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Acidogenic Fermentation of Palm Oil Mill efluent (POME) on Volatile Fatty Acids production as Precursor

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ABSTRACT

Acidogenic fermentation of palm oil mill efluent (POME) can serve as a precursor in a process for production of polyhydroxyalkanoates (PHA) since the produced volatile fatty acids (VFAs) are preferred precursor for PHA production. Acidogenic fermentation of POME was studied in a 2-L reactor with semi-continuous mode operation (once-a-day feeding and draw-off) for optimal volatile acid compositions. Main fermentation products were acetic acid, propionic acid and butyric acid. The results showed acetic acid, propionic acid, and butyric acid with concentrations are 2.79 g/L; 1.18 g/L, and 3.04 g/L, respectively. VFAs that serves as a precursor on PHA production. The result of feeding of synthetic of VFAs or VFAs from POME at the 20th and 40th in a batch show the concentrations DCW and PHA are 2.38 g/L and 0.74 (g PHA/g DCW) or 2.76 g/L and 0.74 (g PHA/g DCW), respectively.

Keywords

Palm oil mill effluent (POME), Volatile Fatty Acid (VFAs), acidogenic, Polyhydroxyalkanoate (PHA)

1. INTRODUCTION

Indonesia is the world largest producer of palm oil [1]. The palm oil extraction from the fresh fruit bunches (FFB) of palm involves a number of processing procedures: sterilization, stripping, digestion, pressing, classification, purification and vacuum drying for which large quantities of water required [2]. The process of one tonne FFB needs about 1.5 m^3 of water, half of this amount ends up as palm oil mill effluent (POME) [2]. In the year 2011, the government is targeting production of FFB for about 35 tonnes/Ha/Y with an area of 7.8 million hectares of plantation, indicating more than 200 million tonnes of POME was generated from around 490 mills in Indonesia [3].

Wastewater treatment in the palm oil industry done by a multistage process that utilizes the pools open. The core component of this process is the biodegradation of organic waste. Anaerobic decomposition of organic matter decomposition compound includes a compound of organic acids and further broken down into gas and water. Methane is formed during wastewater treated in open ponds. Methane off the air will increase the threat of global warming, because methane in air will react with water to form carbon dioxide and water. Reactions that occur in the air causes the accumulation of methane gas and carbon dioxide as well. Methane and carbon dioxide gases are gases that contribute to the greenhouse effect causing global warming synergism [4].

POME has a high organic content (more than 20,000 ppm BOD) and non-toxic that could be as a carbon source in the fermentation system [5]. POME containing high levels of organic matter that could potentially contaminate the environment so that the necessary degradation of organic matter is greater POME substances are usually in complex forms that cannot be directly consumed or production other product such as polyhydroxyalkanaote (PHA).

There is a great potential to bioconvert POME to volatile fatty acids (VFAs), the followed by the recovery of acids for biosynthesis of PHA. On the other hand, species such as *Ralstonia eutropha* as a representative bacterium for PHA synthesis [6]. In a previous study, reported by Aznury [7, 8] the production of PHA *with Ralstonia eutropha* JMP 134 used VFAs POME as a precursor in batch and fed batch fermentations. The study is that effect of feeding time of VFAs POME affected of

DCW and PHA concentrations. In this paper, we present our result on effect of feeding time of VFAs POME on PHA production by *Ralstonia eutropha* JMP 134. It has been demonstrated that the composition of VFAs produced under acidogenic fermentation can be affected by environmental.

2. MATERIAL AND METHODS

2.1 Material

The main material used in the experiments was palm oil mill effluent (POME) taken from PT. Mitra Ogan South Sumatra.

