



## Evaluasi Kinerja Bio-CSTR Untuk Produksi Biohidrogen dari *Palm Oil Mill Effluent* (POME)

### Performance Evaluation of Bio-CSTR for Biohydrogen Production from Palm Oil Mill Effluent (POME)

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#### Abstract

Hydrogen production from biomass is a prospectus energy carrier. Biohydrogen so far only shares 8% of total hydrogen production. Therefore, the production of biohydrogen still has to be increased for its contribution of the total required hydrogen, especially in Indonesia, which is a tropical country and rich in biomass. This research and development would utilize POME, Palm Oil Mill Effluent, as the substrate to produce biohydrogen. The utilization of POME will give added value and solve the environmental problem as well. Based on a modified existing bio-reactor, a bio-Continuous Stirred Tank Reactor (CSTR), the production of biohydrogen was successfully conducted at a scale of 1,000 dm<sup>3</sup> working volume. The bio-CSTR worked with impellers on 4 different levels and the substrate flew laminafly and non-stagnant. As in the first test, biogas production from POME with the majority content of CH<sub>4</sub>, the pH, and COD was measured to assess the quality of this POME utilization. The product was also analyzed, especially to monitor the existence of CH<sub>4</sub> and to assure the product bio H<sub>2</sub>. Bio CSTR was applied in the method fed-batch system. POME and some additional nutrients were fed daily. The work was conducted for at least 2 weeks based on working planning. As the result, biohydrogen is still stable for the duration of 18 days of operations, and no CH<sub>4</sub> exists. The pH was smaller at overflow POME, decreasing maximum, from 4.9 to 4.8. This condition was considered tolerable. The H<sub>2</sub> concentration in the gas product was reached 26% and stable at 12% until the end of the experiment. **Keywords:** POME; bio H<sub>2</sub>; bio CSTR 1 m<sup>3</sup>; laminar and non-stagnant; Scaling bio H<sub>2</sub> production

#### Abstrak

Hidrogen yang diproduksi dari biomassa merupakan *energy carrier* yang menjanjikan. Hingga saat ini, produksi hidrogen dari biomassa baru menyumbang 8% dari keseluruhan produksi hidrogen. Oleh karena itu, kontribusi produksi biohidrogen terhadap kebutuhan hidrogen total masih harus terus ditingkatkan, khususnya di Indonesia yang merupakan negara tropis yang kaya akan biomassa. Penelitian ini akan mengembangkan upaya utilisasi POME, *Palm Oil Mill Effluent*, sebagai substrat untuk memproduksi biohidrogen. Utilisasi POME ini selain akan memberikan nilai tambah, juga akan mengatasi permasalahan lingkungan. Produksi biohidrogen telah berhasil dilaksanakan pada skala volume kerja 1.000 dm<sup>3</sup> dengan menggunakan bioreaktor eksisting yang telah dimodifikasi, yang selanjutnya disebut *Bio-Continuous Stirred Tank Reactor* (Bio-CSTR). Bio-CSTR bekerja menggunakan *impeller* pada 4 level yang berbeda dan substrat akan mengalir secara laminar dan non-stagnan. Sebagaimana pada uji pertama yaitu produksi biogas dari POME dengan kandungan utama CH<sub>4</sub>, pada pengujian ini nilai pH dan COD diukur untuk mengkaji kualitas pemanfaatan POME. Analisa produk juga dilakukan, khususnya untuk memantau keberadaan CH<sub>4</sub> dan memastikan produk bio H<sub>2</sub>. Bio-CSTR dioperasikan dengan metode sistem *fed-batch*. POME dan nutrisi tambahan diumpangkan setiap hari. Keseluruhan pekerjaan dilaksanakan dalam sekurang-kurangnya 2 minggu. Hasilnya, biohidrogen masih stabil selama durasi 18 hari operasi dan tidak ditemukan CH<sub>4</sub>. Nilai pH pada overflow POME lebih kecil, menurun dari 4,9 ke 4,8. Kondisi ini masih dianggap dapat ditolerir. Konsentrasi hidrogen dalam produk gas mencapai 26% dan stabil pada 12% hingga berakhirnya eksperimen.

**Keywords:** POME; bio H<sub>2</sub>; bio CSTR 1 m<sup>3</sup>; laminar dan non-stagnan; *scaling* produksi bio H<sub>2</sub>

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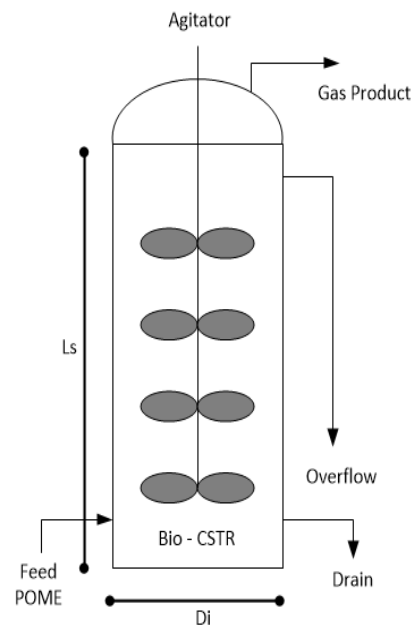


