Simultaneous Saccharification and Fermentation of Oil Palm Empty Fruit Bunch Fiber Kraft Pulp to Produce Ethanol

Yanni Sudiyani[1)], Irni Fitria[1)], Tami Idiyanti[1)], Wawan K Haroen[2)], Euis Hermiati[3)]

[1)] Research Center for Chemistry, Indonesian Institute of Sciences
Kawasan Puspiptek Serpong, 15314, Tangerang, Indonesia
[2)] Balai Besar Pulp dan Kertas, JL Dayeuh Kolot Bandung, Indonesia
[3)] R&D Unit for Biomaterials- Indonesian Institute of Sciences
JL.Raya Bogor Km 46, Cibinong, Bogor 16911, Indonesia

Corresponding outhor: email *ysudiyani@ yahoo.com* (Yanni Sudiyani)

Abstract

The effects of substrate concentration on ethanol production from simultaneous saccharification and fermentation (SSF) of oil palm empty fruit bunch (EFB) kraft pulp by cellulase and *Saccharomyces cerevisiae* MK1 were studied. Substrate concentration was varied from 10 to 20%. Cellulase was added at 4 g l^{-1} substrate, while the yeast inoculum was 10% (w v^{-1}). The SSF process was carried out at 37°C, pH 4.5, 100 rpm for 76 hours. Ethanol production were obtained after 24 hours, however, ethanol concentration was decreased after 60 hours of fermentation. The highest ethanol concentration was obtained from samples with substrate concentration of 15% (w v^{-1}). A much higher ethanol concentration was produced when that particular substrate was supplemented with 2% of sugar.

Key words: ethanol, oil palm empty fruit bunch, pretreatment, simultaneous saccharification and fermentation

Introduction

Energy-related problems are likely to become more and more important in the near future. The increasing energy demand from emerging economies versus the day by day decreasing storage of energy resources, the rising cost of fossil fuels and the considerable environmental impact connected with their exploitation are implications the policy makers can not any further disregard. The most important consideration for development of energy in the near future is how to find more energy alternatives from renewable resources. One of them is to develop bio-fuel, such as ethanol, from biomass.

Oil palm empty fruit bunches (EFBs) consist of lignocellulosic material accompanying oil palm fruits. The oil palm used in this study is known as African palm (*Elaeis guineensis*) because it is originated from West Africa. Indonesia is currently the greatest world producer of palm oil, with 51% of the world output; therefore, palm oil constitutes a great asset for this country. The major biomass byproduct from the palm oil industry is EFBs, the fibrous residue remaining after the palm fruits are separated in the mills. Generally, the palm oil industry disposes about 1.1 ton of EFB for every ton of crude palm oil (CPO) produced. Indonesia now produces about 5 million metric tons of EFBs (at 60% moisture). Currently, EFBs are often incinerated at the plant site, which causes some air pollution.

Being abundant lignocellulosic waste, oil palm EFB has a great potency as a source of cellulose. Cellulose is the major component of lignocellulose and can be hydrolyzed to glucose, which can be fermented to bioethanol. Therefore, bioethanol from lignocellulose has been interest in recent decades (Ballesteros *et al.* 2004; Han *et al.* 2008; Tang *et al.* 2006). Enzymatic hydrolysis of cellulose to glucose is of central importance in corvension of lignocellulose to bioethanol (Curreli *et al.* 2002; Yang *et al.* 2006). Since enzymatic hydrolysis of native lignocellulose usually results in lower cellulose solubilization, pretreatment are generally applied to enhance substrate accessibility (Saha *et al.* 2005).

Pulping is a process to recover cellulose by removing as much as possible lignin and hemicellulose from lignocellulosic materials. The cellulose then becomes a substrate that would be much easier to be hydrolyzed by cellulasae enzymes. Another factor that might affect the hydrolysis and fermentation is substrate concentration. Further, according to our previous research, initial glucose in the substrate increased the rate and production of ethanol. Therefore, in this study the SSF process was used the EFB kraft pulp and the effects of substrate concentration and the addition of initial sugar in the substrates on ethanol concentration and yield were studied.

