Steam explosion pretreatment of Oil Palm Empty Fruit Bunch

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Abstract

Oil Palm Empty Fruit Bunch (OPEFB), a lignocellulosic agricultural waste, composes of cellulose 62 %, hemicelluloses 28 % and lignin 18 % w/w in general. To maximize fermentable sugars released from the OPEFB by enzymatic hydrolysis, an effective pretreatment method is required. Due to high content of lignin, NaOH impregnated OPEFB steam explosion was studied. Optimal condition of the pretreatment method was hammer milled OPEFB (2 mm particle size, 10 % (w/v) loading) which was soaked in 2 M NaOH for 16 h, steam explosed at 200°C for 5 minutes. Maximum reducing sugars liberated was 22.21 g/l after the pretreated OPEFB was hydrolysed by Accellerase 1500 (13,404 CMC U/g, 3281 pNPG U/g) at 50°C for 6 h.

Keywords: oil palm empty fruit bunch, steam explosion, enzymatic hydrolysis

Introduction

Oil palm is an important economic plant of Thailand. Their seeds were raw materials for palm oil production. In the palm oil production process, fresh fruit bunch was subjected to steam sterilization to reduce microbial contamination and separate seeds from its bunch for extraction of oil. Large amount of oil palm empty fruit bunch (OPEFB) was left as an agricultural waste. These waste compose of cellulose, hemicelluloses and lignin as a major component. Due to their chemical composition, availability in large quantity, renewable and low in cost (Sarkar et al., 2012), They are interesting source of fermentable sugar for second generation bio-ethanol production. Bio-ethanol production from lignocellulosic substrate can be categorized into three steps. First, pretreatment, hemicelluloses and lignin were removed or separated from cellulose. Second, hydrolysis of the cellulose to glucose by cellulolytic enzymes. Third, fermentation of the glucose to ethanol by microorganism. To hydrolyze OPEFB to fermentable sugar, pretreatment is require to separate hemicelluloses and lignin from cellulose. Since OPEFB composed of 18.5% (w/w) lignin (Law et al., 2007), therefore the OPEFB was pretreated by steam explosion method. Steam explosion is a physico-chemical pretreatment method which raw material is subjected to high temperature (160-200°C) under high pressure for short time (Sun et al., 2002). Under this condition, moisture in raw material explored which is degradation of lignin and change of acetyl group in hemicelluloses to acetic acid which degrades glycosidic bond of cellulose. After crystalline structure of cellulose is disrupted, enzyme accessibility to cellulose is increased which entailed in high liberation of glucose (Ramos et al., 2003). OPEFB pretreated by NaOH gave highest reducing sugar (Umikalsom et al., 1998) after enzymatic hydrolysis when compared to OPEFB pretreated by HNO3, HCl, EDA and EDTA. In addition, the reducing sugar liberated improved by 2-fold by performing the NaOH pretreatment at 121°C (5 min).

Microscopic observation revealed silica-body penetrated randomly along surface of OPEFB. These silica-body decreased enzyme accessibility to OPEFB. However, grinding and washing of the OPEFB could remove an effect of the silica-body (Law et al., 2007). Comparison of physical, chemical and thermal pretreatments on the enzymatic hydrolysis of OPEFB, indicated that combination method (NaOH treated OPEFB, then autoclave at 121°C (5 min)) gave the highest sugar (Ariffin et al.,2008). In this study, NaOH-impregnated OPEFB was pretreated by steam explosion. Pretreatment condition was optimized by various method. Condition for enzymatic hydrolysis of OPEFB pretreated at the optimal condition was also optimized.

Methodology

Oil palm empty fruit bunch

Oil palm empty fruit bunches (OPEFB), 7.7% (w/w) moisture content, were collected from Thai Tallow and Oil Co. Ltd., in Suratthani province, Thailand. The OPEFB were shredded by Hammer-mill, sieved for 0.25-2 mm, 2-10 mm and 1 cm of fiber length and kept at 4°C until used.

Chemical compositions analysis

Chemical composition of the OPEFB was analyzed by TAPPI (Technical Association of the Pulp and Paper Industry) analytical method for Alpha-, Beta- and Gamma-cellulose (TAPPI T203 cm-99) and acid insoluble lignin (T222 om-02) contents at Department of Science Service, Ministry of Science and Technology, Bangkok.

