Advances in Engineering Research, volume 202
Proceedings of the International Conference on Sustainable Biomass (ICSB 2019)

The Effect of Alkaline Pretreatment on Biogas Productivity and Kinetic from Cocoa Pod Husk Waste Using Batch Reactor

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ABSTRACT
Cocoa pod husk (CPH) as a potential plantation waste was used for the biogas production. Pretreatment using NaOH (N), NaOH with addition of H_2O_2 simultaneously (NHs) and consecutively (NHc) were carried out to remove lignin content in CPH and enhance methane yield. 2% NaOH was used for N pretreatment for 30 minutes at 121oC. In other hand, NaOH with H_2O_2 concentration of 5% (w/w) was used for NHs and NHcpretreatment for 75 minutes in shaker incubator (30 oC, 130 rpm) where the pH was set at 11.5 using 6 M NaOH. All pretreatment was done in three different biomass concentrations (5, 10, 15%). The highest delignification of 79.26%, 91.73% and 89.09% were obtained from N, NH_S and NHcpretreatment, respectively. Biogas production from CPH pretreatedNH_S resulted in higher methane yield (0.0389 m3CH4/kgVS) than other pretreatments which 181.76% improvement corresponding to non-pretreatment CPH and the productivity was 0.0009 m3CH4/kgVS.day. Kinetic study for each variable using the modified Gompertz equation was investigated which the coefficient of determination (R2) values above 0.99 were obtained..

Keywords: *Cocoa Pod Husk, alkaline pre-treatment, biogas, kinetic study*

1. INTRODUCTION

The use of non-renewable energy can cause energy crisis problems. This has occurred lately is the scarcity of fuel oil such as kerosene, gasoline, and diesel. In this situation, the search, development, and dissemination of environmentally friendly non-fossil energy technologies are very important. One of which is biogas technology. Biogas (methane) can be produced from the decomposition of organic waste containing protein, fat, and carbohydrates. This decomposition is carried out by anaerobic bacteria through the fermentation process. Anaerobic digestion was used as a waste to energy technology that suitable to treat solid waste and wastewater [1].

Biogas can produce from agriculture or plantation waste such as rice husk, coffee pulp, cocoa pod husk (CPH), etc. Indonesia has been known as one of the large producers of cocoa. In 2018, it produces 280 thousand tons of cocoa (Cocoa Barometer, 2018) where 70% of which is CPH. CPH contains cellulose (35%), hemicellulose (11%), lignin (14.6%), pectin (6.1%), crude fiber (22.6%), raw protein (5.9%) and ash (9.1%) [2]. The high composition of hemicellulose and cellulose found in CPH makes it can use as a substrate of biogas. These are fermentable after hydrolysis by bacteria. While the presence of lignin can inhibit the process of biogas formation. Lignin plays the role as cement for the cross-linking between cellulose and hemicellulose to form a rigid three-dimensional structure of the cell wall, therefore it must be reduced through the pre-treatment [3]. The content of pectin in CPH affects biogas production [4]. Based on the pectin matrix, it can be seen that pectin covers cellulose and or hemicellulose and closes exposure to cellulose and hemicellulose to be degraded by enzymes [5]. However, the presence of pectin does not interfere with the degradation process after pre-treatment because it has been released from the lignin matrix.

The most used agents for pre-treatment were NaOH, H_2SO_4, and H_2O_2. Reference [6] used these agents for pre-treatment of CPH where delignification results were 56.89%, 45.72%, and 74.84%, respectively. In another one, reference [7] pre-treated CPH with H_2O_2 and

H_2SO_4 which resulted in 71.34% of delignification using H_2O_2, while lignin content was increased by 31.8% after H_2SO_4pretreatment. However, the study that relates to the effect of alkali (NaOH) and alkali hydrogen peroxide (NaOH-H_2O_2) pre-treatment on biogas yield from CPH was less in literature.

This work aims to study the effect of chemical pre-treatment using NaOH (N) and NaOH with the addition of H_2O_2 simultaneously (NH_S) and consecutively (NHC) in various ratio of biomass to CPH composition on lignin and biogas productivity and yield. Kinetic study for each variable was investigated also and was expected to be a reference for biogas production from CPH on a commercial scale.

