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Start-Up of Biohydrogen Production from Palm Oil Mill Effluent under Non-Sterile Condition in 50 L Continuous Stirred Tank Reactor

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Abstract: Feasibility study of biohydrogen production from Palm Oil Mill Effluent (POME) using POME sludge as a mixed culture of natural inoculum was conducted. The experiment was done using a 150 mL serum bottle and 50 L Continuous Stirred Tank Reactor (CSTR) in batch and continuous modes, respectively. The biogas produced from both fermentations was free from methane due to heat treatment of the sludge prior to inoculation. The results obtained showed that the biohydrogen content in 150 mL serum bottle was higher (70%) than that of 50 L CSTR (25%). The biohydrogen rates for serum bottle and 50 L bioreactor were 74 and 33 NmL/h/L, respectively. Butyrate, propionate and acetate were the main soluble metabolites produced during the fermentation and reduced the pH of broth.

Key words: Biohydrogen, palm oil mill effluent, non-sterile condition, continuous stirred, tank reactor

INTRODUCTION

Various attempts have been made to produce biohydrogen as alternative renewable energy from wastewater and solid waste, like waste from food processing (Han and Shin, 2004; Shin *et al*., 2004), sugarbeet (Hussy *et al*., 2005), domestic waste (Van Ginkel *et al*., 2005), Palm Oil Mill Effluent (POME) (Atif *et al*., 2005) and tofu (Zhu *et al*., 1999). These intentions have received considerable attention during the recent years. In Malaysia, palm oil industries play an important role and deemed one of the major contributions in agro-industries beside others commercial crops like rubber and cocoa. In common processing of palm oil industries, 1 tonne of crude palm oil production requires 5-7.5 t of water. From the used water, half of it ends up as a POME. On an average 0.9-1.5 m^3 of POME is generated for each tonne of crude palm oil produced (Vijayaraghavan and Ahmad, 2006). The POME is characterized as brownish in color, viscous, containing about t 95-96% water, 0.6-0.7% oil, 4-5% total solids and it is acidic (pH 4-5) with high organic content, COD and BOD of 50,000 and 25,000 mg L^{-1}, respectively (Najafpour *et al*., 2006) nitrogen content around 0.2 and 0.5 g L^{-1}, as ammonia nitrogen and total nitrogen, respectively (Vijayaraghavan and Ahmad, 2006).

The objective of this study was to investigate the feasibility of biohydrogen production in a pilot scale under non-sterile condition. The efficiency of the biohydrogen production was evaluated based on biogas generated and soluble metabolite content.

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MATERIALS AND METHODS

Seeds Microorganisms

The seed sludge was obtained from an anaerobic digester of a palm oil mill treatment plant at FELDA Serting Hilir Palm Oil Mill, Negeri Sembilan, Malaysia. The characteristic of sludge were 33.3 g L^{-1} Volatile Suspended Solids (VSS), 65.1 g L^{-1} Total Solids (TS), pH 7.2 and 1,350 mg L^{-1} alkalinity as $CaCO_3$. In order to inactivate hydrogenotrophic bacteria and to harvest anaerobic spore-forming bacteria such as *Clostridium* sp. (Lin and Chang, 2004), the sludge was subjected to heat treatment for 20 min at 80°C.

Sludge Efficiency

Preliminary run to observe the sludge efficiency was done using 150 mL serum bottle. Heat-treated sludge was undergo fermentation with initial pH 5.5 and temperature control at 37°C using POME as a substrate after acclimatization with enriched medium, Reinforced Clostridium Medium (RCM), containing 10 g L^{-1} meat extract, 5 g L^{-1} peptone, 3 g L^{-1} yeast extract, 5 g L^{-1} glucose, 1 g L^{-1} starch, 5 g L^{-1} NaOH, 3 g L^{-1} sodium acetate, 0.5 g L^{-1} L-cysteine and 0.5 g L^{-1} agar. The pH was adjusted to 5.5 with 1 M NaOH.

Substrate

The POME was collected from palm oil mill at Dengkil, Selangor, Malaysia and was preserved at a temperature less than 4°C, but above melting point in order to prevent biodegradation due to microbial actions. The POME used in term of COD was 50, 000-60, 000 mg L^{-1}.

