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REVIEW

UTILIZATION OF OIL PALM EMPTY FRUIT BUNCH (OPEFB) FOR BIOETHANOL PRODUCTION THROUGH ALKALI AND DILUTE ACID PRETREATMENT AND SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

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ABSTRACT

Lignocellulosic biomass is a potential alternative source of bioethanol for energy. The lignocellulosics are abundantly available in Indonesia. Most of them are wastes of agriculture, plantation and forestry. Among those wastes, oil palm empty fruit bunch (OPEFB) is one of a potential lignocellulosics to be converted to bioethanol. This EFB, which is wastes in oil palm factories, is quite abundant (around 25 million tons/year) and also has high content of cellulose (41-47%). The conversion of OPEFB to ethanol basically consists of three steps which are pretreatment, hydrolysis of cellulose and hemicellulose to simple sugars (hexoses and pentoses), and fermentation of simple sugars to ethanol. Acid and alkali pretreatments are considered the simplest methods and are potentially could be applied in the next couple of years. However, there are still some problems that have to be overcome to make the methods economically feasible. The high price of cellulose enzyme that is needed in the hydrolysis step is one of factors that cause the cost of EFB conversion is still high. Thus, the search of potential local microbes that could produce cellulase is crucial. Besides that, it is also important to explore fermenting microbes that could ferment six carbon sugars from cellulose as well as five carbon sugars from hemicellulose, so that the conversion of lignocellulosics, particularly EFB, would be more efficient.

Keywords*: OPEFB, lignocellulosics, pretreatment, fermentation, ethanol*

INTRODUCTION

The need and consumption of energy increases steadily year by year. Meanwhile, natural resources that produce energy depleted continuously, because most of energy sources are from non-renewable resources, such as oils, gas and coals. National Energy Commission of World Energy Council [1] predicted that oil sources in Java, Sumatera, and Kalimantan would be dried up in 2018, 2014 and 2017, respectively. Besides that, Indonesia has become an oil importing country, especially for products, such as middle distillate oil and gasoline, since early of this decade. The total of oil imported to Indonesia increased around 10% per year after 2005. It is predicted that supply of oil as a major source of fuels will be end up in the next 18 years.

So far, fossil fuels have been major sources of energy in Indonesia. In fact, there are some other alternative sources of energy, such as biomass of agricultural or forestry wastes, which are renewable. Utilization of these biomass as sources of energy are very limited. Biomass, mostly lignocellulosics, can be converted to energy through different kinds of processes and it can produce different from of energy, among others, ethanol [2]. Ethanol can be used as substitute of gasoline for vehicles.

The use of ethanol as fuel has been studied and implemented in Brazil and in The United States since crisis of fossil fuels hit the countries in early 1970-s. Nowadays the implementation of ethanol utilization for vehicles in Brazil and in The United States reaches 40% and 85%, respectively.

The use of ethanol as fuel has some advantages as well as disadvantages over petroleum derived fuels. The advantages are: a) ethanol has a high oxygen content (35%), so that it will produce cleaner air when it is combusted, b) ethanol is more environmentally friendly due to its 19-25% lower emission of carbon monoxide [3] and greenhouse gas [4] than that of petroleum derived fuels, so that it is not contributed to the accumulation of carbon dioxide in the atmosphere, c) lignocellulosic ethanol is renewable. Emission testing conducted at the laboratory of Center for Thermodynamics, Motor and Propulsion, Agency for the Assessment and Application of Technology (BPPT) revealed that carbon monoxide and hydrocarbon emissions from vehicles that used E10 fuel (10% ethanol, 90% gasoline) were generally lower than those used gasoline only. Emission of carbon monoxide from

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vehicles that used E10 was 0.31 g per km, while that of gasoline was 0.50-0.58% g per km [4]. Cellulosic ethanol produced 8-10% lower of greenhouse gas when it was used in E10, and 68-91% lower when it was used in E85 [5]. Among the disadvantages of ethanol [6] are: a) ethanol is very hygroscopic and it is easily absorb dirt; therefore, if the two contaminants are not completely removed, it can cause damages and corrosions in the engines, b) combustion efficiency of ethanol is lower than gasoline, so that it needs more ethanol than gasoline to run in the same distances, c) molecules of ethanol are relatively polar and they are rather difficult to mix well with gasoline, which is unpolar, especially in the liquid state; therefore, it needs engine modifications to enable the two kinds of fuels to be well mixed in the combustion chamber. The use of up to 10% ethanol substitution does not need engine modification, though. Recently there are more and more flexible fuel vehicles (FFV) produced in order to be able to use ethanol substitute fuels, for example E85 which is mostly used in the United States. Nevertheless, the use of ethanol as fuel increases steadily. In 1997 there was 13,000 tons of ethanol produced and used for fuel in different parts of the worlds [7]. Ten years later, 2007, world ethanol production reached 13,113 million gallons (equals to 49,630 kL) [8].

