

DEVELOPMENT OF A METHANE-FREE, CONTINUOUS BIOHYDROGEN PRODUCTION SYSTEM FROM PALM OIL MILL EFFLUENT (POME) IN CSTR

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Abstract

This study aimed to develop the start-up experiment for producing biological hydrogen in 2 L continuous stirred tank reactor (CSTR) from palm oil mill effluent (POME) by the use of mixed culture sludge under non-sterile conditions. Besides using different source of starter culture, the effects of acid treated culture and various operating temperature from 35 °C to 55 °C were studied against the evolved gas in terms of volumetric H₂ production rate (VHPR) and soluble metabolite products (SMPs). The formation of methane was closely observed throughout the run. Within the studied temperature, VHPR was found as low as 0.71 L/L.d and ethanol was the main by-products (70-80% of total soluble metabolites). Attempts were made to produce biohydrogen without methane formation at higher thermophilic temperature (45-55 °C) than the previous range. The average of 1.7 L H₂ of 2 L working volume per day was produced at 55 °C with VHPR of 1.16 L/L.d. The results of soluble metabolites also are in agreement with the volatile fatty acids (VFAs) which is higher than ethanol. Higher VFAs of 2269 mg/L was obtained with acetic acid being the main by-product. At this time methanogen has been deactivated and no methane was produced. From this study, it can be concluded that thermophilic environment may offer a better option in a way to eliminate methane from the biogas and at the same time improving hydrogen production rate as well.

Keywords: POME, Biohydrogen production, Methane formation, Thermophilic fermentation.

1. Introduction

Hydrogen is a clean fuel as no carbon emissions is produced when it combusts and only water is produced [1]. The gas is considered as a source of renewable energy as it can be produced from sustainable biomass resources and available in abundance. Organic substrates such as carbohydrates are the preferred substrate in a fermentative biohydrogen process. Palm oil mill effluent (POME), liquid agricultural biomass of palm oil industry, has been reported containing amino acids; inorganic nutrients such as sodium, potassium, calcium, magnesium etc.; short fibers; organelles; nitrogenous constituents; free organic acids and a mixture of carbohydrates (hemicelluloses to simple sugars) [2] thus making it potential substrate for biohydrogen production.

Continuous fermentation of hydrogen is preferable compared to batch since batch processes involve regular downtime and non-steady state conditions [3]. However, challenges in optimizing continuous processes are something to ponder. Continuous mixed culture cultivation to produce biohydrogen is influenced by process conditions such as temperature, pH, hydraulic retention time (HRT) and configuration of the reactor used as well as the cultural conditions, e.g., the type of inoculums, substrate loading and nutrient supplements [3, 4]. The need to prevent methanogenic activity dominated during the development of the mixed culture has to be considered.

Many research has highlighted the advantages of conducting continuous biohydrogen from palm oil mill effluent (POME), however, none has yet touched on the development to eliminate the domination of methanogen microorganism for the reduced biohydrogen production from mixed culture. This study was conducted to identify several pre-treatment factors prior to preparing the inoculum and consequently the performance of biohydrogen production was evaluated by manipulating the selected temperatures over a period of time.

2. Materials and Methods

2.1. Preparation of H₂-inoculum and source of feed stock

The seed culture of microbial sludge was taken from anaerobic pond of Palm Oil wastewater treatment plant located at Dengkil, Selangor, Malaysia. The sludge was subjected a pretreatment by adjusting 1 M HCl acid to pH 3.0 at 35°C and the mixture was acclimatized for 3 months with added medium consisting POME and operated in a 1L modified Scott Duran bottle. The POME medium was enriched with 100mg/L glucose together with phosphate-buffer medium containing 20.0 g/l NH₄NO₃, 8.0 g/l MgCl₂·6H₂O; 0.04 g/l MnCl₂·4H₂O; 0.6 g/l CaCl₂·2H₂O, 45.42 g/l KH₂PO₄ and 94.4 g/l Na₂HPO₄.

The source of POME was originated from two different locations: Pulau Carey Oil Mill, Klang, Selangor and Labu Oil Mill, Negeri Sembilan, Malaysia. Samples collected were stored at 4 °C before usage. Table 1 shows the main composition of the POME used.

