL LAP-008 with a modification of enzyme loading to 60 FPU and 30 CBU/g glucan and 5% (w/v) of biomass input in the SSF medium (Hayward et al., 1995).

2.5. Analytical methods

Carbohydrate composition of EFB was analyzed by NREL LAP-002 for two-stage sulfuric acid hydrolysis method (Ruiz and Ehrman, 1996). The hydrolysis products were determined by high performance liquid chromatography (HPLC; Agilent 1100, Agilent Technologies, Waldbronn, Germany) using a Pb^{2+} cation-exchange column (SP0810, Shodex, Showa Denko K.K., Kawasaki, Japan). The lignin content of EFB was determined by NREL LAP-003 (Templeton and Ehrman, 1995). Ethanol in SSF broth was analyzed by a gas chromatograph with a flame ion detector (Agilent 7890, Agilent Technologies, Wilmington, NC). All the analyses were conducted in triplicate.

2.6. Binding of carbohydrate-binding module to EFB

To examine the possible change in the accessibility of EFB to cellulase after ammonia pretreatment, the binding capacity of EFB to carbohydrate-binding module (CBM) was determined using a Type A surface-binding CBM cloned from *Clostridium thermocellum* (i.e., *Ct*CBD3) (Boraston et al., 2004). *Ct*CBD3 was produced as a recombinant protein in the purified form. The cloning, expression, and purification of the recombinant *Ct*CBD3 were conducted using a previously described method (Lee et al., 2010).

Binding experiments were conducted as follows. Five milligrams of substrates were incubated with the excessive amount of bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO; 12.5 μg/mL) as a control protein or *Ct*CBD3 (495 μg/mL) at 4 °C in 500 μL of 50 mM potassium phosphate buffer (pH 7) for 3.5 h. Subsequently, the reaction mixture was centrifuged at 16,000 rpm for 5 min to separate the unbound protein in the supernatant from bound protein in the pellet. The amount of unbound protein was then determined by the Bradford method (Bradford, 1976). The amount of bound protein was determined by the difference of amount between the initially added protein and the unbound protein.

3. Results and discussion

3.1. Effect of pretreatment conditions

To study the effect of temperature and S/L ratio on the aqueous ammonia pretreatment, pretreatment was conducted at 40, 60, and 80 °C and at S/L ratios of 1:6 and 1:12, while the pretreatment time and ammonia concentration were fixed at 12 h and 14% (w/w), respectively. The insoluble solids recovery yields remained approximately at 70% regardless of temperature and S/L ratio, in which these values were similar to those of other studies conducted on sugarcane bagasse, rice straw, and oil palm trunks using ammonia (Kim et al., 2010; Ko et al., 2009; Jung et al., 2011). In general, glucan recovery yield decreases as pretreatment severity such as an increase in temperature (Ko et al., 2009). In this study, the glucan recovery yields ranged from 82.3% to 84.6% and 78.0% to 89.6% at S/L ratios of 1:6 and 1:12, respectively, and the decrease of glucan yield with increasing temperature was not shown at both S/L ratios. At temperatures of 40–80 °C, the lignin removal ranged from 36.4% to 43.6%, and the amount of lignin removal slightly increased with an increase in temperature. Such removal of lignin is the typical chemical change induced by alkali pretreatment using materials such as ammonia (Kim et al., 2010; Ko et al., 2009; Jung et al., 2011). The comparison of the enzymatic digestibility of untreated and pretreated EFB using 15 FPU of enzyme revealed that the pretreatment of EFB at 60 °C and an S/L ratio of 1:12 increased the digestibility from 10.9% (untreated) to 22.7% (pretreated).

At the fixed conditions of 60 °C, an S/L ratio of 1:12, and ammonia concentration of 14%, when the effect of various time durations from 4 to 24 h was studied, the length of the pretreatment time did not have a significant effect on the recovery yields of the insoluble solids and glucan or the amount of lignin removal. A pretreatment time of 12 h, which gave the highest enzymatic digestibility of 21.9% and a lignin removal percentage of 40.9%, was selected as the optimum condition for additional experiments.

Catalyst concentration is another important factor confirming the effectiveness of pretreatment. In this study, 7%, 14%, 21%, and 28% (w/w) of ammonia concentrations were applied while fixing the other conditions at 60 °C, 12 h, and an S/L ratio of 1:12. Although pretreatment with increasing ammonia concentrations induced no significant trends in terms of insoluble solids and glucan recovery yield in the tested ammonia concentration range, 21% ammonia concentration was selected as the optimum condition based on the high lignin removal and enzymatic digestibility of pretreated EFB.

3.2. Effect of cellulase loading

Fig. 1 shows the results of EFB pretreated at optimal conditions and then hydrolyzed using 15, 30, 60, 100, and 150 FPU Accellerase 1000/g of glucan. When untreated EFB were digested with 15 FPU, the enzymatic digestibility after 96 h was 10.0% of theoretical maximum, but the 10-fold increase in cellulase loading from 15 to 150

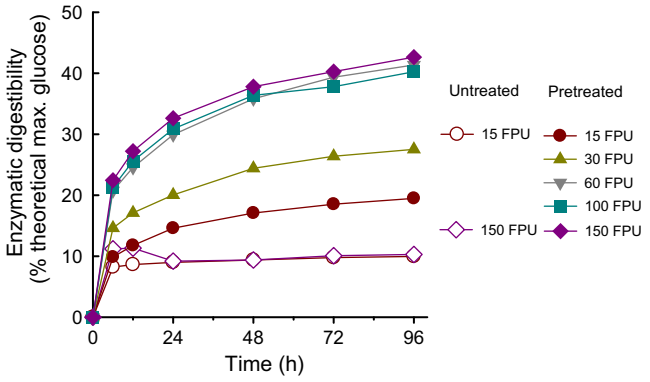

Fig. 1. Effect of cellulase loading with optimally pretreated EFB. The enzymatic hydrolysis was conducted using 15, 30, 60, 100 and 150 FPU of Accellerase 1000/g of glucan at pH 4.8, 50 °C, and 170 rpm for 96 h.