Most of bioethanol produced in Indonesia currently is from molasses, or starchy raw materials, such as cassava and corn. These materials are important raw materials for producing food and supporting food industries. On the other side, there are large amount of lignocellulosics biomass that has not been used. These abundantly available materials are wastes of agricultural, estate crops and forestry industries. There are three main components in lignocellulosic biomass, lignin, cellulose, and hemicellulose, which contents depend on the source of biomass. Either cellulose or hemicellulose can be converted to ethanol after going through some processes, namely pretreatment, hydrolysis and fermentation. Of the many lignocellulosic biomass available in Indonesia, oil palm empty fruit bunch is the most potential to be used as raw material for ethanol production, because this material is produced continuously in large amount throughout the year and is easy to be collected from oil palm factories that produce crude palm oil (CPO).

There are still some drawbacks on the conversion of lignocellulosics to ethanol, either in pretreatment, hydrolysis or fermentation process. Various kinds of lignocellulosics make optimization processes also different for each type of material. Of the many pretreatment processes, the use of alkaline and dilute acid solution are processes that are considered close to commercialization, although optimum conditions of using these solutions for each type of lignocellulosic are not yet available. Hydrolysis and fermentation could be conducted separately or simultaneously, which are known as SHF (Separated Hydrolysis and Fermentation) and SSF (Simultaneous Saccharification and Fermentation). Although there are also some weaknesses in the latter, many researchers considered that the latter was more advantageous than the former. This paper will discuss the potency of oil palm empty fruit bunch as raw material for ethanol production and its conversion to ethanol through alkaline and dilute acid pretreatment, continued by simultaneous saccharification and fermentation.

POTENCY OF OIL PALM EMPTY FRUIT BUNCH FOR BIOETHANOL PRODUCTION

Oil palm is among estate crops that play important roles in Indonesia's economy. It is one of the main sources of currency from non petroleum and gas sector for Indonesia besides rubber and cacao. Indonesia is also the largest producer of palm oil in the world. Crude Palm Oil (CPO) is the main product of oil palm industries in the country. Plantation areas of oil palm in Indonesia increases year by year, and so does the production of CPO (Table 1). In 2009 the plantation

Table 1. Plantation area and production of crude palm oil in Indonesia, operated by different types of business classes

Year	Plantation area (ha)				Production of CPO (tons)			
	SH	GE	PE	Total	SH	GE	PE	Total
2004	2,230,338	606,865	2,458,520	5,284,723	3,847,157	1,617,706	5,365,526	10,830,389
2005	2,356,895	529,854	2,567,068	5,453,817	4,500,769	1,449,254	5,911,592	11,861,615
2006	2,549,572	687,428	3,357,914	6,594,914	5,783,088	2,313,729	9,254,031	17,350,848
2007	2,752,172	606,248	3,408,416	6,766,836	6,358,389	2,117,035	9,189,301	17,664,725
2008*	2,903,332	607,419	3,497,125	7,007,876	6,683,020	2,124,358	9,282,125	18,089,503
2009**	3,204,022	617,169	3,500,706	7,321,897	7,209,067	2,253,358	9,977,867	19,440,291

Source: Directorate General of Estate Crops, Ministry of Agriculture of Republic of Indonesia (2009), *Statistical Estate Crops of Indonesia*. *) Temporary **) Estimated
SH = smallholders; GE = government estate, PE = private estate.

