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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (16): 1127-1133, 2009
ISSN 1028-8880

Pretreatment Evaluation and its Application on Palm Oil Mill Effluent for Bio-Hydrogen Enhancement and Methanogenic Activity Repression

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Abstract: Pretreatment evaluations of biological sludge were performed to observe the enhancement of hydrogen production and repression of methanogenic activity using anaerobic sludge and sucrose as substrate. The treatments include heating (H), ozonation (O), drug (D) application using fluvastatin, Na_2SO_4(S) dosing and their combinations to make up for the total of 9 pretreatment methods. Heat treatment at 95°C for 45 min provided a complete methanogen repression and good enhancement of hydrogen production activity. The remaining pretreatments were imperfect either for repression or enhancement based on the application conditions and concentration ranges. The order of methane repression was: H>HO>HD>HS>O>D>OD>OS>S while hydrogen production was: HO>H>HS>OD>HD>OS>D>O>S. Heat treatment at the specified condition was considered sufficient and suitable for pretreatment of anaerobic sludge. The practical application was explored using Palm Oil Mill Effluent (POME) containing different amounts of solid content (15.830 g VSS L^{-1}, namely LPOME and 21.445 g VSS L^{-1}, namely HPOME) as substrate. Beside the difference in COD of nearly 16% which is higher for HPOME, a higher specific hydrogen production rate was obtained at 0.81 and 0.17 mL H_2 g^{-1} COD h for LPOME and HPOME, respectively. It should also be noted that a prolonged lag-time during start-up was observed for HPOME as well as hydrogen suppression which may be associated with the solid content mainly lipids in wastewater. Thus, high solid content of wastewater may be of concerned for bio-hydrogen production.

Key words: Mixed culture, pretreatment, palm oil mill effluent, hydrogen producing bacteria

INTRODUCTION

Hydrogen gas has been deemed the fuel of the future and it is believed that hydrogen energy would be less pollution than a fossil fuel. The combustion of hydrogen produces only water vapor without CO, CO_2, hydrocarbons or fine particles. Moreover, hydrogen can be produced without causing any environmental problems. At present, hydrogen gas is mainly produced from natural gas reforming. Alternative route of hydrogen productions that are cost effective and pollution free are still demanded (Das and Iu, 2001). There are many methods which can generate hydrogen, such as, water electrolysis, thermo-chemical processing, photo-chemical processing, photo-catalytic processing and photo-electro-chemical processing. The two methods for hydrogen production from microorganisms are photo and dark hydrogen fermentations. The photo-fermentation produces hydrogen by photosynthetic microorganisms, such as algae and photosynthetic bacteria. The dark hydrogen fermentation is carried out by fermentative hydrogen-producing microorganisms, such as facultative anaerobes and obligate anaerobes. The most promising and environmentally friendly method seems to be dark fermentation from organic wastes as it combines the hydrogen generation with waste treatment. The hydrogen fermentative process has the advantage of high bio-hydrogen production rate (Van Ginkel *et al*., 2005). Dark hydrogen fermentation can use wastewater (Mohan *et al*., 2008; Zhu *et al*., 2002; Van Ginkel *et al*., 2005; Atif *et al*., 2005) or solid waste (Chen *et al*., 2006; Fan *et al*., 2006; Kapdan and Kargi, 2006) as substrate by mixed cultures.

Hydrogen can be produced from pure cultures and mixed cultures. In mixed culture, bacteria contain various types of microbes, including hydrogen-producing bacteria and hydrogen-consuming bacteria (especially methanogen). Many pretreatments to inhibit methanogen have been investigated including heat treatment (Zhu and Beland, 2006; Baghchehsaraee *et al*., 2008), acid treatment

