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Evaluation of three methods for enriching H_2-producing cultures from anaerobic sludge

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Abstract

Hydrogen can be harvested from the microbial fermentation of organic substrates when methanogenesis is suppressed in an anaerobic digestion system. In this study three methods, heat-, acid- and alkaline-treatment, were used to suppress methanogenesis in mixed cultures and to enrich H_2-producing inoculum. Highest H_2 yield of 2.00 mol-H_2/mol-glucose was achieved with the heat-treated sludge, while lowest yield of 0.48 mol-H_2/mol-glucose was obtained with the alkaline-treated sludge. A butyrate-type fermentation was found for both heat- and alkaline-treated sludge, while a mixed-type fermentation occurred for the acid-treated sludge. A model was established to describe the kinetics of H_2 production process and the yield coefficients of various products were estimated for the three cases with this model. The relationships among NADH/NAD^+, oxidation–reduction potential and the H_2 partial pressure were established and the evolvement of NADH/NAD^+ and oxidation–reduction potential in the fermentative process for the three cases was also evaluated. The comparative experimental results show that the heat-treatment method was better than the two others for enriching H_2-producing inoculums from mixed anaerobic cultures.

Keywords: Acid-treated; Alkaline-treated; Anaerobic sludge; Heat-treated; Hydrogen; NADH/NAD^+

1. Introduction

Research on alternative energy sources has gained renewed interest, due to global awareness of accumulated carbon dioxide in the atmosphere as a potential cause of climate change [1]. Combustion of H_2 produces no greenhouse gases, and has a high-energy yield of 122 kJ/g, which is 2.75-fold greater than that of hydrocarbon fuels. Thus, utilization of H_2 as a clean energy source seems to be promising. The feasibility of applying acidogenesis of organic wastes to produce H_2 has been widely demonstrated at various laboratories [2–4]. Compared with photosynthetic bacteria, dark fermentative bacteria produce H_2 at a lower cost, because they do not need light provision and have simple requirements for microbial growth. In previous studies both pure cultures such as *Clostridium* sp. and mixed cultures of anaerobic bacteria, have been used to convert carbohydrates (e.g., glucose) to H_2 [5–8].

To scale-up biological H_2 production processes, getting a large amount of anaerobic H_2-producing inoculums economically becomes crucial [7,9]. Previous studies have demonstrated that operation at a low pH and short sludge retention time, or seeding with heat-shocked sludge, is able to suppress the growth of methanogens and accordingly to enhance H_2 production. The acid-treated method and heated-shocked method have been, respectively, used in previous studies [7,8,10]. The effectiveness of pH control for enhancing H_2 production have been demonstrated in batch tests [10]. The heat-treatment has been found to be an alternative for enriching H_2-producing inoculum from natural anaerobic cultures [7]. The heat- and acid-treated methods have been also compared for hydrogen production and a greater H_2 yield was observed in the heat treatment than that in the acid treatment [11]. Recently, it was found that H_2 production could also be enhanced from sewage sludge with alkaline pretreatment [12]. However, little information is available to evaluate the effectiveness of these three enrichment methods for H_2-producing cultures in parallel. Moreover, the evolvement of NADH/NAD^+ and oxidation–reduction potential (ORP) with the H_2 partial pressure in the fermentation process has not been elucidated in these previous studies.

Therefore, in this study H_2-producing inoculum was enriched by using heat-, acid- and alkaline-treatment in parallel, and their effectiveness was evaluated and compared in a fermentor oper-

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ated in a batch mode. The profiles of H_2 production, substrate consumption and product formation were monitored for the three cases, and the kinetics of H_2 production was also explored. Moreover, the relationships between NADH/NAD^+, ORP and the H_2 partial pressure, and the evolvement of NADH/NAD^+ and ORP in the fermentation process for the three cases were investigated.

2. Materials and methods

2.1. Seed sludge

The anaerobic microflora used in this study was obtained from a full-scale upflow anaerobic sludge blanket reactor located in Benpu, China. This reactor is being operated for the treatment of soybean-processing wastewater and production of CH_4. Prior to use, the seed sludge was first washed five times with tap water, and was then sieved to remove stone, sand and other coarse matters. Thereafter, heat-, acid- and alkaline-treatments were, respectively, performed to inactive hydrogentrophic methanogens and to enrich H_2-producing bacteria. For the heat-treatment case the seed sludge was heated at 102 °C for 90 min [13]. For the acid-treatment, the sludge was adjusted to acidic pH (pH 3.0–4.0) with 0.1N HCl for 24 h, and was then adjusted back to pH 7.0 with the addition of 0.1N NaOH [10]. For the alkaline-treatment, the sludge was pretreated with adding 4 M NaOH and the pH was kept at 12.0 for 24 h, and was then adjusted back to pH 7.0 with the addition of 0.1N HCl [12]. The temperature was around 25 °C for both acid- and alkaline-treatments.

