Proceedings of Science and Mathematics
Faculty of Science, Universiti Teknologi Malaysia

Vol. 12, 2022, page 147-156

Biohydrogen Production Performance of Different Pre-treated Inocula

Khayrin Ilyana binti Mohd Lotfi[1], Dr. Mohd Firdaus bin Abdul-Wahab[1*]
[1]Department of Biosciences,
Faculty of Science, Universiti Teknologi Malaysia,
81310 Johor Bahru, Johor, Malaysia
*e-mail: firdausw@utm.my

Abstract
The purpose of this study is to determine the suitable pre-treatment parameters for biohydrogen production from anaerobic digested sludge. In biohydrogen production, the pre-treatment allows the selection of a group of acidogenic bacteria thereby inhibiting the methanogenic microbial community in themixed culture. Organic wastes such as POME are considered as the source of substrate for biohydrogen production due to their abundance in Malaysia. Thus, in this study, the POME anaerobic sludge was pre-treated using several pre-treatment methods, such as heat, acid, base and aeration. Pre-treatment allows the selective enrichment of the specific group of bacteria by inhibiting the activity of the hydrogen-consuming microbial community. This study investigated the performance of different pre-treated POME anaerobic digested sludge to be used as inoculum for biohydrogen production. The results in this study showed that the highest maximum hydrogen production was achieved by acid pretreatment with 637.72 ml. The production of hydrogen is optimum at condition pH value 5.0-6.5. The biogas produced contained hydrogen, carbon dioxide and methane gas. However, methane production was suppressed with heat and acid pretreatment. Acetic acid, propionic acid, butyric acid, lactic acid and formic acid were identified in volatile fatty acids (VFAs) analysis.
Keywords biohydrogen; acidogenic bacteria; methanogenic; POME anaerobic sludge

Introduction

One of the main obstacles in the production of hydrogen is the synthesis of large numbers of active and stable inocula from natural sources. The seed sludge must be processed to reduce the activity of hydrogen-consuming bacteria while maintaining the activity of hydrogen-producing bacteria in order to recover hydrogen from a mixed culture system (Chang et al., 2011). As a result, this project employs a variety of pre- treatment techniques.

Hydrogen-consuming bacteria such as methanogens, homoacetogens, and sulphate-reducing bacteria, on the other hand, may receive energy from molecular hydrogen; their presence in mixed microorganisms may influence hydrogen generation. The seed sludge must be pre-treated to minimise the activity of hydrogen- consuming bacteria while retaining the activity of hydrogen-producing bacteria to extract hydrogen from a mixed culture system.

Recent study has attempted into possibilities of biohydrogen synthesis using a variety of microbial cultures. Mixed microflora has used inoula derived from the natural sources, such as sewage sludge and soils, cow dung and compost, and river sediment, to produce biohydrogen. For different microorganisms, the pretreatment procedures are carried out under different conditions. Different pretreatment procedures result in different hydrogen yields and specific hydrogen production rates (SHPR), as well as affecting the optimal initial pH for hydrogen production (Sinbuathong et al.,2020).

Materials and methods

The POME digested sludge was collected from Felda Maokil Palm Oil Mill, Johor. Then, the anaerobicallydigested sludge undergoes different pretreatments to inactivate the methanogenic and other hydrogen-consuming bacteria. The heat-shock pre-treatment was conducted by heating the sludge at 95°C for 30 min with a water bath under atmospheric pressure. For the acid pre-treatment, sludge was conducted by adjusting the pH of the sludge to 3.0 with 1 mol/L HCl (Merck, Germany) and maintaining for 24 hours, then adjusting back to pH 7 with the addition of 1 mol/L NaOH (Spectrum, U.S.). Next, the base pretreatment was conducted by adjusting the pH of the sludge to 10.0 with 1 mol/L of NaOH and maintaining it for 24 hours, then adjusting back to pH 7 with the addition of 1 mol/L HCl. The aeration pre-treatment was carried out by aerating the sludge with air for 24 hours. The acid, base, and aeration pre-treatments will all be conducted at room temperatures ranging from 20 to 24°C (Chang et al., 2011).