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(Zhu and Beland, 2006; Ren *et al*., 2008; Chen *et al*., 2002), alkaline treatment (Mohan *et al*., 2008; Cai *et al*., 2004; Lin and Chen, 2006), aeration (Zhu and Beland, 2006; Baghchehsaraee *et al*., 2008), 2-bromoethane-sulfonate acid (BESA) (Zhu and Beland, 2006; Baghchehsaraee *et al*., 2008), freezing and thawing (Ting and Lee, 2007). Heat-shock treatment has been widely used. The heat pretreatment method reported in the literature varies in a wide range of temperature from 65 to 121°C and exposure times ranging between 15 to 120 min. Hydrogen yield was improved to 3.18 mol H_2 mol^{-1} sucrose by boiling the sludge for 20 min. Successful preparation of hydrogen producing seeds by acid treatment has been reported by a number of researchers (Mohan *et al*., 2008; Ting and Lee, 2007). Anaerobic sludge was treated with perchloric acid ($HClO_4$) and orthophosphoric acid (OPA) at pH of 3 for 10 min and 24 h, respectively. In the subsequent second-step batch, the seed sludge prepared from base treatment exhibited the highest hydrogen production of 6.12 mol H_2 mol^{-1} sucrose. Successful preparation of hydrogen producing seeds by BESA treatment (0.0317 mmol H_2 g^{-1} COD) has been reported by Mohan *et al*. (2008).

Although hydrogen can be efficiently produced from simple sugar in small-scale laboratory experiments, application of this technology in organic waste or wastewater is a major challenge (Ren *et al*., 2006; Mu *et al*., 2007). One difficulty is that these waste or wastewater contains complex substances composition, e.g., insoluble organic substances, oil and fat. It is hard to convert directly real waste and wastewater into bio-hydrogen gas by microbe anaerobic fermentation. Some researchers indicated that methods for seed and wastewater preparation can affect both the start-up and overall efficiency of the hydrogen-producing reactor (Hawkes *et al*., 2002; Mohan *et al*., 2008). Therefore, the preparation of organic waste or wastewater before fermentation might be one way to improve the yield of hydrogen from substrates.

The objective of this study was to enrich hydrogen producing mixed bacteria from anaerobic sludge with different pretreatment methods. The bio-hydrogen evolution rate and repression efficiency of the methanogenic activity were compared utilizing synthetic wastewater as a main substrate. Additionally, the practical application was explored using Palm Oil Mill Effluent (POME) containing various degrees of suspended solid contents as substrate.

MATERIALS AND METHODS

Seed sludge: Anaerobic sludge was obtained from the bottom portion of upper anaerobic sludge blanket reactor (UASB) treating brewery wastewater. The pH, Volatile Suspended Solids (VSS) and Total Suspended Solids (TSS) concentrations of the anaerobic sludge were 7.11, 7,750 and 14,298 mg L^{-1}, respectively.

Table 1: Characteristics of POME used for the experiment

Parameters	LPOME[a]	MPOME[b]
pH	4.87	4.1
Chemical oxygen demand (COD) (mg L^{-1})	35,340	42,000
Total suspended solids (mg L^{-1})	18,007	23,610
Colatile suspended solids (mg L^{-1})	15,830	21,445

[a]Palm oil effluent with low solid content (LPOME); [b]Palm oil effluent with high solid content (HPOME)

Wastewater compositions: Sucrose was used as a main substrate for the pretreatment evaluations in batch experiments. Sucrose solution was prepared by dissolving 20 g of sucrose in 1 L of distilled water. The POME was obtained at final discharge point prior to any treatment from the palm oil processing industry. The main characteristic properties of the POME used are given in Table 1. It is important to mention that the physico-chemical properties of POME highly depend on local and seasonal factors. The obtained POME was slightly acidic with pH values around 4.1-4.9. It possessed a significant amount of COD of 35-42 g L^{-1}, total suspended solids of 18-23 g L^{-1} and volatile suspended solids of 15-21 g L^{-1}. The dark color of POME corresponds with the high amount of solids. These solids are known to consist of hardly biodegradable substances. The POME was preserved at a temperature of less than 4°C in order to prevent the wastewater from undergoing biodegradation due to microbial action. The substrate contained sufficient inorganics for bacterial growth. The nutrient solution was composed of (mg L^{-1}): NH_4HCO_3 160; KH_2PO_4 80; $FeCl_2 \cdot 4H_2O$ 70.5; NaCl 0.4; $MgSO_4 \cdot 7H_2O$ 4; $CaCl_2 \cdot 2H_2O$ 0.4; $MnSO_4 \cdot 7H_2O$ 0.6; and $Na_2MoO_4 \cdot 2H_2O$ 0.4 (Chen *et al*., 2006).

