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Biohydrogen Production from Palm Oil Mill Effluent Pretreated by Chemical Methods Using *Thermoanaerobacterium*-Rich Sludge

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Abstract: The effect of alkaline and acid pretreatment on solubilization of solid organic matter in palm oil mill effluent (POME) and hydrogen production by *Thermoanaerobacterium*-rich sludge was studied. Organic matter solubilization of alkaline and acid pretreatments were increased up to 28 and 15.7% of initial soluble COD in POME, respectively. That corresponds to carbohydrate solubilization of 41 and 32% of initial soluble carbohydrate in POME, respectively. A maximum hydrogen production yield of 4.6 l H_2/l-POME was achieved from POME pretreated with 1.5% w/v NaOH, which was 3-fold greater than raw POME and 1-fold greater than POME pretreated with 1.5% w/v HCl. Optimum conditions for biohydrogen production from alkaline pretreated POME using *Thermoanaerobacterium*-rich sludge was found at initial pH 5.5 and temperature of 60°C, which gives a maximum hydrogen production yield of 5.2 l H_2/l-POME and increased 51% as compared to raw POME. The biogas was mainly composed of hydrogen and carbon dioxide with the percentage of hydrogen ranging from 55-60% of biogas and free of methane. During the conversion of alkaline pretreated POME into hydrogen, the acetic and butyric acids were main by-products in the metabolism. The results showed that alkaline pretreatment is an effective pretreatment methods for enhancing the hydrogen production yield from POME. The use of *Thermoanaerobacterium*-rich sludge is promising for future engineering practice of biohydrogen production from alkaline pretreated POME.

Key words: *Thermoanaerobacterium*-rich sludge · Palm oil mill effluent · Biohydrogen · Thermophilic condition · Alkaline pretreatment

INTRODUCTION

Biohydrogen is a promising clean fuel as it is ultimately derived from renewable energy sources. It is efficient and environmental friendly since its combustion converts to water, gives high energy yield with less energy intensive processes [1, 2]. One possible approach for biohydrogen production is to convert negative valued organic wastes into hydrogen gas by anaerobic microbial flora [3]. Disposal of agricultural and industrial wastes and residues are economic burden on communities and industries. Therefore, hydrogen production by dark fermentation of wastes can both reduce waste disposal problem and decrease raw material cost [4, 5]. Besides the major advantages of dark fermentative process are high hydrogen production capacities, operation without light sources and no oxygen limitation problems. These characteristics make it more competitive than other biological conversion of organic wastes into hydrogen gas [6]. Dark fermentative hydrogen production gives relatively high theoretical values of hydrogen production. Theoretically, four moles of hydrogen are produced from glucose concomitantly with 2 moles of acetate and only 2 moles of hydrogen are produced when butyrate is the

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main fermentation product. Typically, 60-70% of the aqueous product during sugar fermentation is butyrate [7]. Among a large number of microbial species, strictly anaerobes and facultative anaerobic, such as *Clostridia, Thermoanaerobacterium* and *Enterobactaceae*, are efficient producers of hydrogen via fermentation process [8-11]. From the engineering point of view, a process using mixed cultures gives a stable yield of hydrogen production from non-sterile organic wastes [12]. The advantages of using mixed cultures over pure culture are lower cost (saving in sterilization cost) and the potential of using septic organic wastes as substrate. The advantage of using mixed cultures for biohydrogen production is that high adaptive capacity owing to the microbial diversity, the capacity to use a mixture of substrate and the possibility of obtaining a stable and continuous process.

The most common bacteria in mixed cultures used to produce hydrogen are *Clostridium* [13] and *Thermoanaerobacterium* [14]. To date, the majority of research has been focused mainly on using organic wastes and wastewater to produce hydrogen with mixed microflora. Whereas the conversion of biomass containing cellulose and hemicellulose, such as palm oil mill effluent (POME) is lacking because of their complex chemicals structures [15]. Characterization of POME is high suspended component, low nutrient content, high organic content (80 g COD/l) and low pH. The fermentation processes to produce hydrogen from POME by anaerobic microflora is possible. Anaerobic microorganisms from palm oil mill wastewater treatment plants have been utilized to generate hydrogen from glucose in a batch cultivation test [16]. Mixed microflora from cow dung was used to produce hydrogen from POME using anaerobic contact filter system under mesophilic condition that can achieve both hydrogen production (0.42 l H_2/g COD destroyed) and COD reduction (40%) [17]. But, only trace amount of hydrogen yield and COD removal efficiency were obtained from previous reports because the fermentative hydrogen production is affected by many parameters such as pH, temperature, the nature of the microbial communities and complexity of POME which contains most of insoluble organic matter. The effect of pH is known to be crucial due to its effects on hydrogenase activity, metabolism pathways and microbial communities [18].