The hydrogen production experiments were conducted in triplicates of 160 mL serum bottles with a working volume of 100 mL. 90 mL of synthetic media and 10 mL of inoculum was added to each serum bottle. The treated sludge was centrifuged at 3500 rpm for 10 minutes. Then, the supernatant was discarded and the pellet was transferred into the 250 mL of Schott bottle. Deionised water or media was used to fill the schott bottle to 250 mL.The medium in all samples contained 10 g/L glucose as a carbon source, as well as the following inorganic salts (g/L): NH_4Cl 0.5; KH_2PO_4 0.5; K_2HPO_4 0.5; $NaHCO_3$ 4.0; $FeCl_2{\cdot}4H_2O$ 0.15;$MgCl_2{\cdot}6H_2O$ 0.085; $ZnSO_4{\cdot}7H_2O$ 0.01; $MnCl_2{\cdot}4H_2O$ 0.03; H_3BO_3 0.03; $CoCl_2{\cdot}6H_2O$ 0.02; $CaCl_2{\cdot}2H_2O$ 0.01; $NiCl_2{\cdot}6H_2O$ 0.02; $Na_2MoO_4{\cdot}2H_2O$ 0.03 and filled with deionised water to make up to one litre. The medium was filtered and sterilized by using filter housing with a 47mm nylon membrane filter. For control, 10 mL of untreated sludge was used in one of the tests. Deionized water also was used to fill the bottles to a total working volume of 100 ml. Then, the pH of the combined solution in each bottle was adjusted to 7.0 with 1 mol/L of HCl or 1 mol/L of NaOH. Next, each bottle was flushed with nitrogen gas for 3 min to provide anaerobic conditions, capped with a rubber stopper, and placed in an incubator shaker (Model SI-50D, Protech, Malaysia) at 35°C and 150 rpm.

The amount of evolved gas was measured by releasing the gas pressure in the vials using a glass syringe of 5 to 50 mL to equilibrate with the room pressure as recommended by Owen et al, 2013. The gas samples were taken once every 12 hours, and the gas composition was determined by gas chromatography (Model 7890B, Agilent Technologies, China). At the end of batch cultivation, volatile fattyacids (VFAs) were determined using a gas chromatograph equipped with a flame ionization detector and,and pH were measured by using pH meter (Chang et al.,2011).

The cumulative hydrogen production profiles were fitted with modified Gompertz equation in Eq.(1):

$$H = P_{max} \times \exp\{-\exp[(R_{ma} \times e P_{max})(\lambda - t) + 1]\} \quad (1)$$

where H (mL) was the cumulative hydrogen production at the reaction time t (h); Pmax (mL) is the hydrogen production potential; Rmax (mL/h) is the maximum hydrogen production rate; and λ (h) is the lag time (Chang et al., 2011). The cumulative hydrogen data were fitted with the Gompertz

using Microsoft Excel software.

Results and discussions

In batch experiments, the various pretreatment procedures impact hydrogen generation. The results as shown in figure 1 revealed that hydrogen generation in the sludge prepared with acid, base, heat-shock, aeration and control was increased, indicating that these pretreatments sustain the hydrogen-producing bacteria activity.

