International Journal on Advanced Science Engineering Information Technology

Vol. 2 (2012) No. 4
ISSN: 2088-5334

Pre-Treatment Effect of Palm Oil Mill Effluent (POME) during Hydrogen Production by a Local Isolate *Clostridium butyricum*

Syafawati Ahmad Kamal[a,1], Jamaliah Md Jahim[a,2], Nurina Anuar[a], Osman Hassan[b], Wan Ramli Wan Daud[c], Mariatul Fadzillah Mansor[a] and Shah Samiur Rashid[a]

[a] *Department Of Chemical Engineering And Process, Faculty Of Engineering And Built Environment, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.*

[b] *Department Of Food Science, Faculty Of Science And Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.*

[c] *Fuel Cell Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.*

[1] *syafa.kamal@gmail.com* [2] *jamal@eng.ukm.my*

***Abstract*— Palm Oil Mill Effluent (POME) could become a very good carbon feedstock for hydrogen production in fermentation process if it is well prepared and being utilised based on its fermentable constituents composed in POME. In this research, the effectiveness of pre-treatment methods on POME was examined based on total carbohydrate (TC) and chemical oxygen demand (COD). Several pre-treatment methods such as heat, acid and alkaline were introduced to POME to increase the total carbohydrate content in POME medium. These mediums were further used in fermentation process to ensure the effect of pre-treatment method in term of hydrogen production. The highest hydrogen yield was 2.18 mol H_2 / mol total carbohydrate given by POME using alkaline-heat-supernatant pre-treatment in bioreactor 2L**

Keywords* — Palm Oil Mill Effluent (POME); Pre-Treatment; Hydrogen; Batch Mode; *Clostridium butyricum

I. Introduction

Nowadays, usage of fossil energy causes lots of environmental pollution problems and serious energy crisis. To overcome this problem, hydrogen seems to be an alternative energy with no carbon emissions since only water evolves as exhaust gas when burnt [1] and can be produced from biomass or organic waste which is available in abundance. Corn stover [2], tofu waste [3], and cassava starch [4], are among the organic wastes used to produce biohydrogen.

In 2009, Malaysia produce 0.6-0.65 m^3 Palm Oil Mill Effluent (POME) with every 1 ton of Fresh Fruit Branch (FFB) [5]. POME contains amino acids, inorganic nutrients such as sodium, potassium, calcium, magnesium, short fibers; organelles, nitrogenous constituents, free organic acids and a mixture of carbohydrates (hemicelluloses to simple sugars) [6]. Therefore, POME is a very suitable substrate for hydrogen production. Table 1 show the general properties of POME [7]. Because of its rich carbohydrate content, pre-treatment of POME is important to breaak-up lignocellulosic materials into simple sugar to be used by bacteria during fermentation Various pre-treatment methods used to pre-treat lignocellulosic materials are acid treatment [8], alkaline treatment [9], and enzymatic treatment [10].

TABLE 1
GENERAL PROPERTIES OF POME

Physical & Chemical Properties	Unit	
Moisture Content	%	93.4
Ash	%	1.06
Total Solid	g	65 - 80
Soluble Fibre	% SS	1.12
Non Soluble Fibre	% TS	3.9
COD	mg/L	15000-100000
BOD_5	mg/L	10250 - 43750
pH		3.8-5.0
Protein	% TS	12.31
Fat	% TS	12.95

Many methods on biomass pre-treatment were developed by several researchers those were used as fermentation substrate for many types of product formation such as ethanol [11], hydrogen [2,3,4] and biofuel [12]. Acid pre-treatment has become the most competent method for hydrolysing the lignocellulosic materials [8] and have been investigated for a few decades. There were many types of acid used in acid pre-treatment of lignocelluloses material such as hydrochloric acid (HCl) [13], nitric acid [14], phosphoric acid[15] and sulphuric acid [8]. Concentrated acids are powerful agents for hydrolysis but these concentrated acids are toxic, corrosive and hazardous. Subsequently, these acids must be recovered after hydrolysis to make the process economically feasible[11]. Comparing to that, dilute acid becomes a successful agent for hydrolysis at high temperature [16]. At moderate temperature, the hydrolysis suffers from low yields because of sugar decomposition. Pre-treatment of dilute sulfuric acid with biomass will shift hemicellulose to xylose and other sugar and further hydrolysis convert the xylose to form furfural [13,17]. There are some disadvantages using this methods such as, the existance of furfural [18] and the presence of Cl^- and SO_4 [19] will inhibit the bacterial growth rate and lead to the decrease in hydrogen production. Other disadvantage is that the acid content in the medium must be neutralized before the substrate can be used for fermentation.