This work was conducted initially to investigate effect of alkaline and acid pretreatment on solubilization of palm oil mill effluent (POME) and fermentative hydrogen production. Furthermore, the effect of initial pH and temperature on hydrogen production, together with their corresponding degradation efficiencies for total carbohydrate and soluble metabolites were investigated. The microbial community of hydrogen producing-sludge was analyzed by analyzed by denaturing gradient gel electrophoresis (DGGE) techniques.

MATERIALS AND METHODS

***Thermoanaerobacterium*-Rich Sludge:** The seed microflora for hydrogen production was obtained by treating anaerobic sludge at short hydraulic retention time (24 h), pH 5.5 and thermophilic condition (60 °C) to remove methanogenic bioactivity. Anaerobic sludge was collected from palm oil mill wastewater treatment plant. The sludge was settled and collected after decanting supernatant and then enriched with synthetic medium consisting of sucrose containing 20 g COD/l [19]. The enriched sludge having a volatile suspended solids (VSS) concentration of 7.0 g/l was acclimatized with 10, 30, 60 and 100% of POME. The microbial diversity of enriched sludge was analyzed by denaturing gradient gel electrophoresis (DGGE) and found to be dominated by *Thermoanaerobacterium* spp. The sludge was employed to investigate the effects of environmental factors on hydrogen production yield under thermophilic conditions.

Chemical Pretreatment of Substrate: Raw POME was collected from the receiving tank of Trang Palm Oil Co, Ltd. in southern Thailand. The characteristics of POME were brown color, pH 4.2, a temperature of 70°C, chemical oxygen demand (COD) 75.2 g/l, total carbohydrate 16.3 g/l, total nitrogen 0.83 g/l, total phosphorus 97 mg/l, oil 8.3 g/l, total solids 35 g/l, suspended solids (SS) 12 g/l and a water content of 86-90%. It was kept at 0-4°C until used. It was pretreated with HCl and NaOH according to tested concentration (0-2.5% w/v) by mixing for 30 min and then adopting pH as the investigating parameter with either 1 M NaOH or 1M HCl solution. The characteristics of raw POME and pretreated POME are shown in Table 1.

Experimental Procedure: The experiment was performed in 1.2 l serum bottles containing 650 ml of POME, 50 ml of inoculum and 50 ml of nutrient solution [18]. The batch reactors were incubated at temperature ranging from 30-80 °C and the ranging of initial pH values from 4.5-6.5. The initial pH values of the batch reactors were adjusted using either 1 M sodium hydroxide (NaOH) or 1 M hydrochloric acid (HCl). The evolved gas was collected with a gas collecting bag (Cali-5-bond, Calibrated

Table 1: Characteristics of raw POME, NaOH treated POME and HCl treated POME

Characteristics	Raw POME	NaOH treated POME	HCl treated POME
Color	Brown	Dark brown	yellow
pH	4.2	5.4	4.1
BOD (g/L)	22.0	54.3	45.5
Soluble COD (g/L)	75.2	96.3	87
Total carbohydrate (g/L)	16.3	23	21.5
Total nitrogen g/L	0.83	0.92	0.87
Ammonium-nitrogen (mg/L)	25	30	28
Phosphate	14.7	21	20
Oil (g/L)	8.3	5.2	7.6
Total solids (g/L)	35.0	36.5	35.5
Suspended solids (g/L)	12.0	8.5	9.2

Instruments, Inc.) and measured by water displacement method [20]. The initial anaerobic condition in the reactor was established by replacing the gaseous phase with nitrogen. Fed batch operations were conducted after 48 hours. 350 ml of reaction medium was removed and 350 ml of fresh POME was added to the reactor every 24 hours. The amounts of evolved gas, soluble metabolites and responsible microbial community were investigated.