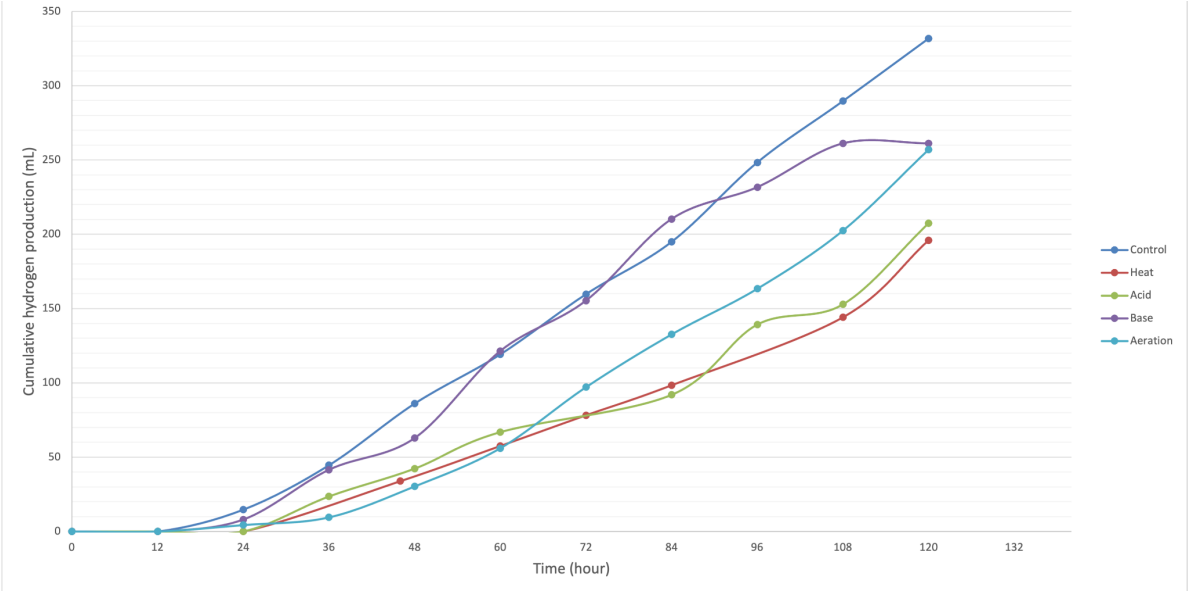

Figure 1 Graph of hydrogen production (mL) against time (hour).

The pouring of POME digested sludge into the Schott bottle for the pretreatment method was done in the laminar flow to avoid contamination. Other than that, the volume of the inoculum exceeds the needed amount to prevent the occurrence of evaporation during the pre-treatment methods. When the pretreatment was done, the sludge had been cooled down to room temperature before storing in the fridge.

The medium contained glucose and inorganic salts as the germination and sporulation of hydrogen producing cultures induced by sugars, amino acids and nutrients. Glucose was known as a biodegradableorganic substrate, and nutrients, such as nitrogen (N) and phosphorus (P), which are important for efficient biohydrogen production.

Table 1 lists the kinetic parameters determined by Eq. (1). The cumulative hydrogen production in the batch experiments of this study could be described using the modified Gompertz model as the determination of coefficient (R2) of all the regressions was over 0.99. The longest lag time was 52.63 hours for acid pretreatment followed by 46.94 hours for aeration pretreatment, 43.93 hours for heat pretreatment, 30.61 hours for base pretreatment and 30.52 hours for control. The highest maximum hydrogen production rate (Req) 4.02 mL/h was observed for the sludge that pretreated by base, followed by control, aeration, acid and heat pretreatment

Table 1: Calculated kinetic parameters of hydrogen production for various pretreatment methods

Pre-treatments	Heat	Acid	Base	Aeration	Control
Maximum hydrogen production, H_{max} (mL)	425.33	637.72	297.37	437.24	503.67
Maximum hydrogen production rate, R_{eq} (mL/h)	2.46	2.97	4.02	3.47	3.82
Lag phase, lambda(h)	43.93	52.63	30.61	46.94	30.52
Methane suppressed	Yes	Yes	No	No	No

Different inoculums pretreatment methods were performed to hydrogen production and found that the acid pretreatment showed the maximum hydrogen production of 637.72 mL. In a previous study, the acid pretreated sludge showed maximum hydrogen production at 83.65 mL.

The graph below is fitted to modify the Gompertz equation. The cumulative hydrogen production increase for all pretreatment methods including control. The best fit curve (modified Gompertz equation) described the formation progress of biohydrogen. Previous biohydrogen researchers have used the Gompertz equation to describe hydrogen evolution by dark fermentation. In this empirical approach,three model parameters such as lag time, hydrogen production potential, and hydrogen production rate are adjusted to fit the Gompertz equation to experimentally measured hydrogen evolution data.

