Analysis of biogas production made from oil palm empty fruit bunches (OPEFB) using anaerobic batch reactor (ABR)

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Abstract. This research aims to analyse the biogas production made from oil palm empty fruit bunches (OPEFB) using anaerobic batch reactor (ABR). In this study three factors were used and each had three levels. The first factor is source of microorganism (liquid waste and sludge of Palm Oil Mill Effluent, and P-Biored), the second factor is preliminary treatment using NaOH (concentrations of 0%, 1%, and 8%) and the third factor is total solids (10%, 25%, and 40%). The research parameters included the volume of biogas, levels of methane gas, and reduced organic matter. The results of this study indicate that the best factor and level are preliminary treatment with 0% NaOH, source of microorganism from liquid waste of Palm Oil Mill Effluent and using 10% total solids which produce as much as 2457 ml biogas with methane yield of 929.01 ml/300 gr OPEFB and reduced organic matter of 138.25 gr/300 gr OPEFB.

1. Introduction
Indonesia is the world's largest producer of crude palm oil (CPO) with total production reaching 31,500,000 tons in 2016 and experiencing an increase to 47,437,000 tons in 2018 [1]. Indonesian CPO is produced from state plantations, private plantations and smallholder plantations [2]. The concern is arising along with the increase of CPO production, which means the waste produced is also getting higher. The total amount of solid waste produced can reach 40% of the total fresh fruit bunches (FFB), including 23% of oil palm empty fruit bunches (OPEFB), 12% fibers and 5% shells, while the volume of liquid waste reaches 2.5 m³ per ton CPO [3].

OPEFB contains 46.5% carbon, 36.7% oxygen, and 5.7% hydrogen which could theoretically be converted to 130.9 m³ methane per ton OPEFB. OPEFB also has cellulose content (42.9%) and hemicellulose (26.1%) of the total solids that have potential as a carbon source [4]. Cellulose and hemicellulose can be hydrolyzed into simple sugars that can be used as energy source for biogas-producing microorganisms.

Biogas is a renewable energy source that is more environmentally friendly than fossil energy. Biogas composition generally consists of 45-70% methane (CH₄) and 30-45% carbon dioxide (CO₂) and several other gases [5]. The heat produced from biogas is 6400 - 6600 kcal / m³ and 1 m³ biogas is equivalent to the heating value produced from 0.62 liters of kerosene, 0.46 liters of LPG, 0.52 liters of diesel oil, 0.08 liters of gasoline or 3.5 kg of firewood[6].

Biogas is produced under anaerobic conditions by a consortium of microorganisms that able to decompose organic matter and convert it into biogas. The process of breaking down organic matter consists of four main stages namely hydrolysis, acidogenesis, acetogenesis and methanogenesis.
Hydrolysis is the initial stage of the biogas production process where complex organic material will be broken down into simple compounds. Acidogenesis is the process of converting simple compounds resulting from hydrolysis into volatile acids by acid-forming bacteria. At the acetogenesis stage volatile acids are converted to acetic acid (CH₃COOH), hydrogen (H₂) and carbon dioxide (CO₂). The last step is methanogenesis, the process of converting CH₃COOH, H₂ and CO₂ into biogas by methane-producing bacteria. In general, 70% of methane is produced from the conversion of acetic acid, while the other 30% comes from H₂ and CO₂ [7].

In addition to cellulose and hemicellulose, OPEFB as lignocellulosic biomass contains lignin polymers which can inhibit the process of lignocellulose degradation into biogas. Therefore, a preliminary treatment is needed to break the structure of the lignin polymer (delignification). In addition to the preliminary treatment, a co-digestion strategy also needs to be able to increase methane production from lignocellulosic biomass feedstock [8]. The purpose of co-digestion is to provide additional substrate for anaerobic microorganisms so that the formation of biogas becomes more stable [9]. Other factors such as total solids can also affect the amount of biogas production [10], with the ideal value in the range of 10-25% [11]. Referring to this matter, this study tries to analyze the production of biogas by considering several factors such as preliminary treatment, co-digestion strategy or source of microorganism and total solids to the biogas volume.

