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Biogas Production from Palm Oil Mill Effluent with Indigenous Bacteria

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***Abstract*— POME or palm oil mill effluent is wastewater from the production of palm oil, which is produced by the sterilization, clarification, and hydrocyclone processes. The POME contains high carbohydrates, lipids, and protein which can contaminate the environment if it is not handled properly. The carbohydrates, lipids, and protein contained in the POME are potential for biogas production through the fermentation process with indigenous bacteria. The research aimed to study the impact of degradation time to the production of biogas in the bioreactor using the indigenous bacteria, KP1.2 (*Stenotrophomonas rhizophila* strain e-p10) capability from palm oil mill effluent. The fermentation process was carried out in the anaerobic bioreactor with ranges of degradation time from 3 to 38 days to produce biogas. The bacterial population was calculated using a haemacytometer in which the number of bacteria was calculated in the small cubicles with a microscope. Biogas was saved in Tedlar bag and the biogas content was analyzed by Gas Chromatography (GC). The bacterial population increased along the fermentation period. The pH values range from 6.8 to 8.3. The highest bacterial population was 7.21 x 10^7 cells/mL and the lowest one was 3.15 x 10^7 cells/mL. The methane content, as well as, carbon dioxide content increased along the fermentation period. The highest methane content was obtained at 63.7 %moles and carbon dioxide was 22.5 %moles, while the lowest methane content was 33.5 %moles and carbon dioxide content was 19.5 %moles.**

Keywords*— palm oil mill effluent; biogas; *Stenotrophomonas rhizophila strain e-p10.

I. INTRODUCTION

Recently, the energy crisis is faced by all countries, especially developing countries. The stock of fossil energy causes it are getting lower. Besides that, the fossil energy can damage the earth, which can cause global warming. That's why there should be a novel innovation to find renewable energy sources as biofuel. Then, the renewable energy source must be low cost and environmentally friendly. One of the innovations in alternative energy is biogas. There are many raw materials for biogas production, such as animal manure, industrial effluents, food wastes, and agricultural wastes. The process for biogas production is fermentation, which is a simple process. So, biogas can be alternative energy for Indonesia because it is cheap and environmentally friendly.

The prospect of CPO trading is still up-and-coming in Indonesia because of the high demand. It is proved by the large area of palm tree plantations that always grow rapidly in Indonesia. As recorded in August 2018, it has reached 30.6 million tons of CPO production and is expected to reach 46.17 million tons [1]. Because of overwhelming CPO production, there will be high volume wastewater produced by palm oil processing. In the process of heating and sterilization, fresh fruit bunches are processed by steam sterilization with a steam pressure of 2.5-3.0 kg/cm^2, a temperature of 135-140^{o}C for 90-100 minutes. First, the wastewater (condensate) produced from each process using a sterilizer in this process. In the extraction process, CPO is pressed by inserting raw materials into the screw press. In the CPO purification process, heating water was added at 90^{o}C, then CPO was purified by extracting impurities in the CPO to the side of the heating water layer. From this process, the oil content in hot wastewater is around 1%.

Because the CPO manufacturing process is steam pressure processing, FFB processing is a batch system, so wastewater is generated every 1 batch. For example, on boiling FFB, the processing time is 90 minutes, so that wastewater (condensate) will also be discharged from this process every 90 minutes. It means that if there are 3 units of boiler in the same facility, wastewater will be discharged as POME every 30 minutes. POME is produced from clarification (60%), sterilization (36%), and hydrocyclone process (4%) [2]. Almost all palm oil industries use an opened pond system to process POME, with economic considerations and simple operation. Even though opened pond system is economical, this system requires more extensive land, wasting time, and liberates methane gas to the environment through the degradation process of organic matters that take place in anaerobic lagoon. The methane releases from the POME treatment system and contributes global warming effect because it produces more than 70% of gas emissions in the overall CPO manufacturing [3]. The conversion of POME to biogas can be an alternative method for palm oil industries to minimize hazardous environments and create renewable energy. Methane gas (CH_4) is a gas that causes greenhouse effects, which can cause the phenomenon of global warming. This is because methane gas has an impact 21 times higher than carbon dioxide gas. POME has high organic materials, so it can be a raw material for biogas production in anaerobic digestion with bacteria because it can convert organic matters into methane. Conversely, in the aerobic process, it cannot be converted to biogas and produces a lot of sludge.