2.1.1 Media for *Ralstonia eutropha*

A mineral salts medium consisted of: 2.0 g/L $(NH_4)_2SO_4$: 2.0 g/L KH_2PO_4, 0.6 g/L Na_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$; 20 mg/L $CaCl_2$, 10 mL/L trace metal solution, 0.1 g/L yeast extract was used. Trace metal solution consisted of: 1.3 mg/L $ZnSO_4.7H_2O$; 0.2 mg/L $FeSO_4.7H_2O$, 0.6 mg/L $(NH_4)\ 6Mo_7O_{24}.4H_2O$ and 0.6 mg/L H_3BO_3. Glucose was used as a source of carbon with a concentration of 40 g/L as a medium for inoculums development and production media. Glucose, yeast extract, and salt solution were sterilized separately at 121^oC and then mixed with the inoculation aseptically. As for the pH adjusted to about 7 using 2 N HCl or 2 N NaOH [9].

2.1.2 The source of carbon, nitrogen, and VFAs

The medium used in the experiment were all the same, i.e 40 g/L as carbon source, with the initial volume of 5.2 L, and urea nitrogen from with a concentration of 2 g/L, then VFAs added at 20th and 40th hours with a volume of 1 L, respectively.

2.2 Methods

2.2.1 Anaerobic fermentation of POME

POME fermented with active anaerobic microbial seed. Active microbial seeds are added to the oil palm industry waste water with a ratio of 1 L: 4 L in a batch bioreactor with a volume of 8 liters. Anaerobic fermentation is taken and replaced with POME with a ratio of 1 L: 1 L per day. Then, POME fermented has destilation to get VFAs.

2.2.2 Analyses and VFAs and Glucose

The content of residual VFAs was determined and described in standard methods [12]. Organic acids and glucose were detected by HPLC (Agilent 1100 equipped with and RID detector, USA) with an Aminex HPX-87 column (300 mm x 7.8 mm, Biorad, USA) at a column temperature of 60 ^oC, and 0.0055 M H_2SO_4 as mobile phase. The injection volume was 20 µl. The eluted time for glucose, acetate, propionate and butyrate was at about 10.7, 15.6, 17.9 and 21.8 min, respectively.

2.2.3 Analyses of PHAs

Samples were collected at every 20 hour intervals during incubation and pH was measured using an Ecoscan Hand-held series pH meter (Eutech Instruments, Singapore). At 20th until 200th hours, 50 ml samples were collected to determine cell dry weights.

In determining the concentration of PHA, biopolymer contained in the cells extracted with the addition of sodium hypochlorite and chloroform on the cell as has been described by Jacquel N. et al [10]. PHA dissolved in chloroform was analyzed by concentration crotonic acid conducted by Slepecky and Law [11].

2.2.4 Experimental Set-Up

The set-up consisted of two bench-scale reactors and a distillation equipment (Fig. 1). The acidogenic fermentation of POME was carried out in a batch reactor under anaerobic conditions.

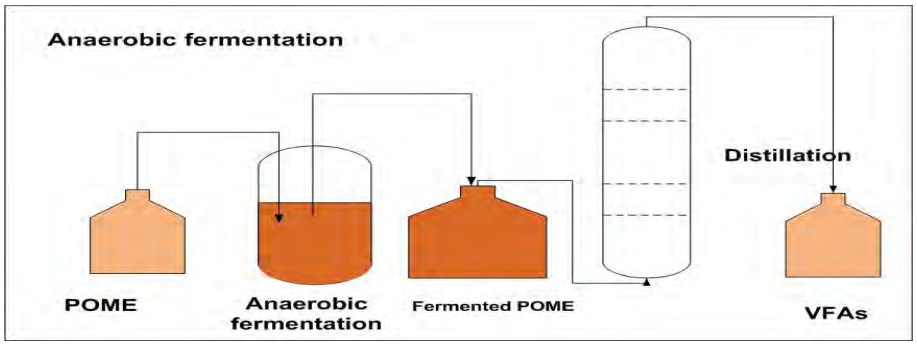

Figure 1: Process POME to VFAs Production

3. RESULTS AND DISCUSSION

3.1 Anaerobic fermentation

The batch anaerobic fermentation was conducted to asses determine complexity organic compounds of POME that could be converted into VFAs. The main products produced from fermentation is VFAs. The results of measurements of VFAs shown in Figure 2. VFAs levels before treatment amounted to 8942.39 mg/L, after 1 day of fermentation increased to 10082.1 mg/L. In fermentation 2 and 3 days had concentration VFAs 9862.93 mg/L and 9766.49 mg/L, respectively. The longer the fermentation time will reduce levels of VFAs produced. The concentration of VFAs was 8978.46 mg/L on 4 days fermentation.