Analytical Methods: The biogas composition was measured by gas chromatography equipped with thermal conductivity detectors (TCD). Hydrogen gas was analyzed by GC-TCD fitted with an 1.5 m stainless steel column SS350A packed with a molecular sieve (80/100 mesh). Nitrogen was used as a carrier gas at a flow rate of 30 ml/min. The temperatures of the injection port, oven and detector were 100, 50 and 100°C, respectively [16]. Methane and carbon dioxide were analyzed by GC-TCD fitted with 3.3 ft stainless steel column packed with Porapak T (60/80 mesh). Helium was used as a carrier gas at a flow rate of 35 ml/min. The temperatures of the injection port, oven and detector were at 150, 50 and 100°C, respectively [21]. The gas sample of 100 µl was injected in duplicate. Volatile fatty acids (VFA) were analyzed by gas chromatography (Hewlett Packard, HP 6850 series) equipped with a flame ionization detector (FID). A column capillary packed with nitroterephthalic acid-modified polyethleneglycol (DB-FFAP) and with a length of 30 meter was used. The temperature of the injection port was 250°C. The chromatography was performed using the following program: 100°C for 5 min, 100-250ºC with a ramping of 10°C/min, 250°C for 12 min. The detector temperature was 300°C. Chemical oxygen demand (COD), pH and suspended solid (SS) were determined in accordance with the procedures described in the Standard Methods [22]. The total carbohydrate content was analyzed by the anthrone method [23].

Microbial Community Analysis: Microbial community analysis, total genomic DNA was extracted from samples collected from batch experiments using the Ultraclean Soil DNA Kit (MoBio Laboratory Inc., USA). The region of the 16S rRNA genes corresponding to the positions 340 to 518 in the 16S rRNA of *Escherichia coli* was PCR-amplified using the forward primer; L340GCf (5'-CCTACGGGAGGCAGCAG-3') with a GC clamp at the 5' end and the reverse primer; K517r (5'-ATTACCGCGGCTGCTGG-3') [24]. The DGGE analysis of the PCR products was performed by electrophoresis for 20 min at 20 V and 15 hours at 70 V through a 7.5% polyacrylamide gel containing a linear gradient of denaturant (100% denaturant corresponds to 7 M urea and 40% (v/v) formamide deionized with AG501-X8 mixed bed resin) ranging from 30 to 60% in 0.5×TAE buffer at a constant temperature of 60ºC. (DGGE unit, V20-HCDC, Scie-Plas Limited, UK).The gel was stained with Sybr-Gold (1000x concentration) for 1 hour and visualized on a UV transilluminator. Most of the bands were excised from the gel and re-amplified with the forward primer without a GC clamp and the reverse primer. After re-amplification, PCR products were purified using E.Z.N.A cycle pure kit (Omega Bio-tek, USA) and sequenced using primer K517r and an ABI PRISM Big Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, USA) in accordance with the manufacturer's instructions. Closest matches for partial 16S rRNA gene sequences were identified by database searches in GenBank using BLAST [25].

RESULTS AND DISCUSSION

Effect of Pretreatment of Substrate on Hydrogen Yield: POME was high suspended solids (12 g/l) wastewater which contains large particle size organic matter and also insoluble. Therefore, pretreatment is necessary in order to enhance the solubilization of POME. The effect of alkaline and acid pretreatment on solubilization of palm oil mill effluent (POME) and fermentative hydrogen production was studied. COD solubilization of alkaline and acid pretreatments were increased up to 28 and 15.7% of initial COD in POME, respectively corresponding to total carbohydrate solubilization of 41 and 32% of initial carbohydrate in POME, respectively (Table 1). Figure 1 depicts the effects of pretreatment by various NaOH and Hcl concentration on cumulative hydrogen production.

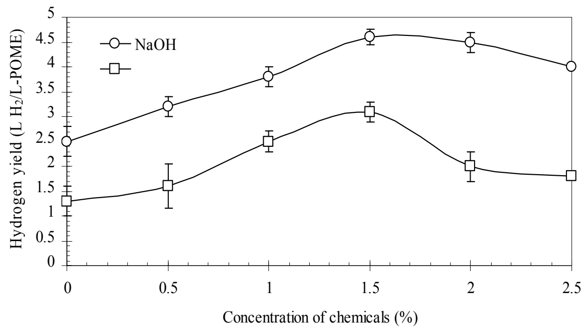

Fig. 1: Effect of NaOH and HCl concentration in POME pretreatment on hydrogen production yield.