Start-Up and Bioreactor Operation

The start-up operation was carried out in two staged manner consisting of (a) seeding stage: carried out using isolated sludge based on heat treatment and (b) acclimatization stage: the seed sludge was acclimatized with RCM. The anaerobic fermentation was commenced by charging 25% v/v of the reactor volume with isolated heat-treated sludge and 25% v/v of RCM medium. Subsequently, the 50 L CSTR was fed with 50,000 mg L^{-1} of POME up to 40 L working volume of bioreactor at HRT 5 days.

The bioreactor was operated in the temperature range 23-25°C and have monitored its pH, Volatile Fatty Acids (VFAs), biogas generation and hydrogen content. In this operation, the fermentation process was carried out at optimum pH 5.5 and Hydraulic Retention Time (HRT) of 4 days.

Data Analysis

Total biogas produced was measured using gas chromatograph (GC, Shimadzu 17 A,) equipped with a Thermal Conductivity Detector (TCD) using a 1.83 m×3.18 mm (inner diameter) stainless-steel column packed with Porapak Q (80/100 mesh) packed with molecular sieve 5A. For organic acids concentration, a broth was analyzed using HPLC Shimadzu equipped with a 300×7.8 mm Amine x HPX-87H column and 4 mM of H_2SO_4 was used as the mobile phase at a flow rate of 0.6 mL min^{-1}. The cumulative batch biohydrogen production, data was fitted to the modified Gompertz equation (Eq. 1). Lay *et al*. (1999) using non-linear estimation function in the Statistic 6.0 software. This equation was a suitable model for describing the progress of cumulative biogas/biohydrogen production in a batch experiment:

$$H = P \times \exp\left[-\exp\left\{\frac{R_m \times e}{P}(\lambda - t) + 1\right\}\right] \quad (1)$$

where, P is the biohydrogen production potential (mL), R_m is the maximum biohydrogen production rate mL h^{-1}, λ is lag-phase time (h) and e is the natural logarithm 2.718281828. The specific biohydrogen production potential (mL g^{-1} COD) was obtained by dividing the P by the POME's COD applied, while the specific biohydrogen production rate (mL g^{-1} VSS) was calculated by dividing the R_m by the sludge weight (g VSS).

RESULTS AND DISCUSSION

Biohydrogen Production in 150 mL Serum Bottle

Sludge efficiency test was done using a 150 mL serum bottle. Fermentation was started by adding 25% v/v of sludge (18.54 g L^{-1} VSS) as inoculum and POME as substrate. Initial pH was fixed at 5.5 as optimum condition for biohydrogen production. At the initial first 8 h of fermentation, no significant effect on pH and no biogas produced. The biogas started released about 6 mL and the pH slightly decreases to an acidic at 10 h. The maximum biogas as well as biohydrogen produced observed at 18 h with biohydrogen content and volume were 70% and 104 mL, respectively. Biogas produced was free from methane as shown by GC chromatogram (data not shown). Cumulative biogas production curves from this fermentation, were obtained over the course of the batch experiment and analysis using the modified Gompertz equation. From the results, the correlation coefficient, R^2 was greater than 0.99 indicating the perfect fit of the experiment of the data. Biohydrogen production potential and biohydrogen production rate were 173 mL and 15 mL h^{-1}, respectively. Meanwhile specific biohydrogen potential was 3.46 mL g^{-1} COD with COD strength was 50,000 mg L^{-1}. The long lag-phase of the fermentation observed with 11 h could be explained by the fact that the seed sludge used was acclimatized with RCM, thus the activity of the seed sludge was slow in order to get adaptation with POME (Fig. 1).

During the fermentation process, pH slowly decreased to 4.8. It was due to organic acid excreted in the fermentation reaction during acidogenesis process. In general for degradation processes, acidogenic bacteria excrete enzymes for hydrolysis and convert soluble organic to volatile fatty acids and alcohols (Reith *et al*., 2003) and accompanied by Acetogenic bacteria to convert a remaining product turn into acetic acid (HAc), hydrogen (H_2) and carbon dioxide (CO_2).

