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Bioethanol From Oil Palm Empty Fruit Bunch (OPEFB): a Review Pretreatment and Enzymatic Hydrolysis

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Article Info	**Abstract**

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The fossil fuel crisis will remain a major topic that needs to be looked for a solution together until other alternative fuels are found. Bioethanol has now become a trend and focus of researchers in Indonesia and various parts of the world as an environmentally friendly alternative energy and given the potential of Indonesia as one of the largest palm oil-producing countries in the world with the amount of lignocellulosic biomass waste such as Oil Palm Empty Fruit Bunch (OPEFB). OPFEB is the most desirable and realistic alternative to substitute fossil fuels as the main energy source. The use of these wastes as renewable fuels is very significant in terms of expanding economic value and solving environmental problems. This review paper offers an insight into the current creative approaches to the development of OPEFB such as characteristics of OPEFB, pretreatment process, and enzymatic hydrolysis.

1. INTRODUCTION

Today, the world has the greatest problem that must be faced in the form of an energy crisis that is a priority for the survival of a country and economy. The predictions for energy demand in 2050 have almost tripled. The energy dilemma seems to remain a subject that needs to be addressed together. Until now, non-renewable fossil fuels have been the main source of energy for humankind, so it is probable that one day the supply will run out. The availability of fossil fuels on Earth is currently limited and it is likely that annual production will peak within this century. In addition, fossil fuels produce CO_2 emissions which result in greenhouse gases which have the largest contribution to global warming (Anser, Hanif, Alharthi, & Chaudhry, 2020; Yoro & Daramola, 2020; Zecca & Chiari, 2010).

Fossil fuels are the main energy consumption which increases every year. Based on data, the total world energy consumption in 2005-2014 has increased from 995.1 million barrels/day to 1,186.2 million barrels/day, while in Indonesia alone in 2000 fuel consumption was 315 million barrels and increased in 2013 to 399 million barrels (Briefing, 2013). Due to concerns about global warming and limited fossil energy resources, the search for renewable energy sources that reduce CO_2 emissions is of widespread concern (Balat, 2009). One of these alternative energy sources is bioethanol.

Bioethanol has several advantages over other alternative energies. Among them, they have a higher oxygen content (35%) (Sebayang et al., 2016), have a higher octane (108) (Azhar et al., 2017), and are more environmentally friendly because they contain 19–25% lower CO2 emissions (Tayyab et al., 2018; Wang et al., 2016). Besides, the substrate for producing bioethanol in Indonesia is very abundant. Bioethanol can be made from cellulose-based materials. An alternative non-food material that has the potential to be developed as a source of biofuel is oil palm empty fruit bunches (OPEFB). OPEFB is waste from oil palm fruit after the fruit is taken. OPEFB includes lignocellulosic biomass which has high cellulose content, is abundant and cheap so that it has the potential for commercial production of the bioethanol industry (Nazir, Wahjoedi, Yussof, & Abdullah, 2013; Rosazley et al., 2016).

The 2nd generation of bioethanol is produced from the process stages, namely pretreatment, hydrolysis, fermentation, and distillation. All of these stages are very necessary in producing it. However, the hydrolysis stage plays an equally important role in the production of bioethanol (Canilha et al., 2012; Elsayed et al., 2018; Talebnia, Karakashev, & Angelidaki, 2010). Broadly speaking, there are 3 types of hydrolysis, namely chemical hydrolysis, fermentative hydrolysis and enzymatic hydrolysis. Chemical hydrolysis is very fast but requires high temperatures so that it requires a large amount of energy which is not environmentally friendly. Enzymatic hydrolysis is more attractive in terms of energy use because it can be carried out at low temperatures and yields glucose up to 70%, but the reaction rate is slow (Cabrera et al., 2014; Zheng, Zhang, Miao, Su, & Wang, 2013). Another difficulty with enzymatic hydrolysis is the high cost of commercial enzymes. Therefore, according to Wilson (Wilson, 2011), enzymatic hydrolysis can be carried out using the help of microorganisms (fermentative hydrolysis) which can hydrolyze polysaccharides into simple monomers such as reducing sugars. The types of microorganisms that can be used in the enzymatic hydrolysis process from biomass waste to reducing sugar are fungus.

