Korean J. Chem. Eng., **29**(10), 1347-1351 (2012)
DOI: 10.1007/s11814-012-0018-z

Comparative study on the effect of various pretreatment methods on the enrichment of hydrogen producing bacteria in anaerobic granulated sludge from brewery wastewater

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(*Received 12 October 2011 • accepted 15 February 2012*)

Abstract−Five pretreatment methods, namely chemical, acid, heat-shock, freezing and thawing, and base, were evaluated for the enrichment of hydrogen-producing bacteria in anaerobic granulated sludge, which will be subsequently used as seed in biological hydrogen production. All the pretreatments showed positive effects towards improving hydrogen (H_2) generation by the microbial population with higher hydrogen production yield and COD removal efficiency as compared to control. The granulated sludge pretreated by heat-shock showed maximum accumulated H_2 (19.48 mLg^{-1}-COD), COD removal efficiency (62%), and biomass concentration (22.5 gL^{-1}).

Key words: Biohydrogen Production, Enrichment, Granulated Sludge

INTRODUCTION

Hydrogen (H_2) is an efficient and eco-friendly fuel due to its higher energy yield (122 kJg^{-1}) and leaving only water as a by-product of its combustion [1-4]. Biological H_2 production can be accomplished with the suppression of methanogenesis by an extensive variety of microorganisms. *Clostridium* sp. (obligate anaerobic) and *Enterobacter* sp. (facultative anaerobic) are major classes of producers with high capability to generate H_2 than other fermentative bacteria from organic sources [5-9]. Various types of organic sources, including real wastes (rice winery, dairy waste, paper mill, palm oil mill effluent, cattle wastewater, food waste, and waste sludge from wastewater treatment plants) [10-17] and synthetic wastewater (glucose, sucrose, starch, and lignocellulosic material) [18-25], have been utilized in the biohydrogen production process.

H_2 production is influenced by a number of factors, including the type of inoculum, pretreatment, pH, temperature, organic loading rate, hydraulic retention time, and wastewater specificity [16, 26-28]. Selection of the microflora for efficient H_2 production is usually started by selecting a particular type of sludge as inoculum followed by its pretreatment. There are several sludge pretreatment methods for enriching H_2-producing bacteria such as heat-shock, acid, base, aeration, freezing and thawing, chloroform, sodium 2-bromoethanesulfonate or 2-bromoethanesulfonic acid and iodopropane [29]. Several studies reported the influence of these pretreatment methods on the enhancement of H_2 production [29-32].

Acid, sodium 2-bromoethanesolfonate, wet heat-shock, dry heat-shock, and freezing and thawing procedures were used as pretreatment methods in batch fermentative reactor inoculated with cattle manure sludge for enriching hydrogen-producing bacteria [29]. They concluded that the acid pretreatment method yielded the highest hydrogen production rate (HPR) (334 mL-H_2 g-VSS^{-1}h^{-1}) among the studied pretreatment methods. The effect of some pretreatment methods (heat-shock, acid, and chloroform) on anaerobic mixed microflora to enhance biohydrogen production utilizing glucose as substrate was reported, where chloroform treatment resulted in the highest H_2 yield (1.34 mol-H_2 mol-glucose^{-1}) compared to the other methods [30]. Zhu and Beland [31] evaluated various pretreatment methods such as heat-shock, aeration, acid and base treatment, 2-bromoethanesulfanic acid, and iodopropane for preparing H_2-producing seeds from digested wastewater sludge. The iodopropane and base treatment gave the highest H_2 yield, i.e., 5.64 and 6.12 mol-H_2 mol-sucrose^{-1}, respectively. Using digested sludge as seed culture, Wang and Wan [32] compared acid, base, heat-shock, aeration, and chloroform pretreatments for enrichment of H_2-producing bacteria with the highest H_2 yield (221.5 mL-H_2 g-glucose^{-1}) obtained from the sludge underwent heat-shock treatment. Heat-shock treatment has also been reported to be a better method of H_2-producing bacteria enrichment from anaerobic sludge as compared to acid and base treatment methods [33].