Optimization of steam explosion pretreatment

The OPEFB was soaked in 0.5 M NaOH at 10% (w/v) dry weight for 16 h, then filtered by sieving to recovery solid particles. NaOH-impregnated OPEFB fiber was further pretreated by steam explosion method using high pressure reactor (Parr Instrument Company, model 4523, USA) at 3% (w/v) substrate loading, 200°C for 2 min. Pretreated OPEFB separated from pretreatment hydrolysate by filtration was washed with distilled water until pH reached neutral then hydrolyzed by cellulose, Accellerase™ 1500 (Genencor, Finland), at 4,468 CMC units/g and 1,094 pPNG units/g (dry weight). The enzymatic hydrolysis was performed by suspending the pretreated OPEFB (10%, w/v (dry weight)) in 100 mM Sodium-citrate buffer pH 6.0 and incubated at 50°C for 6 h. Reducing sugar liberated into enzymatic hydrolysate was analyzed by Somogyi-Nelson method (1952). The pretreatment condition was optimized by univariating of OPEFB fiber length (0.25-2 mm, 2-10 mm and 1 cm), substrate loading (2, 3 and 4% (w/v)), NaOH concentration (0-2.5 M), pretreatment time (2,5 and 10 min) and pretreatment temperature (160, 200 and 240°C). Condition which gave highest reducing sugar liberation was used in next experiments.

Optimization of enzymatic hydrolysis

The cellulolytic hydrolysis of pretreated OPEFB was optimized by sequential varying of enzyme dosage and pH reaction. The condition which gave highest reducing sugar was used in next experiments.

Results

Morphology and chemical composition of OPEFB

Outer surface of OPEFB fiber was smooth, while inner surface was rough as shown in Fig.1A and Fig.1B.

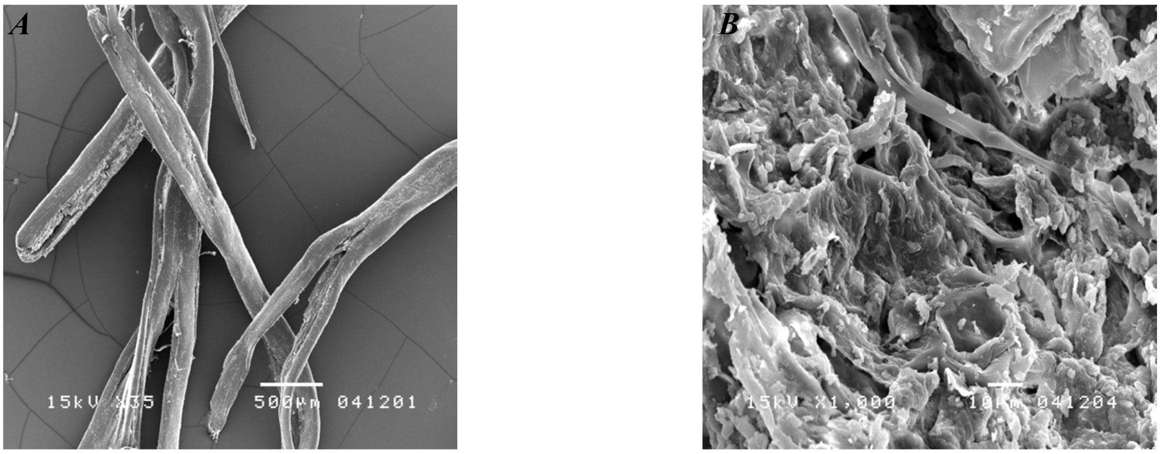

Figure 1: OPEFB observed under scanning electron microscope. (A) Outer surface (35X). (B) Inner surface (1,000X)

The OPEFB had alpha-cellulose as major component (47.9%, w/w) (Table 1). Its hemicellulose content calculated from difference between holocellulose and alpha-cellulose content was 16.8% (w/w). An acid-insoluble lignin was 18.3% (w/w).

Table 1: Chemical compositions of OPEFB

Component	**Dry weight (%)**
Lignin	18.3
Holocellulose	64.7
Alpha-cellulose	47.9
Beta-cellulose	9.2
Gamma-cellulose	7.6

Optimal condition for steam explosion pretreatment

Various lengths of OPEFB fiber (0.25-2 mm, 2-10 mm and 1 cm) were soaked in 0.5 M NaOH at 10% (w/v, dry weight) for 16 h, then subjected to steam explosion pretreatment. After the pretreatment, pretreated OPEFB was washed with distilled water and hydrolyzed by Accellerase^{TM} 1500 for 6 h. It was found that the OPEFB fiber (2-10 mm in length) liberated highest reducing sugar (6.031 g/l) (Fig. 2).

