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Wastewater Processing Technology Opportunities for Palm Oil Mill Effluent as a Raw Material for Renewable Bioenergy

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Highlights:

- Wastewater from palm oil mill effluent (POME) can be used as a substrate for hydrogen and ethanol production.
- A simple circulating bed reactor configuration can be used for POME wastewater treatment to produce bioenergy.
- Proper pH control in the circulating bed reactor can increase hydrogen and ethanol production.
- The reactor with an initial pH of 5.5 produced the highest hydrogen compared to the other initial pH conditions. In contrast, the highest ethanol production occurred in the reactor, with an initial pH of 6.5.

Abstract. The effect of pH on the formation of hydrogen and ethanol using POME as the substrate has not been widely studied. Indonesia, which is the largest producer of palm oil, has a high potential for the utilization of this liquid waste as a substrate for the formation of hydrogen and ethanol. This study determined the optimum hydrogen and ethanol production conditions by controlling pH. POME was used as substrate in an anaerobic reactor and operated in feed batch mode for 72 hours. Mixed culture anaerobic bacteria as biomass were used in the reactor. The pH of the reactor was adjusted to 4.5, 5.5, 6.5, and 7.5 using NaOH 0.1 N and HCl 0.1 N. The reactor's performance was investigated by measuring hydrogen production, ethanol production, and volatile fatty acid product. It was found that with an initial pH of 5.5, hydrogen production was higher than for the other pH conditions, at about 14.7% v/v. In contrast, the most increased ethanol production occurred in the reactor with an initial pH of 6.5 with a concentration of 347.7 mg/L. Based on the results of this study, the right pH setting can optimize hydrogen and ethanol production.

Keywords: *anaerobic processes; ethanol production; hydrogen production; palm oil mill effluent; resource recovery.*

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1 Introduction

Increases in population and living standards in developed countries have put unprecedented stress on energy demand. The current average energy consumption per capita is about 2.5 kW worldwide and is predicted to increase to 6.5 kW by 2050 [1]. Fossil fuels in human activities are very intense for electricity, transportation, material for industries, etc. However, there is apprehension that the world's fossil fuel resources are declining and soon will be depleted. Other problems with fossil fuels as an energy source are the environmental damage and public health problems it causes through CO_2 emissions and particle inhalation. The transport sector is highly dependent on fossil fuels and contributes to 21% of greenhouse gas emissions [2]. Atmospheric CO_2 concentrations (379 ppm in 2005) have increased by almost 100 ppm compared to pre-industrial levels [3]. Global warming is an evident result of fossil fuels and severely impacts ecosystems, food production, water resources, human health, and economics.

The use of renewable energy can be expanded to reduce fossil fuels. This will not only decrease CO_2 emissions but also improve energy security. In 2050, about 60% of energy demand will be satisfied from renewable sources [4]. Renewable fuels include, for example, bioethanol, bio-methanol, biodiesel, biomethane, and biohydrogen, which can be used to replace fossil fuels as an energy source [5]. Hydrogen and ethanol are renewable energy sources that are commonly used. Hydrogen has been indicated as one of the most promising fuels for the future [6]. Hydrogen has the highest energy content per mass unit of all compounds (its heating value is 142 MJ/kg compared to 55 MJ/kg for methane), about three times higher than hydrocarbon. At the same time, the combustion residue is environmentally friendly (H_2O and O_2) [7]. Some of the advantages of using ethanol are its renewable and sustainable nature, accessible storage, and high oxygen and octane numbers [8].

Hydrogen and ethanol can be produced from renewable raw materials with high organic contents, such as palm oil mill effluent (POME), using anaerobic processes. POME contains high concentrations of organic compounds such as carbohydrates, proteins, nitrogen, oil, grease, minerals, and other organic compounds, such as cellulose, hemicellulose, and starch [9]. Currently, the potential of this waste product is not utilized optimally; for example, wastewater treatment in the palm oil industry uses a system of open ponds with anaerobic/facultative release of methane into the environment at 33 kg/ton of palm oil produced [10]. Recent studies have proven the feasibility of POME as a substrate to produce bioenergy through anaerobic processes [11-14]. However, the main product of the anaerobic processes is volatile fatty acids (VFAs), while hydrogen and ethanol are produced in smaller amounts. At pH 4.5, it can inhibit the formation of hydrogen because of the accumulation of volatile acids [15]. Hydrogen can be produced in a pH range of 5.5 to 6.8 [16] with an optimum pH of 6.0 [17]. This study aimed to determine the effect of initial pH on hydrogen gas and ethanol production from organic-rich wastewater (POME) in an anaerobic reactor.