2.2. Seed culture preparation and biohydrogen production experiment

Details of experimental parameters for the treatment of the sludge are shown in Table 2. The mixed culture cultivation was conducted in 2 L continuous stirred

tank reactor (CSTR) with working volume 1.5 L. The medium was inoculated with 25% acclimatized seed sludge and was daily fed with 50% diluted POME (1:1). Prior to feeding, the stirrer was switched off for few minutes for cell biomass to settle down. Then, the decanted effluent was taken out according to the respective volume associating the retention time. After feeding was done, the medium mixture was adjusted with 5 M NaOH to the required initial pH of around pH 6.1-6.3. The reactor was stirred at 200 rpm and purged with nitrogen gas every time after feeding to maintain anaerobic condition.

Table 1. Characteristics of POME.

Parameters*	Pulau Carey	Labu
pH	3.75	4.5
Total solids (TS) (mg/L)	50 600	45 750
Volatile solid (VS) (mg/L)	46 100	30 900
Total Chemical oxygen demand (TCOD) (mg/L)	114 700	86 800
Soluble Chemical oxygen demand (SCOD) (mg/L)	57 400	49 300

* Oil content of both POME source contains less than 1% of total mixture

Table 2. Growth parameters for continuous biohydrogen production system.

Run	Fermentation days	POME sludge source	Inoculum treatment	HRT (Day)	Organic loading rate (g COD L ⁻¹ D ⁻¹)	Temp. (°C)
1	1-20	Pulau Carey	Acclimatize sludge	3	20	35
2	21-29	Pulau Carey	Acid (pH 3, 24 hrs)	3	20	35
3	30-39	Labu	Acid (pH 3, 24 hrs)	3	16.6	35
4	40-46	Labu	Heat (80°C, 1hr)	3	16.6	35
5	47-52	Labu	-	2	25	35
6	53-67	Labu	-	2	25	45
7	68-82	Labu	-	2	25	55

2.3. Analytical methods

The harvested fermentation samples were centrifuged at 4000 rpm for 20 min and the samples were analysed for total suspended solid (TSS), volatile suspended solid (VSS), total chemical oxygen demand (TCOD) and soluble COD (SCOD) were analysed according to standard APHA method [5]. Total carbohydrate was determined using phenol-sulfuric method [6].

The biogas produced was collected by water-displacement method and analysed using gas chromatography (model SRI 8600C, USA) with helium ionization detector (HID) and thermal conductivity detector (TCD). Helium gas (99.99%, MOX Malaysia) was used as carrier gas for this GC at 25 mL/min. While the initial oven temperature was set at 40 °C with the pressure of 2.7 psi. The temperature at 40 °C was held for 5 minutes and followed with a ramped at 30 °C per minute until the temperature achieved to 220 °C and finally held for 10 minutes.

The volatile fatty acids (VFAs) were analyzed using gas chromatography (model SRI 8810C, USA) with flame ionization detector (FID) and a Stabilwax-DA (Restek, Bellefonte, PA, USA) column with 30 m x 0.25 mm i.d., 0.25 μm film thickness. Temperature of GC detector was functioned in gradient mode which started at 70 $^{\circ}\text{C}$ for 1 min then raised to 180 $^{\circ}\text{C}$ at 6 $^{\circ}\text{C}$ per min and to 195 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}$ per min before it finally held at 195 $^{\circ}\text{C}$ for 5 min.

3. Results and Discussion

3.1. Continuous biohydrogen production system

Figure 1 illustrates total biogas produced (L) in bar chart as well as the composition of hydrogen (H_2) and methane (CH_4) gas (%), with the reactor operated at mesophilic temperature 35 $^{\circ}\text{C}$. The other biogas found was carbon dioxide (CO_2), which was not shown in the chart. The start-up experiment of H_2 production utilised acclimatized sludge and diluted POME medium that was adjusted to pH 7.0 and the pH of the fermentation was not controlled throughout the process (Run 1- Table 2). Of the 3.6 L of the total biogas produced, 51% was H_2 gas that was produced without any methane formation in the first day. The feed of POME medium was continued at a flow rate of 0.5 L day^{-1} at HRT of 3 days. Feeding of the new medium into reactor had increased the production of biogas to 5.3 L, however, the percentage of H_2 was slightly reduced to 49%. As the media feeding continued, the biogas gradually increased up to 10L in 5th day. Even though the production of biogas had improved, the percentage of H_2 decreasing as the fermentation continued. After one week operation, a small amount of CH_4 (3%) was first time detected from the total biogas in the system. At this stage, the percentage of H_2 had already reduced to 32.5%. The reactor was continuously fed with the medium every day, however, the percentage of CH_4 kept increasing until day 10th when it almost stable at average 26%, higher than that H_2 of 12%. Total biogas at this moment also had reached the steady state where around 2.2-2.4 L biogas was produced per day. Therefore, with increasing amount of CH_4 detected and fraction of H_2 was obviously dropped, then the system was stopped and considered no longer suitable to produce H_2 .