2. MATERIAL AND METHODS

2.1. Raw Material Preparation

CPH was obtained from PTPN XII Jember, East Java. It was dried using sunlight for 3 days, then its size was reduced to 100 mesh. The initial composition of CPH was 17.33% of cellulose, 15.09% of hemicellulose and 45.36% of lignin.

2.2. Pre-Treatment

2.2.1.N Pre-Treatment

5 grams of the prepared CPH was introduced into an Erlenmeyer flask containing 95 mL of the 2% NaOH solution (5% biomass concentration) was added until it was homogeneous. Then the solution was heated at 121°C for 30 minutes using an autoclave. The pretreatment was repeated with different biomass concentrations (10% and 15%).

2.2.2.NH_S Pre-Treatment

9.5 gram of the prepared CPH was introduced into an Erlenmeyer flask and 1% NaOH of 180.5 mL was added (5% biomass concentration). Then H_2O_2 of 10 mL was added into that Erlenmeyer and stirred until homogeneous. pH of solution was set 11.5 using 6 M NaOH. The solution was then put into an incubator with a rotary shaker at 30°C and 130 rpm for 75 minutes. The pretreatment was repeated with different biomass concentrations (10% and 15%).

2.2.3.NH_c Pre-Treatment

This pre-treatment was done for the best results of variable of biomass concentration in NH_S pre-treatment. Suppose a variable of 5% biomass was used. 9.5 g CPH was inserted into an Erlenmeyer. 1% NaOH of 180.5 mL was added to the Erlenmeyer and stirred until homogeneous. Then 6 M NaOH was added in the same amount of 5% biomass concentration NH_S pre-treatment to the solution. Then it was incubated at 30°C and 130 rpm for 37.5 minutes. Then 10 mL of H_2O_2 was added into the Erlenmeyer and stirred until homogeneous and incubated again at the same condition for 37.5 minutes.

2.3. Preparation of Inoculum

Inoculums were prepared for each variable with adding 2 g of the CPH, 100 mL aquadest and anaerobic active-sludge (starter) with the amount of 10 % working volume into 500 mL Erlenmeyer flask. Then, some of the chemicals such as 4 g/L NH_4Cl. 0.06 g/L KH_2PO_4, 0.005 g/L $NiCl_2$, 0.005 g/L $CoCl_2$, 2 g/L CH_3COONa and 0.025 g/L $MgCl_2$ were added as microbial nutrition into that Erlenmeyer respectively. Subsequently, the top of the Erlenmeyer was covered by a rubber stopper, incubated and stirred in an incubator shaker at 37 °C and 130 rpm for 12 h.

2.4. Biogas Production

Biogas production had 4 substrate variables: three from the best CPH's delignification in each of N, NH_S and NH_C pretreatment and one from the untreated CPH. This process was done in a 500 mL Erlenmeyer reactor, the top closed by the modified glass with a valve and connected with Tedlar Bag to collect the gas. After the preparation of inoculum, then 10 grams of CPH (total substrate of 12 g) from each variable were added to the reactor and purged with N_2. All reactors were incubated at 37 °C and 120 rpm until the volume of biogas was constant.

2.5. Analytical Method

The results of the pretreatment will be carried out by lignin analysis using the TAPPI method, analysis of polysaccharide and cellulose by Ioelovich method, and analysis of pectin by [8] method. Chemical oxygen demand (COD) was analyzed by the APHA method. Analysis of concentrations of CH_4 and volatile fatty acid (VFA) in biogas was analyzed using gas chromatography (GC-FID).

2.6. Kinetic Study

The kinetic study of this research was restricted to the cumulative biogas generation using the modified Gompertz in (1).

$$Bt=B\ \exp\left(-\exp\left[\frac{Rb \times e}{B}(\lambda-t)+1\right]\right) \qquad (1)$$

where Bt represents the cumulative biogas produced (mL), B is the maximum biogas production (mL), Rb is the maximum biogas production rate (mL/d), λ means the lag time (d) which is the min time taken to produce biogas or time taken for bacteria to acclimatize to the environment in days, t refers specifically to the incubation time (d), and e (the natural logarithm constant) equals to 2.72. All parameters (B, Rb, and λ) were estimated by fitting the experimental results into the models via the solver function of the MS Excel Tool Pack [9].