Pretreatment methods: Sludge pretreatment suppresses methanogenic activity bacteria which may, in turn, enhance bio-hydrogen production. The pretreated sludge was used as inoculum in the hydrogen fermentation tests while untreated sludge was used as a control. The individual pretreatment methods and their possible combinations were explored on the anaerobic sludge. The heat treatment (H) was conducted by baking the sludge at 95°C for 45-120 min and then cooled down to room temperature. The temperature was earlier found to be effective for both bio-hydrogen enhancement and methanogen repression (Wimonsong and Nitisoravut, 2009). Ozone treatment (O) was performed by bubbling ozone into the sludge at the concentration of 1 to 5 mg-O_3 mL^{-1} of the sludge by varying exposure time (min). Na_2SO_4 (S) was added at the concentrations of 0.05 to

0.3 mg SO_4^{2-} mL^{-1} of the sludge. Drug treatment (D) was conducted by adding fluvastatin in the concentrations range of 1 to 4 μg mL^{-1} of the sludge. Based on the individual pretreatment study, the inhibitors dosage of each method which did not completely repress methanogenic activity was chosen for combined treatment to enhance the efficiency of repression. The combined pretreatments include heat treated sludge at 85°C for 60 min, ozonation of sludge at 2 mg-O_3 mL^{-1} of sludge, addition of 0.05 mg SO_4^{2-} mL^{-1} of sludge and 3 μg drug mL^{-1} of sludge.

Experimental procedures: The experiment was conducted from October 2007 to March 2009 at Environmental Laboratory, Sirindhorn International Institute of Technology (SIIT). In order to investigate the hydrogen production potential of various treated-sludge, the batch hydrogen production experiments were carried out in 120 mL serum bottles with a working volume of 60 mL. Nine pretreatment methods were applied to anaerobic sludge and used as inoculum for hydrogen production in triplicate. Treated sludge and untreated sludge (control) were added into serum bottles 10 mL each enriched with 45 mL of synthetic medium comprised of sucrose at the concentration of 20 g L^{-1}, together with 5 mL of essential growth nutrients. The POME was used as a representative for actual wastewater based on the obtained conditions for effective bio-hydrogen enhancement and methanogen repression. The initial pH of 5.5 was adjusted using 1N NaOH or 1N HCl. The bottles were capped with a butyl rubber stopper, sealed with an aluminium crimp. Anaerobic condition was created by sparging with nitrogen gas for 3 min. To inhibit the activity of photosynthetic bacteria, the serum bottles were wrapped with aluminum foil. The incubation temperature was 37°C using a water bath placed on an orbital shaker running at 90 rpm. The amount of evolved gas was measured at room temperature by syringes. The gas composition was determined by gas chromatography. Each experimental condition was carried out in triplicate.

Analysis: The accumulated gas in the head space of the serum bottles was measured and sampled every 12 h. The total gas volume was measured by evacuating gas pressure from the vials using a glass syringe (60 mL) to equilibrate with the room pressure as recommended by Owen's method (Owen *et al*., 1979). The components of biogas (hydrogen, methane and carbon dioxide) were analyzed by a gas chromatograph (PerkinElmer, USA) equipped with a Thermal Conductivity Detector (TCD) and fitted with a Porapak Q, 50/80 mesh column. Helium gas was used as a carrying gas at a flow rate of 25 mL min^{-1}. The operating temperatures of column, detector and injector were 45, 100 and 100°C, respectively.