Materials and Methods

Raw material

Oil palm EFB was collected from an Oil Palm Plantation belongs to PT Perkebunan Nusantara VIII, in Pandeglang, Banten, Indonesia. The EFB was dried and ground to particle sizes that passed 30 mesh sieves, then it was stored in sealed plastic bags at room temperature until it was used for pretreatment.

Analyses of raw material

The EFB were characterized as follows: moisture content (gravimetric), oil content (Soxhlet extraction), its lignin, cellulose and hemicellulose contents (Goering & van Soest 1970).

Pulping and pulp analyses

The EFB fiber was cut into 3-5 cm length and then it was digested using kraft process (NaOH and Na_2S 22% as active alkali, sulfidity 30%, fiber : cooking liquor = 1:5). Digestion was conducted in two steps. First, 1.5 hours to reach temperature of 165°C, and then the temperature was kept at 165°C for the next 1.5 hours. The soften coconut fiber was separated from the cooking liquor and it was washed thoroughly until free from sodium hydroxide. The soften coconut fiber was then fibrillated in a disc refiner 2 times. Yield and kappa number of pulp were determined based on the standard test methods TAPPI T 236 cm-85.

SSF

Stock culture of yeast

The yeast *Saccharomyces cerevisiae* MK 1 available at our laboratory was used in SSF experiment. Active culture of yeast was pre-cultured in Potatos Dextrose Agar (PDA) 3%, agar (0.25 g), H_2O (50 ml) and was incubated for 2 days at 37°C. This pre-cultured yeast was used for yeast inoculum in SSF.

Preparation of yeast inoculum

S. cerevisiae MK 1 was pre-cultured in 50 ml of medium (glucose, 10 g l^{-1}; yeast extract 10 g l^{-1}; KH_2PO_4, 0.1 g l^{-1}; $MgSO_4$. $7H_2O$, 0.1 g l^{-1} and $(NH_4)_2SO_4$., 0.1 g l^{-1}) in 200 ml flask at 37°C for 24 hours using an orbital shaker at 100 rpm.

Conditions for SSF

SSF reaction mixtures contained the EFB kraft pulp (10, 15, 20% w v^{-1}), cellulase 4 g l^{-1}, glucose (0%, 2%, and 4%), 0.05M acetate buffer pH 4.5 and 10% (v v^{-1}) yeast inoculum. The sample, nutrient and buffer were sterilized (121°C, 20 min), but the enzyme solution was added without sterilization. The nutrient medium was composed of 10 g l^{-1} $(NH_4)_2PO_4$,; 0.05 g l^{-1}; $MgSO_4$. $7H_2O$, and 2 g l^{-1} yeast extract. SSF experiments were carried out in 250 ml Erlenmeyer flask, that contained 200 ml of the growth medium as described above and then cultivated on an orbital shaker at 100 rpm for 72 hours at 37°C. In the SSF experiment, flask were inoculated with 10% (v v^{-1}) yeast inoculum and sugar (2-4%) were supplemented at different substrate concentration. Samples were taken at regular intervals for analysis reducing sugar and ethanol content.

The ethanol yields were calculated according to the method described by Shendong Zu *et al.* (2006) using the equation as follows :

$$\text{Yield (\%)} = \frac{\text{Ethanol Concentration } (g/l) \times 0.9}{\text{Carbohydrate Content (g/l)} \times 0.51} \times 100$$

Where carbohydrate is cellulose and hemicellulose content in the substrate (g l^{-1})

Ethanol analysis

Reducing sugars were determined by Nelson-Smogyi method using spectrophotometer U-2000 Hitachi Japan. Ethanol was estimated by gas chromatography GC-9A Shimadzu, Japan, with column PEG, SE 30 Chromosorb W 80-100 mesh. The column oven was operated isothermally at 120°C and the detector and injection port were kept at 150°C. Nitrogen was used as the carrier gas at 30-60 ml min^{-1} flow rate, and the combustion gas was a mixture of hydrogen and air. All experiment were conducted in duplicate and average values reported.