Table 2. Neutral sugar (% relative dry weight) and uronic acid (% dry weight) contents in cellulose and hemicellulose fractions of OPEFB

Fractions	Neutral sugars							Uronic acid
	Rha	Rib	Ara	Xyl	Man	Glu	Gal	
Cellulose (insoluble fraction of 10% NaOH extraction)	ND	ND	ND	3.50	1.02	95.48	ND	-
Hemicellulose (soluble fraction of 10% NaOH extraction)	0.50	0.36	2.22	88.59	0.79	5.74	1.79	4.38

Source: Sun et al. 1999 [12]
ND : Not Detected

Table 3. Estimated ethanol produced from cellulose of OPEFB

	Indonesia	Palm oil factory (60 tons of FFB/h capacity)
Amount of dry OPEFB (1,000 tons / year)	10,000	37.8
Potency of cellulose (1,000 tons /year) (assuming cellulose content is 43.9 %)	4,390	16.6
Potency of glucose (1,000 tons /year) (assuming conversion of cellulose to glucose is 0.76)	3,336.4	12.6
Stoichiometric ethanol produced (1,000 tons / year) (x 0.51)	1,701.6	6.4
Ethanol produced (million L/year) (asuming fermentation efficiency 0.75; ethanol density 0.789)	1,617.5	6.2
Ethanol produced (million L/ day) (assuming 1 year = 300 working days)	5.4	0.02

area of Palm Oil were estimated around 7.3 million Ha, total of CPO production are estimated around of 19.4 million ton. CPO rendemen is 20%, and empty fruit bunch fiber (EFB) results are 21% with moisture content >70% [9]. By these assumption, to produce CPO with quantity above mentioned, it is need 97 million ton of FFB (Fresh Fruit Bunch) of oil palm and would be resulted 20 million of EFB that is equal to 10 million ton of dry EFB, which are potentially processed to be any products as well as pulp, particle board, cushion, and so on.

As a lignocellulosic biomass OPEFB contains cellulose, hemicellulose and lignin. Cellulose content of OPEFB is 41.3-46.5%, while hemicellulose and lignin content are 25.3-33.8% and 27.6-32.5%, respectively [10]. The types of main carbohydrates in OPEFB are glucan, xylan, and arabinan, each is 31.0; 17.3, and 0.5% [11]. Extraction of OPEFB using 10% sodium hydroxide solution revealed that major neutral sugar in cellulose (insoluble fraction) was glucose (95.48%), while that in hemicellulose (soluble fraction) was xylose (88.39%) [12]. The content of other neutral sugars in the two fractions can be seen in Table 2.

Due to its high cellulose content (41-47%), OPEFB is potential to be converted to ethanol through hydrolysis of the cellulose to glucose, and continued by fermentation of the glucose to ethanol. One ton of OPEFB that contained 45% of cellulose could produce 151 L of ethanol [13], so that it can be estimated that as much as 3,775 million liter of ethanol could be produced from OPEFB in Indonesia. From an oil palm factory of 60 tons of FFB (Fresh Fruit Bunch) per hour production capacity, running 20 h per day and 300 days per year, there would be around 300 tons of OPEFB per day or 90.000 tons of OPEFB per year produced. Thus, the potency of ethanol produced from these amounts of materials from this factory is 45,300 L per day or around 13.95 million L per year. Estimation of conversion of OPEFB to ethanol is presented in Table 3.

Besides cellulose, xylose which is major component of hemicellulose in OPEFB, is also a potential source of ethanol. Xylose alone or together with glucose can be fermented to ethanol. Study on xylose extraction from OPEFB using sulfuric acid was reported [14]. Potency of ethanol that can be produced from hemicellulose of OPEFB in Indonesia is 844.6 million L per year or 2.82 million liters per day, while the same potency from a palm oil factory of 60 tons of FFB per hour capacity is 3.2 million liter per year or 0.01 million L per day (Table 4).

Table 4. Estimated ethanol produced from hemicellulose of OPEFB

	Indonesia	Palm oil factory (60 tons of FFB/h capacity)
Amount of dry OPEFB (1,000 tons/year)	10	37.8
Potency of hemicellulose (1,000 tons/year) (assuming hemicellulose content is 29.5%)	2,950	11.15
Potency of xylosa (1,000 tons/year) (assuming xylose content in hemicellulose is 88.59%)	2613.4	9.9
Stoichiometric ethanol produced (1,000 tons/year) (x 0.51)	1,332.8	5
Ethanol produced (million L/year) (asuming fermentation efficiency 0.5; ethanol density 0.789)	844.6	3.2
Ethanol produced (million L/day) (assuming 1 year = 300 working days)	2.82	0.01

