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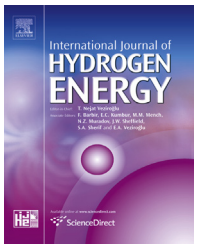

Enriching hydrogen-producing bacteria from digested sludge by different pretreatment methods

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ABSTRACT

The pretreatment of digested sludge by different methods, including ionizing irradiation, heat-shock, acid and base, was performed for enriching hydrogen-producing bacteria. These methods were evaluated and compared based on their suitability in the enrichment of hydrogen-producing bacteria in dark fermentation with glucose as a substrate in batch tests. The experimental results showed that the seed sludge pretreated by ionizing irradiation achieved the best hydrogen production among the different pretreatment methods, and the maximum hydrogen production potential, maximum hydrogen production rate, hydrogen yield and substrate degradation rate were 525.6 mL, 37.2 mL/h, 267.7 mL/g glucose (2.15 mol/mol glucose) and 98.9%, respectively. Ionizing irradiation can be a good optional pretreatment method for enriching hydrogen-producing bacteria from digested sludge. The effect of ionizing irradiation on the microbial community structure dynamics of the pretreated sludge deserves further study, which will help us to understand the mechanisms leading to the effect of high bio-hydrogen production.

Introduction

Energy crisis and environmental degradation is prominent in contemporary society. Growing population and economic development lead to more energy demand as well as environmental deterioration, climate change and global warming. It is urgent to develop renewable and clean energy. Hydrogen (H_2) shows really broad prospects in the process of fuel evolution: it is the most plentiful element in the universe, which ensures various conversion pathways for its production, and has the highest energy yield with 122 kJ/g and its combustion is totally clean with water as the sole end product [1,2].

Biological hydrogen production attracts more and more attention because it is mild, environmental-friendly and economical. Furthermore, it supplies the potential of energy production from variety of renewable sources, especially the degradation of organic wastes. Biological hydrogen production consists of dark fermentation, photo fermentation and biophotolysis. Dark fermentation process is more favorable for its independency of light, generally high rate of hydrogen generation, simple reactor as well as easy control.

Hydrogen production with dark fermentation depends on the obligate and facultative anaerobic bacteria (pure or mixed) presenting in the system and the hydrogen producing efficiency is greatly affected by the microbial communities. As

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the elimination of hydrogen consuming bacteria as well as the distribution of microorganisms, particularly dominant hydrogen-producing species in mixed system are all closely connected to the pretreatment method of mixed culture, different pretreatment methods for enriching hydrogen producers are widely studied by researchers from all around the world. Pretreatment methods reported include: heat-shock [3–11]; addition of chemical inhibitors such as acid, alkali, chloroform, 2-bromoethanesulfonic acid (BESA) etc. [12–16]; ultrasonication [17–20]; load shock [21]; microwave [21]; and electric field [22].

Ionizing irradiation technologies use gamma sources and electron accelerators for different applications, including material modification, food sterilization, environmental protection, and so on. Ionizing irradiation may modify physical, chemical and biological properties of materials. It has been widely used in the field of pollution control [23], especially for the removal of hardly degradable substances [23–26]. When pure water or aqueous solutions are irradiated by gamma irradiation, highly reactive products were produced and react with other substances in the aqueous environment. Extensive literature search indicates that few researches have adopted ionizing irradiation as the pretreatment method for enriching hydrogen-producing bacteria, and considered that the spores of spore-forming bacteria own low core water content, which may helpful to reduce the ability of gamma irradiation to generate hydroxyl radicals. Thus, we expect ionizing irradiation as a pretreatment method helps enriching spore-forming hydrogen producers. Some researchers have pointed out that spores have a better chance to survive under ionizing irradiation than growing cells [27].

The objective of this study was to explore the effect of ionizing irradiation on the hydrogen-producing ability of seed sludge, and to compare it with the most commonly used pretreatment methods including heat-shock, acid and base pretreatment.

Materials and methods

Seed sludge and pretreatment

Anaerobic sludge was obtained from a primary anaerobic digester at Beijing Xiaohongmen Sewage Treatment Plant (China). It was used as seed sludge. The concentration of mixed liquor volatile suspended solids (MLVSS) of the digested sludge was 2.42 g/L.