2 Materials and Methods

2.1 Substrate and Biomass Preparation

Palm oil mill effluent as a substrate of anaerobic bacteria was taken from the influent wastewater treatment plant of PT Condong Garut, Indonesia. Based on previous studies [11-13], the biomass for the inoculum of anaerobic bacteria was enriched from the sludge of POME and cow manure with a ratio of 50:50 (v/v). The biomass was then acclimated to the POME for about 80 hours. The enrichment was conducted in a series of batch reactors, as shown in Figure 1. The typical pH, total chemical oxygen demand (COD), volatile suspended solids (VSS), and total suspended solids (TSS) concentration of the biomass were 5.27, 45.84 g/L, 15.77 g/L, and 18.38 g/L, respectively.

Figure 1 Seeding reactor for anaerobic microorganism enrichment.

2.2 Bioreactor Construction and Running Process

This research was conducted on a laboratory scale using an anaerobic reactor with a working volume of 5.0 L, operated in feed batch mode (Figure 2). Firstly, the anaerobic reactor was flushed with nitrogen gas (N_2) for the first 24 hours to purge oxygen gas (O_2) from the headspace. The anaerobic reactor then continued to operate with internal biogas circulation until 72 hours [11-13]. The anaerobic reactor was filled with POME as substrate and the acclimated biomass at a ratio of 4:1 and with a total volume of 5 L. The reactor was operated at room temperature (25 ± 2 °C). The pH of the reactors was adjusted to 4.5; 5.5; 6.5, and 7.5 using NaOH 0.1 N and HCl 0.1 N. Samples were collected every 6 hours and analyzed for the parameters of ethanol, hydrogen, soluble and total COD, pH, VSS, and volatile fatty acids.

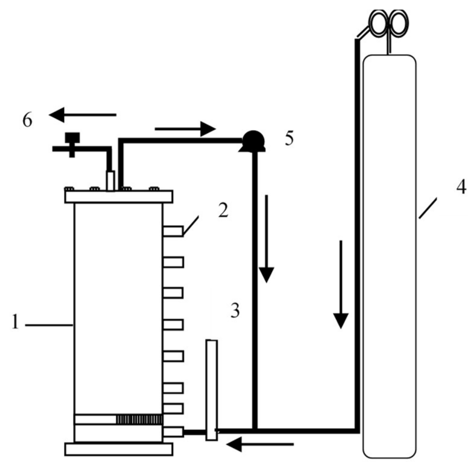

Figure 2 Configuration of anaerobic reactor: (1) bioreactor; (2) sampling port; (3) flowmeter; (4) N_2 tube; (5) circulation pump; (6) gas outlet.

2.3 Analytical method

Soluble COD, VSS, and pH were analyzed using the Standard Method for the Examination of Water and Wastewater, 23rd edition [15]. Ethanol and volatile fatty acids were analyzed using a GC-FID Shimadzu 2010 Plus equipped with an analytical Stabilwax PEG (polyethene glycol) column. Hydrogen gas was analyzed using a gas chromatograph (Shimadzu GC 8A) equipped with a fitted Porapak P 8A serial D-4167 column and a TCD type detector, operating at 100 °C injectors and detector temperature at 130 °C, helium pressure at 60 kPa for a sample injection of 1 µL. The volume of biogas produced during the anaerobic process was measured using the displacement water method, and pressure measurement was done using a Lutron PM9100 manometer was measured.

2.4 Calculations

The degree of acidification (DA) and degree of ethanofication (DE) were determined according to Eqs. (1) & (2) [18]:

$$\text{DA} = \frac{\text{Total Volatile Fatty Acid (TVFA)[mg COD/L]}}{\text{Influent concentration of soluble COD [mg COD/L]}} \quad (1)$$

$$\text{DE} = \frac{\text{Ethanol [mg COD/L]}}{\text{Influent concentration of soluble COD [mgC OD/L]}} \quad (2)$$

The acidogenic product was determined using Eq. (3):

$$\text{COD}_{\text{theoritical}} = \frac{8 \text{ x } (4x+y-2z)\text{g COD}}{(12x+y+16z)\text{g CxHyOz}} \quad (3)$$

where, x = C, y = H, and z = O.

The formation rate product (mg COD/L/hour) was determined using the following Eq. (4):

$$\text{Formation rate product} = \frac{\text{PC}_{\text{end}} - \text{PC}_{\text{in}}\text{[mg COD/L]}}{\text{time (hour)}} \quad (4)$$

where PC_{end} is the concentration of product at the end of the processes and PC_{in} is the concentration of development at the beginning of the processes.