2. Methods
2.1. Raw Material and Preliminary Treatment
OPEFB is obtained from the Palm Oil Mill located in Banten, West Java. OPEFB that used as raw material is washed, sun dried and downsized up to 3-5 cm in size. The OPEFB was then immersed in a solution of NaOH (delignification) indifferent concentration from 0%, 1% and 8% which adjusted to the experimental design for 24 hours. The characteristics of OPEFB can be seen in Table 1 below.

| No | Parameter         | Contents (%)       |
|----|-------------------|--------------------|
| 1  | Water Content     | 77.44±1.20         |
| 2  | Fat               | 0.744±0.02         |
| 3  | Reducing Sugar    | 0.077±0            |
| 4  | Cellulose         | 32.37±0.22         |
| 5  | Lignin            | 28.14±2.40         |
| 6  | Hemicellulose     | 27.02±0.60         |

Inoculums (microorganisms) were obtained from the Treatment Plant of Palm Oil Mill Effluent (liquid waste and sludge) as well as inoculums derived from P-Biored. Table 2 shows the concentration of microorganisms in each inoculum used. The highest TPC is in the P-Biored inoculum and the lowest is in the liquid waste. A side from being a source of microorganisms, sludge and liquid waste are also used as additional substrates (co-digestion) with total solid values as presented in Table 2.

| Source of Inoculum | TPC          | Total Solids (%) |
|--------------------|--------------|------------------|
| Liquid Waste       | 6.65x10⁴±4±0.25 | 17.23±3.10       |
| Sludge             | 1.98x10⁷±0.05  | 1.26±0.03        |
| P-Biored           | 2.34x10⁷±0.00  | -                |

2.2. Experimental Design
This study uses the L9 (3⁴) orthogonal matrix design with three factors, namely the source of microorganism (inoculum), concentration of NaOH and total solids of substrate. Levels of inoculum
source are liquid waste, sludge and P-Biored. The level of NaOH concentration are 0%, 1%, and 8%, while the levels of total solid factor are 10%, 25% and 40%. Table 3 presents the experimental design carried out in this study.

Table 3. Experimental Design

| Experiment Number | Inoculum      | Concentration of NaOH | Total Solids | Code   |
|-------------------|---------------|-----------------------|--------------|--------|
| 1                 | Liquid Waste  | 0%                    | 10           | LcN010 |
| 2                 | Liquid Waste  | 1%                    | 25           | LcN125 |
| 3                 | Liquid Waste  | 8%                    | 40           | LcN840 |
| 4                 | Sludge        | 0%                    | 40           | LpN040 |
| 5                 | Sludge        | 1%                    | 10           | LpN110 |
| 6                 | Sludge        | 8%                    | 25           | LpN825 |
| 7                 | P-Biored      | 0%                    | 25           | PbN025 |
| 8                 | P-Biored      | 1%                    | 40           | PbN140 |
| 9                 | P-Biored      | 8%                    | 10           | PbN810 |

2.3. Anaerobic Batch Reactor

The reactor used in this study is laboratory scale model consisted of a digester connected to a container and a graduated cylinder (Figure 1). The capacity of digester is 4 L of total solid. The principle is to drain the gas produced from the digester into a container that filled with water. The pressure by the gas causes the water in the container to come out. The volume of water that coming out of the container then measured using a graduated cylinder. With this principle, the volume of gas produced is assumed to be the same as the measured water volume. Furthermore, the methane content in the gas is measured using Gas Chromatography-MS.