The new run was prepared with pre-treatment of the sludge and POME solids with adding 5 M HCl until it became pH 3.0. The mixture was stirred for 24 hours at 200 rpm (refer to Run 2 in Table 2). After the pre-treatment end, freshly prepared medium of 750 ml of POME diluted water (1:1) was fed into reactor and pH was adjusted to 7.0. At this time, the pH of the medium was not controlled during fermentation. The results are shown in Fig. 1 with the run started from day 21. About 2 L biogas was collected with 41% H_2 and no production of CH_4 was detected after 24 hour. A two-fold volume of about 4.7 L increased in biogas in day 23, however, the H_2 percentage was only 16%. Until day 25th, 2% CH_4 was detected which it gradually increasing while on the other hand, H_2 portion had started to drop. About 20% methane was detected at day 29 in the system.

The system was again replenished with the same pre-treated sludge but fed with different source of POME. The medium originally from Dengkil area was changed to Labu Oil Mill, Negeri Sembilan, Malaysia. The parameters were kept identical as the above run (Run 3 – Table 2). Total biogas produced from day 30 to 39 had not much different than the last two runs except the percentage of H_2

was comparable. The emergence of CH₄ could not be avoided even though the source of POME was from different location. Therefore, it can be concluded that substrate was not an issue in eliminating methanogens in the system.

A different strategy was then applied by changing the sludge pre-treatment method. Previously acid addition was used to pre-treat the sludge but at this stage, heat shock treatment was chosen. Heat shock treatment was preferred for inoculum preparation because *Clostridia sp.* can easily obtained by activating spores to commence germination as well as reducing the presence of non-spore-forming microorganisms [3, 7]. According to Chong et al. [8], heat treatment can be done at temperature ranging from 75 to 121°C within the duration between 15 min to 2 hour. Therefore, in our case, we selected 80 °C for 1 hr. Meanwhile, the OLR and HRT of the system were remained the same as the previous run and it was assigned as Run 4 (see Table 2). Biogas were analyzed from day 40 to day 42 indicates more than 50% of biogas was H₂ without any presence of CH₄ was observed. Nevertheless, H₂ percentages gradually decline day by day and the presence of CH₄ was perceived on day 45. Once again the reactor system failed generating sole product of H₂. Thus, sludge pre-treatment method alone was found not to be a significant factor making the H₂ producing system successful.

Raw POME contains high solids (Table 1) which, even in diluted form may pose obstacles to the H₂ producers. Low flow rate (0.5 L D⁻¹) applied in this study resulted in the accumulation of solids inside the reactor. To overcome this problem, next strategies applied were to reduce HRT to 2 days and increased the flow rate (Run 5 – Table 2). However, as can be seen from Fig. 1 day 47 to 52, these approaches were also unsuccessful in producing the H₂ without presence of CH₄.

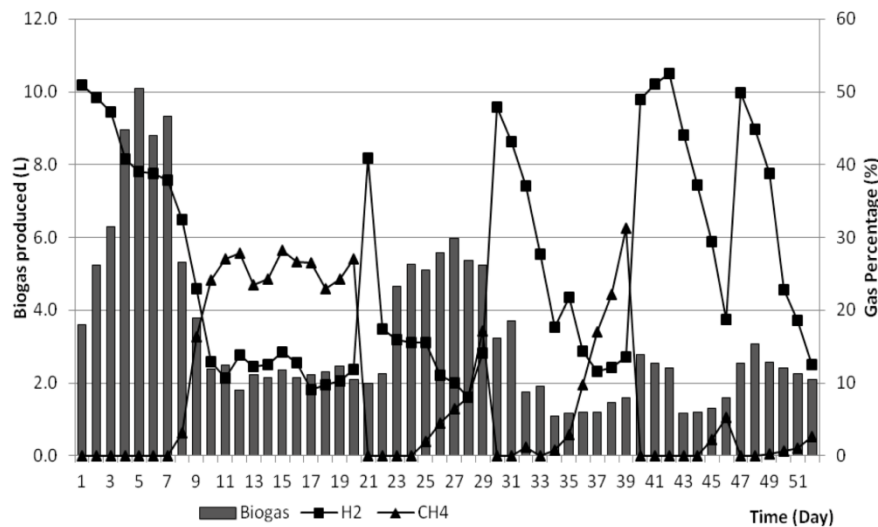


Fig. 1. Profiles of biogas produced (L) in bar graph and composition of hydrogen and methane (%) in line graph. (Refer to Table 2: Run 1 - Day 1 – 20; Run 2 – Day 21- 29; Run 3 – Day 30 – 39; Run 4 - Day 40 – 46; Run 5 – Day 47 – 52).