POME or palm oil mill effluent is wastewater from the production of palm oil. Most of POME production comes from the sterilization, clarification, and hydro-cyclone processes. POME is a promising renewable energy source because it contains high carbon to produce methane. Fresh POME is usually in brownish, with high solid content, oil and lipids, COD and BOD. The characteristics of POME are had low pH at 4-5, COD value was achieved up to 102,696 mg/L, BOD value was up to 65,714 mg/L, containing total solids up to 72,058 mg/L, including 46,011 mg/L of suspended solids, 49,300 mg/L of volatile solids, and containing 9,431 mg/L of oil and grease. Moreover, ammoniacal nitrogen was contained up to 103 mg/L and total nitrogen was achieved at 770 mg/L [4].

Biogas mostly includes gas produced from various industrial organic wastes. Biogas is not a synthetic gas. It is usually known as a mixture of gases that result from anaerobic decomposition of organic substances, that have the same characteristics as natural gas. Biogas technology is one of the appropriate techniques for treating waste, both livestock, agricultural, industrial, and household waste to produce energy. This technology utilizes microorganisms that are available in nature to overhaul and treat various organic wastes that are placed in airtight spaces (anaerobic condition). Generally, biogas compositions are 50-70% of methane (CH_4), 25-45% of carbon dioxide (CO_2), 0-2% of hydrogen (H_2), and other gases in small quantities. There are two essential processes in biogas production, such as mesophilic conditions and thermophilic conditions. Mesophilic digester operates at a temperature of 20–40^oC (68-104^oF), while thermophilic digester operates at the temperature over 50^oC (122^oF) to produce biogas [5]. Biogas is approximately 20% lighter than air. Biogas has a combustion temperature between 650-750^oC, odorless, and colorless. If it is burned, it will produce a bright blue flame, like LNG. The heating value of methane gas is 20 MJ/m^3. The potency from 600-700 kgs of palm oil mill effluent can be produced approximately by 20 cubic meters of biogas and 1 m^3 of methane gas can be converted to energy, which achieved at 4,700-6,000 kcals or 20-24 MJ [3]- [6].

To produce biogas, a reactor/digester is needed. The digester is an important apparatus in minimizing the emissions of methane (CH_4) production of the decomposition of organic materials produced from the agricultural or livestock sector. The principle of the digester is to create an airtight chamber (anaerobic) that is integrated with the input line and the output line. If solid waste is in a clotted condition, stirring is needed that is easier to get into the digester and so does the decomposition processes. The reservoir aims to accommodate the remaining materials (sludge) from the decomposed of organic materials in digesters and the nutrients will increase.

Both anaerobic and aerobic digestion can degrade organic matters effectively, but they have advantages and disadvantages. The anaerobic processes occur conditions without oxygen (without aeration), low energy consumption, and have low sludge growth (5-10%). While aerobic processes take place when there is oxygen, but high energy consumption, and have high sludge growth (30-60%). POME applications for energy sources are better in anaerobic processes [3]. The main reason for choosing an anaerobic process is their ability to produce biogas well. The aerobic process does not convert organic substances into methane. However, it produces more sludge, and the wastes can be discharged directly to the environment. In contrast, anaerobic processing produces methane and residues that are rich in nitrogen and phosphorus.

The kind of organic material used as raw material is a critical factor to be noticed because it affects the duration time of decomposition of materials to produce biogas. Organic materials from agricultural wastes usually take a longer time to decompose than livestock manure. The composition of carbon and nitrogen in organic materials is an important component of biogas production. It because microorganisms need carbon and nitrogen as an energy source for degradation. Microorganisms can decompose optimally in C/N ratio on 25/30. The C/N ratio may not be too high because it can be hampered in the performance of microorganisms. Meanwhile, if the C/N ratio is too low, there will form a high content of organic acids [3].