Biohydrogen Production in 50 L CSTR

For a pilot scale study, a set of start-up fermentation was performed using 50 L CSTR for 26 days with pH control. Slow feeding of POME was applied at initial operation stage, based on HRT 4 days. The data of biohydrogen evolved and content during fermentation with the 50 L CSTR was

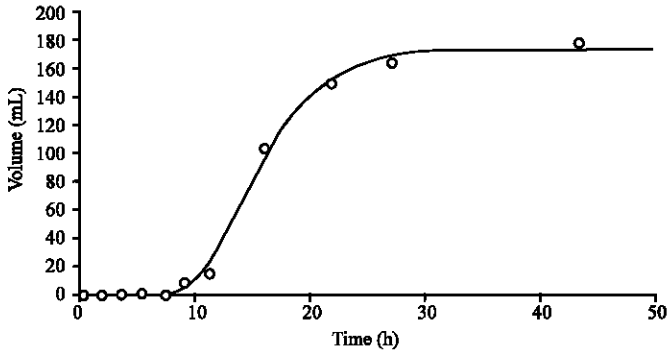

Fig. 1: Cumulative biohydrogen production from the sludge efficiency test using POME as a substrate

Table 1: Performance of biohydrogen fermentation in sludge efficiency test in serum bottle and start-up operation in 50 L CSTR

Experiments (days)	CO_2/bio H_2 ratio	Bio H_2 yield NmL bio H_2/L	Bio H_2 generation rate (NmL/h/L)	Specific bio H_2 generation rate (NmL/h/g VSS)	Bio H_2 content in biogas (%)
Serum bottle					
1	0.43	1773	74	4	60
50 L bioreactor					
2	7.3	120	5	4	12
6	3.0	450	19	10	18
12	1.5	840	35	20	21
20[a]	1.4	970	40	23	23
26[a]	1.2	1130	47	27	25

NmL: The volume was adjusted to standard temperature and pressure (STP); temperature of 273.15K (0°C) and absolute pressure of 101.325 kPa (14.696 psi)

presented in Table 1. The biogas content consisted of CO_2 and H_2 while CH_4 was undetected, however CO_2 generation was dominant in the initial fermentation, the CO_2/H_2 ratio was higher than expected. According to the stoichiometric correlation, it should be expected that the best ratio must close between 0.5-1 and it will give a higher yield to the biohydrogen production. When the fermentation was operated at the same condition, CO_2/H_2 ratios slowly decreased to 1.2 with the H_2 content kept increasing in the biogas.

An initial 4 days, biohydrogen content in a total gas evolved was about 10-12% and later it kept increasing to 20-25% due to adaptation with complex and high strength of POME (60000-70000 mg L^{-1} of COD). The same trend was also shown during sludge efficiency test, where it took long lag phase for adaptation with substrate and inoculum to start reactions. Highest biohydrogen production was observed at day 26 with yield and rates were of 1130 Nml L^{-1} POME and 47 NmL/h/L POME, respectively. Overall biohydrogen generation rated nearly 33 NmL/h/L with biohydrogen content of 20-25%, which was the rate measured during steady state, when biohydrogen content remained constant (less than 15% variations) (Chang and Lin, 2004). The steady state was observed after 16 days of fermentation.

The biohydrogen evolved and biohydrogen content showed not significant fluctuation during the steady state. From the observation, the of microorganism activity which involved in hydrolysis, acidogenesis and acetogenesis reactions was maximum, but not optimum due to certain conditions, especially temperature, as reported by Reith *et al*. (2003). Due the slower conversion processes and bacterial growth under low temperature, the biohydrogen content only increased up to 25%. The temperature of 20-24°C is not the optimum temperature for biohydrogen fermentation and it was frequently a critical factor to the performance of a fermentation process (Mu *et al*., 2006). This is also applicable for fermentative biohydrogen production with a variety of mixed bacterial consortia, which is required for optimum conditions for enzymatic reaction. Compared to the sludge efficiency test in 150 mL serum bottle, the highest biohydrogen content was about 70% from the total gas produced with temperature control at 37°C. The total yield and rate obtained were 1,773 N ml L^{-1} POME and 74 NmL/h/L POME, respectively. Another factor that play an essential action for biohydrogen production are pH, HRT, substrate strength, carbon source, etc. However, the optimum parameters from this study were adopted from others works by Shin *et al*. (2004), Lee *et al*. (2006), Han and Shin (2004) and Atif *et al*. (2005).