The pH value was measured by a pH meter (WTW, Model pH 330). Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and Volatile Suspended Solid (VSS) were determined in accordance with the procedures described in the Standard Methods (American Public Health Association, 1995).

RESULTS

Individual pretreatment methods: Four individual methods for preparation of hydrogen-producing seeds including heat, ozonation, additions of Na_2SO_4 and drug were investigated. Based on the previous study (Wimonsong and Nitisoravut, 2009), the heat treatment of anaerobic sludge was conducted at 95°C for 45-120 min. For all heat exposure time, hydrogen productivity was slightly enhanced while methanogenic activity was entirely eradicated from anaerobic sludge (Fig. 1a). The hydrogen production yield after heat treatment at 95°C for 45 min was 1.38 mmol H_2 $mmol^{-1}$ sucrose. Heat treatment at the specified temperature of 95°C was found to be effective for methanogen repression for a prolong exposure time of greater than 45 min. This was later confirmed through the use of POME as a substrate. In this study, ozone dosages of less than 5 mg-O_3 mL^{-1} of sludge were applied. As can be seen in Fig. 1b, there is no significant change in the methanogen repression within the applied dosages. At a lower dose, ozone caused a reduction of hydrogen yield as compared to the control but recovered at high doses. The highest hydrogen yield of 1.28 mmol H_2 $mmol^{-1}$ sucrose was obtained after sludge ozonated at 5 mg-O_3 mL^{-1} of sludge. Figure 1c shows the effects of Na_2SO_4 concentration on the hydrogen and methane yield. It is noteworthy that a higher hydrogen yield was observed in this study for both control and treatments. The hydrogen yield decreased sharply from 1.62 to 0.68 mmol H_2 $mmol^{-1}$ sucrose with an increase in sulfate concentrations, while a slight enhancement of methanogenic activity was observed. Drug treatment was conducted by adding fluvastatin at the concentrations range of 1 to 4 μg mL^{-1} of the sludge. Within the applied dosages, the methanogenic activity was relatively constant as compared to control (Fig. 1d). Hydrogen yields were slightly decreased with an increase in drug concentration, but may not significant.

Influence of combined treatment methods for repression of methanogenic activity and enhancement of hydrogen production: Figure 2 shows normalized methanogen repression efficiency for various pretreatment methods. Among the pretreatment methods used, heat treatment completely repressed methanogenic activity, which achieved 100% in methanogen repression efficiency. Other treatments provided with an incomplete methanogen repression which allowed detection of