## INTRODUCTION

Climate change is become a big issue to be overcome globally and has drawn more attention day by day. The utilization of fossil fuels for decades was proven to give a negative impact on the environment. Indonesia was paying serious attention to reducing fossil fuel utilization with the declaration of Net Zero Emission in 2060. Along with that, the alternative for clean fuel for transportation is also continuously explored, including hydrogen. Hydrogen has a high energy density (122 kJ/g) (Lubitz and Tumas, 2007) and produces only water as a by-product of its combustion (Levin et al., 2004). Hydrogen was a heat and electricity source (Mishra et al., 2019). Nowadays, hydrogen production was dominated via Steam Methane Reforming (SMR) using fossil sources and water electrolysis. Hydrogen production from biomass or biohydrogen only shares 8% of total hydrogen production (Schoots et al., 2008), which needs to be increased to make a real carbon-neutral and renewable hydrogen. Biohydrogen was produced either using the photosynthesis route or the fermentative route. Moreover, biohydrogen can be produced by utilizing waste of the palm oil industry such as EFB and POME (Kuswardini et al., 2018).

The Bio-Continuous Stirred Tank Reactor (Bio-CSTR) was developed for biohydrogen production via the dark fermentative route. It is an anaerobic fermentation that proceeds in the absence of light, where the microbes (such as *Clostridia sp.* and *Enterobacter sp.*) will break down carbohydrates into hydrogen and intermediates compounds, including volatile fatty acids (VFA) and alcohols (Levin et al., 2004). The Bio-CSTR used in this experiment was a cylindrical tank with an ellipsoidal top and flat bottom lid. The liquid working volume of the reactor is 1,000 dm<sup>3</sup>, with the tank dimension: H = 2.3 m and Ø = 0.8 m. POME was being fed from the bottom of the reactor. Along with that, the gas product will stream out from the top of the reactor. To allow the system to work continuously, a

liquid overflow outlet was set at a height of 2 m of the reactor.



**Figure 1.** The Bio-CSTR Design for Biohydrogen production from POME (Finalis et.al. 2021)

The important part in the development of the Bio-CSTR for biohydrogen production lies in its mixing system. In general, CSTR has a turbulent flow pattern inside the reactor so the concentration of the effluent will be exactly the same as the concentration of the liquid inside the reactor. However, in this Bio-CSTR, POME is designed to flow up laminar and not stagnant inside the reactor. Multiple impellers were set up on 4 different levels with a 450 mm space between impellers. The impellers used are turbine types with the 6-flat blade, which are suitable for POME that has low viscosity below 100 cp. This type of turbine is also excellent for gas dispersion due to its ability to fragment the gas produced into gas bubbles. The arrangement of the impellers will allow each impeller to form a radial discharge flow and produce large-scale circulation loops that are independent (Doran, 2013) but uniform at each level.

The Bio-CSTR was being operated in the fed-batch mode for anaerobic digestion and proven to have a laminar and no stagnant flow inside the reactor as indicated by the



difference in COD and BOD values at the bottom and overflow point. Meanwhile, the pH value showed no significant difference at those two points, indicating that the operating condition at every point in the tank is homogeneous (Finalis et al., 2021). In the previous work by Finalis et al. (2021), the experiment was still limited to proving the performance of the Bio-CTSR in producing a laminar and no stagnant flow inside the reactor. Those type of flow is crucial in the biohydrogen production process. However, the production of biohydrogen itself has not been conducted yet. So far, the Bio-CSTR was proven to succeed in biogas production with methane-dominated gas products. In this research, the performance of the Bio-CSTR for producing biohydrogen was evaluated. Palm Oil Mill Effluent (POME) was used as the substrate in the process.

## MATERIALS AND METHODS

The experiment for biohydrogen production was conducted using a bio-CSTR system located at 225 Building, Puspiptek, Serpong, South Tangerang. The Bio-CSTR has a liquid working volume of 1,000 dm<sup>3</sup> and is equipped with multiple impellers that were set up on 4 different levels with a 450 mm space between impellers. The impellers used are turbine types with a 6-flat blade. Along with the bio-CSTR (T-02) as the primary equipment, the system also includes a pre-treatment tank (T-01), a gasometer for measuring the volume of gas product, and a gas holder for temporarily storing the gas product, and a flare.