Pretreatments such as base, acid, 2-bromoethanesulfonic acid (BESA), load-shock and heat-shock have been compared for the preparation of hydrogen-producing seeds under thermophilic condition (60 °C). The load-shock procedure resulted in the highest H_2 yield (1.96 mol-H_2 mol-hexose^{-1}) [34]. Mohan and co-workers [15] compared the effect of various pretreatment methods (acid, heat-shock, chemical, and their various combinations) on anaerobic mixed microflora to enhance biohydrogen production utilizing a real wastewater (dairy wastewater) as substrate. The chemical treatment procedure gave the highest H_2 yield (0.0317 mmol-H_2 g-COD^{-1}) among the studied pretreatment methods.

It is clear that different pretreatment methods show varied effects towards enriching the H_2-producing bacteria from a different inoculum source and its subsequent biohydrogen yield when supplied with different types of substrates. Thus, the objectives of this study

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were to compare the efficiency of five pre-treatment methods (chemical, acid, heat-shock, freezing and thawing, and base) in enriching H_2-producing bacteria from sludge obtained from up-flow anaerobic sludge blanket (UASB) reactor, and concomitantly increase biohydrogen production and COD removal efficiency when palm oil mill effluent (POME) is provided as substrate.

MATERIALS AND METHODS

1. Wastewater Collection

Palm oil mill effluent (POME) was collected from a local palm oil mill in Felda Sendirian Berhad, Kuala Lumpur, Malaysia. The wastewater collected had the following characteristics: biochemical oxygen demand (BOD_5), 24 gL^{-1}; chemical oxygen demand (COD), 49 gL^{-1}; total suspended solids (TSS), 24.5 gL^{-1}; total volatile solids (VSS), 25.5 gL^{-1}; total Kjeldahl nitrogen (TKN), 227 mgL^{-1}; total phosphorous (TP), 75 mgL^{-1}, and pH 4. The wastewater was immediately transferred to the laboratory and stored at 4 °C before use.

2. Reactor Setup

Six (6) 500 mL Schott bottles were used in this study (Fig. 1). The bottles were filled with 50 mL acclimated (at temperature room using POME as substrate for 20 days before pretreatment) and pretreated (with different pretreatment methods selected) sludge and 150 ml of POME. One bottle with untreated sludge was used as a control. The pH of the mixture was adjusted to 5.5 using NaOH (6 N) and HCl (6 N). To obtain an anaerobic condition for the bacteria, the entire bottle's volume (headspace plus liquid) was sparged with nitrogen for 5 minutes at a flow rate of 10 mLS^{-1}. The reactors were incubated at 35±2 °C, 120 rpm for 72 hours in an incubator shaker (DAIHAN LABTECH Co., Singapore). All experiments were performed in three replicates.

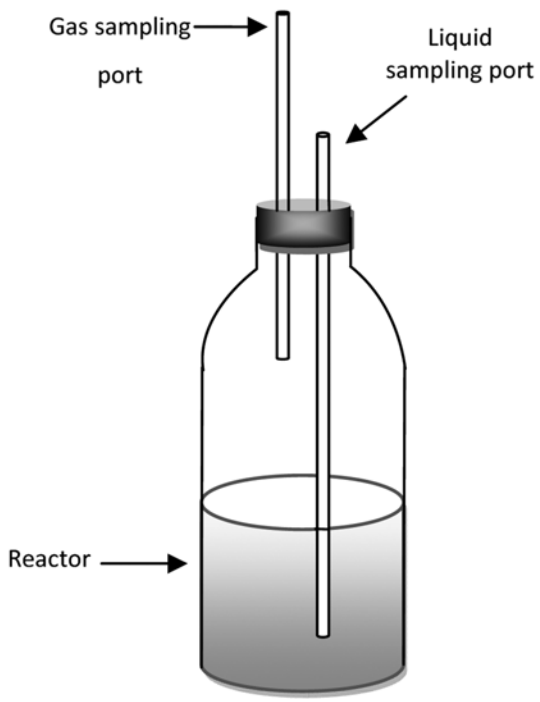

Fig. 1. Schematic diagram of a batch reactor used in the study.

3. Seed Sludge and Pre-treatments

The inoculum for seeding was a granular sludge collected from an up-flow anaerobic sludge blanket (UASB) reactor of Carlsberg Brewery Company (Kuala Lumpur Malaysia Berhad). The sludge was initially passed through a screen (size 2.0 mm) to remove fragments and washed with tap water. Then, the sludge was acclimated to ensure that the bacteria would adjust to their new environment by feeding gradually of brewery wastewater as organic source.