Aspergillus niger is a good fungus used in bioethanol production from lignocellulosic biomass, because Aspergillus niger is a potential source of β-glucosidase. (Singhania, Patel, Sukumaran, Larroche, & Pandey, 2013; Treebupachatsakul et al., 2016; Xue et al., 2020). This is due to the ease with which it can be cultivated in agro product media by fermentation of solid media or fermentation of liquid media. β-glucosidase is a cellulase enzyme that can hydrolyze cellulose by hydrolyzing cellobiose (an intermediate product of cellulose hydrolysis) to become glucose monomers. This makes it possible to support the optimization of the hydrolysis of oil palm empty bunches (EFB) which produces cellulase enzymes that will be more economical in producing bioethanol.

2. METHODS

Basically this section explains how the various methods used in the production of bioethanol. The general methods used are hydrolysis and anaerobic fermentation methods. Then the raw material used comes from the waste of empty palm oil bunches. According to Nurdin *et al*. (Nurdin et al., 2021) studies related to the production of bioethanol from OPEFB by the fungus *Aspergillus niger*. OPEFB waste Sugarcane bagasse is processed by drying, and sieved through a 30 mesh sieve. This stage is called non-pretreatment because there is no addition of chemicals. The pretreatment process was carried out with sodium hydroxide solution at 150◦C for 3 hours in a chemical reactor before enzymatic hydrolysis with the enzyme *Aspergillus niger*. The result obtained that the amount of reducing sugar from OPEFB pretreatment was 0.94 $mg.mL^{-1}$, while for non-pretreated EFB it was 15.83 $mg.mL^{-1}$.

Previous research has been conducted by Richana *et al*. (Richana, Winarti, Hidayat, & Prastowo, 2015) production of bioethanol from OPEFB waste through delignification, enzymatic hydrolysis and fermentation processes. The method used is OPEFB delignification using a solution of H_2SO_4 and NaOH. After that, pretreatment was carried out using a solution of sulfuric acid and sodium hydroxide for 15 minutes to obtain a small amount of sugar. *Saccharomyces cerevisiae* was also used in experiments for 6 day fermentation process in a chemical reactor. In this study, it was shown that pretreatment with a solution of sulfuric acid and sodium hydroxide efficiently and effectively breaks down OPEFB components to produce bioethanol. In addition, the fermentation process by Saccharomyces cerevisiae was able to produce 90% bioethanol from weight of 3.82 kg OPEFB.

Other studies have also been reported by Dahnum *et al*. (Dahnum, Tasum, Triwahyuni, Nurdin, & Abimanyu, 2015) production of bioethanol by a combination of two enzymatic hydrolysis methods, namely Separate Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) with OPEFB as a substrate. The method used is pretreatment with 10% NaOH solution for 30 minutes at a temperature of 150 °C in the reactor. In addition, four concentrations of the enzyme Cellic® CTec2, 10, 20, 30, 40 FPU per gram were prepared for the fermentation process. The maximum bioethanol concentration was obtained at 4.74% with the SHF process and 6.05% with the SSF process. So it can be concluded that the SSF process is more efficient than the SHF process.

Recent studies have been reported by Manmai *et al*. (Manmai, Unpaprom, Ponnusamy, & Ramaraj, 2020) with different pretreatment designs to optimize bioethanol production from sorghum stalk waste. In this work, the pretreatment process is carried out for 1-3 days using a 2% NaOH solution with a heating time of 30°C - 40 °C. Response surface methodology (RSM) was used to optimize the fermentation process. The maximum bioethanol concentration reached 8.374 ± 1.813 g/L with a fermentation time of 2 days.

3. RESULT AND DISCUSSION

Characteristic of Bioethanol from Oil Palm Empty Bunch (OPEFB)

OPEFB is solid waste produced by Crude Palm Oil (CPO) factories. In one day of processing, hundreds of tonnes of OPEFB can be produced depending on production capacity. OPEFB is the most frequently investigated biomass for generating biofuels among the oil palm biomass. OPEFB's conversion into biofuels, such as biogas, hydrogen, ethanol, butanol, and bio-oil, is a good choice and has less effects on the atmosphere. The main components of OPEFB are cellulose, hemicellulose, and lignin (Mondylaksita et al., 2020; Simatupang, Nata, & Herlina, 2012; Tahir et al., 2019). Physically, OPEFB has a water content of around 60% and a maximum oil content of 2.5% and fiber of 23-35%. EFB waste produced is known to still contain large enough cellulose. OPEFB contain 44.4% cellulose, 30.9% hemicellulose and 14.2% lignin. Cellulose is a major part of biomass, especially plants. Cellulose forms a third to a half of the total trunk tissue (Afriani & Kardiansyah, 2015). In addition, based on research by Kresnawaty et al. (Kresnawaty, Putra, Budiani, & Darmono, 2017) which analyzed the macro and micro nutrient content of OPEFB biological charcoal and presented in Table 1.