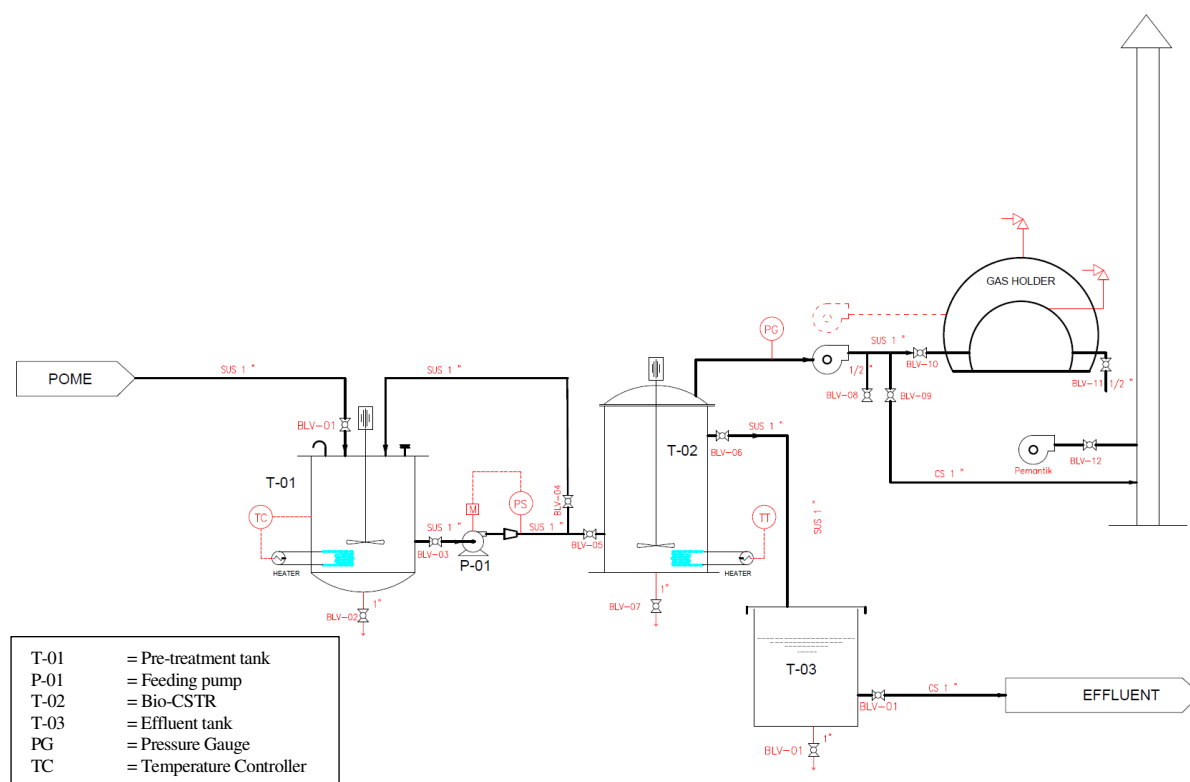


Figure 2. The 1 m<sup>3</sup> Bio-CSTR system

## Materials

POME feed for biohydrogen production was supplied from Cikasungka Ltd, a palm oil mill under PTPN VIII - a state-

owned palm oil company. POME was taken from the palm oil mill periodically as needed to ensure the freshness of the POME, usually 1 m<sup>3</sup> volume of POME in every delivery.



POME was filtered to remove coarse impurities before being fed into the system to prevent clogging in the diaphragm pump.

As the nutrition source, some fertilizer was added into the POME in the pre-treatment tank, including Urea and DAP fertilizer. The fertilizer will provide the micronutrient for the microbes inside the bio-CSTR. Some soda ash and caustic soda were also added in the specified amount according to the pH value of the fresh POME so the pH target value of the POME feed can be achieved.

## Methods

The performance evaluation of bio-CSTR consists of several steps.

### *Pre-cleaning of the Bio-CSTR system*

Pre-cleaning of the bio-CSTR system was conducted prior to the experiment to prevent any contamination that may be caused by prior experiments using this equipment. The pre-cleaning process consists of several steps involving NaOH solution, HCl solution, and hypochlorite solution consecutively with water flushing in between.

### *Starter Preparation*

The initial step in biohydrogen production was preparing the starter. The starter being used in this experiment was slurry from the prior biohydrogen production experiment. The slurry was added with Urea and DAP fertilizer with the dosage of 5 g and 2 g, respectively for  $\pm 125$  L of starter, and then heated in the pre-treatment tank at 95°C for 1 hour. The heat treatment of the starter was aimed to remove the methanogenic bacteria that naturally dominated the bacterium consortia. The starter was then cooled down to 70°C before being introduced into the Bio-CSTR. The starter being used was  $\pm 125$  L.

### *Biohydrogen Production*

The fresh POME was pre-treated in the pre-treatment tank, including the pH value adjustment into 5.0 by adding soda ash and caustic soda, adding the nutrition, and heating the POME at 95°C for 1 hour. The nutrition

used was Urea and DAP fertilizer with the dosage of 5 g and 2 g, respectively for  $\pm 125$  L of POME feed. After the heat-treatment, POME feed was cooled down to 70°C and then fed into the Bio-CSTR.

The feeding was carried out daily starting one day after introducing the starter. POME will accumulate in the Bio-CSTR along with the starter until the overflow condition was achieved. Inside the Bio-CSTR, the mixing system will ensure the fluids flow laminarly and non-stagnant. At the overflow condition, the effluent will stream out from the Bio-CSTR in the same amount of POME fed.

Along with POME feeding, the sampling and measurement were carried out including gas sampling from the top of Bio-CSTR, gas volume measurement, and liquid sampling from the bottom of Bio-CSTR and from the overflow outlet. The fresh POME feed was also sampled to be analyzed.

The performance evaluation was conducted in Hydraulic Retention Time (HRT) of 7 days with an average feeding volume of POME at 125 L/day. Overflow condition will be achieved on day 7. The experiment was performed at mesophilic temperature (30°C, ambient temperature).