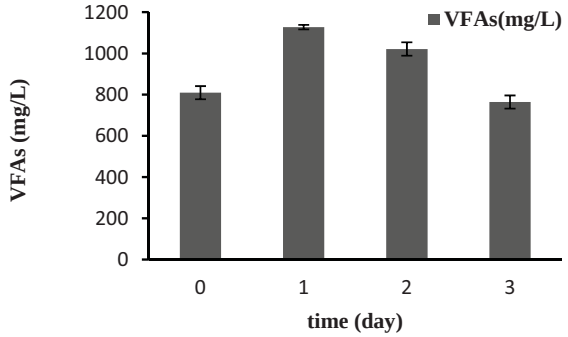

Figure 2: Concentration VFAs from fermented anaerobic POME

Anaerobic fermentation applied as a pretreatment to convert the organic acids into the various components of VFAs which increases the potential for producing PHA from wastewater [12]. Hydrolysis and acidogenesis were early stage to produce organic acids, such as acetic acid, propionic, and butyric which can be used for the synthesis of PHA. Hydrolysis of complex organic components in the POME more soluble compounds. Through the process of acidogenesis, the compound breaks down into VFAs and other monomers. Acidogenesis microbes can produce an acidic solution comprising a mixture of acetic acid and propionic acid or acetic acid and butyric acid [13].

Levels of acid produced in POME correlated with pH value. The results of pH shown in Figure 3, the pH of POME before fermentation was 4.48. The pH value decreased was 4.43 on 1 day fermentation. Fermentation for 2 days at pH values up to 4.67. For fermentation 3 and 4 days was an increase in pH to 4.71 and 4.82, respectively. This resulted in a pH value tends to increase when the fermentation time be longer.

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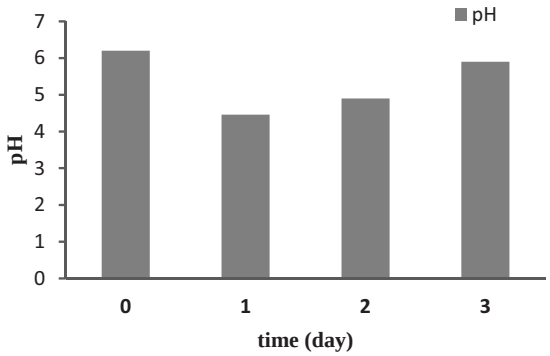

Figure 3: The change of pH with time from anaerobic fermentaion

Data anaerobically fermented POME shows the time to produce the fermentation VFAs on the first day because of the acid produced is quite high, which is about 10000 mg/L or 10 g/L . VFAs concentrations used in these experiments is similar to the acid content 10.3 g/L in batch fermentation POME [14].

POME was also analyzed for total nitrogen content. Analysis of total nitrogen content using a Buchi (Buchi 412 scrubber, Buchi 435 digestion unir, Buchi 339 Distillation unit, Germany). Total nitrogen content of POME early, after fermented and fermented wastewater and distilled after each is 136.68 g/L , 183.07 g/L , and 0 g/L, respectively . The ratio of total nitrogen content can be seen in Figure 4.