Anaerobic fermentation is a profitable biological treatment for high carbon waste like palm oil mill effluent and converts to biogas [4]. Anaerobic digestion often supported by bacteria because bacteria have a crucial role in enhancing biogas. The bacteria which can produce methane are *Bacteroidetes, Clostridiales,* and *Actinobacteria* [7]. The bacteria can be isolated from substrates, for example, *Proteus spp.* and *Enterobacter spp.,* which isolated from crude palm oil [8].

There are many attempts to get high quality of biogas production, POME treatment, bioreactor configuration, and digestion process supporting factors (initial pH, thermophilic condition, temperature, and bacterial communities).

Many researchers had researched the modification of bioreactors in biogas production. Ref. [8] stated that UAMAS (Ultrasonic-assisted Membrane Anaerobic System) is the best design for methane production from palm oil mill effluent throughout anaerobic treatment, which obtained the methane composition maximum was 77%, COD removal efficiency was achieved 98.7%, and the hydraulic retention time was 0.5 day. Ref. [6] used an anaerobic up-flow sludge blanket bioreactor (UASB) to made biogas from POME, which consists of acidogenic and methanogenic reactors. An acidogenic reactor as organic acids forming, like acetate acid and butyrate acid. Then, liquid wastes were streamed into the methanogenic reactor. Their research was obtained 22.8-26.4 liters of biogas in 16 days incubation, with COD reduction was 98% (983 mg/L) and TSS reduction was 99.4% (331.6 mg/L).

The research was conducted by Ref. [9] used palm oil mill effluent in acidified conditions for methane production under thermophilic conditions in ASBR (anaerobic sequencing batch reactor). The research was done in the various hydraulic retention time of 1,2,3,5,8, and 10 days. The highest methane content was obtained at up to 82.42% in 5 days HRT.

Inoculum also the primary factor for improve biogas production. Biogas production could be made by hydrolysis of POME with xylanase enzyme under the optimum condition at 60^oC for 45 days. They used seed sludge from receiving pond as the inoculum. The dominant bacteria from palm oil mill effluent hydrolysate were *Methanocaldococcus sp.* and *Clostridium sp., which* obtained methane yield was 914 CH_4/g VS and can reduce COD was 78-89% [10]. Biogas production can be done by mixing POME and active microbial in a beam-shaped digester with fed-batch, which obtained the highest methane content when the POME was changed every 5 days through 70 fermentation days. It was obtained at 30.0951% moles of methane [11]. To get some methanogenic microbes, the initial low pH is important to produce more methane. The low initial pH until 3.5 can produce methanogenic populations, namely *Methanotrix soehngenii*, which can live in low pH conditions and produced methane yield of 94% [12]. It means that there is a correlation between pH and methane content.

Dairy manure also can be a promising inoculum for biogas production rate. It is mixed with palm oil mill effluent in CSTR with a pH of 6.8 and 37^oC temperature anaerobically. The CSTR operated in five days in batch circumstances at 10 days of retention time. This research was obtained methane content at 59% and COD reduction percentage at 48% [13]. Besides pH, the availability of nutrition, especially nitrogen also affects the biogas production. Nitrogen is a very important component for anaerobic bacteria to grow well. But the dosage of nitrogen must be appropriate because if the nitrogen is too much, it will be form ammonia in biogas production. Ref. [14] found that biogas production was greater than that of without giving nitrogen nutrition (urea) and biogas was produced until 26 days.

One of the anaerobic methods for biogas production is co-digestion. Ref. [15] conducted biogas production by co-digested empty fruit bunches (EFB) and palm oil mill effluent (POME), which obtained the high methane yield was 320 mL CH_4/g VS and with 63-70% biodegradability. Another anaerobic co-digestion research also was done by Ref. [16]. The methane content was obtained at 64.13 % by co-digested palm oil mill effluent (POME) and cattle dung in solar-supported bioreactor semi-continuously at mesophilic temperature (35^oC).