According to some researchers [9], alkaline treatment was also able to hydrolyse lignocellulosic materials effectively and it is good for fermentation processes because alkaline pre-treatment can delignify biomass thus improving the reactivity of the remaining polysaccharides; also remove acetyl and the various acid substitutions on hemicelluloses that lower the accessibility of the enzyme to the hemicelluloses and cellulose surface [20]. The advantage of alkaline pre-treatment process is that it can be utilized at lower temperature and pressures compared to other pre-treatment technologies but the disadvantages of this method is the alkaline pre-treatment can produce some irrecoverable salts or incorporated as salt into biomass by the pre-treatment reactions [17].

Dark fermentation is a favourable method to produce hydrogen where anaerobic bacteria utilize organic compounds in the absence of light [21]. The shift in metabolic pathway changed substrates to simpler compounds such as lactate, acetate and butanol when the concentration of hydrogen increased [2]. Most common bacteria used in hydrogen production were Bacillus sp. [22], Enterobacter sp. [23], and Clostridium sp. [24].

In these studies, effect of pre-treatment on POME, effect of initial pH and effect of suspended solid content in media ware investigated to observe the hydrogen production rate using locally isolated *Clostridium butyricum*. All of the experimental studies were done in non sterile condition. The best result obtain will run in 2L fermenter with pH controller.

II. Material And Methods

A. Palm oil mill effluent (POME)

POME was collected from Sri Langat palm oil mill, Dengkil, Selangor. The collected POME was stored in cold room at 4^{o}C for further use.

B. Inoculums Preparation of Hydrogen-producing bacteria

Clostridium *butyricum* was isolated from POME sludge by previous researcher [25]. The single culture was incubated in Reinforced Clostrial Medium (RCM) plate and broth at 37^{o}C and maintained in every month. For preparation of inoculums, five colonies was taken from RCM agar plate and transfer to 100 mL RCM broth. After incubation at 37^{o}C with agitation speed 150 rpm for around 15 to 20 hour until the optical density (OD) was achieved approximately 0.9 to 1.1. The incubation period depends upon the age and size of the colony used. The culture was transferred to POME medium for preparation of seed culture. The culture was also incubated for 20 hour with same condition as inoculum.

C. Pre-treatment of POME

The Fig.1 summarises five types of pre-treatment methods used to pre-treat POME. About 10 % of 1M H_2SO_4 was added into raw POME for acid pre-treatment. While for alkaline pre-treatment, 10% of 1M NaOH was added into raw POME. After that, the mixed solution was stirred to make sure the solution was well mixed. For heat treatment, the raw POME was heated at 80^{o}C for 1 hour without addition of chemical. For combination of chemical and heat treatments, the POME solutions were prepared as chemical treatments reported above. Then, the solutions were further treated with heat treatment. All of the treatment methods above were repeated and the solutions were further centrifuged to get the supernatant. The supernatant was used to investigate the effect of suspended solid in fermentation broth in term of hydrogen production.