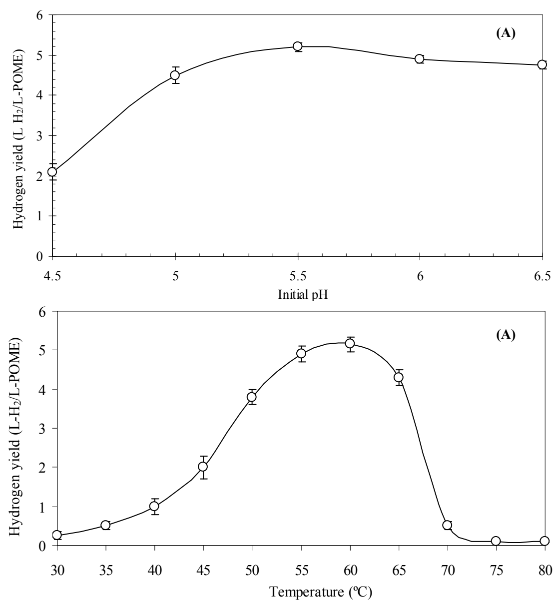

Fig. 2: The effect of initial pH value on hydrogen yield (A) and effect of temperature on hydrogen yield (B).

POME pretreated with alkaline gave the higher hydrogen production yield than POME pretreated with acid. Maximum hydrogen production yield from POME pretreated with alkaline and acid were 4.6 and 2.56 l H_2/l-POME. A maximum hydrogen production of 4.6 l H_2/l-POME was achieved from POME pretreated 1.5% w/v NaOH, which was 3-fold greater than raw POME and 1-fold greater than POME pretreated with 1.5% w/v HCl. The hydrogen production yield gradually increased when the concentration of NaOH was increased until 1.5%w/v and decreased when the concentration of NaOH was increased higher than 1.5%w/v. Vrije *et al*. [26] also reported that base pretreatment shown high solubilization of solids from *Miscanthus* and high hydrogen production yield. Although the high concentrations of NaOH were favored the hydrolysis of POME, but at concentrations higher than 1.5% w/v gradually inhibited hydrogen production. The cumulative hydrogen yield of HCl pretreatment was lower than NaOH pretreatment. Low hydrogen yield in HCl pretreatment may cause from the effect of Cl^- anion, high concentration of Cl^- anion heavily inhibited the hydrogen production [15]. Pretreated POME with NaOH was suitable to use as substrate for hydrogen production by *Thermoanaerobacterium*-rich sludge.

Effect of Initial Ph and Temperature on Hydrogen Yield: The effect of initial pH and temperature on hydrogen production from alkaline pretreatment, together with their corresponding degradation efficiencies for total carbohydrate and soluble metabolites were investigated. Optimum conditions for biohydrogen production from alkaline pretreated POME using *Thermoanaerobacterium*-rich sludge was found to be at initial pH 5.5, temperature of 60°C, which gives a maximum H_2 yield of 5.2 l H_2/l-POME and increased 51% as compared to raw POME. The biogas was mainly composed of H_2 and CO_2 with the percentage of H_2 ranging from 55-60% of biogas and free of CH_4. During the conversion of POME into hydrogen, the acetic acid and butyric acid were main by-products in the metabolism of hydrogen fermentation. The changes of hydrogen production yield over the pH range of 4.5-6.5 were plotted as shown in Figure 2. As shown in Figure 2, while the initial pH level increased from 4.5 to 5.5, the cumulative hydrogen yield increased from 2.1 to 5.2 l H_2/l-POME and slightly decreased in the initial pH range of 6.0-6.5. The initial pH value of 5.5 was represented the optimum point of pH for hydrogen production from alkaline pretreated POME. Hydrogen production yield was still high in the pH range of 6.0-6.5 and this range of pH may promote growth of methanogens thus not selected as optimum pH for hydrogen production. The results implied that a control pH at 5.5 could stimulate *Thermoanaerobacterium* bacteria to produce hydrogen and would enhance the system ability to have a maximum hydrogen yield. The adjustment initial pH of substrate to 5.5 can save the cost of chemicals than adjust to 6.0-6.5 and enhance hydrogen production ability [27]. This is because the activity of hydrogenase was inhibited by low pH or high pH in the overall hydrogen fermentation. Fang *et al*. [18] reported that the biogas was free from methane at pH 5.5 or lower, due to the suppression of methanogenic activity under acidic condition, but considerable quantities of methane were produced with further increase in pH.