3 Results and Discussion

The characteristics of the palm oil mill effluent samples are listed in Table 1. Raw POME is a thick, brownish, viscous, oily and acidic liquid. POME contains amino acids, inorganic micro and macronutrients (Na, K, Ca, Mg, Mn, Fe, Zn, Cu and Co), carbohydrates and organic acids, total nitrogen, a low pH value, and suspended solids.

The effect of pH on the hydrogen gas production in the anaerobic reactor for the different initial pH conditions is shown in Figure 3. In the experiment, the anaerobic reactor was operated with an initial pH of 4.5, 5.5; 6.5, and 7.5, respectively. The influent COD concentration was 18.433 mg/L. The hydrogen gas production was the highest (14.7 % v/v) in the anaerobic reactor, with an initial pH of 5.5 compared to the other operating conditions (initial pH of 4.5, 6.5, and 7.5).

As shown in Figure 3, as the initial pH increased from 4.5 to 5.5, the hydrogen gas production increased from 8.72% to 14.7%, while it decreased from 14.7% to 10.5% and 3.5% as the initial pH was increased to 6.5 and 7.5, respectively. These results are similar to the previous study [17], which found that hydrogen gas can be produced at a pH of 5.5-6.8 [16], with 5.5 to 6.0 as the optimum pH. In this study, the anaerobic reactor with an initial pH of 5.5 exhibited the optimum condition for hydrogen production from POME. The previous study [19] found that the activity of hydrogenase was inhibited at low pH (lower than 5.5) or high pH (higher than 5.5) in the overall hydrogen fermentation. Moreover, in the anaerobic reactor with an initial pH of 4.5, the accumulation of volatile acids caused inhibition of hydrogen production [20].

Table 1 Characteristics of palm oil mill effluent from PT Condong Garut.

Parameter	Unit	Results
Total COD	mg/L	30367 ± 2155
BOD	mg/L	14500 ± 1868
Soluble COD	mg/L	18433 ± 473
pH	mg/L	4.30
Oil and grease	mg/L	630 ± 56
NH_3	mg/L	26.27 ± 1.29
NTK	mg/L	265.67 ± 7.77
TSS	mg/L	6200 ± 300
VSS	mg/L	3967 ± 189
Ethanol	mg/L	44.4 ± 8.65
TFVA	mg/L	566.67 ± 42.52
Iron (Fe)	mg/L	14.96 ± 0.35
Manganese (Mn)	mg/L	2.09 ± 0.355
Molybdenum (Mo)	mg/L	< 0.001
Nickel (Ni)	mg/L	0.025 ± 0.006
Copper (Cu)	mg/L	0.073 ± 0.008
Zinc (Z)	mg/L	0.051 ± 0.007

Note: The results are given in data means average ± standard deviation

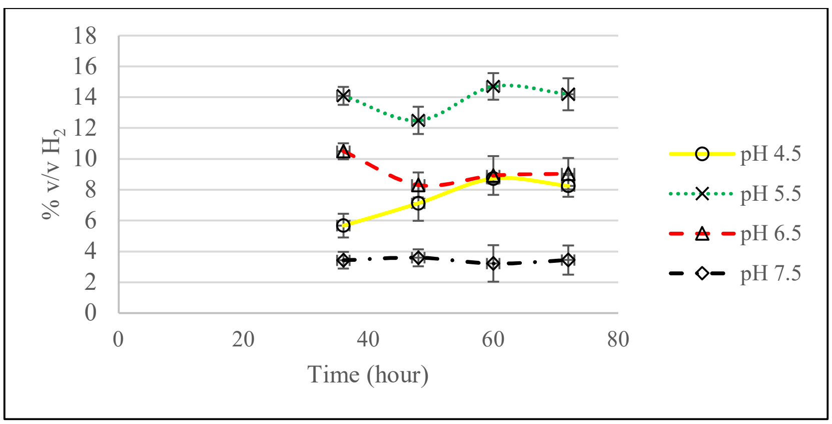

Figure 3 Hydrogen production during the anaerobic process under the different pH conditions.

The ethanol production during the anaerobic process is shown in Figure 4. It can be seen that ethanol is immediately produced after 6 hours of fermentation. For every initial pH condition, the ethanol concentration increased gradually until 42 to 54 hours of fermentation. The highest ethanol concentrations found in the reactor with a pH of 4.5; 5.5; 6.5 and 7.5 were 274.64; 369.53; 256.50 and 253.71 mg/L, respectively, with a rate of formation of 6.55; 6.68; 4.24 and 4.65 mg/L/hour.