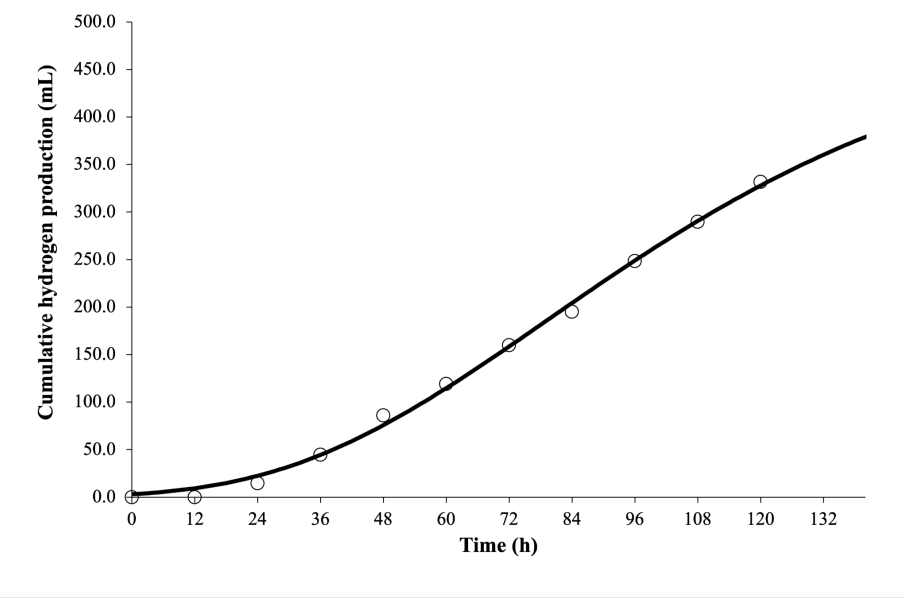

Figure 2 Graph of cumulative hydrogen production against time (hour) for no pretreatment

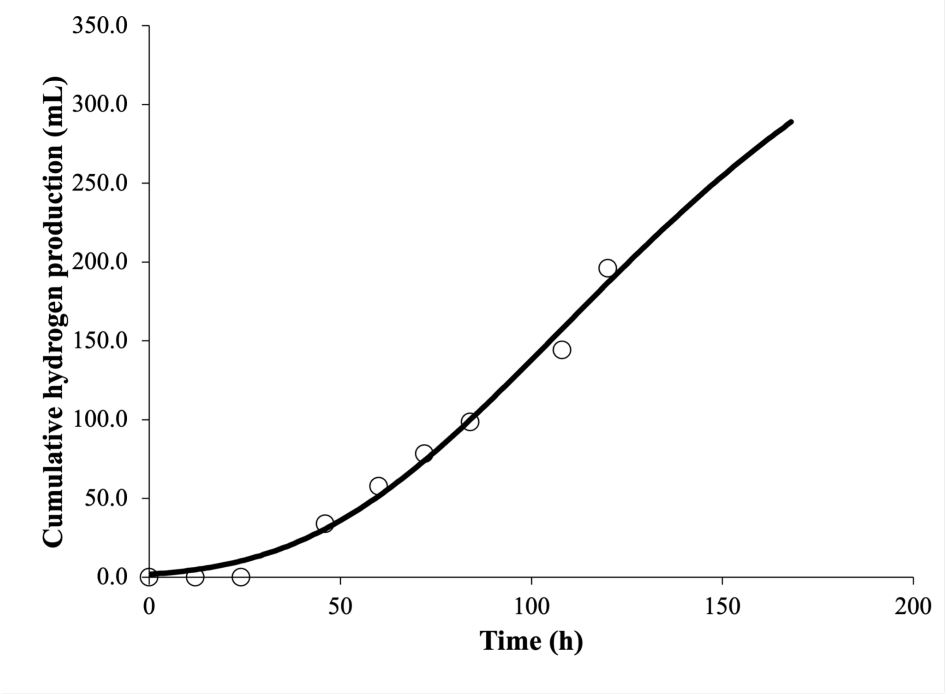

Figure 3 Graph of cumulative hydrogen production (mL) against time (hour) for heat pretreatment