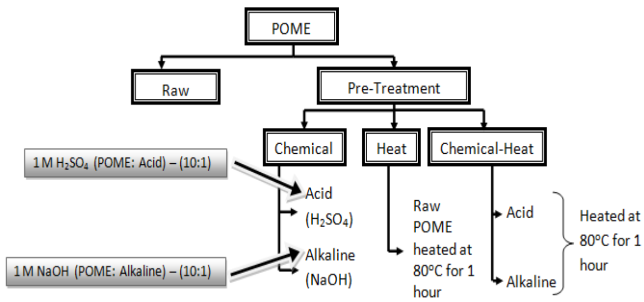

Fig. 1. Overview of pre-treatment process on POME

D. Batch test set-up in 100 mL serum bottle

Hydrogen production rate were depend on substrate used in fermentation process. To study the effectiveness of pre-treatment methods on hydrogen production, the batch tests were conducted under mesophilic condition in 100 mL serum bottle. 10% (v/v) of seed culture was transferred to each treated and raw POME medium was incubated at 37^{o}C with agitation speed 150 rpm. Two parameters were investigated in this study where as the effect of initial pH (pH 5.5 and pH 7) and the effect of suspended solid existence in fermentation broth using treated POME. To investigate the effect of initial pH, the initial pH of the medium was adjusted to pH 5.5 and pH 7 using 5M NaOH and 5M H_2SO_4. The high concentration of pH buffer was used to avoid the total carbohydrate concentration diluted in the medium. For study on effect of suspended solid content in fermentation broth, all of treatments methods above were repeated and the solutions were further centrifuged to get the supernatant. The pH of the medium was adjusted to pH 7 and the duration of fermentation was 24 hours.

E. Batch Fermentation in 2L bioreactor

Six pre-treatment methods such as heat (H), Acid-Heat (Ac-H), Alkaline-Heat (Al-H), Heat-Supernatant (H-S), Acid-Heat-Supernatant (Ac-H-S) and Alkaline-Heat-Supernatant (Al-H-S) were prefer for further fermentation process in bioreactor (2L) with working volume of 1.8L. The initial pH of POME solution was adjusted to 7 and control at 5.5. The temperature used during fermentation was 37^{o}C with agitation speed at 300 rpm to facilitate rapid diffusion of hydrogen and to ensure the medium was well mixed. In this research, the agitation (rpm) has not been studied

F. Analytical Methods

Reducing sugar contain in the media was analysed using *dinitrosalicylic* (DNS) assay [26], while total carbohydrate (TC) content was measured using phenol-sulphuric acid assay [27] and the Chemical Oxygen Demand (COD) was measured using HACH reagents. The amount of organic acid was measured using High Performance Liquid Chromatography (Agilent 1100 series, Agilent Technologies, USA). Total Suspended Solid (TSS) was measured using standard method [28]. Biogas produced was collected using water displacement method and analyzed using gas chromatography (GC) model SRI 8610C with HID and TCD detectors equipped with 6 foot long molecular sieve 13X and 6 foot long silica gel packed column. The gas carrier for GC is helium (MOX 99.99%) at 20 mL/min. The pressure for the carrier was set at 29 psi while the injection port, detector and oven temperature were set at 40°C, 150^{o}C and 40°C respectively. The valve was on at 0.05 minutes and turned off at 6 minutes.

G. Kinetic modeling

The cumulated hydrogen production was estimated by the Gompertz equation as shown in Eq. (1)

$$H = Pexp\,\{-\exp[\frac{R_m e}{P}(\lambda - t) + 1]\} \qquad (1)$$

Where H is cumulative hydrogen production (mL), λ is the lag phase (hr), P is maximum hydrogen production (mL), Rm is maximum hydrogen production rate (mL/hr) and e is constant value 2.718281828. The value of P, Rm, and λ for each batch were estimated from simulation of experiment data with eq. (1) using statistic nonlinear regression wizard function of Sigma Plot® (version 10).

III. Result And Discussion

A. Pre-treatment of POME

H_2SO_4 was selected as acid pre-tretment agent while NaOH for alkaline pre-treatment in this study. H_2SO_4 was selected because dilute H_2SO_4 can effectivly converted hemicellulose to xylose rather than other acid [17] while, dilute NaOH was preferred because the fibre can be delignified by NaOH up to 70% [29]. After pre-treatment of POME using acid or alkaline, both of the mixture showed the colours changed. The POME mixture becomes more brownish when treated with acid while the POME mixture turned into black when it was treated with alkaline. Fig. 2 showed the comparison characteristics on carbon sources of each pre-treatments type in this study. After the pre-treatment, POME were analysed for TC, and COD to observe the effectiveness of the pretreatment methods compared to raw POME. Table 2 indicated that amount of TSS reduced after the pre-treatment, directly porpotional to the increment of carbon sources in the medium. The higher increment of total carbohydrates, the lower TSS value in POME. This shows that, in presence of acid or base-ion can rupture the lignin seal to simple sugar [17]. In presence of temperature, heat can facilitate the cellulosic biomass to make cellulose more accessible to convert the carbohydrate polymers into fermentable sugars, so the conversion became more effective [30].