3.2. Effect of temperature

3.2.1. Hydrogen production

Reactor temperature was taken into consideration in order to eliminate methanogens in hydrogen production system as other aspects tried before were unsuccessful. The temperature varied from 35 °C to 55 °C in this study. This factor was chosen from our observation during culture heating up process (Run 4 – Table 2) in order to treat the inoculums, which some biogas had evolved during the process before the temperature reached at 80°C. The biogas contained H₂ without the presence of CH₄ (data not shown) could be achieved. Therefore, increased temperature to some extent might resolve the problem with methanogens and can provide a sole system producing hydrogen.

Figure 2 shows biogas profiles from temperature 35 °C to 55 °C at HRT of 2 days. This is a repeated experiment data of fermentation day 47 to day 52 from Fig. 1 to compare the fermentation result of 35°C with other temperature. Fermentation at 45°C (Run 6 – Table 2) was commenced on day 53 where the biogas produced trim down to 1.06L and gradually increased until day 58 and fluctuated again as it tried to reach the production steady state. The system was stable after day 62 and fermentation was continued for the 5 days to make a total of 15 days of fermentation at 45°C with average biogas produced almost 1.5 L per day. Although the biogas production fluctuated, when its compositions were analyzed, total of about 60% H₂ and the remaining CO₂ was observed without detection of CH₄. The VHPR at steady-state was 0.71 L H₂/L.d, which was slightly higher than VHPR at 35 °C (Table 3). Thus, rising up the fermentation temperature turned out well in eliminating methanogens from the H₂ production system.

Even though the system generates only the H₂ and CO₂ but the VHPR was too low. Therefore, fermentation temperature was further raised to 55 °C to study the effect of temperature on H₂ production from day 68 (Run 7 – Table 2). The biogas collected was similar on the first day fermentation at 55 °C but the production enhanced further and 3.2L biogas was produced on day 71. The production dropped after that as the system adapting the circumstances to reach the equilibrium state. The biogas produced was stable after day 74 where in an average 2.6 L of biogas was produced per day. The composition of H₂ was also slightly higher than 45 °C where 65% H₂ analyzed and 1.7 L H₂ produced per day in average.

Table 3 shows VHPR at 55°C was 1.16 L H₂/L.d at their steady state. Consequently, increasing the fermentation temperature from 35 °C to 45 °C removed the CH₄ producers hence changing fermentation from mesophilic (45 °C) to thermophilic (55 °C) temperature increased the VHPR and improved production stability. A study by Shin et al. also reported the presence of methane when serum bottles containing food waste as a substrate were incubated at 35°C while incubation at thermophilic temperature (55 °C) evolved biogas was free from methane [9]. Lin et al. [10] demonstrated that hydrogen production from xylose using enriched sewage-sludge microflora was temperature-dependent. Furthermore, this study portrays the significant effect of temperature in continuous mode since previous investigations were mostly in batch mode.

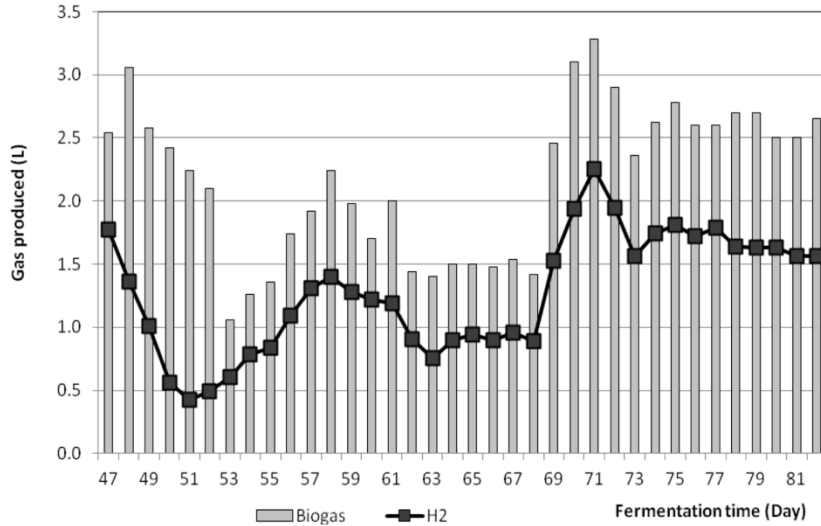


Fig. 2. Biogas (bar graph) and hydrogen (line graph) profiles in liter (L) at different temperature: Day 47 – 52 at 35 °C; Day 53 – 67 at 45 °C; Day 68 – 82 at 55 °C.