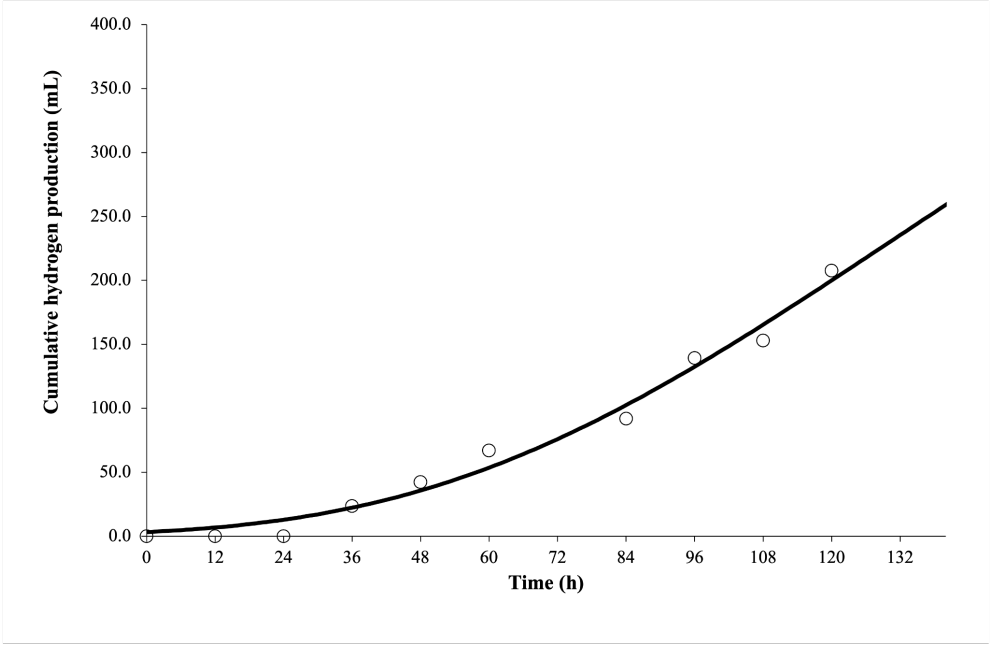

Figure 4 Graph of cumulative hydrogen production (mL) against time (hour) for acid pretreatment

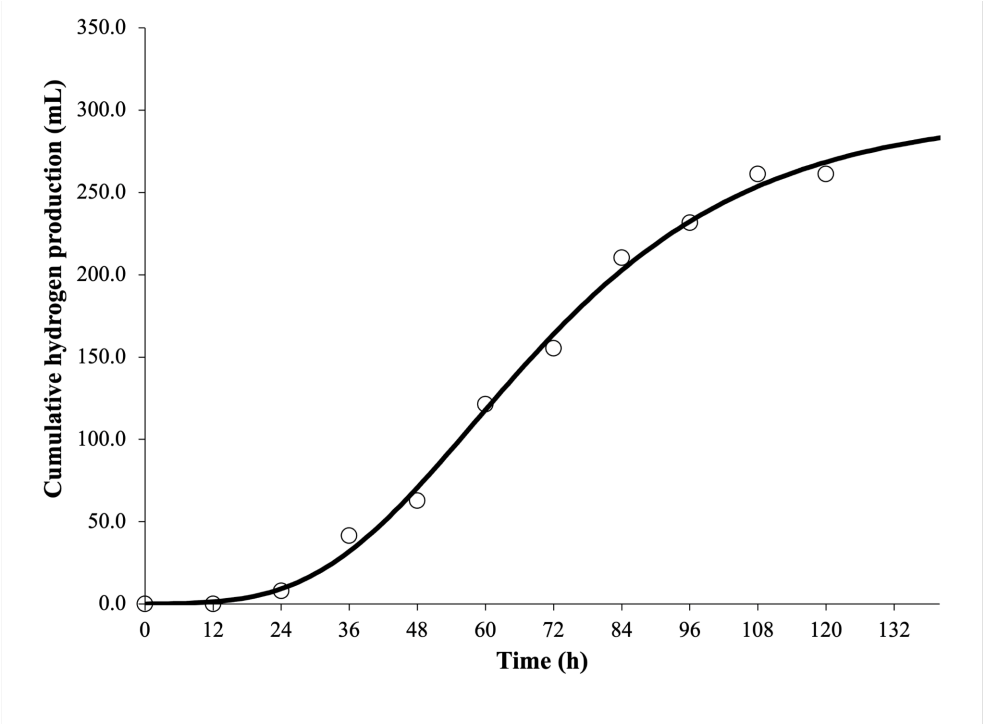

Figure 5 Graph of cumulative hydrogen production (mL) against time (hour) for base pretreatment