Soluble Metabolites Production

In the acidogenesis phase for common anaerobic fermentation of glucose, VFA and alcohol were simultaneously produced as main soluble metabolites. The composition of soluble metabolites was often closely related with the yield and performance of the biohydrogen production. According to the stoichiometric correlations, production of 2 mol of HAc or 1 mol of butayric acid (HBr) as end

Table 2: Distribution of soluble metabolites in sludge efficiency test and start-up biohydrogen fermentation

Experiments (days)	HAc (mg L^{-1})	Hpr (mg L^{-1})	Hbu (mg L^{-1})	Ratio HBu/HAc
Serum bottle				
1	2 267	3 206	1 016	0.5
50 L bioreactor				
2	1 401	1 808	1 400	1.0
6	1 966	2 557	4 350	2.2
12	1 350	2 903	4 973	3.6
20	1 361	3 788	5 581	3.6
26	1 449	2 808	4 361	3.2

product, is accompanied with 4 mol and 2 mol of H_2, respectively, as shown in Eq. 2 and 3 (Wang *et al*., 2007). Since, the composition of the VFA allows vital conclusion on the biohydrogen production process, HAc was consented as a main soluble metabolite instead of HBr or propionic acid (HPr). If HPr turned as the end product, only 1 mol of hydrogen produced from 1 mol of glucose as shown in Eq. 4.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \quad (3)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2COOH + H_2 + CO_2 \quad (4)$$

Table 2 shows the distribution of VFA during the sludge efficiency test in serum bottle and start-up of biohydrogen fermentation in 50 L bioreactor. For sludge efficiency test, 9 386 mg L^{-1} of VFA accumulated during batch fermentation, HPr was dominant, followed of HAc and HBr. Meanwhile, a different trend observed in 50 L star-up operation, the production of HBr dramatically increased and dominated as the soluble metabolite after 4 days of fermentation. Once that HBr started increasing, simultaneously biohydrogen content as well as biohydrogen evolved in total gas produced increased about two folds from initial 3 days. The biohydrogen volume generated totally depends on metabolites concentration, as shown on day 14, 18 and 24, respectively (data not shown). When HPr suddenly arose dramatically, biohydrogen volume simultaneously decreases as much as 20%.(v/v). The HAc/HBr or HBr/HAc ratio has been frequently used as a monitoring parameter for biohydrogen yield (Wang *et al*., 2007). Theoretically, high HAc/HBr ratio might give higher yield of biohydrogen, however in the start-up operation. Once that HBr becomes dominant as soluble metabolite, the fermentation pathway seems to follow Eq. 3. In this study, high HBr/HAc ratio was observed. It was probably due to different bacteria communities involved, once that the bacteria composition obviously depends on the fermentation condition as well as carbon sources.

Generally, of HBr/HAc ratio alone was not a significant evaluation to justify the performance of biohydrogen fermentation. Zheng and Yu (2005) reported that, soluble metabolites in assessing might cause severe inhibition to glucose degradation pathway that may interrupt the acidogenesis and acetogenesis pathway in anaerobic digestion process, especially when HBr concentration is up to 25.08 g L^{-1}. To overcome the effect of low pH due to VFA accumulation, the pH kept controlled at 5.50 to give optimum condition for biohydrogen fermentation process.

CONCLUSION

This study demonstrated that anaerobic degradation of POME for biohydrogen production was influenced by the temperature and pH. Biohydrogen production rate in sludge efficiency experiment

and during start-up operation in 50 L CSTR were 74 and 33 N mL h L^{-1}, respectively. When fermentation was operated in 50 L CSTR with uncontrolled temperature, biohydrogen content in total biogas produced decreased as compared to sludge efficiency experiment. The start-up operation for biohydrogen was successfully conducted under non-sterile condition in large scale using 50 L CSTR.

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