Table 3. Experimental analysis under steady-state conditions at different temperature.

Temp. (°C)	VHPR (L/L.d)	Substrate degradation (%)	SCOD removal (%)	pH effluent
35	0.33 ± 0.04	87.2 ± 2.65	24.8 ± 3.32	5.80 ± 0.059
45	0.71 ± 0.14	65.3 ± 5.53	28.7 ± 8.38	5.44 ± 0.254
55	1.16 ± 0.14	78.4 ± 4.74	26.4 ± 9.82	5.15 ± 0.073

3.2.2. Soluble metabolite components

Data of samples collected for soluble components after substrate degradation after fermentation and the COD removal were shown in Table 3. Substrate degradation calculated was based on total carbohydrates using glucose as standard. Highest substrate degradation obtained when CSTR operated at 35 °C (87%) followed by 55 °C (78%) and 45 °C (65%). It clearly shows that hydrogen production is not dependent of substrate degradation. Chong et al. [8] stated that microorganisms consume the substrates for only cell growth and maintenance therefore high rate of sugar utilization does not correspond with the hydrogen production. Whereas, the SCOD removal analysed for all studied temperatures show almost similar percentage. Less than 30% of COD was removed and therefore more COD as more soluble metabolites still remained in the medium.

Higher pH was recorded on effluent of mesophilic temperature, while pH of the effluent under thermophilic temperature was approaching pH 5.0 (Table 3). The research by Fang and Liu [11] disclosed a presence of methane in

biogas whenever the pH was higher than 5.5 due to the bioactivity of hydrogenotrophic methanogens.

The formation of detected soluble metabolites products (SMPs) at steady state condition was summarized in Table 4. Only ethanol and propionic acid were detected when reactor was operated at 35 °C. As shown in Fig. 1, 80% of soluble metabolites were comprised of ethanol and propionic acid when methane was the dominant gas. The presence of ethanol and propionic acid concurrently will suppress the hydrogen generation. The main product in SMPs at temperature 45 °C was ethanol (70%) even though acetic, butyric and propionic acid were also detected. Reactor operated at thermophilic temperature (55 °C) showed only 11% ethanol in SMPs.

The performance of hydrogen production is usually monitored by ratio of acetic and butyric acid (HAc/HBu) and total volatile fatty acids (TVFAs). The fermentation conducted at 55 °C showed highest value of TVFAs and HAc/HBu ratio was 2269 mg/L and 1.191, respectively. This result also shows that temperature shifting from mesophilic to thermophilic regime can enhance the formation of acetic acid and suppress butyric acid formation which is a favorable situation for producing hydrogen. Therefore, by comparing the value of HAc/HBu ratio and TVFAs between operations running at 45 °C and 55°C obviously demonstrated that temperature of 55 °C was the most suitable condition to produce hydrogen. These findings are in line with Mohan et al. [12] where low value of TVFAs attributed to the uncompassionated environment for acidogenic bacteria and therefore lowers the hydrogen performance.

Table 4. Distribution of soluble metabolites at steady-state conditions.

Temp (°C)	Soluble Metabolites (mg/L)						Et-OH/ SMPs (%)	HAc/HBu
	Ethanol	Acetate	Butyrate	Propionate	TVFAs	SMPs		
35	855 ± 159	nd	nd	205 ± 38	205	1060	81	-
45	947 ± 115	108 ± 15	200 ± 49	106 ± 13	414	1361	70	0.541
55	284 ± 170	1197 ± 225	1006 ± 126	66 ± 22	2269	2553	11	1.191

*nd: not detected; TVFAs: total volatile fatty acids (Acetate + Butyrate + Propionate); SMPs: soluble microbial products (TVFAs + Ethanol); HAc/HBu: Acetate/Butyrate

4. Conclusions

The study presents the experimental development of a methane-free, continuous production system for production of biohydrogen from POME. Several factors taken into consideration were substrate source, pre-treatment of inoculum and HRT. After few attempts had been tried and failed with system dominating methane produced, the effect of temperature was considered and it was able to generate sole hydrogen production system. Operating CSTR at 45 °C resulted of yield biohydrogen free of methane. Further increase of temperature to 55 °C produced highest VHPR value (1.16 L/L.d) where 1.7 L hydrogen was generated per day at steady state condition. This result was also supported by high value of TVFAs of 2269 mg/L and highest HAc/HBu ratio of 1.191.

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