Results and Discussion

Chemical compositions of EFB before and after pretreatment

Before pretreatment total carbohydrate in the oil palm EFB fiber was 56.49% of the total biomass. It consisted of 33.25% alfa-cellulose and 23.24% hemicelluloss. Thus, major component of EFB was alfa-cellulose (33.25%), a polymer of glucose which is very potential as sugar source for ethanol production.

Lignocellulosic biomass requires pretreatment, mainly because the lignin in plant cell walls forms a barrier against enzyme attack. An ideal pretreatment reduces the lignin content and crystallinity of the cellulose and increase surface area (Takagi *et al.* 1977). In order to degrade and reduce the lignin and non cellulose component, EFB was made into pulp using kraft process, which involved treatment with alkali solution and heat at certain times and followed by fibrillation using disc refiner. After this treatment lignin and hemicellulose content was decreased significantly to 9.96% and 10.25%, respectively, while the holocellulose and alfa cellulose increased to 94.37% and 84.12%, respectively, as shown in Table 1. It can be seen that pulping process for EFB with alkaline treatment was effective for decreasing lignin and non cellulose component. Alkaline pretreatment of lignocellulosic biomass is one of the most effective pretreatment methods which predominantly affect lignin content of biomass. The main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by breaking the

ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass (Zhao *et al.* 2008).

The effects of substrate concentration on ethanol concentration

SSF process combine enzymatic hydrolysis of cellulose with simultaneous fermentation of glucose obtained to ethanol. Thus, the presence of yeast together with cellulase reduce the accumulation of glucose, thereby increasing saccharification rate and ethanol yield (Saha *et al.* 2005). Some important factors that affect the SSF process are substrate concentration, temperature, enzyme loading, reaction time, pH and yeast inoculum. According to our previous research, optimum pH for SSF of EFB fiber was 4.5 (data not shown). This study would evaluate the effects of substrate concentration in SSF on the yield of ethanol. The SSF of oil palm EFB kraft pulp was carried out under optimal conditions based on previous research conducted. SSF results in Fig. 1 shows that ethanol concentration increased significantly during the first day of the process, then the ethanol produced slightly decreased as the incubation time increased. The greatest increase was observed in the substrate of 15% concentration, while the lowest was in that of 10% concentration. These results indicated that the saccharification and fermentation efficiency of cellulose was affected by the substrate concentration. When the substrate concentration was higher than 15% the mass transfer was also higher. The highest ethanol concentration (7.86%) was achieved in the SSF medium broth with substrate 15% using cellulase 4 g l^{-1}, incubated for 24 h.

The effects of sugar addition in the substrate on ethanol concentration and yield

Sugar addition in the substrate could increase ethanol concentration and ethanol yield (Fig.2). Even after the ethanol concentration and ethanol yield were corrected with the ethanol that might be produced from the sugar (glucose) added, those value were still higher than those without sugar addition. This suggested that the sugar added to the substrate could trigger the fermentation of glucose to ethanol by *S. cerevisiae*, so that the SSF could produce more ethanol. After 24 h incubation the uncorrected values of ethanol concentration and yield shows that addition more sugar (4%) produced higher ethanol than those without sugar addition and 2% sugar addition. However, after the values were corrected, it was found that after 24 h incubation, either ethanol concentration or ethanol yield of the samples with two different sugar additions were almost the same, around 54-57 g l^{-1} and 68-71%, respectively. Thus, 2% sugar addition in the substrate was sufficient for triggering production of more ethanol in the SSF.