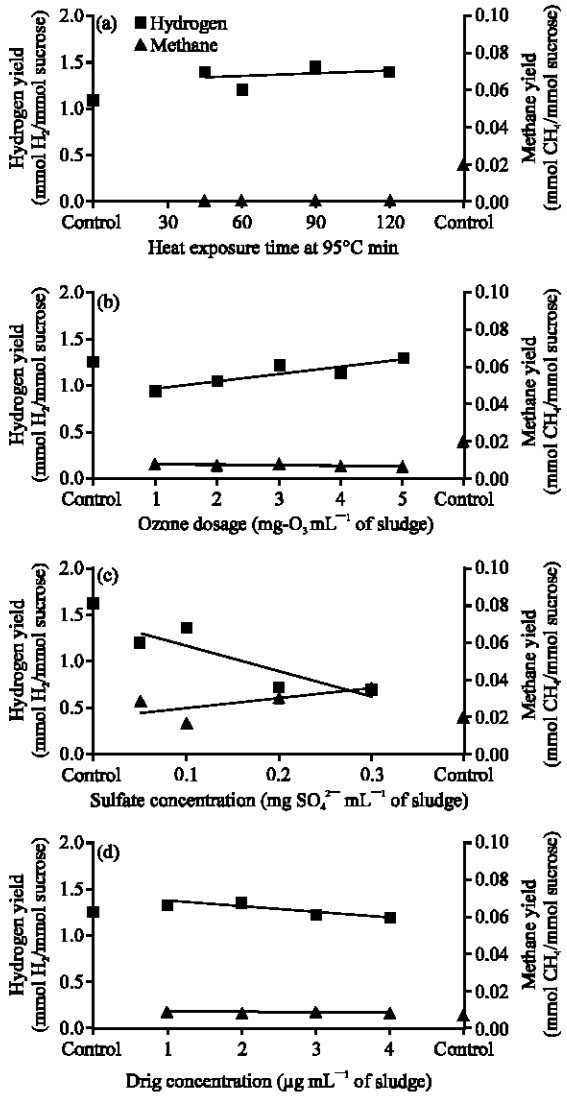

Fig. 1: Hydrogen and methane yields after sludge pretreatment by (a) heat, (b) ozonation, (c) addition of Na_2SO_4 and (d) drug dosing

methane in the accumulated gas. There were two treatment systems resulted in methanogen enhancement which are a combined treatment of ozonation and sulfate dosing (OS) and sulfate dosing only (S). The methanogen repression efficiency was in the order of: H>HO>HD>HS>O>D>OD>OS>S. Heat treatment and all its combinations are the most effective treatment for methanogenic activity control for bio-hydrogen production.

Based on the results obtained, heat and ozonation treatment (HO) shows the highest increment in hydrogen yield of 29% followed by 25% for heat-treated sludge and 19% for heat and sulfate treatment (HS) (Fig. 3). Heat

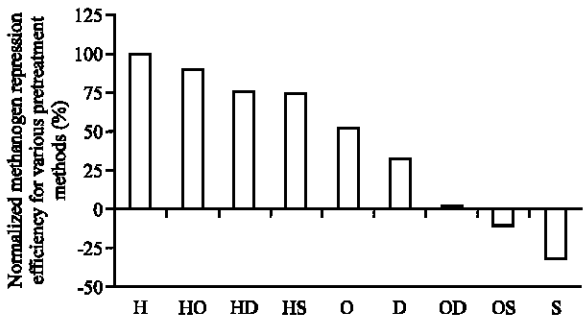

Fig. 2: Normalized methanogen repression efficiency for various pretreatment methods

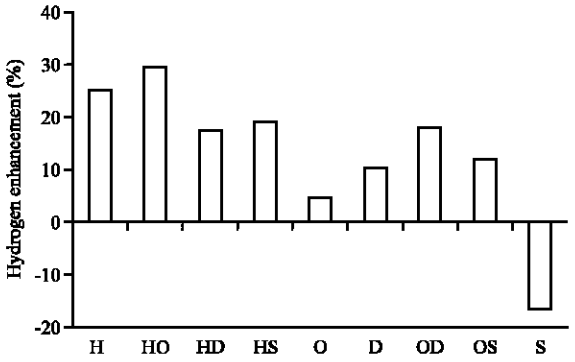

Fig. 3: Percent hydrogen enhancement related to control

treatment and its combinations show a positive enhancement of bio-hydrogen production. Sludge treatment with Na_2SO_4 was the only treatment resulted in hydrogen repression causing a reduction of hydrogen yield of about 16% in related to control.

Hydrogen fermentation from POME: The performance of bio-hydrogen production from POME using the untreated sludge and the heat treated sludge was compared in batch fermentation. The result showed that application of heat treated sludge resulted in a significant increase in the hydrogen yield. For heat treated sludge, a cumulative hydrogen production of 60 mL was obtained, while 21 mL was obtained from untreated sludge. This is about 3 times difference in hydrogen production. Thus, heat treated sludge was used as inoculum for different characteristics of POME.