Biogas production can be obtained from the integrated process, which produces methane and hydrogen at the same time. Recently, Ref. [17] conducted methane and hydrogen production from POME utilizing CSTR and electrolysis cells of microbial. They used alkali-treated sludge as inoculum for methane production, which was obtained at 2700 mL CH_4/g COD with 90% COD removed at eight days of retention time. Mostly, *Methanobacterium beijingense* and *Methanobacterium formicicum* were obtained in microbial electrolysis cell.

The other nutrition for increasing biogas rate from POME is calcium, which is also important for anaerobic microbes. Ref. [18] used nano calcium from eggshell by mixing palm oil mill effluent and cow manure through 20 days. They found that nano calcium concentration of 10 g/L had the highest efficiency in COD removal into 60%. While adding 5 g/L nano calcium into anaerobic bioreactor produced in 1.5 times higher of biogas production than giving calcium. So, it concluded that the particle dimension of calcium affects the biogas production and COD removal.

Toxic substances must be noticed because they can poison microorganisms and decrease the biogas rate. The toxic substances are mineral ions and heavy metals. The substrates for fermentation contain some heavy metals. For example, cow dung has high Zn^{2+} content, at 75.9 – 4333.8 mg/kg. Heavy metals in substrates can affect the biogas and methane content on anaerobic digestion because they contributed to enzyme activity. Zn^{2+} could influence the methanogenic process, which enhanced concentration at 5 mg/L in biogas yield and 0-100 mg/L in methane content. The content of Cu^{2+}, Ni^{2+}, and Fe^{2+} could affect cellulase activity [19].

There are many pre-treatment methods for enhancing biogas production, such as biological pre-treatment, mechanical pre-treatment (microwave, ultrasonic, and mechanical milling), chemical treatment (acid pretreatment, alkali pretreatment, ozonolytic, oregano solvent, and ionic liquid) biological pre-treatment, and hydrothermal pretreatment. The pretreatment method is one of the crucial techniques for biofuel production [20]. Ref. [21] had pretreatment of POME by ozonation, then, produced biogas by combining fermentation and ozonation continuously through 110 hours. That research resulted that 95% of COD can be removed and increased methane production. Ozonation process also was done by Ref. [22] to produce biomethane from POME by comparing the fresh POME and ozonation of POME in a batch reactor using UASB in mesophilic condition (37^oC) and neutral pH. It was concluded that the ozonated POME supplies the production of methane at 624.4 mL. Hydrothermal pre-treatment was conducted by Ref. [23] in the anaerobic process of the organic fraction in municipal solid waste (OFSW) then added cow manure as inoculum. OFSW was pre-heated in various temperatures (80, 100,120,140, and 160^oC) and heating duration of 0,15,30,60, and 120 minutes for each temperature. Pre-treatment at 140^oC for 30 minutes produced the high methane content of 68.6% in digestion periods of 18 days, while the untreated OFSW produced 50.3% methane in 28 days. Ref. [24] stated that biological pre-treatments could be combined with thermal, mechanical, or chemical treatments. Rice straw could produce a 165% increase in methane yield in biological pre-treatment combinations.

Many innovations to get high biogas production. One of the methods is bioaugmentation, which can enhance microbe populations and the *methanogenesis* stage. One of the microbes which can enhance methanogenic stages is *Metahospirillium hungatei*, which obtained from synthetic industrial waste composed of dry milk [7]. Suksong, et al. [25] conducted the production of biogas from empty fruit bunches and bio-augmentation of cellulolytic bacteria consortium. The consortium bacteria are *Clostridiaceae* and

Lachnospiraceae. The best methane production was reached by pre-hydrolysis EFB with *Lachnospiraceae* and obtained at 113 m^3 CH_4 for one tonne of empty fruit bunches.

So, it can be said that POME has a potential raw material for biogas production by anaerobic decomposition with bacteria. In this research, indigenous bacteria, KP 1.2, namely *Stenotrophomonas rhizophila strain e-p10* is used in degradation to degrade lipid components in substrates. Indigenous bacteria are potential bacteria in the biodegradation process that can be isolated from the waste itself. Several indigenous bacteria were successfully isolated from various wastes. It shows that bioremediation is a promising method for waste treatment that can prevent the negative effects of waste on the environment. The research aimed to study the impact of degradation time to the production of biogas in the bioreactor using the indigenous bacteria, KP1.2 (*Stenotrophomonas rhizophila strain e-p10)* capability from palm oil mill effluent.