Table 1 The chemical compositions of EFBs before and after pretreatment with alkaline in kraft pulping process

EFB	Water (% wb)	Extractives (% db)	Lignin (% db)	Holocellulose (% db)	Alfa cellulose (% db)	Hemicellulose (% db)
Initial EFB	8.56	4.19	25.83	56.49	33.25	23.24
EFB Kraft Pulp	73.79	0.63	9.96	94.37	84.12	10.25

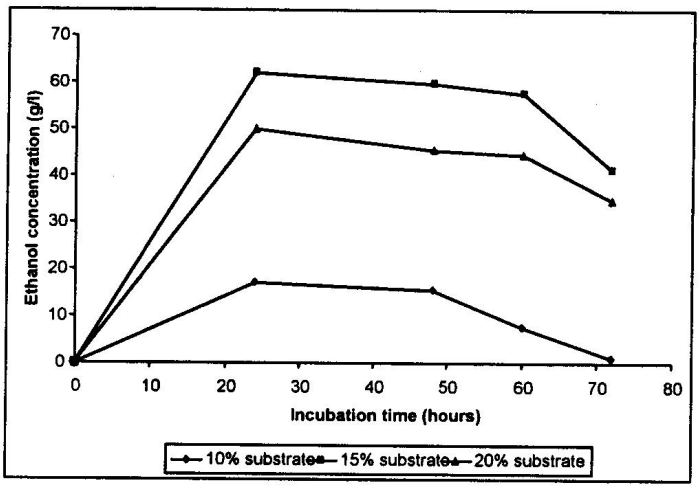

Figure 1 Ethanol concentration produced from SSF of EFB kraft pulp at different substrate concentration with 4% sugar addition in the substrate

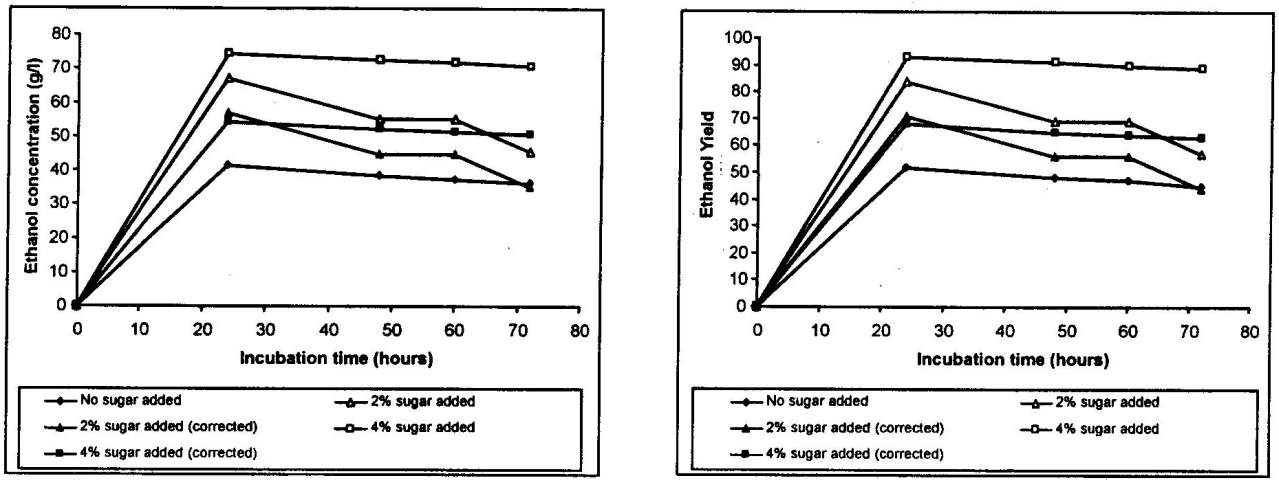

Figure 2 Effects of sugar addition on etahanol concentration (a) and ethanol yield (b) of SSF at 15% substrate concentration

The effects of sugar addition in the substrate on ethanol concentration and yield

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Conclusions

The kraft pulp of oil palm EFB could provide cellulose that could be hydrolyzed much easier in the SSF process. Substrate concentration of 15% could produce much higher ethanol than 10 or 20% substrate concentration. Sugar (glucose) addition to the substrate of SSF could trigger and enhance production of ethanol.

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