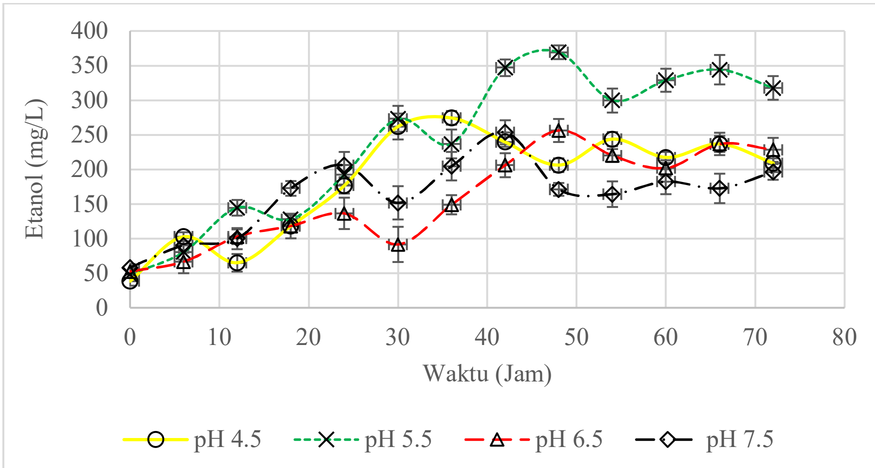

Figure 4 Ethanol production in an anaerobic reactor under different pH conditions.

Based on the present study results, the highest amount of ethanol was formed in the reactor with a pH of 5.5, reaching an ethanol concentration of 369.53 mg/L, which was achieved after 48 hours. After 48 hours, the ethanol concentration decreased to 299.76, probably caused by the conversion of ethanol to acetate and other acidogenesis products. Thus, it can be concluded that the optimum ethanol formation occurred in the reactor with a pH of 5.5 for 48 hours. These results are in line with previous studies, which stated that ethanol could be produced at pH 4 to 5 [21], 5 to 6 [20], and pH 8 [22].

Compared to previous studies, the formation of hydrogen and ethanol from this research was still insignificant. A comparison of hydrogen and ethanol formation can be seen in Table 2. A one-way ANOVA test was used to see the difference in the effect of initial pH on the formation of ethanol and hydrogen. The test consisted of a normality test (data is called normal if the significance value > 0.05) and a homogeneity test (data is called homogeneous if the significance value > 0.05). Meanwhile, in the ANOVA test, significance > 0.05 indicates no difference between the average measurement results of ethanol and hydrogen, whereas when significance < 0.05 there is an average difference.

Table 2 Comparison of hydrogen and ethanol production in several studies.

Substrate	Biomass	Reactor condition		Product		Ref.
		Temp. (ºC)	pH	mol H_2/mol substrate	Ethanol (mg/L)	
POME	Pre-treated digested sludge	55	5.5	2.65	715	[23]
POME	Pre-treated digested sludge	37	5	0.45	820	[24]
POME	Pre-treated digested sludge	55	5.5	2.15	1670	[25]
POME	Pre-treated digested sludge	room temp.	6.5	0.14	1820	[26]
Mannitol	Anaerobic fermentative bacteria	37	4-10	1.82	1910	[27]
Xylose	Activated sludge	75	6-7	2.8	1250	[28]
Glycerol	*Thermotoga neapolitana*	80	6-7	2.86	1400	[29]
Mannitol	*Vibrio tritonius*	25-42	7.5	1.7	785	[30]
POME	Mixed culture bacteria	room temp.	4.5-7.5	0.38	347	this study

The normality and homogeneity test results can be seen in Table 3. It can be seen that the significance value was higher than 0.05 (significance > 0.05), which indicates that the data is normally distributed and homogeneous. Meanwhile, based on the results of the ANOVA test, the significance value of the test was 0.000, which means that there was a significant difference from the average ethanol formed. In other words, the initial pH affected the concentration of ethanol produced.

The results of the ANOVA test on the effect of initial pH on the formation of hydrogen can be seen in Table 4. The same as in the ANOVA test for the formation of ethanol, based on the significance value from the ANOVA test, it produced a significance value of 0.000, which means that there was an effect of pH on the formation of hydrogen.

Figure 5 shows the production of volatile fatty acids (VFA) during the anaerobic processes under different pH conditions, with acetate as the main product. Based on the distribution of the products, the pathways of anaerobic metabolism can be divided into six types: acetate-ethanol type; propionate-type; butyrate type; mixed-acid; lactate-type, and homoacetogenic pathways [31]. Volatile fatty acids (acetate, propionate, butyrate, and valeric) were produced with a higher concentration than ethanol. The dominant volatile fatty acid was acetate, with a concentration of 4335.8 mg/L in the reactor with an initial pH of 4.5. In contrast, in the reactor with an initial pH of 7.5, the dominant volatile fatty acid was butyrate with a concentration of 2950.3 mg/L. The initial pH affects the hydrolysis and acidification processes due to the changes in the microbial community [32].

