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Pretreatment of empty fruit bunch from oil palm for fuel ethanol production and proposed biorefinery process

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ABSTRACT

This study evaluates the effects of some pretreatment processes to improve the enzymatic hydrolysis of oil palm empty fruit bunch (EFB) for ethanol production. The experimental results show that the bisulfite pretreatment was practical for EFB pretreatment. Moreover, the optimum pretreatment conditions of the bisulfite pretreatment (180 °C, 30 min, 8% $NaHSO_3$, 1% H_2SO_4) were identified. In the experiments, a biorefinery process of EFB was proposed to produce ethanol, xylose products, and lignosulfonates.

1. Introduction

Most supplies of oil palm are currently produced in South East Asia. Oil palm was introduced to South East Asia in the 19th century. Malaysia and Indonesia are the two largest producers of oil palm, collectively accounting for approximately 85% of the world oil palm production (Sulaiman et al., 2011). Lignocellulosic fibers can be extracted from the trunk, frond, fruit mesocarp, and empty fruit bunch (EFB) of an oil palm tree (Hasamudin and Soom, 2002). The palm oil industries of Indonesia and Malaysia collectively generate approximately 55.73 million tons of lignocellulosic agricultural waste and by-products every year (Akhtar et al., 2010; Abdullah et al., 2011).

EFB has been usually burned in incinerators of palm oil mills, causing environmental pollution (Rahman et al., 2007). EFB has been made into pulp as early as 1977. Furthermore, EFB can be chemically or biologically hydrolyzed to sugar to become a source of valuable carbon for the production of various chemicals (Kim et al., 2010). Sukiran et al. (2009) investigated the pyrolysis of oil palm EFB to produce bio-oils, as well as the effects of the biomass particle size and the parameters of the fast pyrolysis process on bio-oil properties. Pimenidou and Dupont (2012) investigated the nature of bio-oils from pinewood and palm EFB and discussed the importance of thermal degradation in refinery upgrading, combustion, and hydrogen production processes. Lignocellulosic EFB has recently been considered as a potential low-cost material, and an alternative renewable bioresource for the production of bioethanol (Sanchez and Cardona, 2008; Gnansounou and Dauriat, 2010; Hamzah et al., 2011; Han et al., 2011; Piarpuzán et al., 2011; Kim et al., 2012). Pretreatment is necessary for reducing the hemicellulose and lignin contents in EFB for effective bioethanol fermentation. Several studies have evaluated the effects of some pretreatment processes, such as alkaline pretreatment (Han et al., 2011; Piarpuzán et al., 2011), aqueous ammonia soaking pretreatment (Jung et al., 2011), sequential pretreatment with dilute acid and then alkali (Kim et al., 2012), on enzymatic digestibility of EFB. Pretreatment processes would ideally enhance the proportion of cellulose in the EFB, remove the hemicellulose or lignin to increase the pore size of the biomass, and increase the accessibility of cellulose (Hamzah et al., 2011; Han et al., 2011; Piarpuzán et al., 2011; Jung et al., 2011). However, to obtain high glucose yield in the enzymatic hydrolysis stage and simplify such process, an effective pretreatment process still needs to be investigated to fulfill the biorefinery of EFB.

Many researchers have investigated many pretreatments, which can be broadly classified into physical, chemical, physicochemical, biological, and combined pretreatments (Itoh et al., 2003; Yang and Wyman, 2004; Wingren et al., 2004; Pan et al., 2005; Mosier et al., 2005; Ewanick et al., 2007; Zhang et al., 2007; Kumar et al., 2009; Yoshida et al., 2004; Yu et al., 2009; Zhu et al., 2009, 2011; Wang et al., 2012). These pretreatments break the internal lignin and hemicellulose bonds and separate the lignin and hemicellulose fractions that can be potentially converted to useful products. Among these pretreatments, wet-oxidation (WO) and sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) have been reported as efficient processes (Zhu et al., 2009, 2011; Wang et al., 2012; Carlos et al., 2008; Efthalia et al., 2012). WO is the process of treating a material with

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water and air or oxygen at temperatures above 120 °C. The mechanism lies on formed hydroxyl radicals, whereas auto-catalyzing lies on formed organic acids. Hemicellulose is degraded and dissolved into water during WO pretreatment, and the addition of alkali can cause lignin degradation and removal. WO can also be performed using oxidation agents, such as hydrogen peroxide (H_2O_2) (Azzam, 1989). The SPORL process was proven to be a robust and efficient pretreatment in terms of enzymatic cellulose conversion and ethanol production, particularly when it is applied to highly recalcitrant softwood species. SPORL can remove hemicellulose or lignin by adjusting the pH value of pretreatment liquor. In our preliminary study, the effects of alkaline and dilute-sulfuric acid pretreatments on the chemical components and enzymatic digestibility of EFB were evaluated. In this study, the oxygen-alkali (OA) and bisulfite pretreatments were selected applied on EFB based on the pretreatment results and on the chemical components of EFB. This study provides the experimental results that describe the feasibility of using EFB for ethanol production. Moreover, a biorefinery process of EFB was proposed.

2. Methods

2.1. Materials

The oil palm EFB used in this study was obtained from Malaysia. EFB was milled to the particle size in the range of 0.30–0.45 mm (diameter) and then stored in sealed bags at room temperature. The glucan, xylan, and lignin contents of EFB were analyzed as follows: 36.8% glucan, 19.3% xylan, and 17.9% lignin.