### *Gas Product Biohydrogen Analysis*

The gas product from the top of the bio-CSTR was then analyzed using Gas Chromatography to get the gas composition data. The biohydrogen gas product mainly consists of H<sub>2</sub> and CO<sub>2</sub>. Its composition was analyzed using Gas Chromatography with Thermal Conductivity Detector (GC-TCD) Shimadzu 8A. This detector can analyze H<sub>2</sub>, CO<sub>2</sub>, and CH<sub>4</sub>. The analysis starts by setting the injection temperature, cooling temperature, and final temperature at 100 °C, 50 °C, and 50°C respectively. The gas sample was collected using a sampling bag and introduced into the GC inlet by pushing the sampling bag smoothly for 30 seconds (Heriyanti et al., 2021).



### Chemical Oxygen Demand (COD)

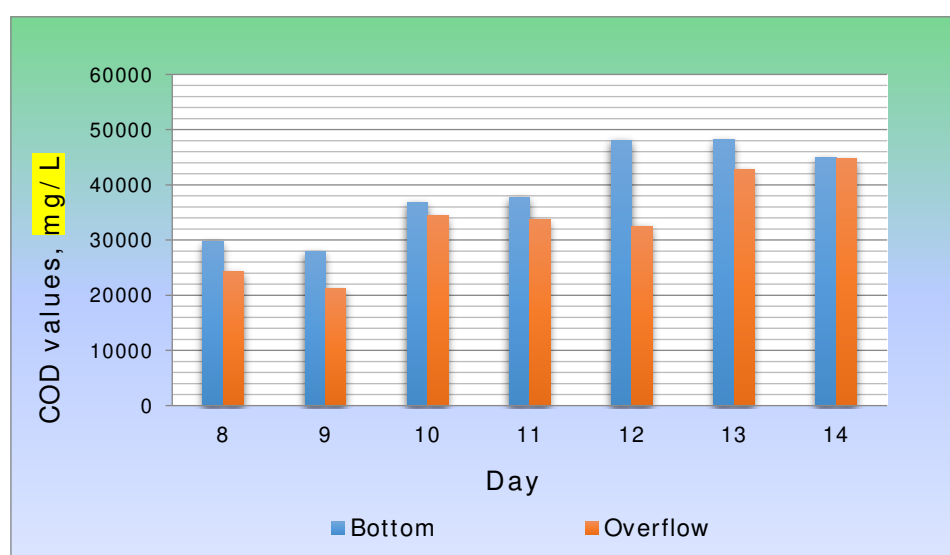
The liquid sample was analyzed to gather its COD content. COD measurement was conducted using Thermoreaktor RD 125 Heater from Lovibond and Photometer system MD 100 from Tintometer-Lovibond. The reagent used was COD kit vials COD / CSB 0 – 15.000 ppm that contain  $K_2Cr_2O_7$ ,  $HgSO_4$  dan  $H_2SO_4$  61%. The sample was shaken in its bottle until homogeneous, and then diluted with aquadest using measuring glass and an Erlenmeyer flask so that the final dilution result was between 0 – 15,000 ppm. A 0.20 ml sample was put into the vial using a micropipette, then mixed until homogeneous. On another vial, a 0.20 ml of distilled water was put as a blank sample. The thermoreaktor was set at a temperature of  $150^\circ C$  and wait until the temperature was achieved. The vials, including the blank, were placed into the thermoreaktor and heated for 120 minutes. The vials were then removed from the thermoreaktor and cooled down to room temperature. The COD value was measured in the vial using the photometer. The COD value of the sample was the result of multiplying the COD value on the photometer with the number of dilutions.

## RESULTS AND DISCUSSION

Prior to the performance evaluation, the Bio-CSTR system was cleaned to ensure there were no contaminants remaining inside. Naturally, methanogen bacteria dominate the microbe consortium in the POME feed. The contamination of the methanogen bacteria will hinder biohydrogen production. Therefore, the system cleaning step was very significant for the succeed of the biohydrogen production process and need to be performed perfectly.

### COD Analysis

The fresh POME from PKS Cikasungka has a COD value in the range of 22,100 - 30,250 mg/L. The addition of nutrients into the fresh POME will increase the COD values in the POME feed. To evaluate the mixing system design of Bio-CSTR, COD analysis was conducted at 2-point levels in the reactor: at the bottom of the reactor and at the overflow outlet that represent the top of the reactor. The collecting of two kinds of data was started after the system achieved overflow condition on day 7. Figure 3 below shows COD values at those two-point levels.



**Figure 3.** Comparison of COD values at bottom and at top (overflow) of the Bio-CSTR

The COD values at bottom of the reactor are shown to be higher than the ones at the top of the reactor (overflow). This finding indicates that the digestion of POME

occurs gradually from bottom to top as designated. The mixing system in Bio-CSTR was therefore proven to build laminar and



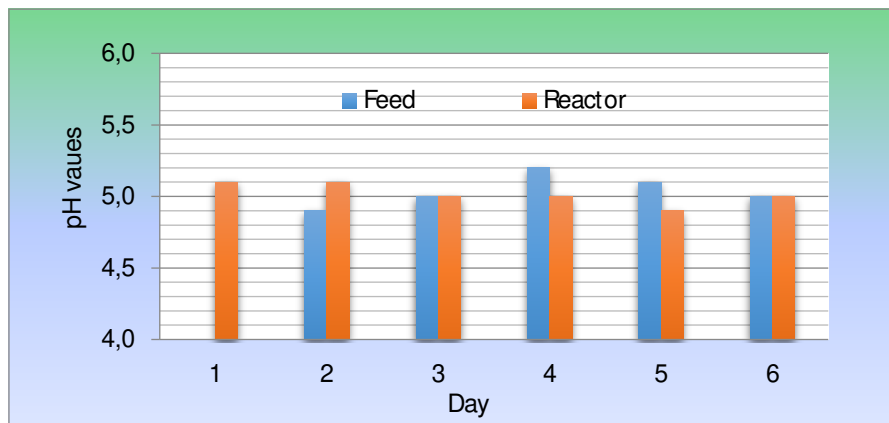
non-stagnant flow inside the Bio-CSTR that was crucial in biohydrogen production.