II. Materials and Method

A. Raw Material Preparation

The raw POME used in this research was taken from the palm oil industry. POME was stored in 30 L plastic jerry can. POME was filtered with filtered-cloth to remove excessive suspended solids.

B. Rejuvenation of Stenotrophomonas rhizophila strain e-p10

To do rejuvenation of bacteria, nutrient agar slant was made first by dissolving 2.3 g of *nutrient agar* powder in 100 mL of aquadest, then heated on stirring hotplate, sterilize in an autoclave with a temperature of 121^{o}C for ± 1 hour. Then, poured into a 3 test tubes (already sterilized in an autoclave). Each tube filled with 7 mL, slanted, and left to make it harden. Took an ose needle and took the bacterial culture with the ose needle and inoculated it zig-zag into test tubes, which were nutrient agar contained. Then, incubated them in the incubator for 24 hours.

C. Preparation of Mineral Medium

The ingredients were used in preparation of mineral medium were 2 liters with the composition of 0.4 $MgSO_4.7H_2O$, 0.2 g $CaCl_2.2H_2O$, 9 g K_2HPO_4, 0.4 g $(NH_4)_2SO_4$, 0.2 g NaCl, 0.04 g $FeCl_3$, 6 g beef extract, 10 g yeast extract, and 40 mL vegetable oil were dissolved in 2 liters of aquadest in Erlenmeyer. After all the ingredients are mixed, then heated on stirring hotplate, then sterilized in an autoclave for 1 hour at a temperature of 121 °C.

D. Preparation of the Stenotrophomonas rhizophila strain e-p10 Inoculum

Preparation of bacterial inoculum was made by adding 4 g *nutrient broth* were dissolved in 500 mL of aquadest in Erlenmeyer, then heated on stirring hotplate. After being heated, *nutrient broth* was sterilized in an autoclave. Then, the suspension of bacteria *(Stenotrophomonas rhizophila strain e-p10)* was poured into Erlenmeyer and shook in shaker at 60 rpm speed for 24 hours.

E. Preparation of Starter

The starter was needed in the bioreactor was 2 liters. To make the 2 liters starter required the 1100 mL mineral medium, the 500 mL inoculum of the *Stenotrophomonasrhizophila strain e-p10*and 400 mL palm oil mill effluent. All the ingredients were put into Erlenmeyer and shook in shaker at 60 rpm for 6 hours.

F. Bioreactor Set-Up

A cylindrical bioreactor was made of fiberglass with a volume of 10 liters and connected to the Tedlar bag as a gas storage. The bioreactor was closed with rubber stopper, to guarantee air did not enter the bioreactor.

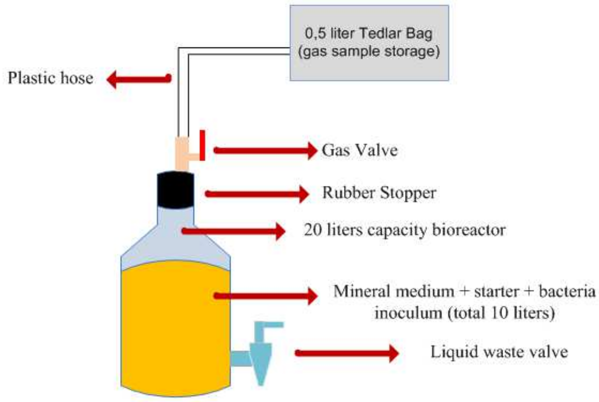

Fig. 1. Bioreactor Set-Up

G. Substrate Degradation Process

In a bioreactor with a capacity of 20 liters, it is filled with 6 liters of palm oil mill effluent, 2 liters of starter (500 mL of *Stenotrophomonas rhizophila strain e-p10* inoculum + 1100 mL of mineral medium + 400 mL of liquid waste), and 2 liters of mineral medium, so overall was 10 liters. The degradation process was carried out up to 38 days. Biogas was stored in Tedlar bag and the biogas content was analyzed by GC (Gas Chromatography). The bacterial population was calculated using a haemacytometer in which the number of bacteria was counted in the small cubicles with a microscope.