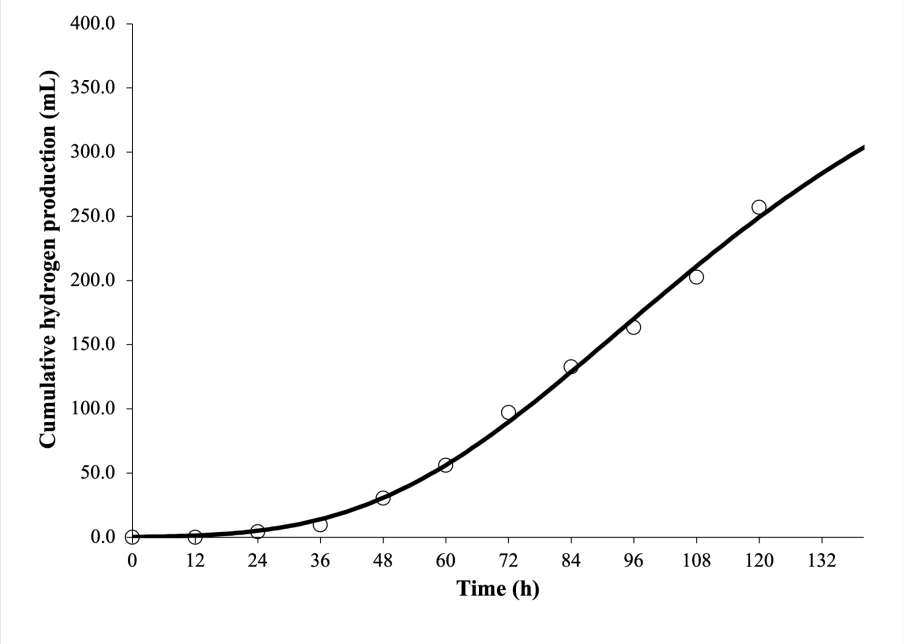

Figure 6 Graph of cumulative hydrogen production (mL) against time (hour) for aeration pretreatment

Due to acidogenic metabolism, volatile fatty acids (VFAs) or solvent synthesis occur concurrently with hydrogen production during the anaerobic digestion process. As a result, the concentration of VFAs works as a helpful indicator for evaluating hydrogen production. These acidic intermediates' formation is a result of changes to the microbes' metabolic pathway (Nazlina et al.,2009). Acetate, butyrate, and propionate were the main soluble fermentation products found in this analysis. The distribution of the main soluble fermentation products using various pretreatment techniques is shown in Table 2. Butyric acid was the most soluble metabolite for the heat, acid, and aeration pretreatments, indicating that the acetic acid-fermentation pathway was being used. Propionic acid was the main soluble metabolite produced during base pretreatment.

Due to acidogenesis during anaerobic fermentation, the pH value decreases during incubation, and the concentrations of organic acids increase during the duration of the fermentation process. Lactate, acetate, and butyrate were the main by-products produced. Acetate dominated at first, but after the synthesis of biohydrogen reached a stable state, butyrate significantly increased and took over as the dominant product. The environment turns acidic as a result of the reduced lactate production, theoretically limiting the synthesis of biohydrogen (Nazlina et al.,2009).

Table 2: Effect of different pretreatment methods on the liquid fermentation end products

Pretreatment methods	Hour	Soluble metabolite concentrations (µg/mL)				
		Acetic acid	Propionic acid	Butyric acid	Formic acid	Lactic acid
Control	0	750	117	220	<10	<10
	120	686	137	1752	<10	<10
Heat	0	445	18	752	<10	<10
	120	458	202	1605	<10	<10
Acid	0	640	60	416	<10	<10
	120	415	44	465	<10	<10
Base	0	447	270	201	<10	<10
	120	562	2734	246	<10	<10
Aeration	0	447	143	109	<10	<10
	120	562	1074	2634	<10	<10

The pH of all samples in batch experiments was adjusted to pH 7. The final pH values for heat pre-treated sludge, acid, aeration, control range between 3.03 to 3.64 respectively, while final pH for base pre-treated sludge is 4.12. This shows that activity of hydrogen-consuming bacteria such as acidogenic bacteria was inhibited when pH decreased to 4. The major pH dropped in all of the samples occurred during the first 24 h of fermentation (Fig. 7). Faster pH drop in the untreated inocula could be because of the higher production of fatty acids (Chang et al., 2011). Decreased pH also enables suppression of hydrogen consuming bacteria and lowering of retention time encourages hydrogen production (Nizzy, Kannan, & Anand, 2017).