The main problem in using the CSTR type reactor for biohydrogen production is the washout of microbes from the reactor due to the short Hydraulic Retention Time (HRT). While in hydrogen production, hydraulic retention time (HRT) needs to be kept short to achieve high hydrogen yield. A short HRT will limit the growth of methanogen so the methanogenesis will be restricted. The Bio-CSTR with a capacity of 1,000 dm<sup>3</sup> has been developed to produce biohydrogen. Multiple turbine types with 6-flat blade impellers were set up on 4 different levels with a 450 mm space between impellers. Each level of the impeller will form a radial discharge flow and act as an independent CSTR. So, the Bio-

CSTR will be like a series of CSTR, and the substrate degradation will occur gradually from bottom to top. In this experiment, this mixing system arrangement was proven to overcome the problem of biomass washout in the CSTR system.

### pH Analysis

In this experiment, pH values in the reactor were maintained at 5.0. Since the pH values of fresh POME were in the range of 4.5 – 4.7, the adjustment of pH was carried out by adding some soda ash and caustic soda into the fresh POME until the pH values reached 5.0 (in the range of 4.9 – 5.2). By doing the pH adjustment, the pH value in the reactor could be maintained at 5.0 below.



**Figure 4.** Adjustment of feed pH value to maintain the pH in the reactor

After the overflow condition was achieved on day 7, the pH analysis was conducted at 2 (two) sampling points: at the bottom of the reactor and at the overflow

outlet that represents the top of the reactor. Figure 5 below shows the pH value at the bottom of the reactor and the overflow.



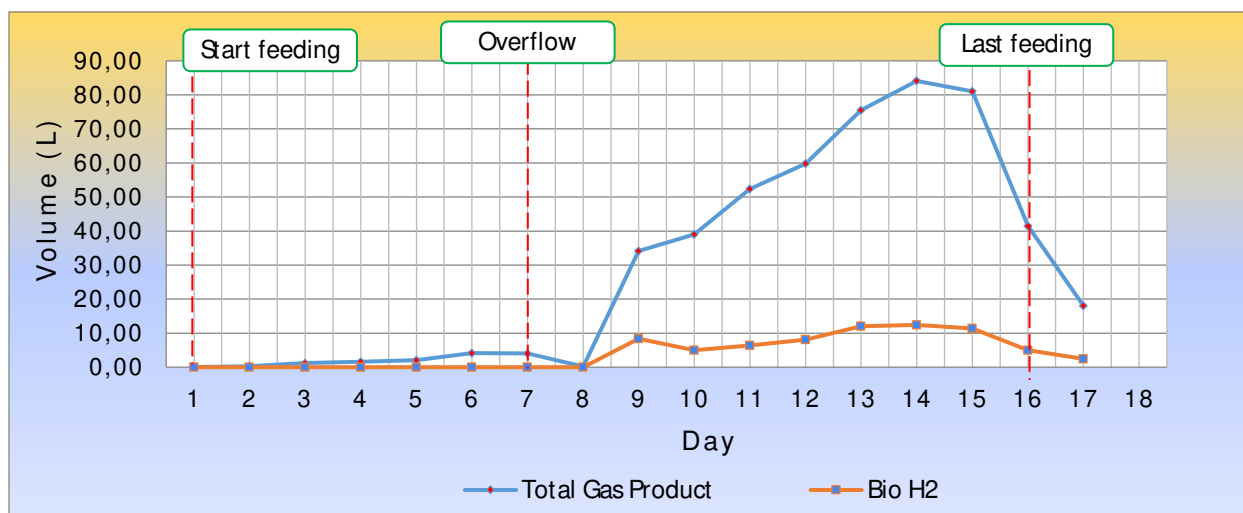
**Figure 5.** The pH value at the bottom of reactor and at the overflow

Figure 5 above shows that the pH value at the bottom of the reactor has the same value as the one at the overflow. It indicates that Bio-CSTR is capable to maintain the uniform pH value throughout the reactor. The pH value in the reactor tends to decrease although the pH of the feed had been adjusted to 5.0. This phenomenon is the result of volatile fatty acids (VFAs) accumulation during the digestion process. The VFAs are the intermediate in the fermentation process, including acetic acid, ethanol, butyric acid, and propionic acid (Lee et al. 2002). Based on the experiment result, the decrease of pH value in the reactor until 4.8 however has not given a negative impact yet on the biohydrogen production. The concentration of biohydrogen in the gas product remains stable in the range of 13.3% - 16.1%. Optimum pH value for biohydrogen production was found to be slightly different according to prior publication, for example, in the range of 4.5 – 9 (Stavropoulos et al. 2016); 5 – 6 (Atif et al. 2005); 5.5 – 6.5 (Ma et al., 2015); 5.5 – 8. At a lower pH value, hydrogenase enzyme activity will be disturbed and hydrogen production will be decreased (Liu et al. 2008).