Figure 1. Laboratory Scale Biogas Reactor

2.4. Observation Parameters

The degree of acidity (pH), biogas volume, methane content, and reducing organic matter are the parameters observed in this study. The observations for biogas volume were carried out every day until the day 35, the observations of methane levels were carried out twice during the fermentation process, on days 15 and 28, while observations of pH and organic matter carried out at the beginning and the end of fermentation. Reducing organic matter observed included fat, sugar, cellulose, hemicellulose, and lignin.
3. Results and Discussion

3.1. Biogas production

The biogas from each experiment is produced started on the 5th day and then increased on the 10th day until the 20th day (Figure 2). On the 10th day to the 20th biogas production tends to be stable with an increase and decrease that is not too large. On day 0 to day 20 is the initial phase of biogas production (lag phase), where microorganisms adapt to environmental conditions to produce biogas. On the 21st day entering the log phase so that biogas production increased significantly, and maximum biogas production was reached on days 25 and 27, after that biogas production tended to decrease until the 30th day. Entering the 31st day of the experiment without delignification using NaOH still experienced a stationary phase, whereas experiments with low concentration NaOH destruction experienced a fairly long lag phase.

At the end of the fermentation, it was found that the highest production (ml) was produced by LpN040, LcN010 and LpN110 at 2524.25 ml; 2457 ml; and 1849 ml respectively (Figure 3), which LpN040, LcN010 and LpN110 are the experiments without NaOH destruction and through destruction of low concentration NaOH. This experiment also used an inoculum of sludge and liquid waste. Liquid waste used in this study has a content of fat and sugar that can be used as co-substrates for microorganisms, and sludge itself has a higher sugar content compared to liquid waste. This organic material is expected to be consumed by microorganisms and contribute to biogas production. In addition, the OPEFB had previously passed the steam explosion treatment in the production line. Steam explosion itself is one of the preliminary treatment methods commonly used for lignocellulosic material. Through this method the biomass will undergo explosive decompression that causes changes in the structure of the material, transformation degradation of hemicellulose and lignin due to high temperatures, which facilitate the hydrolysis of cellulose [12]. This is thought to be the cause of biogas that can still be produced even though the preliminary treatment is not carried out or only by using low concentration of NaOH. In the treatment of low concentration NaOH also produced pH with ideal values for living microorganisms so that the process of biogas formation can run optimally.
Meanwhile, the lowest biogas production was produced by LcN840, LpN825 and PbN810 for 76.631 ml, 20.5 ml and 12.5 ml respectively. Each of these three experiments has N8 code which shows that one of the combinations is the destruction using NaOH with a concentration level of 8%. NaOH destruction alone releases Na\(^+\) ions which can be inhibitors for methanogenic bacteria \[^{13}\]. The destruction process using 8% NaOH also made the initial pH of LcN840, LpN825 and PbN810 very alkaline, which was 11; 11.5; and 12 (Figure 4). The growth of bacteria is very dependent on pH conditions, such as acidogenic bacteria which can only develop optimally at pH 5-6 and methanogenic bacteria at pH 6.5-8 \[^{14}\]. Beyond this value, the bacteria cannot adapt well and biogas production does not run optimally.

**Figure 3. Cumulative Volume of Biogas**

Figure 4 shows the pH value of each experiment at the beginning and end of fermentation. LcN010 which is an experiment with the highest methane yield having a stable pH that is in neutral conditions. Under this condition microorganisms can develop well to produce methane gas which is seen from the high amount of reducing organic matter (Table 4).
3.2. Methane Contents
Figure 5 shows the levels of methane from each experiment. It is known that the highest methane content produced by LpN110 which is 44.13%, followed by the LcN010 experiment with a methane content of 43.04%. Both experiments in addition to producing high levels of methane also produced large volumes of biogas. Whereas in LpN825, LcN840 and PbN810, methane levels were low when compared with other experiments, namely 1.2%, 0.3% and 0.1%. As with volume, these three experiments also produced a low total biogas volume.

