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Effects of different pretreatment methods on anaerobic mixed microflora for hydrogen production and COD reduction from palm oil mill effluent

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Introduction

Dark fermentative hydrogen (H_2) production is one of several alternatives for non-renewable energy source like fossil fuels. H_2 is viewed as a sustainable energy carrier with negligible or zero use of hydrocarbons and high-energy yield (122 kJ g_1) (Mu et al., 2006; Mohan et al., 2008; Özgür et al., 2010). In dark fermentative H_2 production, carbohydrate-rich substrates are converted to H_2 and other products by a number of different microorganisms. H_2 yield from microbial fermentation is dependent upon microbial communities present, type of substrate and operational and environmental factors (i.e. organic loading rate, initial pH, temperature, etc.) (Hallenbeck and Ghosh, 2009; Peintner et al., 2010). Bacterial species such as Clostridium, Escherichia coli and Enterobacter have been employed for H_2 production (Chen et al., 2005; Chong et al., 2009a). These groups of microorganisms are ubiquitous in natural environments e.g. sediments, soil, wastewater sludge, compost etc. (Nandi and Sengupta, 1998; Das and Veziroglu, 2001; Cheong and Hansen, 2006; Zhu and Beland, 2006; Hu and Chen, 2007; Wang and Wan, 2009).

Pretreatment of sludge that is used for inoculum preparation in anaerobic system is one approach which helps to increase growth of bacteria and their H_2 evolving activity (Kim et al., 2003; Zhu and Beland, 2006; Mohan et al., 2007). The three main stages for the microbial H_2 production include (i) the selection of material containing bacterial population of interest; (ii) enrichment of these H_2 producing bacteria and (iii) acclimatization of the bacteria to specific substrates (Zhu and Beland, 2006). Some reported pretreatment methods for enriching H_2 producing bacteria include heat-shock, load-shock, acid, base, 2-bromoethanesulfonate acid (BESA), aeration, freezing and thawing, chloroform, and iodopropane (Hawkes et al., 2002; Mohan et al., 2008; Wang and Wan, 2008; Sompong et al., 2009). Several studies have been conducted to compare the influence of different pretreatment methods on the enrichment of H_2 producing bacteria in anaerobic H_2 production processes using various inoculants. Studies have shown that the application of heat-shock pretreatment method resulted in the

highest H_2 production rate (HPR) (Mu et al., 2007; Wang and Wan, 2008). Sompong and co-workers (2009) reported that the loadshock treatment method was successfully applied in enriching thermophilic H_2-producing inocula and resulted in a maximum H_2 production yield of 1.96 mol H_2/mol hexose with a HPR of 11.2 mmol H_2/(L·h). The highest H_2 yield (0.0317 mmol g_1 COD)was obtained by applying BESA pretreatment method among other methods (Mohan et al., 2008). Cheong and Hansen (2007) on the other hand reported that acid pretreatment method resulted in the highest HPR. Nevertheless, most researchers used the heat-shock treatment of sludge preparation in their experiments. Depending on the utilized bacterial source, they employed temperature values in the range of 80e121 _C for an exposure time between 15 and 120 min (Lay et al., 1999, 2003; Ginkel and Sung, 2001; Logan et al., 2002; Chang and Lin, 2004; Han and Shin, 2004). Nevertheless, a decline in H_2 production after one month operation of batch experiment was observed for microbial populations initially exposed to heat-shock treatment. Therefore, regular repetition of sludge treatment with heat-shock was needed to avoid the aforementioned problem (Duangmanee et al., 2007; Sompong et al., 2009).

In other to remove H_2 consuming bacteria from the inocula, various methods for inocula preparation were applied based on the physiological differences between H_2 producing bacteria and H_2 consuming bacteria (Zhu and Beland, 2006).

The main objectives of this study were to assess the efficiency of several pretreatment methods on anaerobic mixed culture for enriching H_2 producing bacteria to increase biohydrogen production and chemical oxygen demand (COD) removal efficiency employing palm oil mill effluent (POME) as substrate. Simultaneous H_2 production and COD removal utilizing microbial process with POME as a major substrate has been shown and optimized by Jamil and co-workers (Jamil et al., 2009).

2. Materials and methods

2.1. Palm oil mill effluent

The POME was collected from Felda Palm Oil Industry Sendirian Berhad, Kuala Lumpur. Wastewater samples were immediately transferred to the laboratory and stored in a cold room at 4 _C before use. The characteristics of POME are summarized in Table 1.

2.2. Seed sludge

The bacterial population used as inoculumin the production ofH_2

from POME in this experiment was obtained from anaerobic treatment plant operated by Felda Palm Oil Industry Sendirian Berhad, Kuala Lumpur. The sludgewas initially passed through amesh screen to remove fragments. The total volatile solids concentration of the sludgewas measured (27 g L_1) and total suspended solids (TSS)was measured (36 g L_1).

2.3. Pretreatment experiments

In this study, experimentswere conducted to assess the influence of different pretreatment methods on anaerobic sludge in order to increase H_2 production efficiency using POME as substrate. The sludge was sieved using grid size of 2.0 mm mesh to remove coarse matters and thenwashed with tapwater. Subsequently, itwas treated to disable H_2 consuming bacteria and promote H_2 production by one of the following methods: chemical pretreatment, acid hydrochloric pretreatment, heat-shock pretreatment, alkaline (base) pretreatment, freezing and thawing pretreatment.

