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Acidophilic biohydrogen production from rice slurry

Herbert H.P. Fang*, Chenlin Li, Tong Zhang

Centre for Environmental Engineering Research, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China

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Abstract

Batch experiment results showed that hydrogen production from rice slurry was found most effective at pH 4.5, 37 °C treating a slurry containing 5.5 g-carbohydrate/L. An anaerobic digester sludge was used as seed after a 100 °C heat treatment for 30 min. After a 36 h acclimation period, the sludge had a maximum specific hydrogen production rate of 2.1 L/(g-VSS d) and a hydrogen yield of 346 mL/g-carbohydrate, corresponding to 62.6% of stoichiometric yield. The effluent was composed mostly of acetate (28.3–43.0%) and butyrate (51.4–70.9%). Based on the 16S rDNA analysis, the 28 clones developed from this acidophilic hydrogen-producing sludge may be classified into nine OTUs, all of which are affiliated with the genus *Clostridium*. Phylogenetic analysis shows that eight OTUs (96.4% of population) form a distinct group with *Clostridium* sp. 44a-T5zd. Results indicate the acidophilic hydrogen-producing bacteria found in this study are unknown, and warrant further studies.

Keywords: Acidophilic; *Clostridium*; Fermentation; Food waste; Hydrogen; Phylogenetic analysis; Rice

1. Introduction

Hydrogen is a high-value industrial commodity with a wide range of applications. It is an ideal fuel, producing only water upon combustion. It may be converted into electricity via fuel cells or directly used in internal combustion engines. It can also be used for the syntheses of ammonia, alcohols and aldehydes, as well as for the hydrogenation of edible oil, petroleum, coal and shale oil [1]. Many believe that hydrogen will replace fossil fuels as the next generation of energy supply [2]. A hydrogen-based economy will impose no risk of global warming, and will significantly improve the urban air quality [3].

Hydrogen is traditionally generated by hydrocarbon reformation or electrolysis of water [1]. It is, however, technically feasible to harvest hydrogen from carbohydrates in waste and wastewater through biological process by fermentative microbes [4]. The hydrogen production characteristics of a number of pure cultures have been studied, including *Clostridia* [5–7] and *Enterobacteria* [8–10]. However, from an engineering point of view, producing hydrogen by mixed cultures is generally preferred because of lower cost, ease of control, and the possible use of organic wastes as substrate. Furthermore, production of hydrogen from organic wastes is a one-stone-two-birds paradigm; it not only cleans up the environment but also produces a clean and readily usable energy in a sustainable fashion [3]. Most studies of bio-hydrogen production so far, however, have been limited to using pure carbohydrates, such as glucose, sucrose and starch. Little information is available on the feasibility of using the carbohydrate-rich agricultural and food wastes.

Fermentative hydrogen production is affected by many parameters such as pH, temperature and feedstock concentration as well as the nature of the microbial community. pH is crucial due to its effects on hydrogenase activity [11], metabolism pathways [12], and microbial communities [13]. Hydrogen production is usually accompanied by the

* Corresponding author. Tel.: +852 2859 2660; fax: +852 2559 5337.
E-mail address: hrechef@hkucc.hku.hk (H.H.P. Fang).

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production of volatile fatty acids (VFA) and alcohols. Some found that lowering the pH to 4.5 or below may shift the VFA-producing pathway to an alcohol-producing pathway [14,15]. Lay et al. [16] found that pH 5.6 was optimal for hydrogen production, and was also the threshold pH for the VFA- and alcohol-producing pathways. Fang and Liu [13] reported an optimal pH of 5.5, and their analysis based on polymerase chain reaction (PCR) and denatured gradient gel electrophoresis (DGGE) indicated that the diversity of microbial community increased with pH. Morimoto et al. [17] demonstrated that the production of hydrogen, which was started at neutral pH, was reduced with lowering pH and ceased at pH 4. Khanal et al. [15] also found the variations in acetate/butyrate ratio, which implied a metabolic alteration due to environmental changes, such as pH, etc. So far in the literature, pH of 5.5 is regarded as the optimal value and little information is known about the hydrogen production under a more acidophilic environment.

Fermentation reactions can be conducted at mesophilic (25–40 °C), thermophilic (40–65 °C), extreme thermophilic (65–80 °C), or hyperthermophilic (>80 °C) temperatures [18]. Most of the studies on hydrogen production by dark fermentative bacteria were conducted at 25–40 °C, with a few exceptions at 60 °C [19] and 55–70 °C [20].