3. RESULT AND DISCUSSION

3.1. Raw Material of Cocoa Pod Husk

Firstly, CPH was dried to remove the water content. The air-dried method can produce high holocellulose content and low lignin in CPH [10]. Then, it was reduced in size to 100 mesh (± 0.149 mm). The solid composition of CPH obtained water content of 13.83 ± 0.05%, total solid of 86.17 ± 0.05%, volatile solid of 83.92 ± 0.08%, and ash of 2.25 ± 0.03%. While the lignocellulose content included cellulose of 17.33%, hemicellulose of 15.09%, lignin of 45.36%, pectin of 7.79%, and extractive compounds of 8.55%. The high lignin content needs to be reduced through a pretreatment process to increase the enzyme hydrolysis of the cellulose and hemicellulose components during the anaerobic fermentation process.

3.2. Pre-treatment Effect on Cocoa Pod Husk Composition

Three variations of pretreatment were used to determine changes in the content of digestion inhibitors in CPH, especially lignin. In N and NH_S pretreatment three types of biomass concentrations were used for pretreatment (5, 10, 15%). While biomass concentrations for NHC pretreatment were used the same as the best results of lignin reduction in NH_S pretreatment. From the results, solid recovery of N pretreatment was generally greater than the NH_S and NHC pretreatment. The greater the solid recovery, the more economically profitable because of the loss of biomass when pretreatment is less. With the higher biomass concentration, the less amount of solvent in the pretreatment is used, so that less solid biomass is dissolved. The same results are shown in a study conducted by [11] where pre-treatment of the remaining brewing brew using NaOH and H_2O_2 solvents produced greater residue when biomass concentrations at pretreatment were also large.

The highest lignin removal occurs in NHs pretreatment with a5% biomass concentration of 91.73%. Lignin removal in the pretreatment of combination NaOH with H_2O_2 was higher than pretreatment using NaOH alone. Lignin removal on N pretreatment obtained the highest results at 15% biomass concentration, i.e., 79.26%. N pretreatment removes lignin through solvolytic reactions. Where the mechanism that occurs is the separation of the lignin aromatic ring by attacking the ether bond [12]. While in NHs and NHc pretreatment delignification through oxidative cracking. The combination of NaOH and H_2O_2 produces hydroxyl radicals and hydro-peroxyl which attack α-benzyl ether bonds between lignin and hemicellulose [13]. Reference [14] stated that biodegradability of lignocellulosic biomass increases with decreasing lignin content, so the greater the lignin content, the lower the production of biogas. Lignin on the surface of the substrate can be a physical barrier to hydrolytic enzymes, which can increase the efficiency of the anaerobic fermentation process. The mechanism of inhibition by lignin is reversing or irreversibly binding the enzyme to hydrophobic lignin. Based on batch tests, lignin hardly produces methane during anaerobic fermentation because lignin biodegradation occurs mainly in aerobic conditions, and in anaerobic conditions lignin can last for a very long time [15].

The results show that cellulose content was increased. The increase in high cellulose content was obtained from the results of NH_S pretreatment then followed by NHC and N pretreatment. This increase is due to the reduced lignin fraction from CPH. Table 1 shows the greater biomass concentration in NaOH pretreatment, the lower cellulose content. While the content of hemicellulose shows the greater the concentration of biomass at pretreatment, the higher the