Table 2
Comparisons on properties of POME after pre-treatment

Parameters	Raw	Pre-treatment				
		Ac	Al	H	Ac-H	Al-H
TSS (g/L)	37.75	32.47	31.67	28.23	26.1	25.05
Reducing sugar (g/L)	11.26	11.79	11.52	12.76	14.54	13.64
Total Carbohydrate (g/L)	22.27	23.25	23.04	25.42	27.19	26.40
Soluble COD (g/L)	58.40	59.60	58.75	56.10	57.05	58.30
Furfural (g/L)	0.30	0.72	0.00	0.40	0.75	0.00
pH	4.5	2.0	7.0	4.6	2.0	7.7

B. Batch test set-up on hydrogen production in 100 mL serum bottle

In this study, POME directly used after pre-treatment without standardise the initial concentartion of TC content in the medium broth only small amount of buffer were added to adjust the initial pH. Fig 2 represented hydrogen production at different initial pH (5.5 and 7), using local isolate, *Clostridium butyricum*. The fermentation was carried out in non-sterile condition. The result showed the maximum hydrogen production for initial pH 7 was given by Al-H but for initial pH 5.5, the higher hydrogen production was given by Ac-H. For most of the gas produced in initial pH 7 was higher than initial pH 5.5 except for POME treated using Ac-H. In this study, hydrogen production was higher at initial pH 7 rather than pH 5.5. Fermentation process will be stoped at lower pH and undissociated form of acid inhibits the hydrogen production [7]. In this case, the initial pH 7 will have longer period to archieve lower compared to initial pH 5.5. With pH 7, hydrogen production in Al-H treatment much more higher than Ac-H treatment.

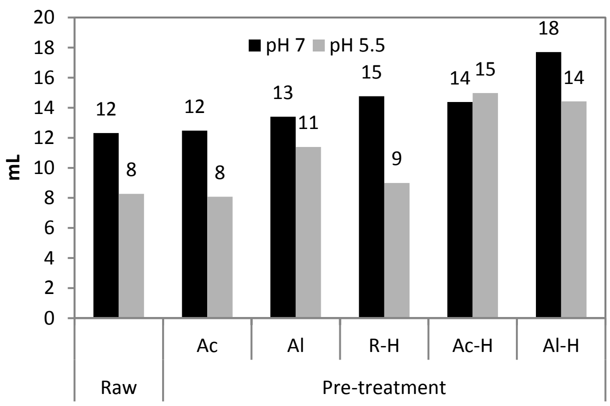

Fig. - 2 Hydrogen production at two diffrent initial pH (pH 5.5 and pH 7) for diffrent POME treatment

Fig 3 shows the effect of suspended solid in POME medium on hydrogen production using treated POME. Suspended solid in medium was represented as insoluble cellulosics in the fermentation broth. The fermentation was carried out in non-sterile condition. Result shows the maximum hydrogen production was 36 mL H_2 in 100 mL medium or approximately 0.12 mol H_2/mol TC using Ac-H without suspended solid (Ac-H-S) treatment method followed by Al-H without suspended solid (Al-H-S). The pattern of hydrogen production showed the existence of suspended solid in the fermentation broth will inhibit the hydrogen production rate. *Clostridum butyricum sp* cannot directly assimilate non soluble carbon source for cell growth and hydrogen production [31]. The fermentation on POME treated using Ac-H-S produced higher hydrogen production because the existence of higher carbohydrate content in POME medium at initial stage as listed in table 3.

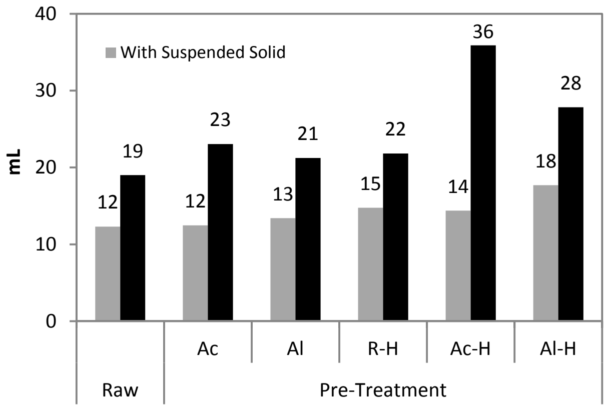

Fig. 3 - Effect of Suspended Solid in POME medium on hydrogen production in serum bottle for 24 hour fermentation process.