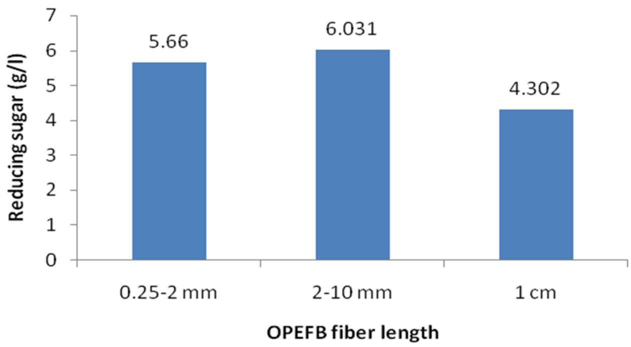

Figure 2: Reducing sugar liberated from various fiber length of pretreated OPEFB

The OPEFB fiber (2-10 mm length) was subjected to steam explosion pretreatment at various substrate loading (2, 3 or 4%, w/v) by the same above condition. It was found that reducing sugar liberated was highest when OPEFB was pretreated at 3% (w/v) substrate loading (Fig. 3).

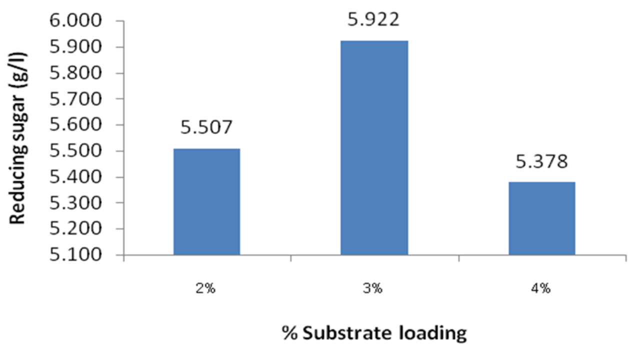

Figure 3: Effect of substrate loading (w/v) on reducing sugar liberation of pretreated OPEFB

OPEFB fiber (2-10 mm in length) was soaked in various concentration of NaOH (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 M), then pretreated by steam explosion using 3% (w/v) substrate loading as described above. Reducing sugar liberated after hydrolysis by Accellerase^{TM} 1500 was highest when the OPEFB was soaked in 2M NaOH (Fig. 4)

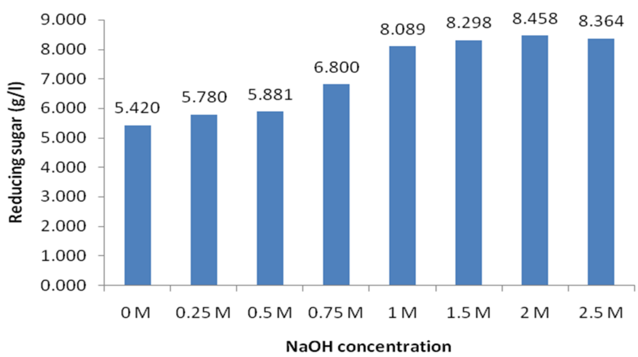

Figure 4: Reducing sugar liberated from pretreated OPEFB soaked in various concentration of NaOH.

The 2 M NaOH - impregnated OPEFB fiber was pretreated by steam explosion as described above, except pretreatment temperature was varied (160, 200 and 240°C). The OPEFB pretreated at 200°C gave the highest reducing sugar (8.398 g/l) after hydrolysis by Accellerase^{TM} 1500 (Fig. 5). The reducing sugar liberated was increased to 8.86 g/l when OPEFB pretreated by the same above method at 200°C, but pretreatment time was extended from 2 to 5 min (Fig. 6).

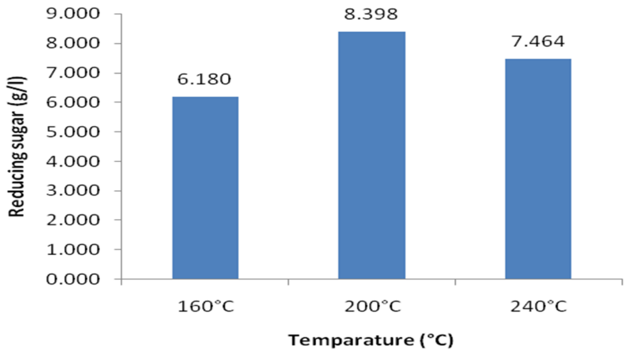

Figure 5: Effect of pretreatment temperature on reducing sugar liberated from pretreated OPEFB