The time course accumulative volume of hydrogen for different characteristics of POME is shown in Fig. 4. For LPOME, a linear relationship between accumulative volume and time was observed after a short lag time of 25 h. This indicates that a hydrogen production rate was constant throughout the period of experiment. There was no substrate limitation as well as product inhibition in the

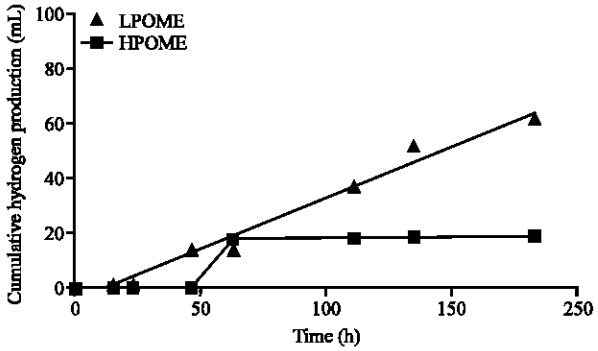

Fig. 4: Cumulative hydrogen production obtained from different characteristic of POME

system. The hydrogen yield was 1.46 mL H_2 g^{-1} COD. The specific hydrogen production rate was calculated at 0.81 mL H_2 g^{-1} COD·h. In contrast, a much longer lag time of nearly double was obtained for HPOME. There was also an indication of inhibition of hydrogen production after 60 h of operation and throughout. The hydrogen yield was much lower as compared to LPOME at 30 mL H_2 g^{-1} COD. The specific hydrogen production rate was calculated at 0.17 mL H_2 g^{-1} COD·h. Moreover, the composition of hydrogen in the accumulated gas was also reduced from 50% for LPOME to 37% HPOME.

DISCUSSION

This study reveals the influence of different pretreatment methods on repression of methanogenic activity and enhancement of hydrogen production. It is suggested that heat treatment of inoculums at 95°C for 45 min is sufficient and suitable for enriching hydrogen producing bacteria from anaerobic mixed culture. On the contrary, the pretreated sludge by ozonation, addition of Na_2SO_4, drug and their combination methods were unsuccessful.

For Na_2SO_4 treatment, the results clearly indicated that the concentrations were insufficient to inhibit methanogenic activity but causing the hydrogen consumption for methanogen and Sulfate Reducing Bacteria (SRB). Lin and Chen (2006) reported the same phenomenon in their study at which sulfate concentration over 500 mg SO_4^{2-} L^{-1} was maintained. They claimed that situation can be recovered by a pH adjustment to be around 5.5. In this study, much lower concentration was used at less than 0.3 mg SO_4^{2-} mL^{-1} of sludge (equivalent to 50 mg L^{-1} of solution) with a pH control of nearly 5.5. Thus, only pH adjustment may not be sufficient for a system containing sulfate concentration lower than the critical concentration for methanogen repression.

A decrease in hydrogen production was also observed in the experiment with ozone treatment. The yield was gradually recovered with an increase in ozone dosages. The consequence was related to a multiple role of ozone as a strong oxidizing agent and disinfectant. The oxidizing property is normally target on substrate causing an oxidation of complex organic into easily biodegradable products thereby enhancing the biological conversion of substrate. The disinfection power will be on a viable microbial cell causing a reduction of microbial population in a system. At low ozone dosages, some of hydrogen producing bacteria might be disinfected. This was later compensated through an increase in biodegradability of organic source allowing a greater hydrogen production at a higher ozone dosage. Nevertheless, there was relatively no bio-hydrogen enhancement observed at the applied ozone dosage of 5 mg-O_3 mL^{-1} of sludge. It should be mentioned that methanogen repression was also insufficient at the provided ozone dosages.

Drug treatment has a potential to specifically inhibit methanogenic bacteria of the rumen (Wolin and Miller, 2006). They reported that lovastatin at concentration of 4 μg mL^{-1} of culture medium was successfully found to inhibit methanogen for pure culture. It should be pointed out that fluvastatin was used in this study but not lovastatin, thought both are closely related analogs. Fluvastatin at nearly the same dosages was used in this study. It was found that fluvastatin was ineffective for controlling methanogenic activity for mixed culture system at the specified dosage of 1-4 μg mL^{-1} of sludge.