Table 3 One-way ANOVA for ethanol production by using IBM SPSS 26.

Test of Normality							
	Reactor code	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Ethanol concentration	pH 4.5	0.244	3	-	0.972	3	0.676
	pH 5.5	0.312	3	-	0.896	3	0.373
	pH 6.5	0.270	3	-	0.949	3	0.564
	pH 7.5	0.260	3	-	0.958	3	0.605

Test of Homogeneity of Variance					
		Levene statistic	df1	df2	Sig.
Ethanol concentration	Based on mean	3.653	3	8	0.063
	Based on median	0.681	3	8	0.588
	Based on median and with adjusted df	0.681	3	3.310	0.616
	Based on trimmed mean	3.280	3	8	0.080

ANOVA					
	Sum of squares	df	Mean square	F	Sig.
Between groups	198.145	3	66.048	113.339	0,000
Within groups	4.662	8	0.583		
Total	202.807	11	-		

Table 4 One-way ANOVA for hydrogen production using IBM SPSS 26.

Test of Normality							
	Reactor code	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Hydrogen concentration	pH 4.5	0.323	3	-	0.878	3	0.318
	pH 5.5	0.344	3	-	0.640	3	0.215
	pH 6.5	0.305	3	-	0.905	3	0.403
	pH 7.5	0.233	3	-	0.979	3	0.724

Test of Homogeneity of Variance					
		Levene statistic	df1	df2	Sig.
Hydrogen concentration	Based on mean	0.267	3	8	0.847
	Based on median	0.023	3	8	0.995
	Based on median and with adjusted df	0.023	3	6.774	0.995
	Based on trimmed mean	0.231	3	8	0.873

ANOVA					
	Sum of squares	df	Mean square	F	Sig.
Between groups	198.145	3	66.048	113.339	0,000
Within groups	4.662	8	0.583		
Total	202.807	11	-		

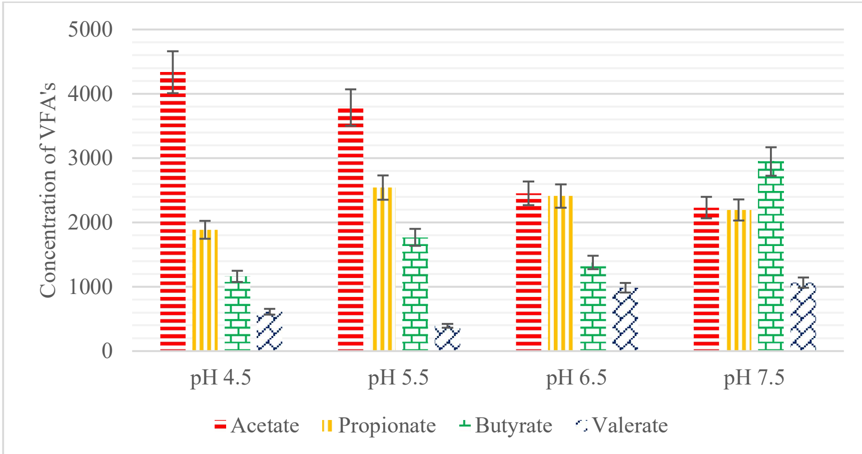

Figure 5 VFA production in the anaerobic reactor under different pH conditions.

The degree of acidification (DA) and the degree of ethanofication (DE) calculated in Eqs. (1) and (2) were used to compare the VFA and ethanol production. The comparison of DA and DE is shown in Figure 6. The result indicates that DA was higher than DE. This means that in the anaerobic processes, VFA was the dominant product. Table 5 shows the concentrations of ethanol and VFA produced at various reactor conditions.

Table 5 Anaerobic product formation during anaerobic processes.

Parameter	unit	Results			
		pH 4.5	**pH 5.5**	**pH 6.5**	**pH 7.5**
Ethanol	mg/L	320.66	341.32	347.74	256.47
Acetate	mg/L	4335.8	3476.9	2336.4	2100.7
Propionate	mg/L	1311.4	1652.3	1581.9	1492.4
Butyrate	mg/L	653.8	1022.6	753.9	2950.3
Valerate	mg/L	275.6	185.6	476.8	512.3
DE	-	0.0371	0.0395	0.0402	0.0297
DA	-	0.4536	0.4583	0.3922	0.4636

Based on the results, the amounts of hydrogen and ethanol produced were much smaller than volatile acids. Another approach that can be pursued to increase the production of hydrogen and ethanol is adding micronutrients. The presence of micronutrients can affect the rate of glycolysis and the conversion of pyruvate to ethanol [33]. Nickel is an essential micronutrient that can increase the growth of methanogenic bacteria and can produce co-factor F340, which affects acidogenesis products [34]. The presence of micronutrients affects the metabolic pathways of the bacterial population involved in the fermentation process and indirectly affects the distribution of acidogenic products [13,21]. Therefore, it is necessary to conduct further research on the effect of adding micronutrients to the formation of hydrogen and ethanol.