Table 1. Macro and micro nutrient content of OPEFB biological charcoal (Nurdiawati et al., 2015)

No	Elements	Concentrations
1	Carbon	60 %
2	Nitrogen	1.07 %
3	Phosphor	1.29 %
4	Potasum	13.37 %
5	Magnesium	1.02 %
6	Iron	0.95 %
7	Boron	31 ppm
8	Zink	248 ppm
9	Calcium	1.71 ppm

Oil palm empty fruit bunches are biomass containing essential minerals and elements such as elements C, N, P, K, Mg, etc. Based on the research of Yahya et al. (Yahya, Sye, Ishola, & Suryanto, 2010) where the results of the analysis show that the OPEFB fibers mostly contain volatile materials (up to 83.86%), followed by fixed carbon (up to 18.3%), moisture (up to 14.28). %) and ash (up to 13.65%). Ash content is the residual EFB after combustion. It consists mainly of metal oxides such as magnesium oxide (0.23%), calcium oxide (0.13%), potassium oxide (2.4%), phosphorus pentoxide (0.18%) and silica (0.19%).

Pretreatment Method for OPEFB Waste

The pretreatment is the lignin degradation (delignification) stage which aims to reduce the amount of lignin so as not to interfere with the hydrolysis process, which is a series of bioethanol production. Lignin degradation is carried out to condition lignocellulosic materials both in terms of structure and size by breaking down and removing lignin and hemicellulose content, damaging the crystal structure of cellulose and increasing the porosity of the material (Agustini & Efiyanti, 2015).

The cross-linked polysaccharide structure makes lignocellulose resistant to enzymatic and chemical conversion. Pretreatment methods need to be done to disrupt the naturally resistant lignocellulose structures that limit the hydrolysis of cellulose and hemicellulose. The pretreatment stage consists of physical and chemical methods and is intended for delignification of lignin in order to release free cellulose and hemicellulose, reduce cellulose crystallinity and increase the surface area and porosity of lignocellulosic materials which result in an increase in the rate of hydrolysis (Ali, Aziz, & Hassan, 2015; Bensah & Mensah, 2013).

Biomass pretreatment is the most important step in enhancing the enzymatic hydrolysis process and determining the amount of fermentable sugar available to produce bioethanol. The purpose of pretreatment is to open the lignocellulose structure so that cellulose becomes more accessible to enzymes that break down saccharide polymers into sugar monomers. Pretreatment provides easier access to the enzyme so that it will experience an increase in glucose and xylose yields (Ni'mah, Ghofur, & Samlawi, 2016). Schematically it can be described as follows:

Table 1. Comparison of pretreated lignocellulosic biomasses compositions

Biomass	Pretreated			Reference
	Lignin (%)	Cellulose (%)	Hemicellulose (%)	
Barley straw	7.6	58.3	30.3	(Ibarra-Díaz, Castañón-Rodríguez, Gómez-Rodríguez, & Aguilar-Uscanga, 2020)
Sugarcane bagasse	5.77	67.8	9.22	(Raghavi, Sindhu, Binod, Gnansounou, & Pandey, 2016)
Rice hull	9.08	37.07	31.15	(Germec et al., 2016)
Wheat straw	6.30	44.30	32.74	(Moset, Xavier, Feng, Wahid, & Møller, 2018)

Corn stover	16.10	30.36	14.68	(Yu, Xiao, Han, & Huang, 2019)
Oil Palm Empty Fruit Bunch	9.7	38.4	43.8	(Arbaain, Bahrin, Ibrahim, Ando, & Abd-Aziz, 2019)
Switchgrass	34.8	33.8	25.5	(Bonfiglio et al., 2019)
Seaweed	9.4	19.4	14.4	(Li et al., 2016)
Sago Palm Bark	25.85	40.79	22.32	(Ethaib et al., 2017)

Chemical Characteristics of Alkali Pretreatment OPEFB

Chemical characteristics of OPEFB Pretreatment are carried out to determine the components contained in the Pretreatment OPEFB which will later be compared with the components contained in the Non-Pretreatment OPEFB so that it can be seen the abundance of cellulose that will be used in the hydrolysis process. Based on Table 2, the analysis method in determining the chemical characteristics of Alkali Pretreatment OPEFB uses the NREL (National Renewable Energy laboratory) method and HPLC.