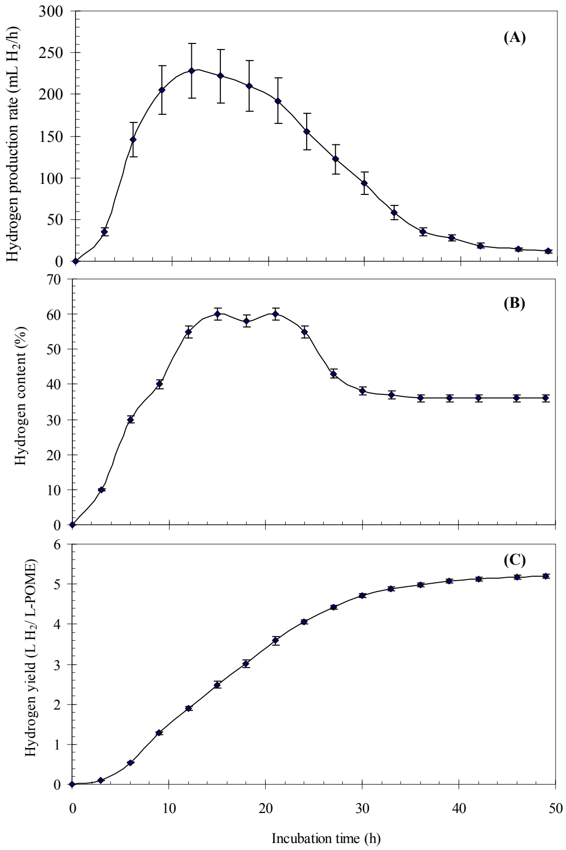

Fig. 3: Development of hydrogen production rate (A), hydrogen content in biogas (B) and cumulative hydrogen (C) in the fed-batch hydrogen production from alkaline pretreated POME by Thermoanaerobacterium-rich sludge under optimum condition.

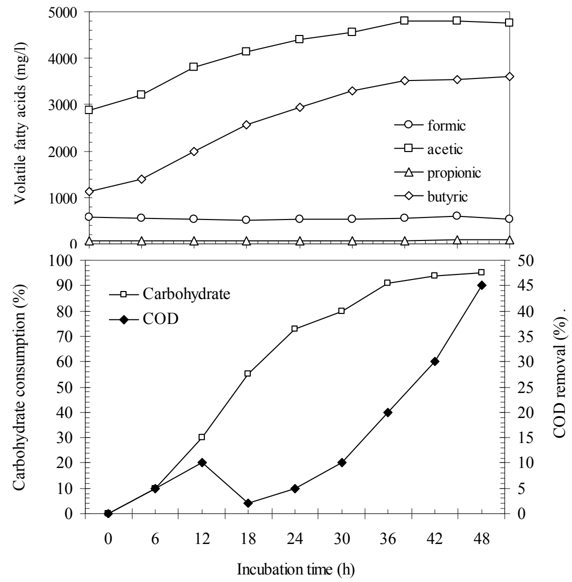

Fig. 4: Development of VFAs, carbohydrate consumption and COD removal in fed-batch hydrogen production from alkaline pretreated POME by Thermoanaerobacterium-rich sludge under optimum condition.

The effect of temperature on hydrogen production by *Thermoanaerobacterium*-rich sludge was tested at a temperature ranged of 30-80°C and fixed pH at 5.5 (Figure 2). The cumulative hydrogen yield sharply decreased when elevated temperature ranged from 65-80°C. Optimum temperature for biohydrogen production from alkaline pretreated POME using *Thermoanaerobacterium*-rich sludge was found at 60°C, which gives a maximum hydrogen production yield of 5.2 l H_2/l-POME and increased 51% as compared with raw POME. The hydrogen yield observed in this study was substantially higher than that reported by other researchers using acidogenesis condition [16, 28]. Figure 3 illustrated the development of hydrogen production rate, hydrogen volume and hydrogen content in biogas during the conversion of alkaline pretreated POME into hydrogen by *Thermoanaerobacterium*-rich sludge under optimum conditions. As shown in Figure 3, the hydrogen evolution started to occur after 3 h of cultivation. The maximum hydrogen production rate of 228 ml H_2/h was observed at 12 h of cultivation and the maximum hydrogen yield of 5.2 l H_2/l-POME was obtained at the end of cultivation. The hydrogen percentage in biogas of 55-60% was observed during 12-24 h of cultivation. The biogas was mainly composed of H_2 and CO_2 with the percentage of H_2 raging from 55-60% of biogas and free of CH_4. This might be due to the deactivation of methonogens by a low pH (5.5) and high temperature (60°C) [9,28]. Carbohydrate consumption efficiency of 95% was observed from the end of cultivation. COD removal efficiency of 45% was also observed from the end of cultivation. In general, COD removal during fermentative hydrogen production is 20-30% that has been reported from POME [10]. Alkaline pretreatment enhanced COD removal up to 45%. However, the COD value of the effluent was still high (53 gCOD/l) and sub sequent treatment is needed, such as anaerobic treatment for methane production in a two-stage process, stabilization ponds, etc., before discharging to the environment. Hydrogen production from alkaline pretreated POME was terminated by low pH, final pH of 4.0 was observed due to the hydrogen production accompanied with the formation volatile fatty acids (Figure 4). The concentration of butyric acid and acetic