Table 1. Composition of cocoa pod husk after pretreatment

Pre treatment	% Biomass	Solid Recovery (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Pectin (%)	Lignin Removal (%)
N	5	41.99	31.36	26.27	25.74	0.768	75.26
	10	44.23	30.74	30.36	24.29	1.932	75.41
	15	45.87	28.87	34.03	20.86	9.254	78.10
NH_S	5	38.56	41.89	28.68	9.73	5.473	91.42
	10	39.02	36.44	28.82	14.07	6.571	87.44
	15	39.87	33.05	34.59	11.17	5.731	89.81
NH_C	5	40.77	36.08	31.02	12.13	4.730	88.68

content of hemicellulose. Decrease in cellulose and hemicellulose content in NaOH pretreatment due to partial dissolution in solvents, especially xylose and arabinose. Greater hemicellulose solubility was achieved using higher NaOH concentrations [11]. Higher NaOH concentrations were found in pretreatment with low biomass concentrations. Cellulose is a major component in the formation of biogas. Unlike cellulose, hemicellulose is more amorphous, random, and branched. Amorphous and branching properties make hemicellulose very susceptible to biological, thermal and chemical hydrolysis of its monomeric [3]. In a study conducted by [15], it was shown that microcrystalline cellulose anaerobic fermentation produced 251.4 mL / g of VS methane which was higher than hemicellulose (arabinogalactan) 223.5 mL / g VS under conditions the same one. This shows that biomass with high cellulose content has a high potential to produce biomethane. However, on anaerobic fermentation with a combination of cellulose substrate (microcrystalline cellulose) and hemicellulose (xylan), it was able to produce more methane gas than if using only cellulose substrates, i.e., 262 mL / g VS. Xylan in biomass can facilitate cellulose swelling. So that it can increase enzymatic hydrolysis to cellulose fibers.

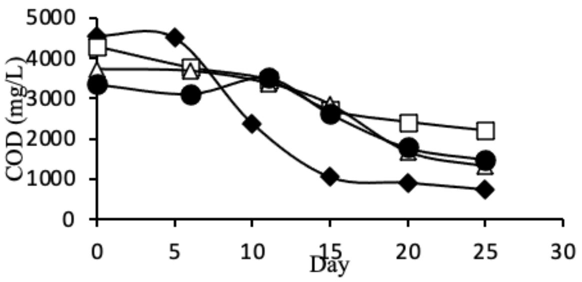

Figure 1. Chemical Oxygen Demand profile in substrate of N (♦), NH_S (Δ), NH_C (●), and untreated (□)

The pectin content in CPH biomass after pretreatment is shown in Table 1. At N pretreatment, the higher the biomass concentration the higher the pectin content in biomass. This shows that pectin will be more soluble in solvents containing more NaOH. While in the combination pretreatment of NaOH-H_2O_2 there was no significant difference in the pectin content. Which the highest pectin removal occurs in N pretreatment with 5% biomass concentration i.e., 96.51%. Eliminating pectin can increase the cell wall surface and improve the access of enzymes to degrade cellulose [16].

3.3. Chemical Oxygen Demand

COD is one of the parameters to measure the activity of microorganisms in degrading organic matter in the biogas fermentation process. The formation of biogas can be indicated by a decrease in the level of COD [17]. In Figure 1 shows that the substrate variable N pretreatment drastically decreases COD on days 5 to 15 and decreases slightly on days 0 to 5 and days 15 to 25 Whereas in the substrate variable the results of NH_S and NHC pretreatment decreased COD occurred significantly on the days 10 to 20 and a slight decrease on days 0 to 10 and days 20 to 25. For untreated substrate, significant COD decreases on days 10 to 15 and a slight decrease occurred before day 10 and after day 15. Significant reduction in COD indicates the formation of methane gas as a result of the process of methanogenesis. Whereas an insignificant reduction in COD in the early days showed that biogas production was still in the stage of hydrolysis and acidogenesis and for the last days the biogas production process had almost been completed [18]. According to [19] COD would decrease significantly on days 2 to 10 of fermentation, it will slowly drop and will stabilize on days 12 to 20. However, this condition also depends on the type of reactor used and the type of biomass used. This is because the anaerobic degradation of organic compounds can be efficient and stable when there is a balance in the rate of metabolism of acid-forming bacteria and CH4 form bacteria. Imbalance can occur due to excessive organic content or changes in operating conditions (the type of reactor used) which will trigger the accumulation of intermediate products that inhibit methanogen bacteria [19].