Table 2. Chemical Composition of OPEFB after Alkali Pretreatment using 10% NaOH

Composition (%)			**Method**	**Reference**
Cellulose	Hemicellulose	Lignin		
73.01 ± 0.2	14.9 ± 0.2	12.1 ± 0.5	NREL	(SERAT, 2018)
72.53	14.22	6.17	NREL	(Muryanto, Sudiyani, & Abimanyu, 2016)
72.59	8.04	14.04	NREL	(Triwahyuni, Muryanto, & Abimanyu, 2015)
73.24	7.81	15.70	NREL	(Dahnum et al., 2015)
68.86	5.69	11.02	NREL	(Sudiyani et al., 2013)

OPEFB pretreatment process is carried out starting with physical treatment which aims to reduce the size of lignocellulose so that it is easy for further processing. Furthermore, the chemical pretreatment process is carried out by mixing 10% alkaline NaOH solution and OPEFB in a chemical explosive reactor (CHIMEX) with a temperature variation of 150°C. Pretreatment using an alkaline solution (NaOH) is able to dissolve the lignin component and some hemicellulose components and increase the accessibility of the lignocellulose surface so that it can increase the amount of cellulose content (Burhani, Putri, Waluyo, Nofiana, & Sudiyani, 2017). Based on the research of Karimi et al. (Karimi, Shafiei, & Kumar, 2013) that sodium hydroxide solution (NaOH) is often used and favored by many researchers because of its relatively high level of alkalinity and its many benefits. Pretreatment using sodium hydroxide (NaOH) solution can improve the poly-ionic properties of lignocellulose which is related to the diffusion of sodium (Na^+) ions into lignocellulose and also acts as an exchange for carboxylate ions and increases swelling so as to reduce lignin levels and increase cellulose levels from berlignocellulosic materials.

Delignification in lignocellulosic biomass with the same lignin content, but different biomass requires different levels of NaOH. As in the description , NaOH is able to break the bonds between cellulose, hemicellulose and lignin, and can break the lignin bonds itself. The broken bond is a hydrogen bond that is connected between lignin and cellulose and hemicellulose and the bond between lignin and hemicellulose in the form of ether and ester groups. NaOH can also break bonds in lignin compounds consisting of ether and carbon groups into lignin fragments (Gargulak, Lebo, & McNally, 2000). The reaction with NaOH also causes the breaking of the bonds in the lignin compound which causes the lignin molecule to degrade. Lignin degradation is preceded by the attack of the H atom attached to the phenolic OH group by the hydroxy ion (OH) from NAOH (Figure 3). The presence of dissolved lignin is indicated by a dark black solution. This black color appears as an indication of the dissolution of compounds that have chromophore groups, namely groups that have conjugated double bonds.

Enzimatic Hydrolysis Process using Aspergillus Niger

Hydrolysis is the breakdown of polymer compounds into monomer compounds. The decomposition of lignocellulosic biomass, specifically cellulose and hemicellulose, into simple sugar monomers is known as polysaccharide hydrolysis (Patil, Cimon, Eskicioglu, & Goud, 2021; Tareen, Punsuvon, & Parakulsuksatid, 2020). Enzymes can speed up the hydrolysis process, which is known as enzymatic hydrolysis. Enzymes are biocatalysts that help biological reactions move faster. The enzyme reduces the activation energy by acting as a catalyst. Generally, temperature, pH, and other factors all have an impact on the performance of enzymes (Naghdi et al., 2018; Nannipieri, Trasar-Cepeda, & Dick, 2018) Enzymes are not resistant to high temperatures, and their activity decreases as a result. Enzyme hydrolysis is often performed at low temperatures, which is one of the benefits of enzyme hydrolysis over acid hydrolysis in terms of energy consumption (Fuadi & Harismah, 2014).