Until the end of the experiment, no methane was detected in the gas product. Heat-treatment for both feed and inoculum can therefore prove to be effectively remove the methanogen. However, the effect of pH value inside the reactor toward the methane generation can not be neglected too, as the literature shows that methanogen microbial was optimally grown at neutral pH (Lettinga et al. 1993). For the pH value outside the range of 6.5 – 7.5, the methane production will be low (De Mes et al., 2003).

### Biohydrogen production

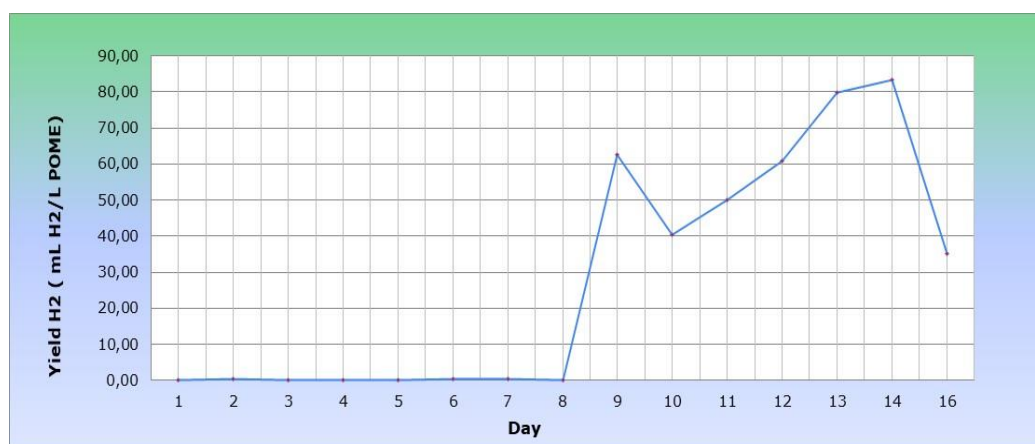
The performance evaluation of Bio-CSTR was conducted for 18 days, not including the preparation of the Bio-CSTR (cleaning) and the starter preparation and introduction into the Bio-CSTR. The volume of the gas product was measured daily before the feeding of POME. The measurement was conducted using a gasometer that works based on water replacement. Figure 6 below shows the volume of the gas product during performance evaluation of Bio-CSTR.



**Figure 6.** Gas production during performance evaluation of Bio-CSTR

The volume of the gas product was increased gradually from 0.04 L on day 1 to 4.07 L on day 7. As designated, the overflow condition was achieved on day 7, and this had an impact on gas production which drop to 0.18 L on day 8. However, this disturbance was only temporary and the gas production bounce back to 34.18 L on day 9 and continuously increase until 84.10 on day 14 and 81.03 L on day 15. The slight decrease on gas product volume on day 15 might be a normal fluctuation in the process that begin to achieve a stable production of biohydrogen

that started since day 13. The difference of the gas product volume was less than 5% both between day 13-14 and day 14-15. This argument needs a further confirmation, but unfortunately there was a condition on day 15 which cause the delayed feeding of POME. This resulted in the decrease in gas production the day after, even though the feeding was done immediately on day 16. The decrease on day 16 and day 17 was much significant compared to the decrease on day 15, that is 50,47% and 56,26% respectively. The similar pattern was shown by the biohydrogen yield.



**Figure 7.** Yield of biohydrogen from POME in Bio-CSTR

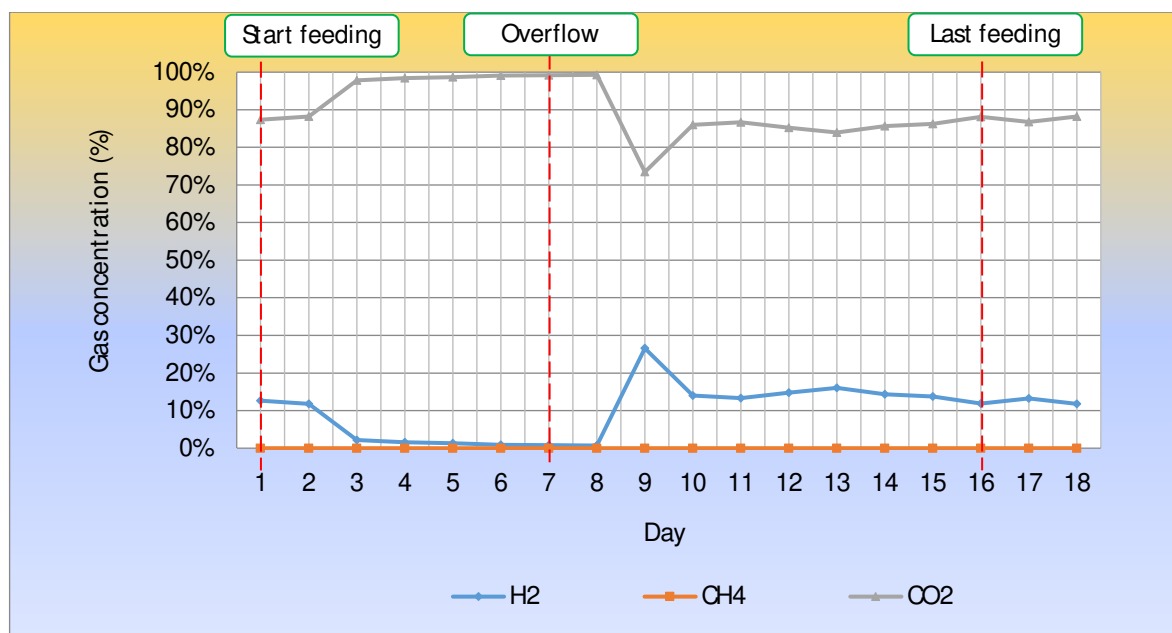
This finding shows that the decrease of the gas product volume on day 16 and 17 were not only a fluctuation but an actual disturbance on biohydrogen production. It