The chemical pre-treatment was conducted by adding 0.1% (v/v) of chloroform into the sludge followed by 24 hours incubation. The acid pre-treatment was done by adjusting the pH of the sludge to 3.0 *via* the addition of concentrated HCl (6 N) with further 24 hours incubation. The heat shock pre-treatment was conducted by heating the sludge to 100 °C for 1 hour in the water bath. The base pre-treatment was conducted by adjusting the pH of the sludge to 12 with concentrated NaOH (6 N) and then incubated for 24 hours. The freezing and thawing pre-treatment was conducted by freezing the sludge to −10 °C for 24 h and then thawing it in a water bath at 30 °C until it reached room temperature.

4. Analytical Methods

The following parameters were analyzed according to Standard Methods [35]: pH, total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), BOD and COD. The biogas volume was measured by replacement water system into the column. The biogas composition was determined using a gas chromatograph (Perkin Elmer, AutoSystem GC) equipped with thermal conductivity detectors (TCD) and digital data acquisition system. Hydrogen gas was analyzed by GC-TCD fitted with a 1.5 m stainless steel column (SS350A) packed with a molecular sieve (80/100 mesh). The temperatures of the injection port, oven and detector were 80, 200, and 200 °C, respectively. Argon was used as the carrying gas at a flow rate of 30 $mLmin^{-1}$. The biogas produced sample (1 mL) was injected in duplicate.

RESULTS AND DISCUSSION

1. Effect of Pre-treatment Methods on H_2 Production

In Fig. 2 is shown the variation of hydrogen production for 64

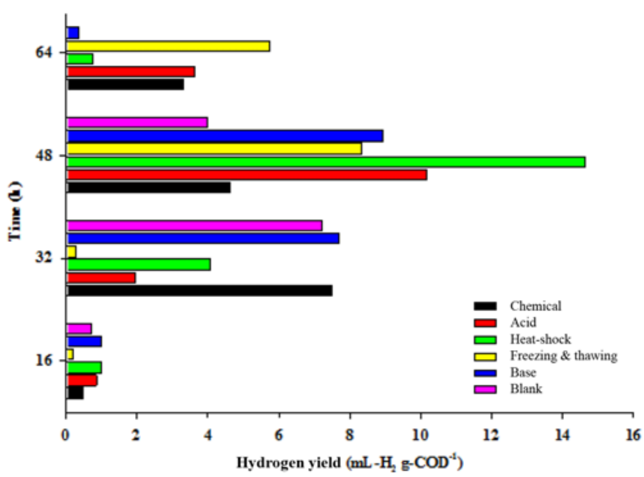

Fig. 2. Hydrogen yield as a function of pre-treatment methods and time.

hours process time with the granulated sludge inocula subjected to different pre-treatments relative to control. Hydrogen and carbon dioxide were the only gases detected from this performed fermentative process. After 32 hours, the biogas components produced from the control reactor consisted of H_2, CO_2 and CH_4. The primary reason for this phenomenon could be as follows: the initial pH of culture was adjusted at 5.5 and at this pH the fermentative bacteria were dominant. However, after 32 hours, the culture pH increased to more than 6.0, under which hydrogen consuming bacterial populations could have developed. In all experiments, the granulated sludge treated by heat-shock showed maximum hydrogen yield at 14.62 mL-H_2 g-COD^{-1} up to 48 hours cultivation. Heat shock treatment was suggested as an effective means to suppress homoacetogens in the sludge, and their suppression allows the growth enrichment of H_2-producing bacteria. On a 48-hour basis, granulated sludge treated by the chemical method (chloroform, 0.1% v/v) produced the lowest H_2 yield (4.64 mL-H_2 g-COD^{-1}) but was higher than the obtained H_2 yield from control experiment at 3.99 mL-H_2 g-COD^{-1}. It appears that the appropriate hydraulic retention time in these experiments was at 48 hours, except for the chemical pre-treatment, base pre-treatment and control that were at 32 hours.