Table 3 indicated the hydrogen yield of each fermentation process using different type of medium pre-treatment. In this research, effect of initial TC concentration has not been studied. POME treatment was directly used for fermentation after adjusted initial pH to pH 7. For fermentation of POME treated using Al-H-S, the hydrogen production was lower then POME treated using Ac-H-S interm of hydrogen production but Al-H-S higher than Ac-H-S interm of hydrogen yield (mol H_2/mol TC). This was because the fermentation using Ac-H-S assimilated more TC concentration compared to other fermentation. The highest yield was given by raw and POME treated using Al with 0.14 mol H_2/Mol TC. To futher confirmation, the effect of suspended solid content in medium, six pre-treatment was chosen for hydrogen fermentation in 2L fermenter such as R-H, Ac-H, Al-H, R-H-S, Ac-H-S and Al-H-S untill no gasses was produced in the fermentation process.

TABLE 3
HYDROGEN YIELD OF EACH FERMENTATION PROCESS USING DIFFERENT TYPE OF MEDIUM PRE-TREATMENT AT INITIAL PH 7

Type Of Pre-Treatment POME	Initial TC (g/L)	Final TC (g/L)	Productivity (mL H_2/mL POME)	Yield (Mol H_2/Mol TC)
Raw	19.69	13.36	0.12	0.14
Ac	21.00	13.98	0.12	0.13
Al	20.26	13.44	0.13	0.14
R-H	23.56	15.26	0.15	0.13
Ac-H	26.85	17.72	0.14	0.12
Al-H	25.34	15.55	0.18	0.13
R-S	25.04	9.68	0.19	0.09
Ac-S	26.69	8.19	0.23	0.09
Al-S	27.65	7.68	0.21	0.08
R-H-S	30.29	9.91	0.22	0.08
Ac-H-S	29.93	7.36	0.36	0.12
Al-H-S	23.86	7.76	0.28	0.13

C. Hydrogen Production Using 2L Bioreactor Using Treated POME

Production of hydrogen from fermentation process was done in bioreactor (2L) with 1.8L working volume as a lab scale study. Fig 4 shows hydrogen production profile and total carbohydrate consumption profile for whole fermentation process. The highest hydrogen production was achieved by the POME using treatment method of Ac-H, followed by Al-H and Al-H-S. For each medium with suspended solid such as R-H, Ac-H and Al-H need longer period to complete the fermentation process compared to medium without suspended solid such as R-H-S, Ac-H-S and Al-H-S. It proved that, the presence of suspended solid can disturbed the fermentation process and spent more time to complete the fermentation process. All of hydrogen production for each medium was in exponent's phase when the concentration of TC content achieved at 10 g/L. The faster hydrogen production was given by Al-H-S at 15th hour fermentation compared to other medium at 24th hour after fermentation process. The reduction of TC concentration in medium extremely at early fermentation process was mostly used for growth of bacteria until the TC concentration achieved optimum concentration for hydrogen production. It showed that initial concentration affected the hydrogen production rate.

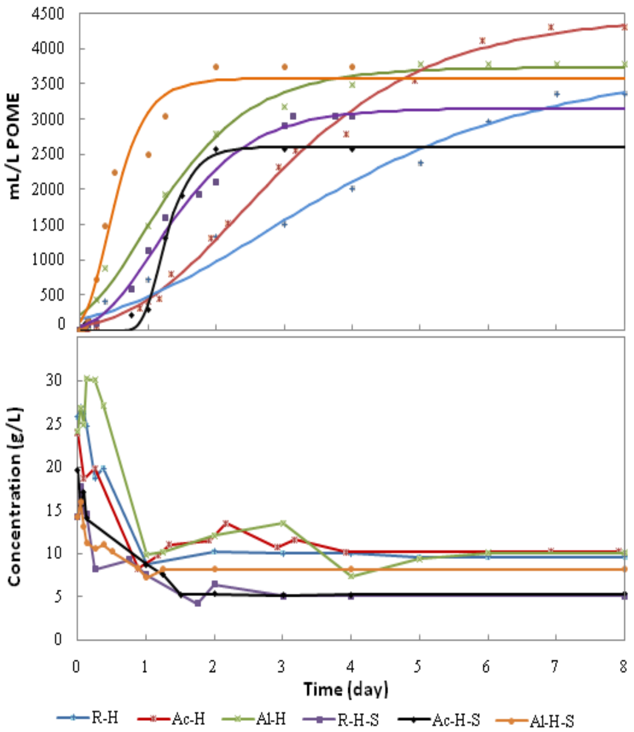

Fig. 4 - Profile of hydrogen production and substrate consumption during fermentation process.