2.2. Experiments

Hydrogen production experiments were conducted in triplicate in a 5-L fermentor (Baoxin Biotech Ltd., Shanghai) equipped with an impeller and four baffles. Agitation of the fermentation broth was provided by using a six-bladed impeller. One litre of heat-, acid- or alkaline-treated anaerobic sludge with volatile suspended solids (VSS) of 18.0 g/L and 3 mL of nutrients solution were added to the fermentor. The working volume was adjusted to 3.0 L with distilled water. The solution in the fermentor was composed as follows (unit in mg/L): sucrose 25,000; NH_4HCO_3 2025; $K_2HPO_4{\cdot}3H_2O$ 800; $CaCl_2$ 50; $MgCl_2{\cdot}6H_2O$ 100; $FeCl_2$ 25; NaCl 10; $CoCl_2{\cdot}6H_2O$ 5; $MnCl_2{\cdot}4H_2O$ 5; $AlCl_3$ 2.5; $(NH_4)_6Mo_7O_{24}$ 15; H_3BO_4 5; $NiCl_2{\cdot}6H_2O$ 5; $CuCl_2{\cdot}5H_2O$ 5; $ZnCl_2$ 5. Prior to operation, the fermentor was purged with nitrogen gas for 10 min to ensure anaerobic conditions.

The pH of the mixed liquor was kept at constant by feeding 4 M NaOH or 2 M HCl solutions via respective peristaltic pumps. The agitation rate in the fermentor was kept at 120 rpm. A biogas sampling port was installed between the meter and the reactor to allow direct biogas sampling with a syringe. In each trail, the pH of the mixed liquor was chosen as 5.5, which was reported to be optimum for H_2 production by mixed anaerobic cultures [14,15]. The temperature was kept at 35.0 °C with a temperature controller, and the initial sucrose concentration was 25 g/L.

2.3. Analytical methods

The amount of biogas produced was recorded using water-replace method. The biogas composition was analyzed by using a gas chromatograph (Lunan, Model SP-6800A) equipped with a thermal conductivity detector and a 1.5 m stainless-steel column packed with 5 Å molecular sieve. The temperatures of injector, detector and column were kept at 100 °C, 105 °C and 60 °C, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min. The concentrations of ethanol and volatile fatty acids (VFA), including acetate, propionate, butyrate, *i*-butyrate, valerate, and caporate in the effluent were determined by second gas chromatograph (Agilent, Model 6890NT) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 μm fused-silica capillary column (DB-FFAP). The liquor samples were first centrifuged at 12,000 rpm for 5 min, and were then acidified by formic acid and filtrated through 0.2 μm membrane and finally measured for free acids. The temperatures of the injector and detector were 250 °C and 300 °C, respectively. The initial temperature of oven was 70 °C for 3 min followed with a ramp of 20 °C/min for 5.5 min and to final temperature of 180 °C for 3 min. Nitrogen was used as carrier gas with a flow rate of 2.6 mL/min. The sucrose concentration was measured using anthrone-sulfuric acid method [16]. VSS concentration was measured according to Standard Methods [17].

A following modified Logistic equation was employed to model the kinetics of biohydrogen production [19]:

$$H = \frac{H_{\max}}{1 + \exp[((R_{\max,H_2} \times e)/H_{\max})(\lambda - t) + 1]} \quad (1)$$

where H (mL) is the total amount hydrogen produced at reaction time t (h), $H_{\max}$ (mL) the potential maximal amount hydrogen produced, $R_{\max}$ (mL/h) the maximum hydrogen production rate and λ (h) is the lag time to exponential hydrogen production.

Once cumulative hydrogen production curves were obtained over the course of an entire batch experiment, a curve was drawn using the modified Logistic equation and the values of $H_{\max}$, $R_{\max}$, λ, $H_{\max}$ and $R_{\max}$ were also determined. The data were analyzed by using software Origin 6.1 in the study.

3. Results and discussion

3.1. Substrate degradation

The degradation profiles of sucrose with acid-, heat- and alkaline-treated sludge are illustrated in Fig. 1. After a lag time, sucrose was degraded rapidly and became nearly depleted within 45 h in the three cases (Fig. 1).

3.2. H_2 production

The H_2 partial pressure profiles in the reactor headspace were in accord with the conversion of sucrose to H_2 for the three cases and no methane was detected (Fig. 2). This might be due to the fact that, in the enrichment, the H_2-utilizing methanogens were inactivated or inhibited, but that the H_2-producing microorganisms, e.g., clostridia, survived. These endospores are resistant to heat, and cannot be inactivated easily even by harsh chemicals [18]. After the end of lag phase, the H_2 partial pressure increased and peaked of 0.61 atm for the heat-treated sludge at hour 26, 0.59 atm for the acid-treated one at hour 23.3, and 0.51 atm for the alkaline-treated one at hour 27. Then, the H_2 partial pressure declined for the three cases. Such a decrease was

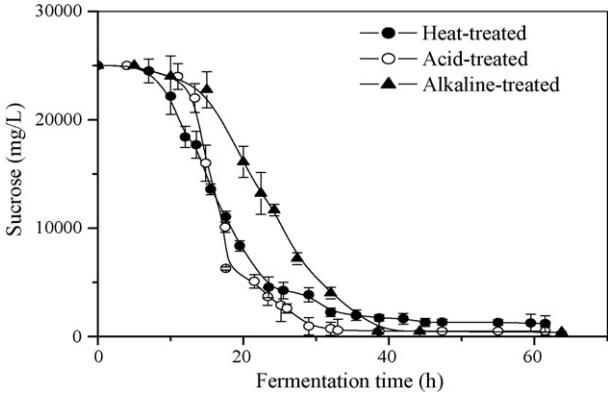

Fig. 1. Sucrose degradation profiles for the heat-, acid- and alkaline-treated sludge.