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Influence of Ni^{2+} concentration on biohydrogen production

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ABSTRACT

In this paper, the effect of Ni^{2+} concentration ranging from 0 to 50 mg/L on fermentative hydrogen production by mixed cultures was investigated in batch test. The results showed that at 35 °C and initial pH 7.0, Ni^{2+} was able to enhance the hydrogen production rate with increasing Ni^{2+} concentration from 0 to 0.2 mg/L, and enhance the hydrogen production potential and hydrogen yield with increasing Ni^{2+} concentration from 0 to 0.1 mg/L. The maximum hydrogen production potential of 288.6 mL and the maximum hydrogen yield of 296.1 mL/g glucose were obtained at the Ni^{2+} concentration of 0.1 mg/L. In all tests, the major soluble metabolites produced by mixed cultures were ethanol, acetic acid and butyric acid, without propionic acid. Ni^{2+} had little effect on the substrate degradation efficiency with increasing concentration from 0 to 50 mg/L. Ni^{2+} was able to enhance the biomass production yield with increasing Ni^{2+} concentration from 0 to 0.1 mg/L. The maximum biomass production yield of 232.5 mg/g glucose was obtained at the Ni^{2+} concentration of 0.1 mg/L. In all tests, the final pH after fermentative hydrogen production was lower than the initial pH.

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1. Introduction

Environmental pollution due to the use of fossil fuels as well as their shortfall makes it necessary to find alternative energy sources that are environmentally friendly and renewable. Hydrogen satisfies the above requirements because it produces only water, when it is combusted as a fuel or converted to electricity. Among various hydrogen production processes, biological method is known to be less energy intensive, for it is carried out at ambient temperature and pressure. Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production. The efficiency of photosynthetic hydrogen production is low and it cannot be operated in the absence of light, while fermentative hydrogen production can produce hydrogen all day long without light using various kinds of substrates such as organic wastes, and has higher hydrogen production efficiency, higher hydrogen production stability, simpler control requirements, lower operating costs and higher feasibility for industrialization. Thus fermentative hydrogen production is more feasible and widely used. Especially, it is of great significance to produce hydrogen from organic wastes by fermentative hydrogen production, because it plays the dual role of waste reduction and energy production (Wang and Wan, 2008; Xing et al., 2008).

Hydrogenases that are able to catalyze the oxidation of hydrogen or the reduction of proton are classified into two major families: the [Ni–Fe] hydrogenases and the [Fe–Fe] hydrogenases, according to the metal content at their active site (Frey, 2002). Among them, the [Ni–Fe] hydrogenases are widely distributed among bacteria, whereas the [Fe–Fe] hydrogenases are restricted to a few bacteria. Moreover, the [Ni–Fe] hydrogenases have a higher substrate affinity than the [Fe–Fe] hydrogenases (Casalot and Rousset, 2001). The [Ni–Fe] hydrogenases basically consist of two subunits, a large one and a small one, and contain 1 nickel atom and usually about 12 iron atoms per molecule, and most [Ni–Fe] hydrogenases possess two [4Fe–4S] clusters and often a [3Fe–4S] cluster (Albracht et al., 1995).

During a hydrogen production process catalyzed by the [Ni–Fe] hydrogenases, electrons are transported through an intra-molecular electron transfer chain from the redox partner of the [Ni–Fe] hydrogenases (such as NADH or NADPH) to the active site, meanwhile, protons are also transferred to the active site, and then the protons are reduced by the electrons at the active site to produce hydrogen (de Lacey et al., 2005; Kim et al., 2008).

Since nickel is a fundamental component making up the [Ni–Fe] hydrogenases, it may influence the fermentative hydrogen production by influencing the activity of [Ni–Fe] hydrogenases and thus may play an important role in fermentative hydrogen production. Even though at a higher concentration, nickel may inhibit the activity of [Ni–Fe] hydrogenases, a trace level of nickel is required for activation or function of [Ni–Fe] hydrogenases and thus is conducive to fermentative hydrogen production (Lin and Lay, 2005; Li and Fang, 2007a).

So far, most of the studies on fermentative hydrogen production have focused on how the substrate and its concentrations, temperature, pH and the like affect fermentative hydrogen production,

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with few researches focusing on how the micronutrients, especially nickel, influence fermentative hydrogen production (Idania et al., 2005; Kim et al., 2006; Levin et al., 2006; Lin et al., 2006; Yokoyama et al., 2007).

Since nickel is essential to the fermentative hydrogen production and the studies on how Ni^{2+} concentration affects the fermentative hydrogen production are rare, the objective of this study was to investigate the effect of Ni^{2+} concentration ranging from 0 to 50 mg/L on the fermentative hydrogen production by mixed cultures from glucose in batch tests at 35 °C and initial pH 7.0, with the purpose of obtaining the optimal Ni^{2+} concentration for fermentative hydrogen production by mixed cultures.

2. Methods

2.1. Seed sludge

The digested sludge collected from a primary anaerobic digester at Beijing Gaobeidian Sewage Treatment Plant (China) was used as the seed sludge. The concentration of the volatile suspended solids (VSS) of the sludge was 11.8 g/L. Heat-shock is simple and effective to repress hydrogen-consuming bacteria (Li and Fang, 2007b; Zhu and Béland, 2006). Moreover, analysis showed that the biogas produced by heat-shock pretreated sludge contained only hydrogen and carbon dioxide, without detectable methane, thus heat-shock was used in this study to enrich hydrogen-producing bacteria by heating the seed sludge at 100 °C for 15 min.

