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Ethanol production from palm pressed fiber by prehydrolysis prior to simultaneous saccharification and fermentation (SSF)

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ABSTRACT

Palm pressed fiber (PPF) is a lignocellulosic material that has potential to be cheap substrate for ethanol production. In this study, the enzymatic hydrolysis of alkali pretreated PPF was investigated. It was found that the hydrolysis with cellulase (10 FPU g^{-1} of PPF) and β-glucosidase (10 U g^{-1} of PPF) gave the higher reducing sugar production than using cellulase alone. In addition, the optimum condition of simultaneous saccharification and fermentation (SSF) with 6 h of prehydrolysis for ethanol production was the PPF concentration of 100 kg m^{-3} with the enzyme loading of cellulase 6.0 FPU g^{-1} of PPF and β-glucosidase 3.0 U g^{-1} of PPF at pH of 5.0 and 35 °C. The ethanol concentration of 10.4 kg m^{-3} and ethanol yield of 192 g kg^{-1} of cellulose was obtained at the optimum condition in 24 h of SSF. Finally, the effect of prehydrolysis of pretreated PPF prior to SSF on the ethanol concentration was examined with batch and fed-batch mode of hydrolysis. It was found that prehydrolysis with the fed-batch mode did not improve ethanol yield. However, the fed-batch mode of prehydrolysis gave the highest ethanol production of 12.1 kg m^{-3} at 12 h of SSF process. Also, it was concluded that the advantages of fed-batch of prehydrolysis prior SSF were the less enzyme requirement and the increasing PPF loading for ethanol production.

1. Introduction

Palm oil mill industries are important companies in Thailand, especially in the south of Thailand. Palm oil mill production generates abundant wastes, such as oil palm empty fruit bunches (OPEFB), palm pressed fiber (PPF), palm kernels and palm nut shell corresponding to the quantity in the dry mass fraction of 50–59%, 12–14%, 1.0–5.0% and 8.0–11%, respectively [1,2]. Especially, PPF is a lignocellulosic material, which is a renewable resource [3,4], that can be processed either chemically or biologically to biofuel such as bioethanol [4]. It is less expensive than molasses and cassava starch and is available in large quantities.

Bioethanol production from lignocellulosic materials involves pretreatment of raw material, saccharification and fermentation [5]. Pretreatment is required to alter the structural and chemical composition of lignocellulosic biomass to facilitate rapid and efficient hydrolysis of carbohydrate to fermentable sugar [4,6]. Compared with acid pretreatment, alkaline processes cause less sugar degradation, less corrosion problems, and many of the caustic salts can be recovered and/or regenerated. Sodium, potassium, calcium, and ammonium hydroxides are suitable alkaline pretreatment agents [7]. However, sodium hydroxide has been frequently studied for biomass pretreatment [7–10]. In the saccharification, biomass is usually enzymatic hydrolyzed to obtain single sugar because of better yields than acid-catalyzed hydrolysis [11], controlled modification and fewer undesirable by-products, making it more suitable for microbial fermentation [12]. The widely mechanism for enzymatic

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cellulose hydrolysis involves synergistic actions by cellulase (including endoglucanase and exoglucanase) and β-glucosidase [13]. Endoglucanases hydrolyze accessible intramolecular β-1,4-glucosidic bonds of cellulose chains randomly to produce new chain ends, whereas exoglucanases processively cleave cellulose chains at the ends to release soluble cellobiose or glucose. Furthermore, β-glucosidases hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition [14]. Consequently, the reaction rate is reduced [15].

Moreover, the process used for saccharification and fermentation is also an important factor affecting the cost of ethanol production from lignocellulose [8]. Recently, there are many reports that the simultaneous saccharification and fermentation (SSF) is superior to the traditional saccharification and subsequent fermentation in the ethanol production because the SSF process can improve ethanol yields by removing end-product inhibition of saccharification process [8] and decrease the enzyme loading [16]. Moreover, SSF requires a single fermenter for the entire process and eliminate the need for separating reactors for saccharification and fermentation leading to reduce the investment cost [8,17]. Öhgren et al. [18] reported that the prehydrolysis is to partly hydrolyze the cellulose to oligomeric and monomeric sugars prior to the yeast addition, which would increase the ethanol production rate during the initial part of the SSF. Prehydrolysis also enables higher temperature during the initial enzymatic hydrolysis, potentially increasing the enzymatic activity and another important feature is that an enzymatic prehydrolysis of the lignocellulosic material decreased the viscosity of the substrate. Only limited researches, which studied the prehydrolysis prior to SSF in ethanol production, have been found in literature. In the previous study, only the batch mode of prehydrolysis was performed with solely cellulase enzyme. Unfortunately, they found that the prehydrolysis of steam pretreated corn stover was found to have no effect on the overall ethanol yield in SSF. The viscosity and mixing before SSF might affect on ethanol production [17]. The fed-batch mode of prehydrolysis might be the new approach to reduce viscosity of substrate and enhance good mixing during prehydrolysis.