also suggests that the gas production in the fermentation process was highly dependent on the stability of substrate availability. The substrate availability was crucial in the



process since the substrate act as carbon resources for microbe's consortium that maintain the microbial life. Supply of the substrate, therefore, had to be kept sustained, which was become troublesome in this performance evaluation of Bio-CSTR with a capacity of 1,000 dm<sup>3</sup>, since it needed POME feed in a quite big volume, while the location

of Bio-CSTR was far from the POME source. POME feeding at day 16 then becomes the last feeding because of the run out of POME. However, the gas analysis was still conducted until two days after. Figure 7 below shows the gas product composition during performance evaluation of Bio-CSTR.



**Figure 8.** Gas composition during performance evaluation of Bio-CSTR

At the first two days of POME feeding into the Bio-CSTR, there were found some hydrogen in quite high concentrations at 12.7% and 11.8% consecutively. Since then, the hydrogen concentration continuously dropped from 2.2% on day 3 to 0.7% on day 8. The gas product was almost fully dominated by CO<sub>2</sub>. In this period of stabilization, it was predicted that the process inside the reactor was dominated by the microbe's growth, so the main product was CO<sub>2</sub> as the respiration product. The hydrogen concentration starts to increase on day 9 when it reached 26.6%, followed by a stable value in the range of 11.8%- 16.1% starting from day 10 until the experiment being stopped at day 18. This H<sub>2</sub> concentration was lower than that of bio H<sub>2</sub> production at fermentor 2.5 L, 26.5% (Prasetyo, 2018). CO<sub>2</sub> as the dominant component in the gas product, can subsequently be converted into other

substances or stored back into biomass, after being separated from the bio-H<sub>2</sub> product. Until the end of the experiment, methane gas was still satisfyingly undetected, indicating that there was no contamination of methanogen bacteria inside the reactor. This proves that the heat treatment process for both inoculum and feed that aimed to selectively eliminate methanogenic bacteria was successful. The temperature of heat treatment of starter at 95°C was also used by Florio et al. (2017) with a slight difference in heat treatment duration, which they conducted in 30 minutes, while in this experiment was 1 hour. Heat pre-treatment and acidic pH inactivate hydrogen consumers (mainly methanogens) whereas spore-forming bacteria (*Clostridium* sp., *Bacillus* sp.) responsible for hydrogen production easily survive (Lee et al., 2010; Sikora et al., 2013).



## CONCLUSION

The main problem in using the CSTR type reactor for biohydrogen production is the washout of microbes from the reactor due to the high Hydraulic Retention Time (HRT). The arrangement of the mixing system that is being used in this Bio-CSTR which allows a laminar flow pattern inside the reactor may solve this bottleneck of washout phenomena in CSTR. The microbes will have sufficient time to digest the feed inside the reactor to produce biohydrogen.

The HRT in the performance evaluation was 7 days and the overflow condition was achieved on day 7. The gas production was significantly increased on day 9 as much as 34.18 L/day and continuously increase until reached 92 L/day on day 14. The hydrogen concentration in the gas product was stable in the range of 11.8% - 16.1%, and the remaining gas as CO<sub>2</sub>. The methane gas was satisfyingly undetected until the end of the experiment. Biohydrogen productivity, however, needs optimization in operating parameters (shorter HRT, pH value inside the reactor, temperature, etc). The Bio-CSTR with mixing arrangement system as described show a promising result for biohydrogen production.

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## REFERENCES

Atif, A.A.Y., Fakhru'l-Razi, A., Ngan, M.A., Morimoto, M., Iyuke, S.E., Veziroglu, N.T. (2005). Fed batch production of hydrogen from palm oil mill effluent

using anaerobic microflora. *Int J Hydrogen Energy* 2005;30:1393e7.