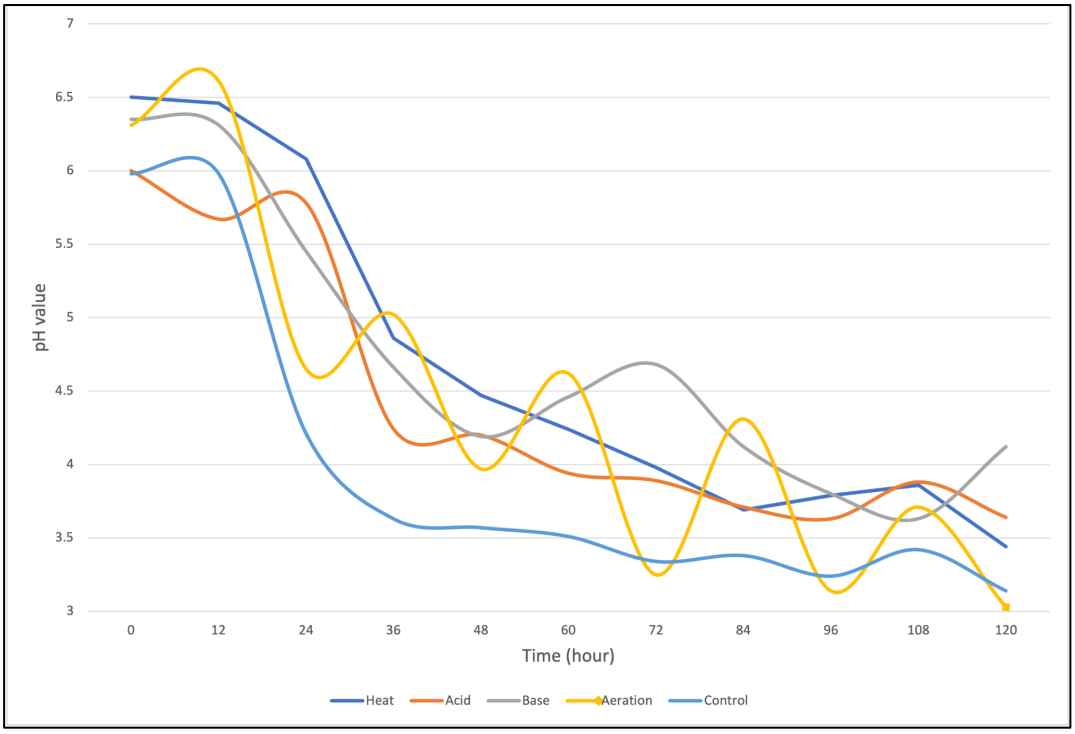

Figure 7 Graph of the effect on pH against time (hour)

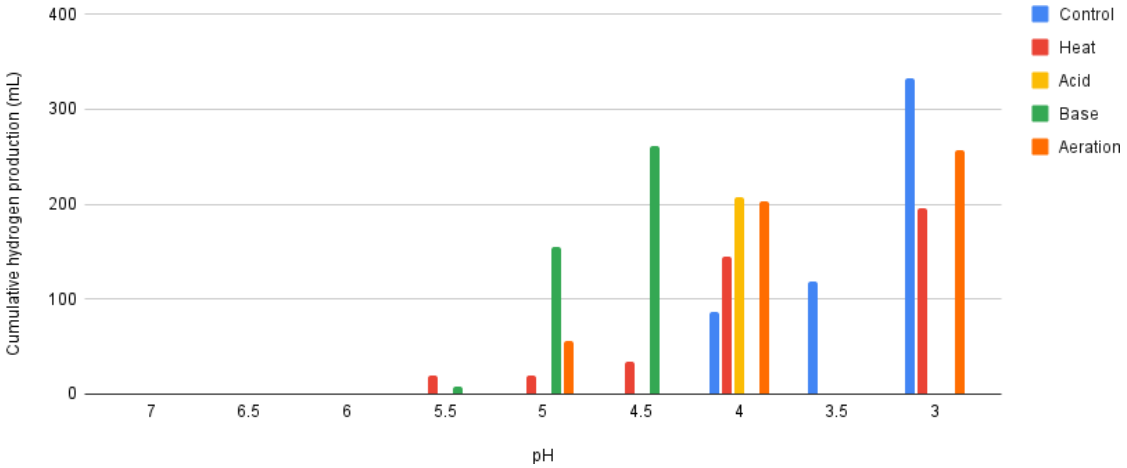

Figure 8 Graph on cumulative hydrogen production (mL) against pH value

Figure 8 shows the comparison of hydrogen production between different initial pH values. For all pH values, heat pretreatment samples were efficient in producing biohydrogen compared to the other samples. The study also shows that pH 3 and 4.5 produces the highest hydrogen if compared to other pH values. This finding is different to previous study by Che Zuhar, Lutpi, Idris, Wong, & Tengku Izhar, 2018 which state that the fermentative anaerobic bacteria were mostly favored to produce hydrogen at optimal pH of 5.5 and 6.0.