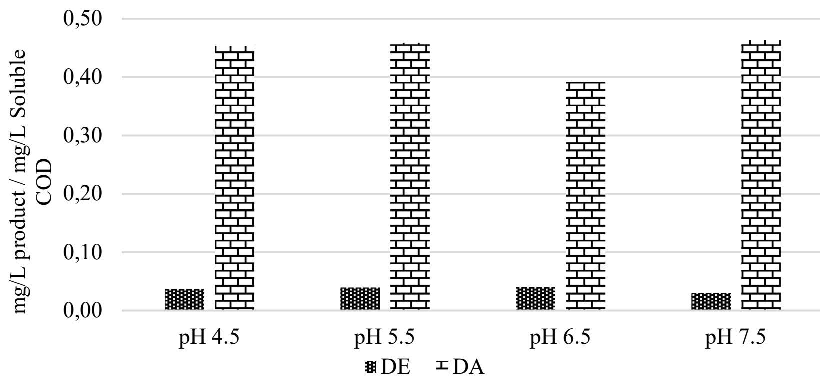

Figure 6 Comparison of the degree of ethanofication (DE) and the degree of acidification (DA).

The acidogenic process in forming ethanol using mixed culture bacteria involves a consortium of anaerobic bacteria consisting of *Clostridium sp. A1*, *Clostridium acidtolerans*, *Serratia marcescens* and swine manure bacteria (close to *Enterobacter asuburiae*). The substrate used in this study was still an organic polymer, which can be hydrolyzed to form simple compounds with the help of exoenzymes such as amylase, which can be produced by *Clostridium acidtolerans* [35]; S marcescens and Clostridium sp can produce lipases and proteases. *A1* [36]. Furthermore, the monomer glucose, glycerol, and amino acids formed will be converted to pyruvic acid via the Embden–Meyerhof–Parnas (EMP) pathway [37].

4 Conclusions

This paper observed the effect of initial pH on the hydrogen gas and ethanol production from organic-rich wastewater (POME) in an anaerobic reactor. The results showed that the initial pH of the reactor affected the anaerobic pathway, both for ethanol and hydrogen production. The reactor with an initial pH of 5.5 produced a higher hydrogen production (14.7% v/v) than the other pH conditions. In contrast, the most increased ethanol production occurred in the reactor with an initial pH of 6.5 with a concentration of 347.74 mg/L. The DA and DE values showed that VFA was the dominant product in this process. From these results, we can conclude that a slight change in pH value affects the hydrolysis process in hydrogen and ethanol fermentation.

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References

[1] Dincer, I. & Zamfirescu, C., *Sustainable Hydrogen Production*, Elsevier, Oxford, pp. 65-66, 2016.

[2] Koskinen, P., *The Development and Microbiology of Bioprocesses for the Production of Hydrogen and Ethanol by Dark Fermentation*, Thesis for the degree of Doctor, Tampere University of Technology, 2008.

[3] Rogner H-H., Zhou D., Bradley, R., Crabbé, P., Edenhofer, O., Hare, B., Kuijpers, L. & Yamaguchi, M., *Introduction. In Climate Change 2007: Mitigation*, Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007.

[4] Ji, C.M., Eong, P.P., Ti,T. B., Seng, C.E., & Ling, C.K., *Biogas from Palm Oil Mill Effluent (POME): Opportunities and Challenges from Malaysia's Perspective*, Renewable and Sustainable Energy Reviews, **26**, pp. 717-726, 2017.

[5] Mshandete, A.M. & Parawira, W., *Biogas Technology Research in Selected Sub-Saharan African Countries – A Review*, African Journal of Biotechnology, **8**, pp. 116-125, 2009.

[6] Rafieenia, R., Girotto, F., Peng, W., Cossu, R., Pivato, A., Raga, R. & Lavagnolo, M.C., *Effect of Aerobic Pre-Treatment on Hydrogen and Methane Production in a Two-Stage Anaerobic Digestion Process Using Food Waste with Different Compositions*, Waste Management, **59**, pp. 194-199, 2017.