III. Results And Discussion

A. The Effect of Degradation Time on Bacteria Populations

The bacteria population can affect the biogas production. Calculation of the bacterial population was done based on the effect of the fermentation time. The growth curve of the *Stenotrophomonas rhizophila strain e-p10* along the fermentation period is shown in Figure 2.

The purpose of the calculation of the bacteria population was to know the correlation between fermentation time and bacteria growth. Calculation of bacteria population was started on the 20th day because the gas was visible on that day.

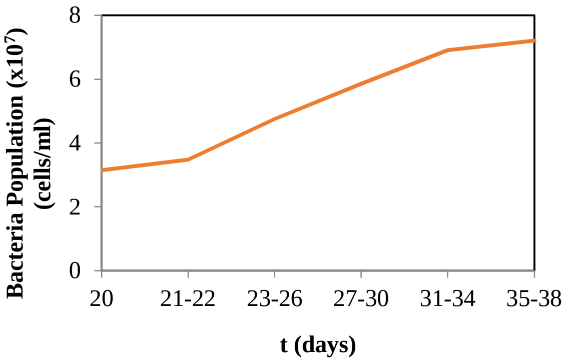

Fig. 2. Bacteria Populations Curve

The length of the degradation process affects the growth performance of bacterial populations. Bacteria grown in this bioreactor were *Stenotrophomonas rhizophila strains e-p10*, which is anaerobic, methanogenic, and lipolytic, which can decompose lipid in POME that produces metabolism in the form of methane gas in anaerobic conditions (Fig.2).

In general, the bacterial population always increases on day 20 until day 38, where the bacteria grow at a constant rate. On the 20th day, the bacterial population was 3.15 x 10^7 cells/mL. On this day, the bacteria undergo the lag phase, where the bacteria adjust to the bioreactor environment. On the31-34th day, the bacteria began to increase significantly to 6.91x10^7 cells/mL and until the 38th day, the bacterial population was 7.21 x10^7 cells/mL. It shows that the bioreactor condition and its supporting factors are suitable for bacterial growth so that bacterial growth was increased along the fermentation period.

Bacteria are the same as other living things, need much nutrition as a source of energy and cell growth. The basic elements are carbon, nitrogen, hydrogen, oxygen, sulfur, phosphorus, iron and a small number of other metals. Some studies have suggested that acidogenic processes require treatment of the inoculum to minimize the growth of methanogenic bacteria, which consume hydrogen. However, if the interest is in biomethane production, this pretreatment is not necessary. Among the techniques adopted, the most important is acid, alkaline, thermal, and chloroform treatments, among others [26]. Methanogenic bacteria are found in organic matters which can produce methane and other gases in the whole process in anaerobic conditions. Media formulations influence the growth of anaerobic bacteria. Like research was conducted by Ref. [27] made biogas from dairy cattle waste and anaerobic bacteria were used in various growth mediums, such as Rumen-Fluid-Glucose-Agar (RGCA), media 98-5, and NA (Nutrient Agar). A lot of bacteria grown much more in NA than the other media, which produced anaerobic bacteria at 2148x10^4 CFU/mL, but the biogas production was 1.06% CH_4 and 9.893% CO_2. For the best biogas production was RGCA, which was 4.644%CH_4 and 9.5356% CO_2.

Methanogenic bacteria grow slowly and are sensitive to sudden changes in chemical and physical conditions. Sudden changes on temperature can cause a decrease in the rate of bacterial growth and have an impact on low methane gas production. Therefore, it is very important to place the digester in the right position and location so that the resulting temperature can support the performance of methanogenic bacteria. Starter also supported the bacterial population growth. The starter is an additional ingredient for a microorganism that is useful for accelerating the decomposition process.

B. The Effect of Degradation Time on Gas Content

Analysis of gas content was done to analyze the biogas content along the fermentation process of POME by bacteria anaerobically. Biogas production is influenced by the presence of methane gas, which is produced by bacteria in POME.