Conclusion

Based on the result obtained, it is concluded that hydrogen-producing bacteria can be enriched by several pretreatment methods. The results of these experiments demonstrated that the hydrogen production rate was influenced by various pretreatment methods. The results showed that the highest maximum hydrogen production was achieved by acid pretreatment with 637.72 mL followed by control, aeration, heat and base pretreatment. The production of hydrogen is optimum at condition pH value 5.0-6.5. In this study, the biogas produced contained hydrogen, carbon dioxide and methane gas.However, methane production was suppressed with heat and acid pretreatment except for base, aeration and no pretreatment. Acetic acid, propionic acid, butyric acid, lactic acid and formic acid were identified in volatile fatty acids (VFAs) analysis. It was concluded that acid treatment is a simple, economic, and effective method for enriching hydrogen- producing bacteria from digested anaerobic sludge.

Acknowledgement

The authors are grateful to the supervisor of the project, Dr. Mohd Firdaus bin Abdul Wahab, for encouragement, and guidance and thanks to the mentors, Jemilatu Omuwa Audu, Eka Latiffah, Dr. Kamilah, friends and family for their guidance, advice and motivation. Without their continued support and interest, this thesis would not have been the same as presented here.

References

Alissara Reungsang, Zhong, N., Yang, Y., & Liao, Q. (2018, April 21). Hydrogen from Photo Fermentation. Chandrasekhar, K., Lee, Y. J., & Lee, D. W. (2015). Biohydrogen production: strategies to improve process efficiency through microbial routes. *International Journal of Molecular Sciences,* 16(4), 8266-8293.

Chang, S., Li, J. Z., & Liu, F. (2011). Evaluation of different pretreatment methods for preparing hydrogen-producing seed inocula from waste activated sludge. *Renewable Energy,* 36(5), 1517-1522.

Che Zuhar, C. N. S., Lutpi, N. A., Idris, N., Wong, Y. S., & Tengku Izhar, T. N. (2018). Enhancement of Biohydrogen Production via pH Variation using Molasses as Feedstock in an Attached Growth System.*E3S Web of Conferences,* 34, 02045.

Chouler, J., & Di Lorenzo, M. (2015). Water quality monitoring in developing countries;can microbial fuel cells be the answer?. *Biosensors,* 5(3), 450-470.

Dzulkarnain, E.L.N., Audu, J.O., Wan Dagang, W.R.Z. et al. Microbiomes of biohydrogen production from dark fermentation of industrial wastes: current trends, advanced tools and future outlook. *Bioresour. Bioprocess.* 9, 16 (2022).

Nazlina, H.M.Y., Nor Aini, A.R., Ismail, F., Yusof, M.Z.M. and Hassan, M.A. (2009). Effect of Different Temperature, Initial pH and Substrate Composition on Biohydrogen Production from Food Waste in Batch Fermentation (2022). *Asian Journal of Biotechnology,* 1: 42-50.

Nizzy, A., Kannan, S., & Anand, S. (2017). Chemical Science Review and Letters Effect of Pretreatment and pH on Biohydrogen Production from Sago Industrial Effluent Containing Mixed Microbes. *Chemical Science iReview and Letters,* 6(23).

Ohimain, E. I., & Izah, S. C. (2017). A review of biogas production from palm oil mill effluents using

different configurations of bioreactors. *Renewable and Sustainable Energy Reviews,* 70, 242–253.

Prasetyo, J., Murti, S. S., Senda, S. P., Latief, A. D., Prasetyo, R. D. H., Hastuti, Z. D., ...& Muis, L. (2018). Palm Oil Mill Effluent (POME) utilization for bio-hydrogen production targeting for biofuel: optimization and scale up. In *E3S Web of Conferences* (Vol. 67, p. 02003). EDP Sciences.

Preethi, Usman, T. M. M., Rajesh Banu, J., Gunasekaran, M., & Kumar, G. (2019). Biohydrogen production from industrial wastewater: An overview. *Bioresource Technology Reports,* 7, 100287.

Sinbuathong, N., Charnnok, B., Sillapacharoenkul, B., & Sakdaronnarong, C. (2021). Preparation of active hydrogen-producing cultures from palm oil mill sludge for biohydrogen production system. *International Journal of Hydrogen Energy,* 46(57), 29100-29107.

Wang, J., & Wan, W. (2008). Comparison of different pretreatment methods for enriching hydrogen-producing bacteria from digested sludge. *International Journal of Hydrogen Energy*, 33(12), 2934–2941.