[7] Guwy, A., Dinsdale, R., Kim, J., Nicolau, J. & Premier, G., *Fermentative Biohydrogen Production Systems Integration*, Bioresource Technology, **102**, pp. 8534-8542, 2011.

[8] Putra, M.D., Abasaeed, A.E., Atiyeh, H.K., Al-Zahrani, S.M., Gaily, M.H., Sulieman, A.K. & Zeinelabdeen, M.A., *Kinetic Modeling and Enhanced Production of Fructose and Ethanol from Date Fruit Extract*, Chemical Engineering Communication, **202**, pp. 1618-1627, 2015.

[9] A-Shorgani, N.K.N., Shukor, H., Abdeshahian, P., Yusuf, M., Nazir, M., Kalil, M.S., Hamid, A.A., Mohtar, W. & Yusoff, W, *Process Optimization of Butanol Production by Clostridium saccharoperbutylacetonicum N1-4*

(ATCC 13564) using Palm Oil Mill Effluent in Acetone–Butanol–Ethanol Fermentation, Biocatalysis and Agricultural Biotechnology, 4, pp. 244-249, 2015.

[10] Schuchardt, F., Wulfert, K., Darnoko, D. & Herawan, T., *Effect of New Palm Oil Mill Processes on the EFB and POME Utilisation*, Proceedings of Chemistry and Technology Conference PIPOC , Kuala Lumpur , pp. 44-57, 2007.

[11] Gumilar, A., Handajani, M. & Syafila, M., *Influence of N_2 Flushing for the Ethanol Production from High Concentration of Organic Waste*, The 5th Environmental Technology and Management Conference 'Green Technology towards Sustainable Environment', 23-24 November 2015, Bandung, 2015.

[12] Handajani, M., Syafila, M., Gumilar, A. & Andrio, D, *Effect of the Iron (II) and Zinc (II) Addition for the Ethanol Production from the Degradation of Palm Oil Mill Effluent (Pome) by Anaerobic Processes*, Proceedings of International Seminar on Chemical Engineering in Conjunction with Seminar Teknik Kimia Soehadi Reksowardojo (STKSR), 27-28 October 2016, Bandung, pp. 595-601, 2016.

[13] Syafila, M., Gumilar, A., Handajani, M., Mecca, B. M. & Puteri, T. W., *Effect of Co, Zn and Mn Metal towards Ethanol Formation in Anaerobic Process of Palm Oil Mill Effluent (POME)*, Proceedings of Sustainable Infrastructure and Built Environment (SIBE) 2017, 26-27 September 2017, ITB, Bandung, 2017.

[14] Salihu, A., & Alam, M., *Palm Oil Mill Effluent: A Waste or a Raw Material?*, Journal of Applied Sciences Research, **8**, pp. 466-473, 2012.

[15] American Public Health Association, *Standard Method for the Examination Water and Wastewater*, 23rd edition, 2017.

[16] Prasertsan, P., O-Thong, S. & Birkeland, NK., *Optimization and Microbial Community Analysis for Production of Biohydrogen from Palm Oil Mill Effluent by Thermophilic Fermentative Process*. International Journal of Hydrogen Production, **34**, pp. 7448-7459, 2009.

[17] Pisutpaisal, N., Nathao, C. & Sirisukpoka, U., *Biological Hydrogen and Methane Production in from Food Waste in Two-stage CSTR*, Energy Procedia, **50**, pp. 719-722, 2014.

[18] Doran P.M.B.T.B.E.P., *A – Conversion Factors*, London: Academic Press, pp. 395-397, 1995.

[19] Fan, Y.T., Li, C.L., Lay, J.J., Hou, H.W., & Zhang, G.S., *Optimization of Initial pH Substrate and pH Levels for Germination of Sporing Hydrogen Producing Anaerobes in Cow Dung Compost*, Bioresource Technology, 91, pp. 189-193, 2004.

[20] Anzola-Rojas, M.DP., Zaiat, M. & Wever, H.D., *Improvement of Hydrogen Production via Ethanol-Type Fermentation in an Anaerobic Down-Flow Structured Bed Reactor*, Bioresource Technology, **202**, pp. 42-49, 2016.

[21] Elreedy, A., Tawfik, A., Enitan, A., Kumari, S. & Bux, F., *Pathways of 3-Biofules (Hydrogen, Ethanol and Methane) Production from Petrochemical Industry Wastewater via Anaerobic Packed Bed Baffled Reactor Inoculated with Mixed Culture Bacteria*, Energy Conversion and Management, **122**, pp. 119-130, 2016.