The total volume of methane is shown in Figure 6. The experiment without preliminary treatment of NaOH and using liquid waste inoculums are known to produce the highest methane yield with 929.01 ml/300 gr OPEFB. This result can be obtained because microorganisms in liquid waste have a complete community including microorganisms that play a role in the process of hydrolysis, fermentation, acidogenesis, and methanogenesis. Biogas produced comes from cellulose and hemicellulose which are integrated by microorganisms in liquid waste. This is indicated by the decrease in cellulose and hemicellulose content as presented in Table 4. In addition, the high biogas production is estimated because liquid waste as co-digestion also contains organic matter such as fat (1.27 ± 0.02%) and reducing sugar (0.246 ± 0%) which were converted to biogas. The use of palm oil liquid waste as co-digestion of oil palm empty fruit bunches can increase the amount of methane gas produced [15]. The pH that is formed is also in an ideal condition for microorganisms to grow optimally so that the degradation of lignocellulose to methane gas runs optimally.

![Figure 5. Methane Content](image-url)
Reduced Organic Matter

Based on Table 4 it is known that the greatest reduction in organic matter occurred in PbN025 with total reduced organic matter of 153.025 grams and followed by LcN010 of 138.256 grams. The lowest was in PbN810 with total reduced organic matter of 51 grams. The average results using the S/N ratio method showed the greatest effect in reducing lignin levels due to the pretreatment factor with a concentration of 1% NaOH solution. The effect of the preliminary treatment also affects the amount of hemicellulose in the material, where the increase in hemicellulose content is proportional to the increase in the concentration of NaOH solution. Whereas the most cellulose reduction occurred in the preliminary treatment using 0% NaOH because microorganisms that digest cellulose cannot digest properly under alkaline conditions caused by the addition of NaOH especially in high concentrations. Experiments with sources of P-Biored microorganisms also showed a high reduction in cellulose because P-Biored contains a lot of cellulolytic microorganisms so that a lot of cellulose is reduced. However, microorganisms in P-Biored cannot convert organic matter into biogas, this is indicated by the low methane gas formed. The amount of reduced cellulose is directly proportional to the production of methane gas produced through experiments LcN010 and LpN040.

Table 4. Reduced Organic Matter

| Code   | Fat  | Sugar | Cellulose | Hemicellulose | Lignin | Total     |
|--------|------|-------|-----------|---------------|--------|-----------|
| LcN010 | 1.379| 0.467 | 53.920    | 38.785        | 43.705 | 138.256   |
| LcN125 | 1.114| 0.479 | 26.325    | 47.325        | 56.145 | 131.389   |
| LcN840 | 0.583| 0.500 | 19.275    | 73.095        | 40.230 | 133.684   |
| LpN040 | 1.798| 0.430 | 45.220    | 27.505        | 35.815 | 110.768   |
| LpN110 | 0.816| 0.467 | 10.845    | 24.840        | 34.755 | 71.722    |
| LpN825 | 0.180| 0.474 | 15.015    | 70.170        | 34.350 | 120.189   |
| PbN025 | 1.993| 0.478 | 57.565    | 45.355        | 47.635 | 153.025   |
| PbN140 | 0.775| 0.477 | 24.690    | 41.385        | 48.945 | 116.272   |
| PbN810 | 0.869| 0.526 | 2.985     | 33.210        | 13.635 | 51.226    |

Figure 6. Total Volume of Methane
3.4. Determination of the Best Factors and Levels

Each factor and level have an optimum condition that is different from the indicators used. The comparison of optimum levels and factors for each indicator can be seen in Table 5.

| Table 5. Comparison of Response to Each Indicator |
|--------------------------------------------------|
| Factor | Volume of Methane | Cellulose | Hemicellulose | Lignin | Fat | Sugar |
|        | mean SNR | mean SNR | Mean SNR | mean SNR | mean SNR | mean SNR |
| Source of Microorganism | 1 | 1 | 1 | 1 | 3 | 3 |
| Pre-Treatment using NaOH | 1 | 1 | 3 | 2 | 1 | 3 |
| Total Solids | 1 | 2 | 2 | 2 | 1 | 1 |

The highest loss function and S/N values were in LcN010 which is 1.0788 followed by LpN110 trial with a value of 0.3705 (Table 6). From all the experiments that have been carried out only LcN010 and LpN110 are positive, showing that there are significant differences from the two experiments compared to 7 other experiments.