PRETREATMENT OF OPEFB USING ALKALI AND DILUTE STRONG ACID SOLUTIONS

Conversion of lignocellulosic materials to ethanol mainly consist of three steps, pretreatment, hydrolysis of cellulose to simple sugars, and fermentation of simple sugars to ethanol using yeasts or bacteria. Pretreatment is needed to remove or reduce lignin content, to reduce cellulose crystallinity, and to increase the material porosity, so that the cellulose can be easily hydrolyzed. The pretreatment of lignocellulosic for ethanol production should consider among others: a) the process could enhance the sugar formation or increase the ability of sugar formation enzymatically, b) the process should prevent degradation or loss of carbohydrates, c) the process should prevent the formation of by products that inhibit hydrolysis and fermentation, and d) the process should be cost effective.

There were many reports regarding the use of dilute strong acid for pretreatment of lignocellulosics [15-18]. The use of acid solution during pretreatment resulted in degradation of hemicellulose in the material. Thus, there were some cavities that can be entered by cellulose enzymes during saccharification. The pretreatment mostly used dilute strong acid, such as sulfuric acid and hydrochloric acid [19]. The cost of this process was higher than was steam explosion or ammonia fiber explosion. Besides that, the pH should be neutralized to achieve good conditions in hydrolysis and fermentation. However, some reports explained that pretreatment using dilute strong acid was one of pretreatment processes, which was potential to be developed and was close to a commercial process [20-22].

There were also reports on the use of sodium hydroxide for pretreatment of lignocellulosic materials [23-27]. In this alkaline process the mechanism was saponification of ester linkages between cross-linked molecules and other molecules, such as lignin and hemicellulose [19]. The loss of these crosslinkings caused increase of material porosity. Dilute sodium hydroxide solution also caused swelling of the material, so that it increased internal surface area in the material, reduced degree of polymerization and crystallinity, caused scission of chains between lignin and carbohydrates, and degraded lignin structures. Calcium hydroxide was also reported can be used for pretreatment of lignocellulosics [28]. Some other alkaline processes reported were alkaline peroxide which combined sodium hydroxide and hydrogen peroxide [29-30] or alkali and microwave [25,31]. Some reports mentioned that pretreatment using alkali was also one of processes, which was close to commercialization [20-22].

Study on pretreatment of OPEFB for ethanol production was reported by some researchers [10,24,32]. Pretreatment of OPEFB using alkali (NaOH) was reported more efficient in providing suitable substrate for saccharification than that using acid solution (HCl or HNO_3) [24]. Furthermore, it was also explained that addition of treatment using autoclave at 121 °C, 15 psi for 5 min to the chemically treated OPEFB could increase cellulose hydrolysis by two fold. Other pretreatment of OPEFB reported was that of using white-rot fungi (*Ceriporiopsis subvermispora*, *Dichomitus squalens*, *Pleurotus ostreatus*) [10]. This study showed that of the three fungi tested, *D. squalens* was the most extensive in degrading lignin in OPEFB. After 8 weeks of incubation, the amount of lignin and holocellulose loss from OPEFB reached 25.7% and 22.8%, respectively. Degree of saccharification of OPEFB treated with *D. squalens* was also higher than those treated by the other two fungi.

SACCHARIFICATION AND FERMENTATION

During saccharification process cellulose was converted to cellobiose, which was then further converted to simple sugars, such as glucose. Hydrolysis of cellulose can be conducted using acid solutions or enzymes, each with its advantages and disadvantages.

Enzymatic hydrolysis usually runs in mild conditions (pH around 4.8 and temperature 45-50 °C) and does not cause corrosion problems. The disadvantage of using enzymes is the high enzyme cost. The cost of enzymes could be as much as 53-65% of total chemical cost, while the chemical cost was around 30% of the total cost. Hydrolysis of cellulose could also be accomplished using microbes that produce cellulase enzymes, for example *Trichoderma reesei*, *Trichoderma viride*, and *Aspergillus niger*. While the usage of enzymes produced breakdown products that are specific, acidic hydrolysis did not. Besides glucose, the latter also produced some other by products, such as furans, phenolics and acetic acid [33]. These by products if not removed could inhibit further process, that is fermentation. Hemicellulose component in lignocellulosics could also be hydrolyzed, and further fermented to produce ethanol. Hemicellulose can be hydrolyzed using enzymes, such as glucuronidase, acetil esterase, xylanase, β-xylosidase, galactomannanase and glucomannanase, that can attack hemicellulose [34].