3.4. Volatile Fatty Acid and pH

In the biogas production process, the substrate is always converted into intermediate products such as VFA (acetic, propionic, butyric acid), hydrogen, and carbon dioxide before being converted to methane. The increase in VFA value shows that the high concentration of VFA produced and the decrease in VFA values indicate the conversion of VFA to methane. However, not always a high concentration of VFA will guarantee efficient biogas formation, because the accumulation of VFA will make a nitrogen-deficient biomass substrate [17]. In this experiment, VFA will increase significantly around days 10 to 15 which indicates the reduction of reducing sugars resulting from hydrolysis to VFA. Then it will drop back between days 15 and 20 which shows the conversion of VFA that has been produced to methane gas [20]. The increase in VFA causes the pH to drop. This condition is beneficial for acidogenic and acetogenic bacteria because it can develop well in the pH range of 4.5 to 5.5 [1]. However, on the day the VFA begins to fall (day 15) the pH needs to be conditioned around 7 to optimize the work of methanogenic bacteria in producing methane gas. Where optimal conditions for biogas production are obtained at pH 6.8-7.6 [20].

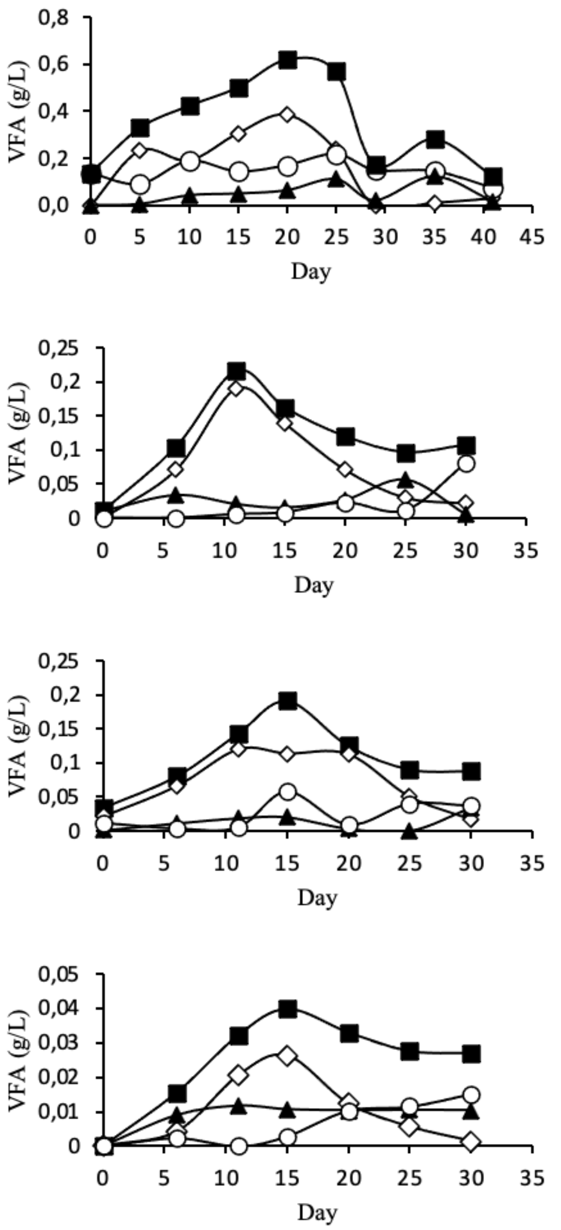

Figure 2. Volatile Fatty Acid (total (■), acetic (◊), propionic (○), butyric (▲)) alteration in substrate of N (A), NH_S (B), NH_C (C), and untreated (D)

3.5. Biogas production and kinetic

Gas production is the result of biomass conversion which is aided by various types of bacteria in the inoculum where methane gas is the most desirable gas because of its high heating value [17]. The highest gas produced is obtained from the NH_S substrate variable (509 mL). Then followed by variables N (500 mL), NHC (434 mL) and untreated substrate (318 mL). On the 5th day for all variables have begun to produce gas, this shows that on the 5th day methanogenic bacteria began to actively convert acetic acid to methane gas and continued to increase until the 20th day. For variables of the untreated substrate, it produces quite a bit of gas and is very long due to the substrate not going through the pretreatment process so that the work of bacteria is inhibited by the content of lignin which blocks the degradation of cellulose.