2.2. Experimental procedures

Batch tests were conducted in 150 mL glass bottles. One liter of the nutrient solution contained $NaHCO_3$, 40,000 mg; NH_4Cl, 5000 mg; $NaH_2PO_4 \cdot 2H_2O$, 5000 mg; $K_2HPO_4 \cdot 3H_2O$, 5000 mg; $FeSO_4 \cdot 7H_2O$, 15,000 mg; $MgCl_2 \cdot 6H_2O$, 85 mg. Fifteen milliliter of the pretreated sludge, 1 g glucose, 10 mL nutrient solution and certain $NiCl_2$ solution were added to each glass bottle, respectively. And then the total working volume of the bottles was filled to 100 mL by de-ionized water, and the Ni^{2+}concentration in each batch test was from 0 to 50 mg/L. The initial pH of the mixed solution in each bottle was adjusted to 7.0 by 1 mol/L HCl or 1 mol/L NaOH. Each bottle was flushed with argon for 3 min to provide anaerobic condition, and capped with a rubber stopper, and finally placed in a reciprocal shaker (reciprocation: 150 strokes/min). The batch tests were conducted at 35 °C and each batch test was done three times. Data shown are representative results of independent tests that were triplicate.

2.3. Analytical methods

The water displacement method was used to collect and measure the biogas produced. The fraction of H_2 in the biogas was determined by a gas chromatograph (Model 122, Shanghai, China) equipped with a thermal conductivity detector (TCD) and a 2 m column packed with 5 A molecular sieves. Helium was used as the carrying gas at the flow rate of 12 mL/min. The operating temperature of the column, detector and injector were 40 °C, 80 °C and 50 °C, respectively. All gas production data reported were standardized to the standard temperature (0 °C) and pressure (760 mm Hg). The soluble metabolites were also analyzed by a gas chromatograph (Model 8000, Italy) equipped with a flame ionization detector (FID) and a 2 m column packed with GDX-103 (60/80 mesh). The temperature of the column, oven and detector were 180 °C, 240 °C and 210 °C, respectively. Nitrogen gas was used as the carrying gas at the flow rate of 50 mL/min. The pH in the solution was measured by a pH meter (Model 526, Germany). The concentration of glucose after reaction was determined by the DNS colorimetric method (Miller, 1959). The concentration of volatile suspended solids (VSS) was determined according to the procedures described in the standard methods (APHA, 1995).

3. Results and discussion

3.1. Effect on hydrogen production

Fig. 1 illustrates the effect of the fermentation time on the cumulative hydrogen production under different Ni^{2+} concentration. The results showed that all the fermentative hydrogen production finished within 48 h. At the first 18 h of the fermentative hydrogen production, hydrogen production rate had a trend to increase with increasing Ni^{2+} concentration from 0 to 0.2 mg/L, but it decreased with further increasing Ni^{2+} concentration from 0.2 to 50 mg/L.

The lag time of hydrogen production was 18 h with increasing Ni^{2+} concentration from 0 to 0.01 mg/L, and it was 12 h at the Ni^{2+} concentration of 0.02 mg/L. The lag time of hydrogen production was just 6 h with further increasing Ni^{2+} concentration from 0.05 to 0.2 mg/L, while it was 12 h with increasing Ni^{2+} concentration from 0.5 to 10 mg/L and was 24 h with increasing Ni^{2+} concentration from 20 to 50 mg/L. This indicated that in an appropriate range, Ni^{2+} was able to increase the hydrogen production rate with increasing concentration, but Ni^{2+} at much higher concentration could inhibit the hydrogen production.

Hydrogen production potential is defined as the maximum cumulative hydrogen production at each Ni^{2+} concentration. The results showed that the hydrogen production potential in batch tests increased with increasing Ni^{2+} concentration from 0 to 0.1 mg/L, however, it trended to decrease with further increasing Ni^{2+} concentration from 0.1 to 50 mg/L. The hydrogen production potential was lower than that of the control test with increasing Ni^{2+} concentrations from 10 to 50 mg/L. In all tests, the maximum hydrogen production potential of 288.6 mL was obtained at the Ni^{2+} concentration of 0.1 mg/L. This suggested that in an appropriate range, Ni^{2+} could increase the hydrogen production potential with increasing concentration.

The study by Li and Fang (2007a) showed that the hydrogen production potential trended to decrease with increasing Ni^{2+} concentrations from 0 to 50 mg/L, which is different from the result of this study. The possible reasons may be due to the different substrates, pH, the Ni^{2+} concentration range and the seed sludge.

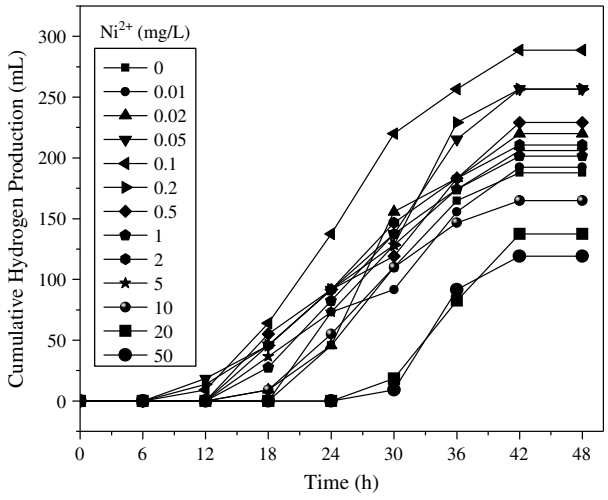

Fig. 1. Effect of the fermentation time on the cumulative hydrogen production under different Ni^{2+} concentration.

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