Technology and equipment needed for fermentation of cellulose derived sugars are basically the same as those for fermentation of sugars from starch or tree sap, which are commercially available. In fermentation process simple sugars formed during hydrolysis were fermented to ethanol by yeasts, such as *Saccharomyces cerevisiae*, or bacteria, such as *Zymmomonas mobilis*. Fermentation was usually executed at temperature around 30 °C, pH 5 with a little anaerobic condition. During glucose fermentation one molecule of glucose produced two molecules of ethanol and two molecules of carbon dioxide (CO_2). Fermentation of sugars obtained from hydrolysis of hemicellulose, such as xylose, to ethanol can use several yeasts, such as *Pichia stipitis* or *Candida shehatae* [35]. During xylose fermentation three molecules of xylose produced five molecules of ethanol, five molecules of carbon dioxide, and five molecules of water [36].

Some microorganisms that are used for fermentation of sugars from lignocellulosics have been patented, for example for conversion of toxic lignocellulose to ethanol using *Pichia stipitis* NPw9 [37] or ethanol production through SSF using bacteria *Eschercia coli* and *Klebsiella* with an addition of ethanologenic enzymes [38].

Hydrolysis and fermentation processes can be accomplished separately, known as SHF (Separated Hydrolysis and Fermentation) or simultaneously, known as SSF (Simultaneous Saccharification and Fermentation). The use of both processes for lignocellulosics have been reported widely, either SHF [15-16,23-24] or SSF [25,28,39-46]. Microbes that were usually used in SSF are cellulase producing fungi, such as *T. reesei* or *T. viride*, and yeasts, such as *S. cerevisiae*. Optimum temperature for SSF was around 38 °C, which was a compromised temperature of optimum hydrolysis temperature (45-50 °C) and optimum fermentation temperature (30 °C) [19]. Substrate concentration was usually around 10% (unsoluble solids), with enzyme loadings 10-20 FPU (Filter Paper Unit) per g cellulose and yeast concentration of 1.5-3.0 g/L, while incubation time was usually 72 hours.

Simultaneous Saccharification and Fermentation has some advantages over SHF, among others: a) the process is environmentally friendly, 2) it increases yield of product, 3) it reduces the need of sterile conditions, since glucose is directly converted to ethanol, 4) it takes shorter time of process, and 5) it needs less volume of reactor since there is only one reactor needed [19]. Study on comparison of SHF and SSF for lignocellulosic from corn stover [47] confirmed previous studies that SSF is more advantageous than is SHF. Some obstacles in SSF that have to be overcome, among others: 1) different optimum temperatures for hydrolysis and fermentation, 2) microbes toleration to ethanol, and 3) enzyme inhibition by ethanol produced.

CONCLUSION

Research that has been done and should be developed regarding the conversion of lignocellulosics through SSF basically consist of 1) selection of lignocellulosic material and its pretreatment, 2) selection and development of microorganisms or enzymes for hydrolysis, 3) selection and development of microorganisms for fermentation, and 4) adapting and developing analysis technique to monitor the advantages of SSF.

Even though there are so many researches regarding utilization of lignocellulosics for ethanol, there are still a lot of chances for its development since there are so many types of different kinds of lignocellulosics, from different sources with different characteristics. Besides, the present technology available is not yet economical. Reports on the use of OPEFB as raw material for ethanol production are only a few. In fact, the materials are abundantly available in Indonesia. Therefore, research on utilization of OPEFB would give alternative solution in handling wastes in palm oil industries, while it also could give some added values to the material. Compare to other pretreatment methods for lignocellulosics, the use of alkali and acid solutions are relatively easy, simple, fast, and are close to be commercialized, even though there are still some disadvantages. Based on research and studies completed and reported, SSF process was more advantageous than was SHF, even though there are still some recalcitrance to be overcome.

The use of local isolate of microorganisms that are potential producers of cellulase enzymes, such as *Trichoderma* sp., and those that play roles in fermentation of sugars to ethanol, such as *Saccharomyces cerevisiae* PA1 and PA2, which are thermotolerant and ethanol tolerant, are expected could reduce production cost by providing alternative sources of cellulase enzymes and could solve one of problems in SSF by providing fermentation microorganisms that are more tolerant to heat and ethanol.

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