The highest hydrogen production was 4304 mL H_2/L POME with highest yield of butyric acid given Ac-H (Table 4). The POME treated by Ac-H produced higher cumulative hydrogen because the existence of higher carbohydrate content in POME medium. It was the reason why the hydrogen yields become lower compare to other treatment using alkaline treatment. For Al-H-S, fermentation process completed after 2 days fermentation differs from Ac-H treatment, the fermentation cycle completed at 7 days. The hydrogen produced from POME in this research was higher than reported in other research using same bacteria [7]. This was because of the use of different initial pH.

Although cumulative hydrogen production from POME treated using Ac-H was the highest, but the hydrogen yield was lower compared to Al-H-S as presented in table 4. The highest hydrogen yield was 2.18 mol H_2/mol TC followed by Al-H, Ac-H, R-H, R-H-S and Ac-H-S. The highest hydrogen produce in this study was lower than reported before with 4.5 mol H_2/mol reducing sugar using same sources of POME but different bacteria [32].

TABLE 4
YIELD OF EACH METABOLITE FOR EACH TREATMENT IN BIOREACTOR 2L

Yield (Mol product / Mol TC)	Pre-Treatment POME					
	R-H	Ac-H	Al-H	R-H-S	Ac-H-S	Al-H-S
H_2	1.40	1.87	1.99	1.36	0.74	2.18
CO_2	0.89	0.82	1.31	1.23	1.91	1.50
Butyric Acid	0.66	1.11	1.08	0.46	1.00	1.08

According to the theory, in acidogenesis phase, 1 mol of glucose will produce 4 mol of hydrogen with 2 mol of carbon dioxide in presence of 2 mol of acetic acid as shown in Eq. 1 or 1 mol of glucose will be converted to 2 mol of hydrogen with 2 mol of carbon dioxide with 1 mol of butyric acid as given in Eq. 2. In this fermentation process, no acetic acid was detected in the fermentation broth. This shows the fermentation process followed the Eq. 2 and type of fermentation was butyric type fermentation.

$$C_6H_6O_6 \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_6O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

Table 5 illustrated the comparison of other study on hydrogen production using POME as substrate. All researchers used same initial pH as 5.5 with many types of microorganism use.

Table 5 - Comparison in hydrogen production using POME with other researchers			
Microorganism	Mode/pH/ Temp	Yield (mL H_2/ L POME),	Ref
Mixed culture	Batch/ 5.5/60^oC	4708	[1]
Clostridium butyricum EB6	Batch/ 5.5/37^oC	3195	[7]
Mixed Culture	Continuous /5.5/23-35^oC	565	[33]
Mixed Culture	Continuous /5.5/60^oC	6500	[34]
Mixed Culture	Continuous/6.8/37 ^oC	6700	[35]
Clostridium butyricum	Batch/ 7/37^oC	4304	This study

IV. Conclusion

This study was investigated the effect of initial pH and effect of suspended solid using POME treated. Five pre-treatment methods were introduced such as acid, alkaline, heat and combine acid-heat and alkaline heat. The study found that, using acid-heat pre-treatment, TC carbohydrate contains achieved higher concentration compare to other pre-treatment method but by using acid pre-treatment, the final product will contain furfural. Furfural became one of the inhibitions for cell growth and hydrogen production. The medium treated and raw medium was adjusted to initial pH 5.5 and 7.0 to investigate the effect of initial pH on fermentation process using treated POME. In this study, the pattern of cumulative hydrogen was higher at initial pH 7, compared to fermentation using initial pH 5.5 but the maximum hydrogen production rate was achieved at pH 5.5. In present of suspended solid in fermentation broth, the insoluble cellulose cannot directly utilized by *Clostridium butyricum* at once become the disturbing agent for hydrogen production. After 24 hour fermentation, the highest hydrogen achieved using medium without insoluble cellulose (suspended solid). The profile of hydrogen production was carried out using fermentation in 2L bioreactor with controlled pH. Experimental result showed, the best hydrogen production was given by Al-H-S with shorter fermentation period with higher hydrogen yield (2.18 mol H_2/mol TC). This work has illustrated that the pre-treatment method for hydrolysis can be introduced to POME to increase the TC content but higher TC content will inhibit the hydrogen production. The fermentation process wills successfully achieved when no suspended solid content in the medium broth. The present study provides valuable information about the pre-treatment methods of POME for growth of *Clostridium butyricum* and hydrogen production to design more effective system for treated POME as substrate for fermentation process.