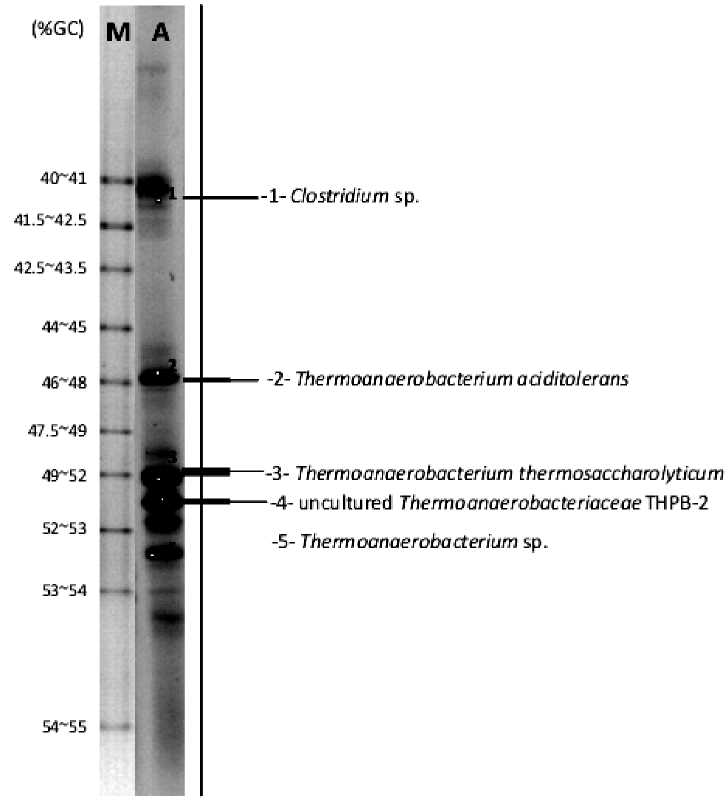

Fig. 5: DGGE profile of 16S rRNA genes fragments. The fragments were PCR-amplified from total DNA extracted from *Thermoanaerobacterium*-rich sludge culture with alkaline pretreated POME (M, DGGE marker and A, bacteria community).

acid achieved the maximum value of 3600 and 4750 mg/l, respectively. The biodegradation of alkaline pretreated POME was similar to that of general carbohydrate, in which the VFAs mainly consists of acetic acid and butyric acid [8]. These phenomena were expected because hydrogen production appears to be usually accompanied with the formation of volatile fatty acids and alcohols.

Microbial Community Composition: The DGGE profile shown that *Thermoanaerobacterium* sp. was identified as the most dominant organism, sharing 97% 16S rRNA sequence identity with *Thermoanaerobacterium thermosaccharolyticum* (Figure 5). According to the intensity of this band, this bacterium has a higher population density under optimal cultivation on alkaline pretreated POME. This indicates that the optimal conditions favor the growth of this organism and that it might play an important role in the conversion of the carbohydrate- and fat-rich substrate to hydrogen. Optimum temperature of *Thermoanaerobacterium*-rich sludge was found to be in the range of 55-65°C. Several species of *Thermoanaerobacterium* are known for their hydrogen production characteristics, including *Thermoanaerobacterium thermosaccharolyticum*, *Thermoanaerobacterium polysaccharolyticum*, *Thermoanaerobacterium zeae*, *Thermoanaerobacterium lactoethylicum* and *Thermoanaerobacterium aotearoense*. All these organisms have optimal growth conditions at 55-70°C and at pH 5.2–7.8 [5, 29]. The use of *Thermoanaerobacterium*-rich sludge is promising for future engineering practice of biohydrogen production from alkaline pretreated POME.