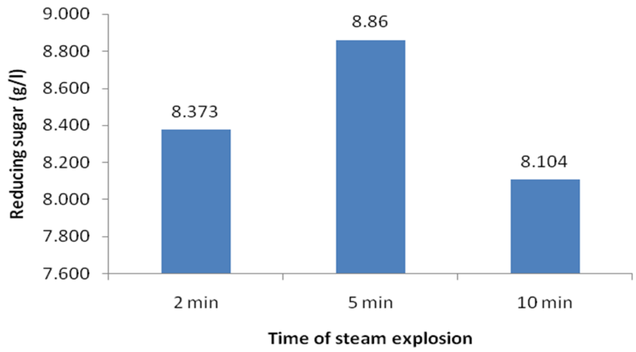

Figure 6: Effect of pretreatment time on reducing sugar liberated from pretreated OPEFB

Optimization of cellulolytic hydrolysis of the pretreated OPEFB

OPEFB pretreated at the optimized condition was hydrolyzed by Accellerase^{TM} 1500 at various pH (4.0, 4.5, 5.0, 5.5 and 6.0). The pretreated OPEFB which was hydrolyzed at pH 4.5 gave highest reducing sugar (13.229 g/l) (Fig. 7). When dose of the Accellerase^{TM} 1500 was increased to 13,405 CMC units/g and 3,281 pPNG units/g), dry weight, the reducing sugar liberated increased to 22.213 g/l or 0.22 g/g OPEFB (Fig. 8).

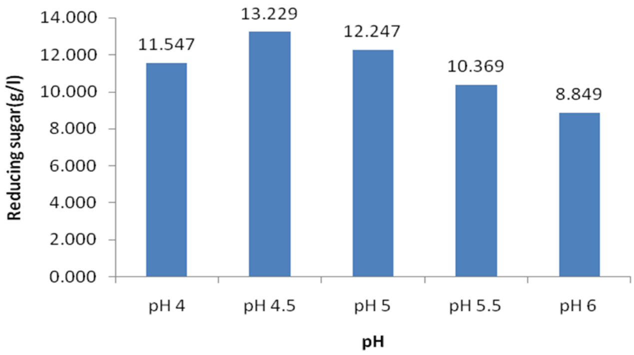

Figure 7: Effect of pH on cellulolytic hydrolysis of pretreated OPEFB

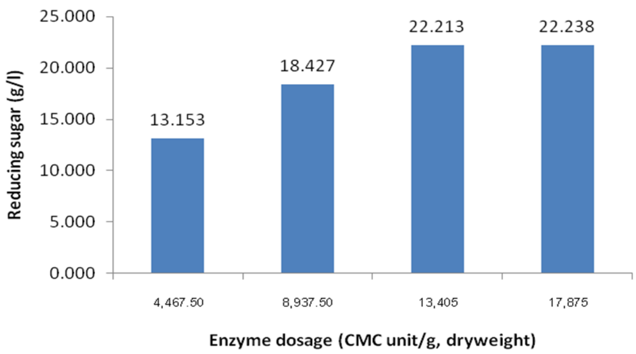

Figure 8: Effect of enzyme dosage on cellulolytic hydrolysis of pretreated OPEFB

Discussion

The oil palm empty fruit bunch (OPEFB) was soaked in NaOH, then subjected to steam explosion pretreatment. The pretreated OPEFB was further hydrolysed by cellulase for 6 h. Reducing sugar released into the hydrolysate was used as pretreatment efficiency index.

Optimal condition for steam explosion pretreatment of the OPEFB was 2-10 mm fiber length, soaked in 2 M NaOH (10 %, w/v) for 16 h then steam explosion pretreatment at 3 % (w/v) loading at 200°C for 5 min. OPEFB fiber length of < 2 mm was easily lost during filtration to recovery solid residue while long fiber length provided low surface area for enzyme digestion.

The OPEFB sample contained 18.3 % (w/w) of lignin which was coincided to previous report (Law et al., 2007). Lignin is a cross-link polymer of phenolic-monomer which covered cellulose and hemicelluloses component in lignocelluloses structure. Soaking the OPEFB in NaOH, the alkalinity caused lignocellulosic structure swollen, the 3major components: lignin, hemicelluloses and cellulose were separated and easy to separate from each others. OPEFB pretreated at the optimized condition released maximum reducing sugar (22.213 g/l or 0.22 g/g OPEFB) after hydrolysed by cellulase.

Conclusion

Based on this study, optimal conditions for steam explosion pretreatment of OPEFB were 2-10 mm fiber length, soaked in 2 M NaOH at 10% w/v (dry weight) for 16 h, then steam explosion pretreatment the NaOH-impregnated fiber at 3 % (w/w), substrate loading, 200°C for 5 min. Pretreated OPEFB at the optimized condition hydrolyzed by Accellerase^{TM} 1500 (13,405 CMC unit/g) at 50 °C, pH 4.5 for 6 h liberated maximum reducing sugar (22.213 g/l).

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