This study was conducted initially to investigate the feasibility of producing hydrogen from rice slurry which is a common carbohydrate-rich food waste. It was soon found that, unlike reported in most studies, acidophilic condition was favored for rice slurry. The focus of this study was then shifted to investigate the performance, optimal operational conditions and microbial community of acidophilic hydrogen-producing sludge, using 16S rDNA-based molecular techniques, including PCR–DGGE, cloning, sequencing and phylogenetic analysis.

2. Materials and methods

2.1. Batch experiments of hydrogen production

Rice, the most common dietary food worldwide, was chosen as the model of carbohydrate-rich food waste after steaming at 100 °C for 30 min. The rice was composed of carbohydrate (78.3%), protein (6.6%), lipid (3.2%) and water (11.9%). An anaerobic digester sludge, sampled from a local municipal wastewater treatment plant, was used as seed. The sludge was pre-heated at 100 °C for 30 min to deactivate the hydrogenotrophic methanogens before it was used to seed the reactors. Three series of batch experiments were conducted in duplicate in 280 mL serum bottles. The wastewater was prepared using the following nutrients (all in mg/L): $NaHCO_3$ 1250; NH_4Cl 2500; KH_2PO_4 250; K_2HPO_4 250; $CaCl_2$ 500; $NiSO_4$ 32; $MgSO_4 \cdot 7H_2O$ 320; $FeCl_3$ 20; $Na_2BO_4 \cdot H_2O$ 7.2; $Na_2MoO_4 \cdot 2H_2O$ 14.4; $ZnCl_2$ 23; $CoCl_2 \cdot 6H_2O$ 21; $CuCl_2 \cdot H_2O$ 10; $MnCl_2 \cdot 4H_2O$ 30; yeast extract 50.

Series I was to investigate the effect of pH on hydrogen production. Experiments were conducted from pH 4.0 to 7.0 with 0.5 increments at 37 °C and 5.5 g-carbohydrate/L. Series II was to compare hydrogen production at 37 and 55 °C treating the rice slurry at pH 4.5 and 5.5 g-carbohydrate/L. After the optimal pH and temperature were identified as 4.5 and 37 °C, respectively, Series III was to further investigate the effect of feedstock concentration at 2.7, 5.5, 8.3, 11.0, 13.8 and 22.1 g-carbohydrate/L. In all batches, 150 mL rice slurry was treated with 85 mg of sludge as measured by volatile suspended solids (VSS). The mixed liquor was first purged with nitrogen for 20 min and capped tightly with butyl rubber to ensure anaerobic conditions. These reactors were then placed in a reciprocating shaker at 100 rpm at controlled temperatures. The mixed liquor pH was periodically adjusted using either 1 M HCl or 1 M NaOH to maintain the desired values.

2.2. Chemical analysis

The amount of biogas produced in each reactor was measured using a glass syringe. Biogas of 50 μL was sampled to analyze the contents of hydrogen, carbon dioxide and methane by a gas chromatograph (GC) (Hewlett–Packard 5890II, USA) equipped with a thermal conductivity detector and a 2 m × 2 mm (inside diameter) stainless steel column packed with Porapak N (80–100 mesh). Injector, detector and column temperatures were kept at 57, 180 and 50 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min.

Concentrations of VFA and alcohols in the mixed liquor were analyzed by a second GC of the same model equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary column. The VFA analyzed included acetate, propionate, butyrate, i-butyrate, valerate, i-valerate and caproate, whereas alcohols included methanol, ethanol, propanol and butanol. Mixed liquor sample of 1 mL was first filtered through a 0.2 μm membrane, acidified by formic acid and measured for free acids. The initial temperature of the column was 70 °C for 3 min followed with a ramp of 10 °C/min and a final temperature of 180 °C for 4.5 min. The temperatures of the injector and detector were 200 and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 25 mL/min.

The biomass concentration was measured by VSS according to the Standard Methods [21].

2.3. Kinetic modeling

The cumulative hydrogen production in the batch experiments followed the modified Gompertz equation [22,23].

$$H = P \exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\}, \quad (1)$$

where H is the cumulative hydrogen production (mL), λ the lag time (h), P the hydrogen production potential (mL),