| Table 6. Total Loss Function and S/N |
|--------------------------------------|
| Experiments | Volume of Methane | Cellulose | Hemicellulose | Lignin | Fat | Sugar |
|             | Wi x Nj | TL | N |
| LcN010 | 2.7554 | 0.3810 | 0.1006 | 0.1696 | 0.0323 | 0.0239 | 3.462 | 0.584 | 7 | 1.0788 |
| LcN125 | 0.0179 | 0.0914 | 0.1492 | 0.2798 | 0.0213 | 0.0251 | 7 | 0.581 | -0.4661 |
| LcN840 | 0.0000 | 0.0487 | 0.3559 | 0.1437 | 0.0061 | 0.0274 | 0.903 | 7 | -0.4706 |
| LpN040 | 0.3932 | 0.2678 | 0.0504 | 0.1140 | 0.0579 | 0.0202 | 0.903 | 5 | -0.0882 |
| LpN110 | 1.3288 | 0.0164 | 0.0411 | 0.1077 | 0.0142 | 0.0238 | 1.531 | 9 | 0.3705 |
| LpN825 | 0.0000 | 0.0295 | 0.3280 | 0.1047 | 0.0006 | 0.0246 | 0.487 | 5 | -0.6241 |
| PbN025 | 0.0035 | 0.4339 | 0.1371 | 0.2014 | 0.0682 | 0.0249 | 0.869 | 1 | -0.1219 |
| PbN140 | 0.0013 | 0.0798 | 0.1142 | 0.2126 | 0.0102 | 0.0249 | 0.443 | 0 | -0.7072 |
| PbN810 | 0.0000 | 0.0015 | 0.0735 | 0.0165 | 0.0142 | 0.0303 | 0.136 | 0 | -17.331 |

The influence response of all factors and levels can be seen in Table 7. The best results of the influence response to the source of microorganisms is at level 1, in the factor of preliminary treatment the best result is at level 1, and the best result on the total solids factor is at level 1. At the factor of source of microorganisms, the most influence is liquid waste followed by sludge and P-Biored. In the preliminary treatment it is known that the higher the concentration will be inversely proportional to
biogas production, this is because high concentration of NaOH will increase the pH value which hardly meet the optimum conditions for microorganisms to grow. Whereas high total solids will inversely proportional to the biogas yield, this is because biogas production still requires water (H₂O) to be able optimizing the biogas yield.

| Influence Response of All Indicators | Source of Microorganism | Pre-Treatment using NaOH | Total Solids |
|-------------------------------------|-------------------------|--------------------------|--------------|
| Level 1                             | 1.543                   | 1.745                    | 1.710        |
| Level 2                             | 0.974                   | 0.853                    | 0.647        |
| Level 3                             | 0.483                   | 0.402                    | 0.643        |
| Difference score                    | 1.060                   | 1.343                    | 1.063        |
| Rank                                | 3                       | 1                        | 2            |

Based on the best response from each factor, it can be seen the best combination is to use microorganisms sourced from liquid waste, preliminary treatment using 0% NaOH and 10% total solids. While the preliminary treatment using NaOH is the factor that gives the highest effect compared to the other two factors.

4. Conclusion
The best combination of treatment is the use inoculum from palm oil liquid waste, preliminary treatment with 0% NaOH and 10% total solids. In this combination biogas can be produced with a volume of 2457 ml with a methane yield of 929.01 ml/300 gr OPEFB and reduced organic matter as much as 138.25 grams/300 grams of OPEFB. Though in this combination the cellulose and hemicellulose of OPEFB were not optimally used as biogas source due the absence of preliminary treatment, the palm oil liquid waste as an inoculum and co-digestion has a complete microorganism community and high enough organic matter content that contributes to the increase in biogas production. In addition, optimal pH conditions make acidogenic and methanogenic bacteria work actively to form biogas which is seen from the degradation of organic matter, especially cellulose and hemicellulose.

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