Nomenclature

Ac	Acid
Ac-H	Acid-Heat
Ac-S	Acid-Supernatant
Ac-H-S	Acid-Heat-Supernatant
Al	Alkaline
Al-H	Alkaline-Heat
Al-H-S	Alkaline-Heat-Supernatant
Al-S	Alkaline-Supernatant
COD	Chemical Oxygen Demand
H	Heat
H-S	Heat-Supernatant
H_2SO_4	Sulphuric Acid
NaOH	Potassium Hydroxide
R-S	Raw-Supernatant
TC	Total Carbohydrate
TSS	Total Suspended Solid

ACKNOWLEDGEMENT

The authors wish to thank the financial support from Universiti Kebangsaan Malaysia and the Ministry of Science, Technology and Innovation (MOSTI) Malaysia, under UKM-AP-PI-13-2010 and the Science-fund Research program of UKM-MGI-NBD16-2007, for funding of this project.

REFERENCES

[1] Atif AAY, Fakhru'l-Razi A, Ngan MA, Morimoto M, Iyuke SE, Veziroglud NT. Fed batch production of hydrogen from palm oil mill effluent using anaerobicmic microflora. Int. Journal of Hydrogen Energy 2005;30:1393-97.

[2] Ni M, Leung DYC, Leung MKH, Sumathy K. An overview of hydrogen production from biomass. Fuel Processing Technology 2006;87:461-72.

[3] Liu CZ, Cheng XY. Improved hydrogen production via thermophilic fermentation of corn stover by microwave-assisted acid pretreatment. Int J of Hydrogen Energy 2010;35:8945-52.

[4] Kim MS, Lee DY. Fermentative hydrogen production from tofu-processing waste and anaerobic digester sludge using microbial consortium. Bioresource Technology 2010;101:48-52.

[5] Hassan WHW, Wahid BM, Choo YM, Menom NR, Lim WS, Aziz AA, Bakar AN. SREP Development in Sabah from MPOB Perspective In 2009, 2009.

[6] Santosa SJ. Palm Oil Boom In Indonesia: From Plantation To Downstream Products And Biodiesel. Clean- Soil, Air, Water. 2008;36:453-65.

[7] Chong ML, Rahim, R A, Shirai Y, Hassan MA. Biohydrogen production by clostridium butyricum eb6 from palm oil mill effluent. Int J Of Hydrogen Energy 2009; 34:764-71.

[8] Nasirian N, Almassi M, Minaei S, Widmann R. Development of a method for biohydrogen production from wheat straw by dark fermentation. Int J of Hydrogen Energy 2011;36:411-20.

[9] Ozkan L, Erguder TH, Demirer GN. Effects of pretreatment methods on solubilization of beet-pulp and biohydrogen production yield. Int J of Hydrogen Energy 2011;36:382-89.

[10] Wong KM, Rahman NA, Aziz S, Sabaratnam V. Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. Research J Microbiology 3 2008;6: 474-481.

[11] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology 2002;83:1-11.

[12] Vicente G, Bautista LF, Rodriguez R, Gutierrez FJ, Sadaba I, Ruiz-Vazquez RM, Torres-Martinez S, Garre,V. Biodiesel production from biomass of an oleaginous fungus. Biochemical Engineering Journal 2009;48:22–7.

[13] Cui M, Yuan Z, Zhi X, Wei L, Shen J. Biohydrogen production from poplar leaves pretreated by different methods using anaerobic mixed bacteria. Int J of Hydrogen Energy 2010;35:4041-47.

[14] Brink, D.K., 1996. Method of treating biomass material us patent 5536325.