The cellulase (Sino Enzymes R) with a filter paper activity of 160 IU/g and β-glucosidase activity of 40 IU/g was purchased from Baiyin Sainuo Technology Ltd. (Gansu province, PR China). The *Saccharomyces cerevisiae* used in the ethanol fermentation was of commercial grade and purchased from Angelyeast Corporation Ltd. (Hubei, China). Sodium bisulfite, sulfuric acid, sodium hydroxide, and hydrogen peroxide were used as analytical reagents.

2.2. Pretreatment of EFB

The pretreatment processes used in the experiment are described below.

2.2.1. Oxygen-catalyzed pretreatment

Oxygen-catalyzed pretreatment was conducted in a rotary electrothermal pressure digester. Oxygen was placed in the digester to stated pressure prior to heating. The pretreated samples were washed, and the chemical components were analyzed using the National Renewable Energy Laboratory (NREL, USA) protocol.

Five oxygen-catalyzed processes, including the WO, Fe^{3+}-catalyzed WO, OA, H_2O_2-enhanced OA, and OA with alkaline impregnation processes were used for EFB pretreatment in an attempt to improve the enzymatic digestibility of EFB. The process conditions in the pretreatment are as follows:

WO process: oxygen pressure of 0.6 MPa, treatment time of 30 min, dry solid weight to liquor volume ratio of 1:8, and treatment temperature of 120 °C;
Fe^{3+}-catalyzed WO process: 0.5% $Fe_2(SO_4)_3$ on dry solid weight, oxygen pressure of 0.6 MPa, treatment time of 30 min, dry solid weight to liquor volume ratio of 1:8, and treatment temperature of 120 °C;
OA process: 1.6% NaOH on dry solid weight, oxygen pressure of 0.6 MPa, treatment time of 30 min, dry solid weight to liquor volume ration of 1:8, and treatment temperature of 120 °C;
H_2O_2-enhanced OA process (OA + H_2O_2): 10% NaOH and 0.5% H_2O_2 on dry solid weight of solid, oxygen pressure of 0.6 MPa, treatment time of 30 min, dry solid weight to liquor volume ratio of 1:8, and treatment temperature of 120 °C;
OA process with alkaline impregnation process: An alkaline impregnation stage was introduced to improve the enzymatic digestibility of EFB, in which the EFB was first impregnated with a diluted soda solution (5 g/L NaOH) at 70 °C for 60 min to improve the permeability of the EFB. After alkaline impregnation, the soaked EFB was further treated using the H_2O_2-enhanced OA process with different dosages of NaOH.

2.2.2. Bisulfite process

The sodium-based liquor was prepared as needed from reagent grade sodium sulfite ($NaHSO_3$) and sulfuric acid (H_2SO_4). In this process, the EFB samples reacted with a solution of sodium bisulfite at 180 °C and pH level in the range of 2–4 for 30 min in batch operations. The pretreatment liquor to wood ratio can be as low as 4. The pretreatment was conducted in 1 L stainless steel reactors with an electrical heating system. After the pretreatment, the spent liquor was separated from the solid (pretreated substrate) via filtration and then stored for chemical analysis. The solid substrate was collected, washed thoroughly with tap water, and then stored in plastic bags for further analysis and enzymatic hydrolysis.

2.3. Enzymatic hydrolysis of pretreated samples

The pretreated EFB samples were hydrolyzed in 100 mL flasks by using a commercial cellulase to evaluate their enzymatic digestibility. The enzymatic hydrolysis conditions are as follows: 2% solid concentration (on dry weight), pH level of 4.8 (0.05 M sodium citrate buffer), 45 °C, 150 rpm in a shaker, and cellulase dosage of 20 FPU/g dry sample. Hydrolysates were taken at predetermined intervals, and then centrifuged. The supernatant was used for glucose content analysis. Glucose was measured using the SBA-40C biological sensor analyzer. Conversion of cellulose to glucose was calculated as follows:

$$\text{Conversion of cellulose } (\%) = \frac{\text{Glucose released from enzyme hydrolysis (mg)} \times 0.9}{\text{Sample weight (mg)} \times \text{Glucan content } (\%)} \times 100\%$$

2.4. Quasi-simultaneous saccharification and fermentation (Q-SSF)

The yeast required activation treatment (0.12 g yeast, with 0.16–0.32 g of glucose, 8–16 mL of sterile water). The cultures were grown in a shaker bath at 30 °C and 200 rpm. The Q-SSF experiments consisted of a pre-hydrolysis phase and an SSF phase. First, 5.4 g of the dry sample of the solid fraction (approximately 18% of solid concentration) were added to the 100 mL fermentation flasks containing approximately 30 mL of citric acid buffer medium (0.05 M, pH level of 4.8). The flasks were then sterilized in an autoclave at 115 °C for 30 min, and then enzyme (the enzyme loading of 20 FPU/g dry sample) was added to the flasks. In the pre-hydrolysis phase, the medium temperature and pH level were maintained at 35 °C and 4.8, respectively. After pre-hydrolysis, 2 mL of *S. cerevisiae* was added into the medium, and then SSF was conducted. Moreover, 200 μL aliquots from the broth were taken periodically and then centrifuged, and the supernatant was used for ethanol content analysis. Ethanol was measured using the SBA-40C biological sensor analyzer.

2.5. Analytical methods

Moisture content and ethanol-extractives were determined according to the analytical procedure of the NREL (NREL, 2006).