



## Dilute acid pretreatment of lignocellulose for whole slurry ethanol fermentation



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### HIGHLIGHTS

- ▶ EFB were pretreated with 1% (w/v) sulfuric acid at 190 °C.
- ▶ Washed and pretreated EFB showed 88.5% glucose yield in the enzymatic hydrolysis.
- ▶ Whole pretreated EFB gave 87.5% ethanol yield based on total glucan of initial EFB.

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### ABSTRACT

Dilute sulfuric acid pretreatment of oil palm empty fruit bunches (EFB) followed by the whole slurry fermentation of the pretreated EFB slurry was investigated. The optimized pretreatment conditions were at 1% (w/v) sulfuric acid with 3 min ramping to 190 °C in a microwave digester. Pretreated and washed EFB exhibited enzymatic digestibility of 88.5% of theoretical glucose yield after 48 h of hydrolysis. When the whole slurry of pretreated and neutralized EFB was used in simultaneous saccharification and fermentation (SSF) using cellulase and *Saccharomyces cerevisiae*, sulfuric acid-pretreated EFB resulted in 52.5% of theoretical ethanol yield based on total glucan in the untreated initial EFB after 72 h of SSF. When pretreated EFB slurry was treated with activated carbon before subjecting to SSF, the SSF furnished 87.5% ethanol yield based on the initial glucan content in untreated EFB (after 48 h of SSF).

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### 1. Introduction

Oil palm empty fruit bunches (EFB) are the main waste products of the palm oil processing industry. They are considered to be one of the most abundant lignocellulosic feedstocks available for the production of biofuels. For instance, the annual production of EFB is approximately 25.9 million tons in Indonesia and 19.8 million tons in Malaysia (Bardant et al., 2012; Daud and Law, 2011). The main carbohydrates of EFB such as glucan and xylan are present in high contents such as 35–50% and 10–20%, respectively; therefore, EFB are considered ideal biomass for ethanol production (Jung et al., 2011; Millati et al., 2011). However, EFB are currently left in waste ponds for self-decomposition or are burnt for generating power and producing ash for fertilizer (Millati et al., 2011).

The cellulosic ethanol production from lignocellulose consists of 3 main steps such as pretreatment, enzymatic hydrolysis of cellulose, and ethanol fermentation (Lynd et al., 2005). Production of fermentable sugar from lignocellulose, which involves pretreat-

ment and saccharification, is the bottleneck in the cellulosic ethanol production (Lynd et al., 2008). To date, various physicochemical pretreatment methods such as acid, alkali, hydro-thermal, steam explosion, and wet oxidation processes have been studied (Agbor et al., 2011; Zheng et al., 2009). Among them, acid or alkali pretreatment is considered the most mature technology for ethanol production from lignocellulose (Garlock et al., 2011; Kim et al., 2005; Ko et al., 2009). However, when using acids or alkalis at severe conditions involving a high temperature or a long time duration of pretreatment, the generation of substances inhibitory to fermentative microorganisms, such as carboxylic acids, sugar degradation products, phenolic compounds, and inorganic salts, is not avoidable (Klinke et al., 2004). Alternative pretreatment processes using fungi, electron beam, or ionic liquids are under consideration, but they are far from commercialization (Bak et al., 2009a,b; Shill et al., 2011).

In the acid or alkali pretreatment of lignocellulose, substantial amounts of sugars are generally solubilized from hemicellulose and cellulose into a liquid phase of pretreated biomass slurry (Kim et al., 2005; Ko et al., 2009). To reduce the cost of the separation process and to utilize all the sugars available in the pretreated

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slurry, it is desirable to ferment the whole slurry (liquid plus solid fractions together) of pretreated biomass. Most pretreatment studies in the literature have been focused on the enzymatic digestibility test of washed pretreated biomass (Kim et al., 2011a; Pallapolu et al., 2011; Selig et al., 2008) or the simultaneous saccharification and fermentation (SSF) of washed pretreated biomass only (Lau et al., 2010; Zaldivar et al., 2000). Only a few studies are available regarding enzymatic hydrolysis and ethanol fermentation of whole slurry of pretreated lignocellulose (Dutta et al., 2010; Tengborg et al., 2001).

In a previous study, aqueous ammonia soaking pretreatment was exploited to evaluate the use of EFB for ethanol production (Jung et al., 2011). Because EFB contain a relatively high amount of lignin, ammonia soaking was found to be effective for removing lignin from lignocellulose (Ko et al., 2009). However, the lignin removal in the previous study (Jung et al., 2011) was less than 50% of its initial amount, and the enzymatic digestibility of pretreated EFB was less than 45% of its theoretical maximum glucose yield. The high recalcitrance of EFB was also shown in other studies that reported a low enzymatic digestibility of EFB after pretreatment using steam or sodium hydroxide (Shamsudin et al., 2012; Sudiyani et al., 2010). In this study, to increase the saccharification and ethanol yields from EFB, the dilute-acid pretreatment followed by the fermentation of whole slurry of pretreated EFB was developed. In this context, the effectiveness of dilute sulfuric acid which was found to increase the enzymatic digestibility of lignocellulose by preferentially solubilizing hemicellulose during pretreatment was investigated for EFB pretreatment, and the simultaneous saccharification and ethanol fermentation of the whole slurry of pretreated EFB containing the liquid phase was studied. The fermentation of the whole pretreated slurry could increase the utilization of sugar in the liquid fraction and minimize the solid/liquid separation cost, which will eventually reduce the production cost of cellulosic ethanol.