CONCLUSIONS

The environmental factors tested in this study, pretreatment of POME, pH and temperature are influenced in the conversion of POME to hydrogen by *Thermoanaerobacterium* species. The alkaline pretreatment of the POME enhanced hydrogen yield and biodegrading properties by *Thermoanaerobacterium*-rich sludge. The maximum hydrogen yield of 5.2 l H_2/l-POME was achieved at the pH 5.5, temperature of 60°C and base pretreatment. This value was about 50% higher than cultivation in raw POME. The hydrogen content in biogas was in the range of 55-60% and no methane was observed. The results shown that alkaline pretreatment is an effective methods for enhancing the hydrogen yield from POME. The use of *Thermoanaerobacterium*-rich sludge is promising for future engineering practice of biohydrogen production.

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REFERENCES

1. Nielsen, A.T., H. Amandusson, R. Bjorklund, H. Dannetum, J. Ejlertsson, L.G. Ekedahl, I. Lundstrom and B.H. Svensson, 2001. Hydrogen production from organic waste. International Journal of Hydrogen Energy, 26: 547-550.
2. Das, D. and T.N. Veziroglu, 2001. Hydrogen production by biological processes: a survey of literature. International Journal of Hydrogen Energy, 26: 13-28.
3. Montgomery, R., 2004. Development of biobased products. Bioresource Technology, 91: 1-29. Bioresource Technology
4. Logan, B.E., S.E. Oh, I.S. Kim and S. Van Ginkel, 2002. Biological hydrogen production measured in batch anaerobic respirometers. Environmental Science & Technology, 36: 2530-2535.
5. Zhang, T., H. Liu and H.H.P. Fang, 2003. Biohydrogen production from starch in wastewater under thermophilic condition. Journal of Environmental Management, 69: 149-156.
6. Hawkes, F.R., R. Dinsdale, D.L. Hawkes and I. Hussy, 2002. Sustainable fermentative hydrogen production: challenges for process optimization. International Journal of Hydrogen Energy, 27: 1339-1347.
7. Liu, W.T., O.C. Chan and H.H.P. Fang, 2002. Microbial community dynamics during start-up of acidogenic anaerobic reactors. Water Research, 36: 3203-3210.
8. Ueno, Y., S. Haruta and Y. Igarashi, 2001. Microbial community in anaerobic hydrogen producing microflora enriched from sludge compost. Applied Microbiology and Biotechnology, 57: 555-562.
9. Shin, H.S. and J.H. Youn, 2005. Conversion of food waste into hydrogen by thermophilic acidogenesis. Biodegradation, 16: 33-44.
10. O-Thong, S., P. Prasertsan, N. Intrasungkha, S. Dhamwichukorn and N.K. Birkeland, 2007. Improvement of biohydrogen production and pollution reduction from palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. Enzyme and Microbial Technology, 41: 583-590.
11. Rachman, M.A., Y. Liasari, M.M. Nasef, H. Saidi, Z. Salam and A. Ahmad, 2011. In situ continuous production of hydrogen gas from molasses using mutated Enterobacter aerogenes ADH43 for fuel cell application. Iranica Journal of Energy and Environment, 2(4): 291-296.
12. Noike, T. and O. Mizuno, 2000. Hydrogen fermentation of organic municipal wastes. Water Science and Technology, 42: 155-162.
13. Shin, H.S., J.H. Youn and S.H. Kim, 2004. Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. International Journal of Hydrogen Energy, 29: 1355-1363.
14. O-Thong, S., P. Prasertsan and N.K. Birkeland, 2009. Evaluation of methods for preparing thermophilic hydrogen producing seeds from anaerobic digested sludge with microbial communities aspects. Bioresource Technology, 100: 909-918.
15. Fan, Y.T., G.S. Zhang, X.Y. Guo, X. Xing and M.H. Fan, 2006. Biohydrogen production from beer lees biomass by cow dung compost. Biomass Bioenergy, 30: 493-496.
16. Morimoto, M., M. Atsuko, A.A.Y. Atif, M.A. Ngan, A.F. I-Razi, S.E. Iyuke and A.M. BaKir, 2004. Biological production of hydrogen from glucose by natural anaerobic microflora. International Journal of Hydrogen Energy, 29: 709-713.
17. Vijayarahavan, K. and D. Ahmad, 2006. Biohydrogen generation from palm oil mill effluent using anaerobic contact filter. International Journal of Hydrogen Energy, 31: 1284-1291.
18. Fan, Y.T., C.L. Li, J.J. Lay, H.W. Hou and G.S. Zhang, 2004. Optimization of initial pH substrate and pH levels for germination of sporing hydrogen producing anaerobes in cow dung compost. Bioresource Technology, 91: 189-193.
19. Lay, J.J., H.S. Fan, J.I. Hwang, J.I. Chang and P.C. Hsu, 2005. Factors affecting hydrogen production from food wastes by Clostridium-rich composts. Journal of Environmental Engineering, 131: 598-602.
20. Owen, W.F., D.C. Struckey, J.B. Healy and P.L. Mccarty, 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Research, 13: 485-492.