[15] Israilides CJ, Grant GA, Han YW, Sugar level fermentable, and acceptability of straw treated with different acids. Applied Environmental Microbiology 1978;36:43-46.

[16] Mc Millan JD, 1994 Pretreatment of lignocellulosic biomass. In Himmel, M.E., Baker, J.O., Overend, R.P (Eds.), Enzymatic conversion of biomass for fuel production. American Chemical Society, Washington , DC, pp. 292-324

[17] Mosier N, Wyman C, Dale BR, Elander R, Lee YY, Holtzapple M, Ladisch M. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology 2005;96: 673-686

[18] Panagiotopoulos I, Bakker R, De Vrije T, Van Niel E, Koukios E, Claassen E. Exploring critical factors for fermentative hydrogen production from various type of lignocellulosic biomass. Journal of the Japan Institute of Energy 2011;90:363-68

[19] Wang Y, Wang H, Feng X., Wang X., Huang J. Biohydrogen production from cornstalk wastes by anaerobic fermentation with activated sludge. Int J of Hydrogen Energy 2010;35:3092-99.

[20] Chang VC, Holtzapple MT. Fundemnetal factors affecting biomass enzymatic reactivity. Applied Biochemistry And Biotechnology 2000;74:135-59.

[21] Levina DB, Pitt L, Love M. Biohydrogen production: Prospectsand limitations to practical application. Int J of Hydrogen Energy 2004;29:173-185.

[22] Kotay SM, Das D. Microbial hydrogen production with Bacillus coagulans IIT-BT S1 isolated from anaerobic sewage sludge. Bioresour Technol 2007:98;1183-90.

[23] Nath K, Chittibabu G, Das D. Continuous hydrogen production by immobilized enterobacter cloacae dm11 using cane molasses as feedstock. Proceedings Int. Hydrogen Energy Congress and Exhibition IHEC 2005. 2005;1-7.

[24] Wang X, Monis PT, Saint CP, Jin B. Biochemical kinetics of fermentative hydrogen production by Clostridium butyricum W5. Int J of Hydrogen Energy 2009;34:791-798.

[25] Kadir MS, Jahim J, Anuar N. Biohydrogen production by local isolate of Clostridium butyricum: Initial nutrient optimization study. Pertanika 2009;12:381-88.

[26] Miller GL. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Analytical Chemical 1959;31:426-427.

[27] Fournier`E. Current Protocols In Food Analytical Chemistry (John Wiley & Sons,2001).

[28] APHA. Standard methods for the examination of water and wastewater, 19th ed., Washington DC, USA: American Public Health Association 1998.

[29] Ren NQ, Cao GL, Guo WQ, Wang AJ, Zhu YH, Liu BF, Xu JF. Biological hydrogen production from corn stover by moderately thermophile thermoanaerobacterium Thermosaccharolyticum W16. Int J Of Hydrogen Energy 2010; 35 (7): 2708-2712.

[30] Taherzadeh MJ, Karimi K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A Review. Int J of Molecular Sciences 2008;9:1621-51.

[31] Lo YC, Huang LF, Cheng CL, Chen J, Chang JS. Biohydrogen production from cellulosic hydrolysate produced via temperature-shift-enhanced bacterial cellulose hydrolysis. Bioresource Technology 2009; 100: 5802-07

[32] Mansor MF, Jahim, J, Rahman RA. Combination effect of anaerobic sludge and palm oil mill effluent (POME) pretreatment on hydrogen production. Proceedings of Int Conference on Advances in Renewable Energy Technologies ICARET 2010. 2010.

[33] Yusuff, M.Z.M., Hassan, M.A., Aziz, S., Rahman, N.A. Start-up of biohydrogen palm oil mill effluent under non-sterile condition in 50L contineous stir tank reactor. Int of Agricultural Research 2009;4:163-69.

[34] O-Thong S, Prasertsan P, Intrasungkha N, Dhamwichukorn S, Birkeland NK. Optimization of simultaneous thermophilic fermentativehydrogen production and COD reduction from palm oil mill effluent by Thermoanaerobacterium-rich sludge. Int J of Hydrogen Energy 2008; 33:1221-31.

[35] Badiei M, Jahim J, Anuar N, and Abdullah SRS. Int J of Hydrogen Energy 2011;36: 5912-19