The chemical pretreatmentwas conducted by adding 0.1% (v/v) of chloroform into the sludge for 24 h at room temperature (Hu and Chen, 2007). The acid pretreatments were conducted by adjusting the pH of the sludge to 3.0 by adding HCl (6 N) and maintained for 24 h and subsequently re-adjusted the pH to 5.5 by NaOH solution (2 N) (Chen et al., 2002; Sompong et al., 2009; Lee et al., 2009). The heat-shock pretreatment was conducted by heating the sludge to 100 _C for 1 h in thewater bath (Ginkel and Sung, 2001;Mohan et al., 2008). The base pretreatment was conducted by adjusting the pH of the sludge to 12 by adding NaOH (6 N) and maintained for 24 h and subsequently re-adjusted the pH to 5.5 by HCl solution (1 N) (Cai et al., 2004; Sompong et al., 2009). Freezing and thawing pretreatment 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 (Cheong and Hansen, 2006). A control experiment using the sludge was also conducted without any pretreatment method.

2.4. Experimental set-up

Fig. 1 showed a schematic diagram of the serum bottle which served as a batch reactor in this study. Six 500 mL serum bottles were used for each run at 10% v/v sludge. The sludge samples in different serum bottles were subjected to the aforementioned pretreatments. After the pretreatments, POME was added into each bottle to obtain a final working volume of 200 mL. The run was conducted at 35 _ 2 _C and an agitation rate of 120 rpm. The pH of the mixture was also adjusted to 5.5 _ 0.3 before initiating the batch test. In order to obtain an anaerobic condition for the bacteria, the entire serum bottle volume (headspace plus liquid)

was sparged with nitrogen for 15 min at a flow rate of 10 mL s_1. The batch runs lasted for 72 h and all experiments were performed in three replicates.

2.5. Analytical methods

The production of biogas was monitored via gas sampling at regular intervals. A syringe was used to extract measured volume of gas sample from each test bottles. The biogas composition was determined using a gas chromatograph (Perkin Elmer, AutoSystem GC) equipped with thermal conductivity detectors (TCD) and digital data acquisition system. H_2 content was analyzed by GC-TCD fitted with a 1.5 m stainless steel column (SS350A) packed with a molecular sieve (80/100 mesh). The temperature values of the injection port, oven and detectorwere 80, 200, and 200 _C, respectively. Argon was used as a carrier gas at a flow rate of 30 mL min_1. The gas sample (1 mL) was injected in replicates. Other parameters, viz. chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), were determined in accordance with the procedures described in the standard methods (APHA, 1999).

3. Results and discussion

3.1. Effect of different pretreatments on H_2 production

The effect of various pretreatment methods on the enrichment of H_2 evolving bacterial population and their H_2 production efficiency by using POME as substrate was studied. Fig. 2 showed the variation of H_2 production yield from the serum bottles inoculated with sludge subjected to different pretreatments and from the serum bottles without any pretreatment (control) during 72 h operation. Experimental results demonstrated the need for inoculum pretreatment for improved H_2 generation by the microbial population as compared to control experiment. Heat-shock pretreatment was shown to be the most effective in enhancing the biological H_2 production up to 48 h cultivation which appeared to coincide with the leveling of the COD reduction efficiency (see next section). The H_2 yield from this method was 0.41 mmol H_2/g COD. H_2 yield from control experiment was the lowest i.e. 0.12 mmol H_2/g COD. The relatively low pH between 5.5 and 6.0 for entire duration of the batch process could have inhibited the growth of H_2 consuming bacteria (Tang et al., 2008; Wang and Wan, 2009; Zhu et al., 2009; Chong et al., 2009b). H_2 yield following the heatshock treatment was 3.4 times higher than that obtained in the control experiment. Among the pretreatment methods applied, the

freezing and thawing method produced the lowest H_2 yield (0.19 mmol g_1 COD) whichwas approximately 58% higher than the control. Although, H_2 production was possible without any pretreatment (control), the application of different types of pretreatment resulted in a significant increase in H_2 yield. The time profiles of the H_2 production following the different pretreatments showed general maxima at 48 h cultivation. Thus, it is suggested that the harvesting of the pretreated sludge for the subsequent H_2 production process should take place after not more than 48 h. The cumulative H_2 productions following different pretreatment methods are shown in Fig. 3. The highest cumulative H_2 production (8.89 mmol) was observed from the heat-shock pretreated sludge. The acid and base pretreatments showed similar influence on the cumulative H_2 production but their H_2 yields were lower than heat-shock treated sludge. The freezing and thawing pretreatment methods produced only 4 mmol H_2 and had the least influence on H_2 production among the different pretreatments.

The lag time before significant increase in H_2 production varied between 8 (minimum) to 40 (maximum) h for heat-shock and freezing & thawing pretreatment methods, respectively. The rest of the pretreatments and control exhibited lag time for H_2 production in between this range. Cultures that were subjected to treatments using heat shock and base leveled off in their H_2 production at 48 h whereas the rest were still showing increase albeit slower in their H_2 evolution.