Hydrolysis is the first stage of the fermentation stage. This stage is the decomposition of organic materials with complex compounds that have soluble properties such as lipids, proteins, and carbohydrates into simpler compounds. This step can also be interpreted as a structural change from the polymer into a monomer form. The compounds that were produced from the hydrolysis process included organic acid compounds, glucose, ethanol, CO_2, and other hydrocarbon compounds. These compounds will be used by microorganisms as an energy source to carry out fermentation activities.

The compounds were formed in the hydrolysis stage will be used as an energy source for microorganisms in the next stage, namely the acidification stage. In this stage, the bacteria will generate organic acid compounds such as lactic acid, butyric acid, propionic acid, and acetic acid along the fermentation process and produce by-products such as alcohol, CO_2, hydrogen, and ammonia.

The amount of energy produced in the biogas formation is very dependent on the concentration of methane gas produced in the methanogenesis process. The higher the content of methane was produced; the more energy was formed. Conversely, if the concentration of methane gas produced is low, the energy produced will also be lower.

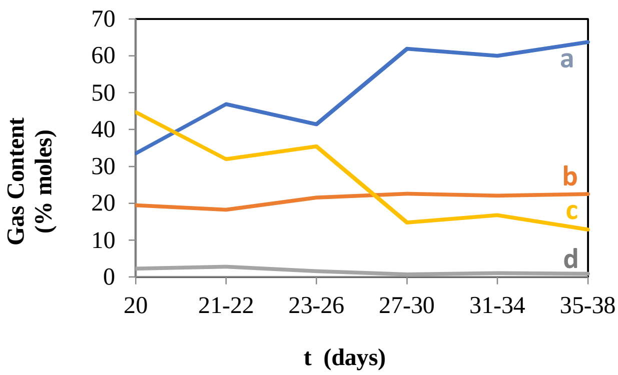

Fig. 3. Gas Content Curve

From Fig. 3, the longer degradation time, the more methane gas (a) was produced. The longer degradation time, the more bacterial population increases, the more lipase enzymes produced to degrade palm oil mill effluent, so that more methane gas (a) will be produced and so did carbon dioxide (b). While the oxygen (d) and nitrogen (c) were decreased through the fermentation period. The biogas production was visible on the 20th day, in which methane content was 33.5 %moles and the carbon dioxide was 19.5 %moles. Then, on the 22nd day, methane content was increased at 46.9% moles. But, on the 26th day, the methane content was decreased at 41.4% moles. It because organic substances as microorganism's nutrients are getting lower. On the 38th day, the highest methane content reached 63.7 %moles and carbon dioxide was 22.5% moles. It means that on the 38th day was the optimal time for microorganisms to produce high biogas. The range of oxygen content (d) was 0.7 – 2.8 % moles, while the nitrogen content was 12.9 – 44.7 % moles.

The process of methane production by bacteria starts from the hydrolysis process which is the initial step for organic material will be decomposed into simpler forms, so bacteria can decompose it in the fermentation process. Bacteria decompose the long chain of carbohydrates, lipids, and proteins into shorter chains. Lipid is hydrolyzed into fatty acids or glycerol. Furthermore, the acidification stage takes place where the product has been hydrolyzed, converted to volatile fatty acid (VFA), aldehydes, alcohols, carbon dioxide, ketones, ammonia, hydrogen, and water by acid-forming bacteria. Then a methanogenesis process occurs where the methane bacteria slowly form methane gas under anaerobic condition.

The temperature during the fermentation process needs to be controlled every day. The action of bacteria and biogas-producing microorganisms depends on the temperature inside the digester. The optimal temperature for biogas production is 32 – 37^oC. Extreme temperature changing in the digester will decrease of microorganism populations and cause the rapid decline in biogas production. Therefore, the placement of a reactor or biogas digester must also be appropriate.