Fig. 3 shows cumulated hydrogen production with respect to the various pre-treatment methods. The heat shock pre-treated sludge yielded the highest cumulative hydrogen production (19.48 mL-H_2 g-COD^{-1}), while freezing and thawing pre-treatment produced only 14.87 mL-H_2 g-COD^{-1} and thus is thought as to exert the least influence on enhancing hydrogen production among the other pre-treatment methods. The cumulative hydrogen production obtained from heat shock pre-treatment procedure in this study was higher than that generally obtained (0.7 mL-H_2 g-COD^{-1}) from dairy wastewater using chemical pretreatment method [15], but it was about 90 percent less than those results obtained from heat-shock pretreatment method (207 mL-H_2 g-COD^{-1}) by Wang and Wan [31] and (233 mL-H_2 g-COD^{-1}) by Mu et al. [33], respectively. The chemical and acid pre-treatment methods had approximately similar effect on the cumulative hydrogen production with 16.22 and 16.38 mL-H_2 g-COD^{-1}, respectively. These results were less than the results obtained from the chemical (80.6 mL-H_2 g-COD^{-1}) and acid (93.5 mL-H_2 g-COD^{-1}) pretreatment methods by Wang and Wan [31].

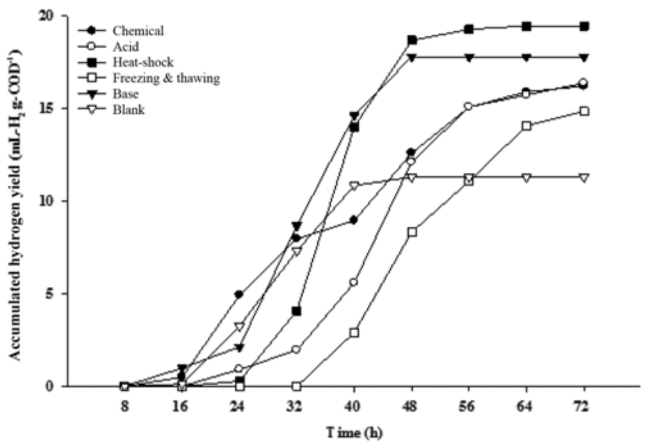

Fig. 3. Cumulative hydrogen yield as a function of pre-treatment methods and time.

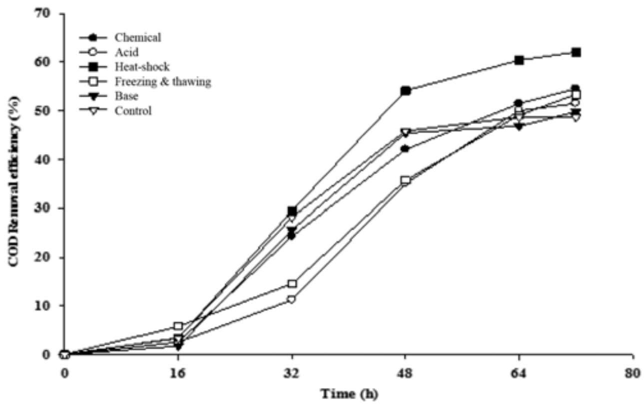

Fig. 4. COD removal efficiency as a function of pre-treatment methods and time.

The results showed that the cumulated hydrogen production of the pre-treated sludge inocula were higher than that of the control during the biohydrogen production utilizing POME as the substrate. Hydrogen evolution by the microbial culture in control experiment leveled off at pH more than 6.0 after 40 h operation.

2. Effect of Pre-treatments on COD Removal Efficiency

The effect of various pre-treatments on COD removal efficiency is shown in Fig. 4. The trend of changes in COD removal efficiency was quite similar to control for all pretreatment methods except heat-shock procedure. The highest COD removal efficiency of 62% was achieved when heat-shock treated sludge was used. The lowest COD removal efficiency (49.8%) was obtained when the base pre-treated sludge was used, close to that of control at 48.7%; their cumulated H_2 productions, however, were calculated at 17.81 and 11.31 mL-H_2 g-COD^{-1}, respectively. This was attributed to the high H_2 content of biogas (average 37.5% for the base pretreatment procedure as compared to the control experiment (average 22.9%).