Commercial cellulase production generally uses isolated fungi or bacteria. Although many microorganisms can degrade cellulose, only a few microorganisms that produce cellulase in significant amounts are capable of hydrolyzing cellulose crystals. Fungi are the main microorganisms that can produce cellulases, although some bacteria and actinomycetes have been reported to also produce cellulase activity. Filamentous fungi such as Aspergillus niger which can produce cellulase enzymes commercially. These fungi are very efficient in producing cellulase enzymes (Adsul et al., 2020; Kumar, Barrett, Delwiche, & Stroeve, 2009; Saroj, Manasa, & Narasimhulu, 2018).

Cellulolytic enzymes play an important role in the natural biodegradation process in which the berlignocellulosic material is degraded efficiently by cellulolytic fungi, bacteria, actinomycetes, and protozoa. The conversion of cellulose to glucose through the role of microorganisms is considered a productive step in the production of biofuels and various other bioproducts from lignocellulosic materials as an alternative due to the high price of commercial cellulase enzymes and their low efficiency (Neesa, Jahan, Khan, & Rahman, 2017).

Based on the description of Figure 1, the hydrolysis of cellulose to glucose involves a multi-complex cellulase enzyme consisting of three types of enzymes, namely endoglucanase, exoglucanase and β-glucosidase. The three kinds of enzymes work synergistically for the complete series of hydrolysis of cellulose to glucose through several hydrolysis stages. In the first stage, the cellulose fibers are hydrolyzed by endogucanases releasing small cellulose fragments with free ends / terminals in reducing and non-reducing forms, which are then further hydrolyzed by exogucanase releasing small oligosaccharides and cellobiose. In the final stage, β-glucosidzase complements the hydrolysis of cellulose by hydrolyzing cellobiose (an intermediate product of cellulose hydrolysis) into glucose monomers (Bai et al., 2017; Treebupachatsakul et al., 2015; Zhou et al., 2012). Table 3 presents the glucose content of various lignocellulosic biomass resulting from the enzymatic hydrolysis process using *Aspergillus niger* where the resulting glucose monomer is influenced by several factors such as the number of substrates and the time of hydrolysis and glucose will be used as raw material in the fermentation of bioethanol production.

Table 3. Glucose content from the enzymatic hydrolysis process of lignocellulose using Aspergillus niger.

Substrate	Mass (g)	Microorganism	Hydrolysis time (h)	Glucose content	Reference
OPEFB	1	Aspergillus niger EFB1	168	1.38 ± 0.10 g/L	(Kamsani et al., 2018)
Water hyacinth	50	Aspergillus niger ITCC 7678	72	13.5 g/L	(Singh & Bishnoi, 2013)
Cassava peel	2	Aspergillus niger	48	23 g/L	(Ajala, Adeoye, Olaniyan, & Fasonyin, 2020)
Potato waste	100	Aspergillus niger	120	9.30 g/L	(Chintagunta, Jacob, & Banerjee, 2016)
Rice residue	1	Aspergillus niger ITCC 302	72	55.6 g/L	(Prasad et al., 2020)
Napier grass	50	Aspergillus niger	45	0.51 g/L	(Liu et al., 2017)
Wood dust	10000	Aspergillus niger BCRC 31,130	72	2.57 g/L	(Chen et al., 2017)

4. CONCLUSION

The world's need for energy is currently still very dependent on the availability of fossil energy. Whereas the availability of fossil energy is inversely proportional to its needs because of the non-renewable nature of fossil energy, so it is likely that one day the supply will run out. OPEFB waste is increasingly present as an alternative energy in the form of bioethanol which is environmentally friendly. Bioethanol production cannot be separated from sugar monomer raw materials by paying attention to the stages in the form of alkaline pretreatment using NaOH with a relatively high level of alkalinity and increasing accessibility on the lignocellulose surface so that it can increase the amount of cellulose content and enzymatic hydrolysis using Aspergillus niger which is cheaper and economical. This review shows how the characteristics of the pretreatment with cellulose content obtained are around 68.86% to 73.24% and the mechanism of OPEFB hydrolysis using Aspergillus niger as a biocatalyst producer of cellulase enzymes in producing sugar monomers as raw material for economic bioethanol production. Based on research that has been reviewed on the hydrolysis of lignocellulose using Aspergillus niger, it was obtained 1.38 ± 0.10 g/L to 13.5 g/L glucose content.

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