Four different pretreatment methods were used in this study: ionizing irradiation, acid, base and heat-shock. For ionizing irradiation, seed sludge was treated with 5 kGy dose in sealed bottle with a working volume of 100 mL at ambient temperature (around 25 °C). ^{60}Co-source gamma ray was used in this study, its radioactivity was around 3.6×10^{14} Bq. The seed sludge was irradiated at dose rate of 286 Gy/min. The heat-shock pretreatment was carried out by boiling the sludge at 100 °C for 15 min. The acid pretreatment was conducted by adjusting the pH of seed sludge to 3.0 with 1 mol/L of HCl and maintained for 24 h at 25 °C. The base pretreatment was performed by adjusting the pH of seed sludge to 10.0 with 1 mol/L of NaOH and maintained for 24 h at 25 °C.

Biohydrogen production

Before the hydrogen production process, pretreated sludge was pre-cultured for 48 h to cultivate the microorganisms. For the inoculum preparation, 30 mL of differently pretreated seed sludge (including 30 mL untreated sludge as the control test) were inoculated in the medium containing 50 g/L glucose, 0.5 g/L yeast extract, 10 g/L peptone, 20 mL of nutrient solution (each liter of nutrient solution contains 40 g $NaHCO_3$, 5 g NH_4Cl, 5 g $NaH_2PO_4 \cdot 2H_2O$, 5 g $K_2HPO_4 \cdot 3H_2O$, 0.25 g $FeSO_4 \cdot 7H_2O$, 0.085 g $MgCl_2 \cdot 6H_2O$, 0.004 g $NiCl_2 \cdot 6H_2O$), then filled with de-ionized water to 200 mL in 250 mL Erlenmeyer flasks. The initial pH was adjusted to 7.0 by 1 mol/L HCl and 1 mol/L NaOH. The flasks were placed in a reciprocal shaker (100 r/min) at constant temperature (36 °C) for 48 h incubation, and the cultures were used as inoculum in the following batch experiments.

Batch experiments of hydrogen producing process were performed in 250 mL Erlenmeyer flasks, silicone rubber stoppers were used to ensure the tightness. Inoculum mentioned above were inoculated in fresh medium, which comprises 2 g of glucose, 20 mL of nutrient solution and filled with de-ionized water to total volume of 200 mL. Initial pH of cultivation media was adjusted to 7.0 with 1 mol/L HCl and 1 mol/L NaOH. Argon gas was passed through the culture medium for 3 min to drive away the residual oxygen before incubation. During the fermentation process, bottles were placed in a reciprocal shaker (100 r/min) at constant temperature of 36 °C for 48 h. Each batch test was conducted in three replicates.

Analytical methods

The volume of biogas produced was determined at room temperature (25 °C) by measuring the water displaced by the gas produced. The fraction of H_2 in the biogas was measured by a gas chromatograph (model 112A, Shanghai, China), equipped with a thermal conductivity detector (TCD) and a packed column (model TDX-01, long 3 m, diameter 3 mm). The temperature of the column, detector and injector were 160 °C, 110 °C and 180 °C, respectively. Argon was used as the carrier gas and the pre-column pressure was 0.2 MPa. All gas production data reported were converted to the standard conditions (0 °C, 760 mm Hg). The volatile fatty acids (VFA) were analyzed using an ion chromatograph (Dionex model ICS 2100) equipped with a dual-piston pump, a Dionex IonPac AS11-HC analytical column (4 × 250 mm), an IonPac AG11-HC guard column (4 × 50 mm), and a DS6 conductivity detector. The concentration of glucose remaining after the reaction was determined using DNS colorimetric method.

Results and discussion

Effect on hydrogen production

Biogas produced in each batch was examined with Gas Chromatogram and the results showed that only carbon dioxide and hydrogen was observed in all tests. Possible reason of no detectable methane is that seed sludge of different pretreatment including control test were obtained from a primary anaerobic digester, which was dominated by the