## 2. Methods

### 2.1. Biomass and composition analysis

Oil palm EFB were obtained from the Tropical Chase plantations (Kuala Lumpur, Malaysia), and they were washed and ground by a high-speed rotary cutting mill (MF 10, IKA, Staufen, Germany) to provide particle sizes in the range of 125–706  $\mu\text{m}$  (25–120 mesh). Avicel (Sigma–Aldrich, St. Louis, MO) was used as pure cellulose for control experiments. Compositional analysis of pretreated or untreated EFB was carried out by following the Laboratory Analytical Procedure (LAP) of National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). Glucose, xylose, galactose, arabinose, and mannose were analyzed by high performance liquid chromatography (HPLC; Agilent 1100, Agilent Technologies, Waldbronn, Germany) equipped with an SP0810 column ( $\text{Pb}^{2+}$  form; Shodex, Showa Denko, Kawasaki, Japan) and a refractive index detector (RID; G1362A, Agilent Technologies). Ethanol and various inhibitors found in pretreatment slurry, including furfural, hydroxymethylfurfural (HMF), acetic acid, glycerol, levulinic acid, and formic acid, were analyzed by HPLC equipped with an Aminex HPX-87H column (Biorad, Hercules, CA) and an RID. All analyses were conducted in triplicate. The initial composition of EFB on a dry weight basis was 39.2% (w/w) glucan, 17.4% xylan, 2.5% galactan, 4.5% arabinan, 31.8% lignin, and 2.8% ash.

### 2.2. Acid pretreatment of EFB

Sulfuric acid (Daejung Chemicals & Metals, Siheung, Korea) was used as the catalyst for the pretreatment of EFB. Ground EFB (2 g)

were suspended in different concentrations of sulfuric acid solution (20 mL) at a solid-to-liquid ratio of 1:10 in an SK-12 type vessel with an internal volume of 100 mL (Milestone, Shelton, CT) that was equipped with a thermocouple. The vessel containing EFB and the acid solution mixture was loaded into an Ethos EZ Microwave Digestion Labstation (Milestone), and the microwave digester was run at 1200 W for various holding times with 3 min ramping to a set temperature. Pretreated EFB slurry was then filtered using a filtration cloth (pore size: 22–25  $\mu\text{m}$ ; Calbiochem, La Jolla, CA) to separate insoluble solids, and the filtered solids were washed with approximately 1 L of distilled water until a neutral pH was reached. The washed insoluble solids from pretreated EFB were transferred into a weighing dish and dried in a drying-oven (SH-450; BioFree, Seoul, Korea) at 45 °C for more than 3 days. The total solids content of the dried insoluble solids was determined in triplicate in a drying oven at 105 °C.

### 2.3. Enzymatic digestibility of pretreated EFB

To evaluate the effectiveness of EFB pretreatments at various conditions, insoluble solids of pretreated and washed EFB were subject to enzymatic hydrolysis with 1% (w/v) glucan loading and 15, 30, or 60 filter paper units (FPU) of cellulase (Celluclast 1.5 L, Novozymes, Bagsvaerd, Denmark) and 30 cellobiase units (CBU) of  $\beta$ -glucosidase (Novozyme 188, Novozymes) in accordance with the LAP of NREL (Selig et al., 2008). For the hydrolysis of EFB, a reaction mixture containing EFB, cellulase,  $\beta$ -glucosidase, and 0.05 M sodium citrate buffer (pH 4.8) was incubated in a shaking incubator at 50 °C and 170 rpm. The reaction mixture was analyzed for the quantity of glucose or reducing sugar at specific time intervals by HPLC or the dinitrosalicylic acid (DNS) assay, respectively.

### 2.4. Activated carbon treatment of pretreated EFB slurry

Activated carbon was used to remove inhibitors generated during the chemical pretreatment. Activated carbon granules (Dae Jung Chemicals & Metals) were washed with water and equilibrated with 0.4 M hydrochloric acid solution (Sigma–Aldrich). The hydrochloric acid-treated activated carbon was washed with water and dried at room temperature. For the activated carbon treatment of pretreated EFB slurry, the liquid fraction was separated using the filtration cloth. Activated carbon was added to the liquid fraction at a solid-to-liquid ratio of 1:10 and stirred for 1 h at room temperature. The activated carbon-treated liquid fraction was separated using the filtration cloth and then merged with the solid fraction before SSF for the whole slurry fermentation.

### 2.5. Simultaneous saccharification and fermentation

To evaluate the ethanol fermentability of pretreated EFB, SSF was carried out in SSF media consisting of 1% (w/v) yeast extract and 2% (w/v) peptone in a 0.05 M citrate buffer (pH 4.8). For the SSF of washed insoluble solids, only pretreated and washed insoluble solids of EFB were added to the SSF media at the final glucan concentration of 3% (w/v). For the whole slurry fermentation of pretreated EFB, it was neutralized to pH 4.8 ( $\pm 0.2$ ) with 28% (w/w) ammonia solution (Junsei Chemical, Tokyo, Japan) before SSF. For the SSF of whole slurry of pretreated biomass, pretreated and neutralized whole slurry of EFB or pretreated, activated carbon-treated, and neutralized whole slurry of EFB were added to the SSF media at the final EFB concentration of 6% (w/v) assessed on the basis of initial EFB before pretreatment. After autoclaving the SSF media containing medium components and pretreated EFB at 121 °C for 30 min, 30 FPU of cellulase (Celluclast 1.5 L) and 30 CBU of  $\beta$ -glucosidase (Novozyme 188), which were both per gram of glucan contained in washed EFB (for SSF of pretreated

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