The final content of methane for NH_S, NHC, N and untreated variables were 50.12%, 43.55%, 31.30% and 29.11%, respectively. The biogas yield (m3CH4 / kg VSremoval) was 0.0389, 0.0265, 0.0309 and 0.0172, respectively. While the productivity of biogas (m3CH4 / kgVSremoval day) was 0.0009, 0.0006, 0.0007, and 0.0004, respectively. Biogas results from pretreatment substrate showed an increase over

Table 2. Kinetic parameter

Sample	Biogas Produced (mL)	B (mL)	Rb (mL / day)	λ (day)	R^2
Untreated	318	322.74	20.086	6.08	0.9955
N pretreatment	500	533.20	21.609	1.41	0.9910
NHs pretreatment	509	527.44	24.525	1.60	0.9940
NHc pretreatment	434	451.16	21.548	2.14	0.9903

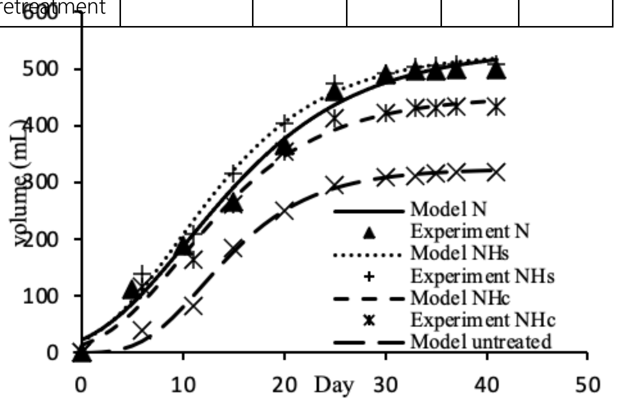

Figure 3. Kinetic model of biogas production

untreated substrate. N, NH_S and NH_C pretreatment sequentially produced more biogas (methane) 69.06%, 175.59%, and 104.18% than untreated substrate.

Modeling of biogas production with experimental results shows high suitability. All determinant coefficient values (R^2) are greater than 0.99 which can be shown in**Error! Reference source not found.** and Figure 3. This shows that the modified Gompertz equation can be used as a model in biogas production. The maximum biogas volume (B) that can be generated is the highest value for N pretreatment substrate and followed by NHs, NHc, and untreated substrate. While the highest maximum biogas production rate was obtained on NHs pretreatment substrate.

This shows that N and NHs pretreatment can increase the rate of bacteria in degrading organic materials properly. However, in terms of the quality of NHs pretreatment is better because it has a higher methane gas content than N pretreatment. While the time of bacterial acclimatization occurs the longest on the untreated substrate which is almost 4 times longer than the N and NHs pretreatment. This shows the influence of lignin as a bacterial inhibitor to degrade organic materials (cellulose and hemicellulose) in CPH.

4. CONCLUSION

The combination of the NaOH-H_2O_2 pretreatment method in this study can effectively remove the lignin content and release the cellulose fibrils in the CPH and therefore enhance the methane progress. The highest methane yield (0.0389 m3CH4/kg VS) was obtained from a substrate that resulted in NHs pretreatment. This pretreatment increased methane yield by 175.59% over that obtained with the untreated substrate. The increase in methane anaerobic fermentation demonstrated that NHs pretreatment is valid in treating CPH. The modified Gompertz equation can use as modeling for biogas production in this research with R^2>0.99.

ACKNOWLEDGMENTS

Authors acknowledge Research and Community Service Foundation of Institut Teknologi Sepuluh Nopember (LPPM-ITS) for the helps and Ministry of Research, Technology and Higher Education of the Republic of Indonesia for financial support with grand number 915/PKS/ITS/2019

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