21. Chang, F.Y. and C.Y. Lin, 2004. Biohydrogen production using an up-flow anaerobic sludge blanket reactor. International Journal of Hydrogen Energy, 29: 33-39.
22. APHA. 1998. Standard Methods for Examination of Water and Wastewater, 19th ed. American Public Health Association, USA: Washington DC.
23. Morris, D.L., 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science, 107: 254-255.
24. Muyzer, G., E.C. DeWaal and A.G. Uitterlinden, 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, 59: 695-700.
25. Altschul, S.F., W. Gish, W. Miller, E. Myers and D.J. Lipman, 1990. Basic local alignment search tool. Journal of Molecular Biology, 215: 403-410.
26. Vrije, T.D., G.G. Hass, G.B. Tan, E.R.P. Keijsers and P.A.M. Claassen, 2002. Pretreatment of *Miscanthus* for hydrogen production by *Thermotoga elfii*. International Journal of Hydrogen Energy, 27: 1381-1390.
27. Fang, H.H.P., T. Zhang and H. Liu, 2002. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresource Technology, 82: 87-89.
28. Fakhru'l-Razi, A., A.A.A. Yassin, S.E. Lyuke, M.A. Ngan and M. Morimoto, 2005. Bio-hydrogen synthesis from wastewater by anaerobic fermentation using microflora. International Journal of Green Energy, 2: 387-396.
29. Liu, H., T. Zhang and H.H.P. Fang, 2003. Thermophilic hydrogen production from a cellulose containing wastewater. Biotechnology Letters, 25: 365-369.

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چکیده

تاثیر پیش تیمار قلیایی و اسیدی بر انحلال پذیری مواد آلی جامد در پساب کارخانه روغن نخل (POME) و تولید هیدروژن توسط لجن حاوی ترموانروباکتریوم (*Thermoanaerobacterium*) بررسی شد. پیش تیمار قلیایی و اسیدی به ترتیب موجب افزایش حلالیت مواد آلی به ۲۸ و ۱۵/۷ درصد COD اولیه محلول در POME گردید. به همین ترتیب انحلال پذیری کربوهیدرات به ۴۱ و ۳۲ درصد کربوهیدرات قابل حل اولیه افزایش یافت. بازده حداکثر تولید هیدروژن حجمی ۴/۶ H_2/POME برای POME پیش تیمار شده با ۱/۵٪ w/v NaOH به میزان ۳ برابر بزرگتر از POME تیمار نشده و ۱ برابر بزگتر از POME پیش تیمار شده با ۱/۵٪ w/v HCl بود. شرایط عملیاتی بهینه برای تولید بیوهیدروژن از POME پیش تیمار شده قلیایی توسط لجن حاوی *Thermoanaerobacterium* در pH ۵/۵ و دمای ۶۰°C به دست آمد، بازده حداکثر تولید هیدروژن حجمی ۵/۲ H_2/ POME حاصل شد که ۵۱ درصد نسبت به POME تیمار نشده افزایش داشته است. بیوگاز به طور کلی مرکب از هیدروژن و دی اکسید کربن با ۶۰-۵۵ درصد هیدروژن و عاری از متان بود. در فرآیند تولید هیدروژن از POME پیش تیمار شده قلیایی، استیک و بوتیریک اسید محصول جانبی عمده متابولیزم بودند. نتایج بدست آمده نشان داد که پیش تیمار قلیایی روش موثری برای افزایش بازده تولید هیدروژن از POME می باشد. استفاده از لجن حاوی *Thermoanaerobacterium* برای اجرای مهندسی آتی طرح در تولید عیدروژن از POME پیش تیمار شده قلیای نوید بخش می باشد.