3. Effect of Pre-treatments on pH

Fig. 5 shows the variation of pH as a function of the different pretreatment methods and time. A slight drop in the pH of the cultures occurred during lag time. Afterwards, the cultures' pH increased and then became constant. For all the pretreatment methods, the final pH of the cultures varied within a narrow range of 5.75 to 6.0

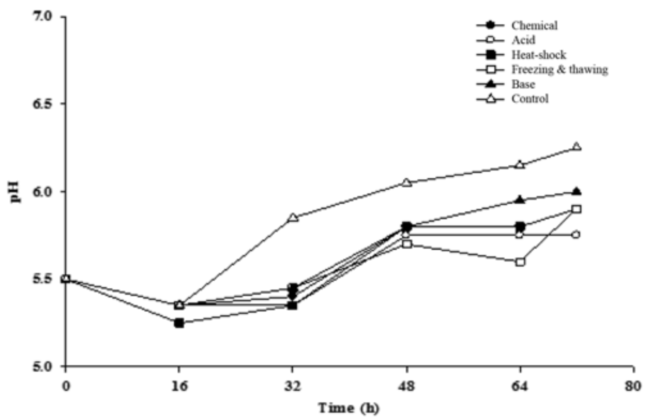

Fig. 5. The variation of pH as a function of pre-treatment methods and time.

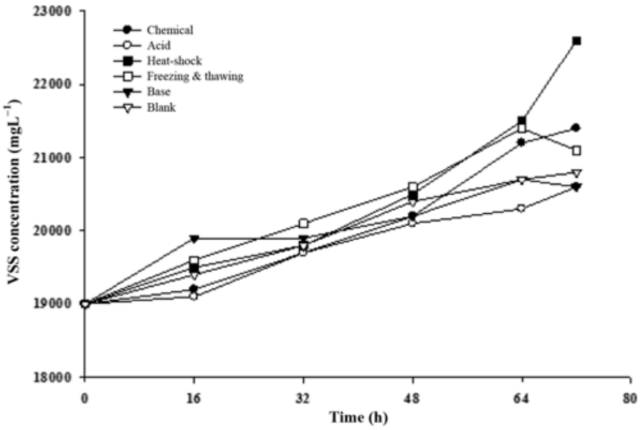

Fig. 6. The variation of biomass concentration as a function of pre-treatment methods and time.

from the initial pH 5.5. On the other hand, the pH in control experiment showed a steeper pH increase after 16 hours as compared to the pretreatment experiments. At the end of the process, the pH in control experiment was 6.3. The increase in pH was attributed to significant growth of the methanogens inside the culture due to the concomitant detection of the presence of CH_4 gas.

4. Effect of Pre-treatments on VSS Concentration

Fig. 6 shows the variation of VSS as a function of different pretreatment methods and time. The results show that the biomass concentration of the sludge samples increased with time. At the end of experiments (72 h), the VSS concentrations in the heat-shock, chemical, and freezing and thawing, were more than the VSS concentration in the control. On the other hand, the biomass concentrations were similar in the acid and base pretreatment methods (20,600 mgL^{-1}) but slightly lower than the control (20,800 mgL^{-1}). The highest biomass concentration (22,500 mgL^{-1}) was obtained from the cultivation using heat-shock treated sludge.

CONCLUSIONS

Chemical, acid, heat-shock, freezing and thawing and base pretreatment methods were used to enrich hydrogen-producing bacteria and its subsequent enhancement of biohydrogen production. The results showed that all pre-treatments had positive influence on H_2 yield and COD removal efficiency as compared to the control. Heat shock pre-treatment is a simple method for enriching hydrogen-producing bacteria from granulated sludge resulting in high yield H_2, COD removal efficiency, and biomass concentration at 14.62 mL-H_2 g-COD^{-1}, 62%, and 22.5 gL^{-1}, respectively.

ACKNOWLEDGEMENT

PM would like to acknowledge financial support from the University of Malaya PPP and Biohydrogen grant from the Malaysian Genome Institute, and the kind assistance of Mrs Kalaiselvi in this project.

NOMENCLATURE

H_2 : hydrogen
UASB : up-flow anaerobic sludge blanket
POME : palm oil mill effluent
COD : chemical oxygen demand
BOD : biochemical oxygen demand
TSS : total suspended solids
TDS : total dissolved solids
TKN : total Kjeldahl nitrogen
TP : total phosphorous

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