The methanogenesis process are as followed [28]:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$
$$4HCOOH \rightarrow CH_4 + CO_2 + 2H_2O$$
$$CH_3COOH \rightarrow CH_4 + CO_2$$
$$CH_3CH_2COOH + ½\ H_2O \rightarrow 7/4\ CH_4 + CO_2$$
$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$$
$$CH_3(CH_2)_2COOH + 2H_2O + CO_2 \rightarrow CH_3COOH + CH_4$$
$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$$
$$4(CH_3)N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$$

C. The Effect of Degradation Time on pH Value

The high acidity (pH) is related to the performance of microorganisms in helping the fermentation process. Microorganisms will be effective in the pH range of 6.5-7.5. During the initial stages of fermentation, the pH will tend to fall below 6 or lower. However, after 2-3 weeks, the pH will rise again along with the growth of methanogenic bacteria. The rate of decrease or increase in pH that is too extreme usually affects microbial populations, especially bacteria will decrease, so that the anaerobic digestion process is disrupted. This can be prevented by adding lime such as Ca $(OH)_2$ or $CaCO_3$ [28].

Treatment of pH control can increase total biogas production because it also affects bacteria activity. Budiyono, et al. [14] conducted biogas production from bioethanol waste and rumen fluid, which was done the pH control. Biogas rates at pH control were 11.0754 mL/g COD, while at no pH control, biogas productions were 2.2781 mL/g COD. While, without pH control, pH substrate decreased so drastically that biogas production decreased. The concentration of H^+ ions is a crucial role in the anaerobic processes because pH value has a direct effect on the activity of various microbes found in a mixed culture. Several studies have focused on the impact of the initial pH on the anaerobic process because, without pH controlling of the effluent, the pH value changes may occur in the metabolic pathways [26]. Syaichurrozi, et al. [29] also investigated the initial pH of anaerobic co-digestion of RS (Rice Straw) and *Salvinia molesta* (SM) to produce biogas. From that research, it concluded that an initial pH of 8 is the best acidity value for biogas production.

The pH of the substrate has a big impact on the life of microorganisms in palm oil mill effluent because bacteria need different pH to grow optimally. In anaerobic conditions, especially the methanogenic bacteria grow optimally at pH 6.8 - 7.2 [30]. The effect of degradation time on pH changes is shown in Figure 4.

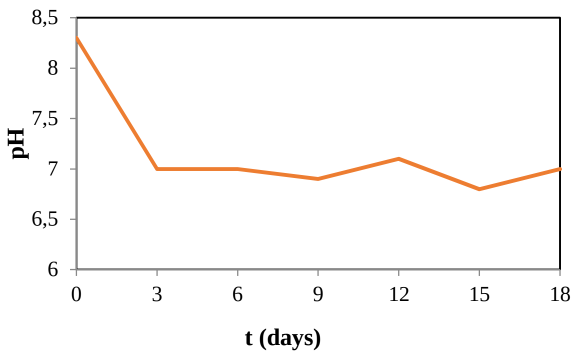

Fig.4. pH Value Curve

From Fig. 4, the pH tends to be stable from day 3 to day 18. On the early day, the pH value is still high at 8.3 because there are no bacteria to help degrade the palm oil mill effluent. On the 3rd day until the 6th day, pH was obtained at 7.0. This condition is neutral and suitable for the growth of anaerobic bacteria, where anaerobic bacteria like to grow in neutral pH conditions. On the 9th day, the pH value was decreased at 6.9 and increased again on the 12th day at 7.1. On the 15th day, the pH decreased slightly, at 6.8. Then, on the 18th day, the pH back to neutral. However, the pH data obtained is still within the range tolerated for anaerobic conditions. The up and down of pH value is influenced by bacteria that produce acid and alkaline that exist in the bioreactor and control the pH value.

IV. CONCLUSIONS

The *Stenotrophomonas rhizophila strain e-p10* bacteria populations always increased through the fermentation period. The highest methane content was obtained at 63.7%moles and carbon dioxide was 22.5 %moles, while the lowest methane content was33.5 %moles and carbon dioxide content were 19.5 %moles. The growth of bacteria increased since it was supported by pH value, which was around 6.8 to 8.3 through the fermentation period. The highest bacterial population was $7.21x10^7$ cells/mL and the lowest was 3.15×10^7 cells/mL.

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