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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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 heatshock, 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) (334mL-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 (1.34mol-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 6.12mol-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 (221.5mL-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 ^{o}C). The load-shock procedure resulted in the highest H_2 yield (1.96mol-H_2 mol-$hexose^{-1}$) [34]. Mohan and co-workers [15] compared the effect of various pretreatment methods (acid, heatshock, 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 (0.0317mmol-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 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), 227mgL^{-1}; total phosphorous (TP), 75mgL^{-1}, and pH 4. The wastewater was immediately transferred to the laboratory and stored at 4 ^{o}C before use.

2. Reactor Setup

Six (6) 500mL Schott bottles were used in this study (Fig. 1). The bottles were filled with 50mL acclimated (at temperature room using POME as substrate for 20 days before pretreatment) and pretreated (with different pretreatment methods selected) sludge and 150ml 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 5minutes at a flow rate of 10mLS^{-1}. The reactors were incubated at 35±2 ^{o}C, 120 rpm for 72 hours in an incubator shaker (DAIHAN LABTECH Co., Singapore). All experiments were performed in three replicates.

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 2.0mm) 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 ^{o}C for 1 hour in the water bath. The base pretreatment was conducted by adjusting the pH of the sludge to 12 with concentrated NaOH (6N) and then incubated for 24 hours. The freezing and thawing pre-treatment was conducted by freezing the sludge to −10 ^{o}C for 24 h and then thawing it in a water bath at 30 ^{o}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 1.5m 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 ^{o}C, respectively. Argon was used as the carrying gas at a flow rate of 30mLmin^{-1}. The biogas produced sample (1mL) was injected in duplicate.