A comparative characteristic of POME on hydrogen production was also explored in this study. A negative performance obtained for HPOME clearly indicates an unfavorable condition for bio-hydrogen production. If one tries to account for the contribution of volatile suspended solids in POME to COD, the soluble COD for both LPOME and HPOME can then be calculated. It was found that the soluble COD for the two samples were in fact not much different. It was, thereby, suspected that a decrease in the hydrogen yield was possibly due to a high solid content in the composition of HPOME.

The high amounts of suspended solids in the POME come from insoluble organic substances including lipids. The lipid-rich waste contains long chain fatty acids, especially palmitate and oleate that have been reported to inhibit bacterial growth (Cirne *et al*., 2007). Oil and grease can be hydrolyzed by microorganisms to volatile fatty acids (VFAs). The VFA in their undissociated forms can freely permeate the bacteria cell membrane. If a high level of dissociated VFA is present in the culture, the ionic strength in the solution will increase. Such an increase can result in cell lysis. As a result, the inhibitory effect will

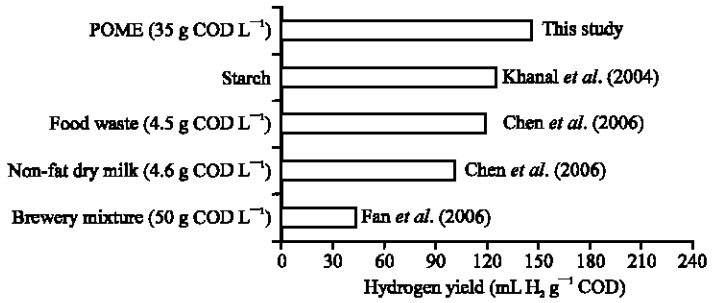

Fig. 5: Bio-hydrogen yields for various waste materials using dark-fermentation

occur (Niel *et al*., 2003). It was suspected that product inhibition by VFAs might cause the decrease in the hydrogen.

The effect of VFAs for hydrogen production was reported by a number of researchers. For example, Zheng and Yu (2005) indicated that the addition of butyrate had a strong inhibitory influence on substrate degradation and hydrogen production. The accumulation of VFAs in the anaerobic fermentation process resulted in a low hydrogen production. Wang *et al*. (2006) introduced a strategy for avoiding propionic acid accumulation in the anaerobic process for bio-hydrogen generation, while Van Ginkel and Logan (2005) found that butyric acid was observed to decrease hydrogen production more than acetic acid by 13% (acetic) and 22% (butyric) after acids added to the feed at a concentration of 25 mM. Nearly 60 mM of either acid decreased hydrogen production by greater than 93%. It was very unfortunate that the VFA as may be the results of fermentation and the key to the answer could not be determined due to the malfunction of instrument during the period of study. Therefore, there was no available data for VFA to support the Fig. 5 summarizes the bio-hydrogen yields from different wastewaters and solid wastes as compared to this study. The maximum hydrogen yield for LPOME in this study was 146 mL H_2 g^{-1} COD. This yield was comparative or relatively higher as compared to many other studies for various types of wastewaters. The obtained hydrogen yield was nearly 3 times greater than the amount reported by Fan *et al*. (2006) for brewery wastewater. Nevertheless, the hydrogen production depends upon the available substrates in wastewater which may vary in their biodegradability and path of reactions.

CONCLUSIONS

Among various pretreatments used, including heat, ozonation, Na_2SO_4, drug and their combination, heat treatment and its combination offered the most attractive performance in terms of bio-hydrogen enhancement and methanogen repression for mixed anaerobic culture. For a complete methanogen repression, heat treatment at 95°C for an exposure time of 45 min was proven for both synthetic and Palm Oil Mill Effluent (POME). High solid content in POME may affect bio-hydrogen production as well as prolong lag time during a start-up of the bioreactor.

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