- De Mes, T.Z.D., Stams, A.J.M., Reith, J.H., Zeeman, G. (2003). Methane production by anaerobic digestion of wastewater and solid wastes. In *Bio-methane & Biohydrogen: Status and perspectives of biological methane and hydrogen production*. Edited by J.H. Reith, R.H. Wijffels and H. Barten. Dutch Biological Hydrogen Foundation
- Doran, P.M. (2013). *Bioprocess Engineering Principles (Second Edition)*. Copyright © 2013 Elsevier Ltd. All rights reserved. ISBN: 978-0-12-220851-5.
- Finalis, ER., Prasetyo, J., Rahmawati, N., Rini, T.P., Hastuti, Z.D., Valentino, N., Patisenda, S. (2021). Development of Bio-CSTR Design for Bio-H<sub>2</sub> From POME as Renewable Fuel, AIP Proceeding
- Florio, C., Pirozzi, D., Ausiello, A., Micoli, L., Pasquale, V., Toscano, G., Turco, M., Dumontet, S. (2017). Effect of Inoculum/Substrate Ratio on Dark Fermentation for Biohydrogen Production from Organic Fraction of Municipal Solid Waste. *Chemical Engineering Transactions*, 57, 175-180 DOI: 10.3303/CET1757030.
- Heriyanti, S.I., Prasetyo, J., Murti, G.W., Sholihah, A., Adiprabawa, A.B., (2021), Scale Up of Biogas Starter Production: Enrichment Of Activated Sludge From Biogas For Bio-Hydrogen Production, *JITK*, Vol. 5 No. 2 , UnPam Press, DOI: 10.32493/jitk.v5i2.11101.
- Kusmardini, D., Prasetyo, J., Saepudin, E., Hudiyo, S., (2018), Biohydrogen Production through Separate Hydrolysis and Fermentation and Simultaneous Saccharification and Fermentation of Empty Fruit Bunch of Palm Oil, *Research Journal of Chemistry and Environment*, Vol. 22(Special Issue II).
- Lee, D.-Y., Ebie, Y., Xu, K.-Q., Li, Y.-Y., & Inamori, Y. (2010). Continuous H<sub>2</sub> and CH<sub>4</sub> production from high-solid food waste in the two-stage thermophilic fermentation process with the



- recirculation of digester sludge. *Bioresource Technology*, 101, 42-47. <https://doi.org/10.1016/j.biortech.2009.03.037>
- Lee, Y.J., Miyahara, T., Noike, T. (2002). Effect of pH on microbial hydrogen fermentation. *J. Chem. Technol. Biotechnol.* 77 (6), 694–698.
- Lettinga, G., Haandel, A.C. (1993). Anaerobic digestion for energy production and environmental protection, in *Renewable energy; Sources for fuels and electricity*, T.B. Johansson, et al., Editors. 1993, Island press, California: London. p. 817-839.
- Levin, D.B.; Pitt, L.; and Love, M. (2004). Biohydrogen production: prospects and limitations to practical application. *International Journal of Hydrogen Energy*, 29, 173-185. DOI: 10.1016/S0360-3199(03)00094-6
- Liu, Y., Yu, P., Song, X., Qu, Y. (2008). Hydrogen production from cellulose by co-culture of *Clostridium thermocellum* JN4 and *Thermoanaerobacterium thermosaccharolyticum* GD17. *Int. J. Hydrog. Energy* 33, 2927–2933.
- Lubitz, W., Tumas, W. (2007). *Hydrogen: an Overview*, ACS Publications, <https://doi.org/10.1021/cr050200z>
- Ma, J., Zhao, Q.B., Laurens, L.L.M., Jarvis, E.E., Nagle, N.J., Chen, S., Frear, C.S. (2015). Mechanism, kinetics and microbiology of inhibition caused by long-chain fatty acids in anaerobic digestion of algal biomass. *Biotechnol Biofuels* 8, 141.
- Mansor, M F., Jahim, J M., Mumtaz, T, Rahman, R A., Mutalib, S A. (2016). Development of A Methane-Free, Continuous Biohydrogen Production System from Palm Oil Mill Effluent (POME) in CSTR . *Journal of Engineering Science and Technology* Vol. 11, No. 8 (2016) 1174 - 1182
- Mishra, P., Krishnan, S., Rana, S., Singh, L., Sakinah, M., Ab Wahid, Z. (2019). Outlook of fermentative hydrogen production techniques: An overview of dark, photo and integrated dark-photo fermentative approach. *Energy Strategy Reviews* 24 (2019) 27–37
- Prasetyo, J., Febijanto, I., Murti, S.D.S., Dewi, E.L. (2018). Preliminary Study of using Palm Oil Mill Effluent to produce Bio Hydrogen as Biofuel, *Research Journal of Chemistry and Environment*, Vol. 22(Special Issue II), 245-248.
- Rittmann, S., Herwig, C. (2012). A comprehensive and quantitative review of dark fermentative biohydrogen production. <http://www.microbialcellfactories.com/content/11/1/115>
- Schoots, K., Ferioli, F., Kramer, G., Van der Zwaan, B. (2008). Learning curves for hydrogen production technology: an assessment of observed cost reductions, *Int. J. Hydrogen Energy* 33 (2008) 2630–2645, DOI: 10.1016/j.ijhydene.2008.03.011
- Sikora, A., Błaszczuk, M., Jurkowski, M., & Zielenkiewicz, U. (2013). Lactic acid bacteria in hydrogen-producing consortia: On purpose or by coincidence? In M. Kongo (Ed.), *Lactic acid bacteria – R & D for food, health and livestock purposes* (pp. 487-514). Rijeka, Croatia: Intech
- Stavropoulos, K.P., Kopsahelis, A., Zafiri, C., Kornaros, M., (2016). Effect of pH on continuous biohydrogen production from end-of-life dairy products (EoL-DPs) via dark fermentation. *Waste Biomass Valoriz* 7, 753–764.