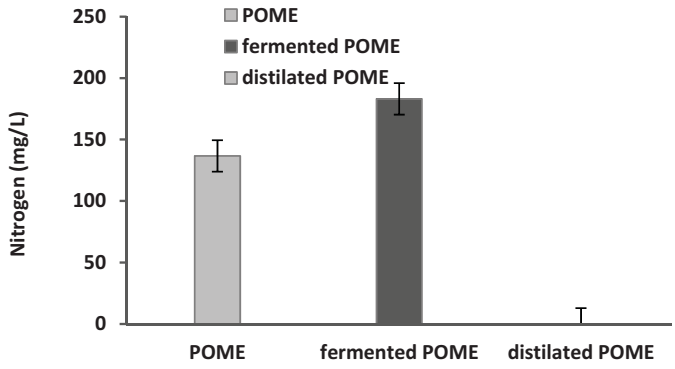

Figure 4: Comparison nitrogen content from POME, fermented POME, and destilated POME

POME has been distilled showed levels of total nitrogen was 0 g/L and containing VFAs. So later in the text will be written is the VFAS from POME. VFAs from POME will be used as a precursor on PHA production.

POME after fermented to start for distilled to obtain pure compounds VFAs. The results of this distillation analyzed using HPLC (Waters, USA) to determine the type and consentration acidic compounds by comparing the standard of acetic acid (JT Baker), propionate acid (Merck), and butyric acid (Sigma-Aldrich). Figure 5 showed concentration of acetic acid, propionate acid and butyric acid from POME, fermented POME, and distillated POME.

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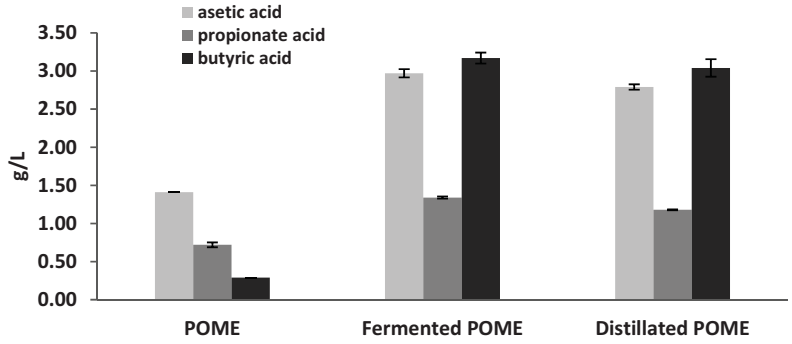

Figure 5: Comparison acetic acid, propionate acid, and butyric acid from POME, fermented POME, destilated POME

POME fermented anaerobic on VFAs production for 1 day and at pH 4.43. POME and fermented POME also contain other fatty acids such as citric acid, formic acid, and malonic acid, while for distillation POME had acetic acid, propionate acid and butyric acid. The results VFAs from POME had acetic acid, propionate acid and butyric acid that the concentration were 1.41 g/L; 0.72 g/L, and 0.29 g/L, respectively. While fermented POME produced acetic acid, propionate acid and butyric acid were 2.97 g/L; 1.34 g/L, and 3.17 g/L, respectively. Destilated POME produced acetic acid, propionate acid and butyric acid, were 2.79 g/L; 1.18 g/L, and 3.04 g/L, respectively. VFAs from destilated POME would used to feed as percursor of Synthetic VFAs on PHA production.

Hassan, et al [15] got the levels of concentration of VFAs from POME depending on the pH of the fermentation. Fermented POME at pH 4 got formic acid, acetic acid, and propionic acid with 1.4 g/L , 4.4 g/L , and 0.5 g/L, respectively. Then fermentation at pH 7 consisted acetic acid and propionic acid with concentration 6.6 g/L and 1.2 g/L, respectively [15].

3.2 Aplication: VFAs as precursor on PHA Production

VFAs from POME were added 1 liter in the batch fermentation at the 20^{th} and 40^{th} hours. Consumption of glucose, acetic acid, propionate acid, and butyric acid analyzed at the 0^{th} to 200^{th} hours. Figure 6 shows the cells to consume glucose and VFAs from POME. The concentration of glucose consumed by bacteria as source of growth at the 0^{th} to 160^{th} hours. Glucose concentration shows a very drastic decrease in the concentration from 20.77 g/L to 3 g/L at the 60^{th} to 80^{th} hours. The concentration of glucose decreased indicates that the cells require a source of the other substrates on PHA production.