Consequently, this study investigated the enzymatic hydrolysis of alkali pretreated PPF using cellulase and β-glucosidase. Moreover, the optimum conditions of SSF process with prehydrolysis, including substrate concentration, enzyme concentration, pH and temperature, for ethanol production from pretreated PPF were studied. Finally, the effect of prehydrolysis of pretreated PPF prior to SSF on the ethanol concentration was examined. The comparison of prehydrolysis with batch and fed-batch systems was also studied.

2. Materials and methods

2.1. Substrate and pretreatment

PPF was residues obtained from a palm oil extraction plant (the Pure Oil Company, Songkhla province, Thailand). Mostly, fruit oil palms were harvested from oil palms (*Elaeis guineensis*) with average age of 3–30 years. After PPF was collected from industry, it was dried in the sunlight for two days. The 100 g of dried PPF consisted of the cellulose, hemicellulose and lignin with the amount of (31.1 ± 1.34) g, (25.2 ± 1.37) g and (23.5 ± 1.98) g, respectively. Dried PPF was pretreated with 2.5 kmol m^{-3} of NaOH aqueous solution (solid to liquid ratio of 100 kg to 1 m^3) by boiling at 100 °C for 15 min. Afterward, the residues were recovered by cloth cheese filtration and washed with tap water until clean and colorless or neutral pH, dried at 60–70 °C in the oven, and ground to 24 mesh with hammer mill. After pretreatment, the 100 g of dried PPF consisted of the cellulose, hemicellulose and lignin with the amount of (54.0 ± 1.79) g, (20.8 ± 2.06) g and (19.8 ± 1.79) g, respectively.

2.2. Enzyme

Cellulase used in this study was the commercial enzyme from *Trichoderma reesei* (Sigma company) with filter paper activity of 217 FPU kg^{-1} of enzyme at pH 4.8 and 50 °C [11].

β-glucosidase was the commercial enzyme from *Aspergillus niger* (Fluka company) with a cellubiose assay of 66 U kg^{-1} of enzyme at pH 4.8 and 50 °C [12].

2.3. Enzymatic saccharification of pretreated PPF

Saccharification of pretreated PPF with enzyme was studied in 3 sets; Set I: cellulose at 10 FPU g^{-1} of PPF, Set II: cellulase at 20 FPU g^{-1} of PPF, Set III: combination of cellulase at 10 FPU g^{-1} of PPF and β-glucosidase at 10 U g^{-1} of PPF.

The pretreated PPF of 50 kg m^{-3} and citrate buffer of 0.05 kmol m^{-3} (pH 5.0) were added in Erlenmeyer flasks, then autoclaved at 121 °C for 15 min and cooled down to room temperature. Subsequently, enzyme with different loading was added. The mixture was incubated at either 35 °C or 50 °C in a rotary shaker at 2.7 Hz. Samples were taken at 0, 24, 48, 72 and 96 h, and heated to 100 °C immediately for 5 min to denature the enzyme. Afterward, the mixture was cooled down to room temperature and centrifuged for 20 min at 5514× *g*. The supernatant was used for determination of the reducing sugar content.

2.4. Preparation of yeast inoculum

Saccharomyces cerevisiae TISTR 5596 used in SSF experiments was maintained on YM slant (malt extract, 3.0 kg m^{-3}; glucose, 10 kg m^{-3}; yeast extract, 3.0 kg m^{-3}; peptone 5 kg m^{-3}; agar 15 kg m^{-3}). Active culture for inoculum was prepared by transferring microorganisms from YM slant into YM broth containing 40 kg $m^{-3}$of glucose and growing on at a rotary shaker 2.7 Hz 30 °C for 24 h. The volume fraction of inoculum used was 10% of the medium in the SSF with the cell concentration of 1.0×10^{14} m^{-3}.

2.5. Simultaneous saccharification and fermentation (SSF) with prehydrolysis

SSF experiments were performed in reaction 250 cm^3 Erlenmeyer flasks containing pretreated PPF, 90 kg m^{-3} to 110 kg m^{-3}; yeast extract, 1.0 kg m^{-3}; $MgSO_4 \cdot 7H_2O$, 0.03 kg m^{-3}; $(NH_4)_2HPO_4$, 0.5 kg m^{-3}; tween-20, 2.5 kg m^{-3};