[22] Carosia, M.F., Dos Reis, C.M., Sakamoto, I.K., Varesche, M.B.A. & Silva, E.L., *Influence Of C/P and C/N Ratios and Microbial Characterization in Hydrogen and Ethanol Production in an Anaerobic Fluidized Bed Reactor*, International Journal of Hydrogen, **42**, pp. 9600-9610, 2017.

[23] Tahti, H., Kaparaju, P. & Rintala J*., Hydrogen and Methane Production in Extreme Thermophilic Conditions in Two-Stage (Upflow Anaerobic Sludge Bed) UASB Reactor System*, Journal of Hydrogen Energy, **38**, 4997-5002, 2013.

[24] Ismail, I., Hassan, M.A., Rahman, N.A.B. & Soon, C.S., *Thermophilic Biohydrogen Production from Palm Oil Mill Effluent (POME) Using Suspended Mixed Culture*, Biomass and Bioenergy, **34**, 42-47, 2010.

[25] Khrisnan, S., Singh, L., Sakinah, M., Thakur, S., Wahid, Z.A. & Sohaili, J., *Effect of Organic Loading Rate on Hydrogen (H_2) and Methane (CH_4) production in Two-Stage Fermentation under Thermophilic Conditions Using Palm Oil Mill Effluent (POME)*, Energy for Sustainable Development, **34**, 130-138, 2016.

[26] Mohammadi, M., Man, H.C., Hassan, M.A. & Yee, P.L., *Review: Treatment of Wastewater from Rubber Industry in Malaysia*, African Journal of Biotechnology, **9** (38), 6233-6243, 2016.

[27] Xia, A., Jacob, A., Herrmann, C., Tabassum, M.R. & Murphy, J.D., *Production of Hydrogen, Ethanol and Volatile Fatty Acids from the Seaweed Carbohydrate Mannitol*, Bioresource Technology, **193**, 488-497, 2015.

[28] Ngo, T.A., Nguyen, T.H. & Bui, H.T.V., *Thermophilic Fermentative Hydrogen Production from Xylose by Thermotoga neapolitana DSM 4359*, Renewable Energy, **37**, 174-179, 2012.

[29] Maru B.T., Constanti M., Stchigel A.M., Medina F., Sueiras J.E., *Biohydrogen Production by Dark Fermentation of Glycerol using Enterobacter and Citrobacter Sp.*, Biotechnology Progress, **29**, 31-38, 2013.

[30] Matsumura, Y., Sato, K., Al-saari, N., Nakagawa, S. & Sawabe, T., *Enhanced Hydrogen Production by a Newly Described Heterotrophic Marine Bacterium, Vibrio Tritonius Strain AM2, Using Seaweed as the Feedstock*, Int. J. Hydrogen Energy **39**, 7270-7277, 2014.

[31] Zhou, M., Yan, B., Wong, J.W.C. & Zhang Y*., Enhanced Volatile Fatty Acids Production from Anaerobic Fermentation of Food Waste: A Mini-Review Focusing on Acidogenic Metabolic Pathways*, Bioresource Technology, **248**, pp. 68-78, 2018.

[32] Lin, L., Wan, C., Liu, X., Lee, D-J., Lei, Z., Zhang, Y. & Tay, J.H., *Effect of Initial pH on Mesophilic Hydrolysis and Acidification of Swine Manure*, Bioresource Technology, **136**, pp. 302-308, 2013.

[33] Cao, W. & Liu, R., *Screening and Optimization of Trace Elements Supplement in Sweet Sorghum Juice for Ethanol Production*, Biomass and Bioenergy, **50**, 45-51, 2013.

[34] Choong, Y.Y., Norli, I., Abdullah, A.Z. & Yhaya, M.F., *Impacts of Trace Element Supplementation on the Performance of Anaerobic Digestion Process: A Critical Review*, Bioresource Technology, **209**, 369-379, 2016. (Journal)

[35] Lee, K.S., Lo Y.C., Lin P.J. & Chang J.S., *Improving Biohydrogen Production in a Carrier-Induced Granular Sludge Bed by Altering Physical Configuration and Agitation Pattern of the Bioreactor*, International Journal of Hydrogen Energy, **31**, pp. 1648-1657, 2006.

[36] Zhao, X.Q., Xue, C., Ge, X.M., Yuan, W.J. Wang, J.Y. & Bai, F.W., *Impact of Zinc Supplementation on the Improvement of Ethanol Tolerance and Yield of Self-Flocculating Yeast in Continuous Ethanol Fermentation*, Journal of Biotechnology, **139**, pp. 55-60, 2009.

[37] Kim, B.H & Gadd, G.M., *Bacterial Physiology and Metabolism*, Cambridge University Press, pp. 252-289, 2008.