VFAs consumption were an after adaptation phase at the 60^{th} and 80^{th} hour. The increase of consumption VFAs were need cell on PHA production. VFAs consumption ceased when glucose had also begun to run out can be seen at the 120^{th} hour.

Butyric acid consumption was faster consumed than acetic acid and propionate acid by the cell. Effect of addition of VFAs as a precursor suggests that the ability of cells to consume each of the different acids. Effect of high concentrations VFAs will be inhibitory or toxic thus may result in a low growth rate and PHA content [16].

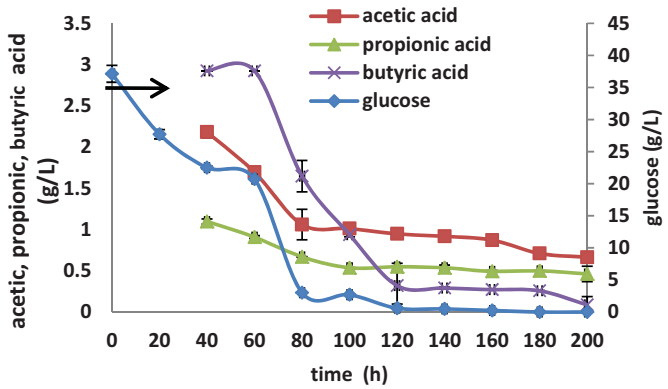

Figure 6. Effect of feeding time of precursor VFAs from POME in batch fermentation at the 20th and 40th of the consumption of glucose, acetic acid, propionate acid, and butyric acid.

Figure 7 shows concentration of DCW and PHA increased during the first reflected the cell growth at the 20th hour. VFAs from POME did not significantly affect the growth of bacteria due to the concentration of DCW at the 20th and 40th hours were 0.29 g/L and 0.4 g/L, respectively. VFAs had not innhibit cell on growth phase. While the highest number of DCW fermentation occurs in the 200th hour was 3.66 g/L. Graph the rate of cell growth has been through a phase when compared to static fermentation at the 160th, and 180th were 3.57 g/L, and 3.66 g/L, respectively. So the increase ranged only from 0.09 g. A small increase was indicates that the cell has experienced a static phase.

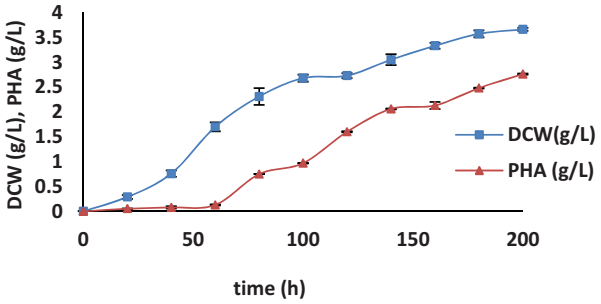

Figure 7. Effect of feeding time of precursor VFAs from POME at the 20th and 40th hours on production of DCW and PHA

The higest consentration and content of PHAs were 2.76 g/L and 0.75 (g PHA/g DCW) at the 200th hour. PHAs were also the rate of increase began to decline from from 160th to 200th hours and ranged from 0.28 to 0.35. PHAs content in the cell begins to decline or already depolymerization by cells for energy. The cells harvest to produce PHA at the 200th hour.

4. CONCLUSION

Acidogenic fermentation of POME in batch resulted in VFAs production. With the condition applied in 1 day and at pH 4.43 the main fermentation products were acetic acid, propionate acid and butyric acid. The composition and concentration of POME acetic acid, propionate acid and butyric acid, were 2.79 g/L; 1.18 g/L, and 3.04 g/L, respectively. For the used VFAs from POME as a precursor in batch and fed batch fermentations. The result of feeding of synthetic of VFAs or VFAs from POME at the 20th and 40th in a batch show the concentrations DCW and PHA are 2.38 g/L and 0.74 (g PHA/g DCW) or 2.76 g/L and 0.74 (g PHA/g DCW), respectively.The application for this research was that effect of feeding of VFAs from POME affected on DCW and PHA concentrations.

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