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Enhanced dark fermentative hydrogen production under the effect of zero-valent iron shavings

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ABSTRACT

Dark fermentative hydrogen production under the effect of zero-valent metal shavings (iron, aluminum and copper) was studied by using a sucrose medium and a mixed bacterial consortium. The iron shavings were found to be unique to promote the hydrogen production, the hydrogen yield obtained from an optimal dose of 8–16 g/L reached 4.2 mol/mol hexose, doubled compared with that obtained from the control without addition of the iron shavings. The effect was more obvious in low pH buffered medium than in higher buffered medium. The aluminum and copper shavings were either inert or toxic to the cultivation. It is evident that the addition of the zero-valent iron helped maintaining the pH to an optimal range for hydrogen production and drove more reducing equivalents to the production of hydrogen. A microbial corrosion system mediated by the hydrogen producing bacteria was proposed to be responsible for the improvement of hydrogen production.

Introduction

In the dark fermentative hydrogen production undertaken by anaerobic bacteria such as *Enterobacter* and *Clostridium*, the pyruvate generated by glycolysis reacts with co-enzyme A to generate acetyl-Co A and either formate or reduced ferredoxin, depending on the bacterium involved. The formate is then cleaved to carbon dioxide and hydrogen; and the reduced ferredoxin directly delivers electrons to hydrogenase where the electrons react with protons to produce hydrogen. Some of the pyruvates are diverted to certain liquid products such as volatile fatty acids and alcohols (typically ethanol) to recover NAD^+ which is necessary for the glycolysis and the followed citric acid cycle. Compared with photo fermentation the dark fermentation has high hydrogen production rate [1–3]. This partly attributes to the fast growth of the anaerobic bacteria and short turnover time of the hydrogenase during the dark hydrogen fermentation [4]. However, it is because of the fast growing nature of the bacteria, a large amount of energy is spent for the anabolic metabolism. In addition, a substantial amount of energy is stored in the intermediate products (such as volatile fatty acids and alcohols). Therefore in the dark hydrogen fermentation, the energy converted to hydrogen

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only takes a small part of the energy contained in the substrate, resulting in poor hydrogen yield. A review [5] revealed that the hydrogen yields by dark fermentative bacteria (pure culture and mixed culture) were less than 2.5 moles H_2 per mol hexose, no matter synthesized media or mixed wastes were used.

Many efforts have been made to improve the hydrogen yield. The methods which have been examined include either genetic manipulation (to optimize the metabolic pathways) or process optimization (to fine-tune the operation parameters such as pH, hydrogen partial pressure, substrate loading, hydraulic retention time, and temperature) [6]. However, these methods did not completely alter the metabolic pathways; therefore the hydrogen yields achieved by these methods would not be substantially increased.

The theoretical hydrogen yield which could be achieved by dark fermentation is 4 moles hydrogen gas from each mole hexose in case 2 mole acetate is co-produced [7]. This yield represents a thermodynamic limitation which the biological metabolism converting the organic substrates to hydrogen could achieve without inputting other physical or chemical energy. Without the limitation of inputting external energy, the maximum theoretic hydrogen yield (also called stoichimetric yield) from glucose which could be achieved by a biological system is 12 moles of hydrogen from each mole of hexose as calculated by the following equation [8]:

$$C_xH_yO_z + (2_{x-z})H_2O \rightarrow (y/2 + 2_{x-z})\ H_2 + xCO_2 \quad (1)$$

By comparing with this stoichiometric yield, the yield obtained by far from the dark hydrogen fermentation only represents a production efficiency of approximate 20%.

While the yield of dark hydrogen fermentation is poor, the photo fermentative hydrogen production system, which is driven by light irradiation, typically has a high efficiency (60–70%) [9]. Liu et al. [10] have reported a dark hydrogen fermentation system assisted with low electrical voltage. With this system, 3 mole of hydrogen were produced from 1 mole of acetic acid. By combining this system with a conventional hydrogen fermentation system, a yield of 8–9 mol of hydrogen per mol of glucose could be achieved. A respiration driven dark hydrogen fermentation in cyanobacteria and algae has been studied by adding a limited amount of oxygen and the hydrogen yield was expected to reach 10 moles per mole glucose [11–13]. All the systems mentioned above provide the pathways to improve the hydrogen yield. However, these methods directly or indirectly depend on light and electricity, their hydrogen production rates are limited by the surface area of light irradiation and electrodes, therefore practically tend to be low.

Zero-valent iron was proposed to reduce halogenated organic compounds in subsurface water in the earlier 1990's [14]; it has been widely accepted as an effective on-site remediation method to degrade various organic pollutants. When zero-valent iron is in an aqueous environment, micro electrochemical cells can be formed on the surface to initiate the corrosion process [15]. The iron is dissolved into water on the anode side of the cell and two electrons are released and transferred to cathodic side where electrons are further transferred to available electron acceptors such as protons, oxygen, and various oxides for depolarization. This metal corrosion can be accelerated by biological process, known as microbial corrosion. Sulfate reductive, methanogenic, nitrate and iron reductive microbial corrosions have been reported [15–18]. In these processes, the microbes participate in the cathodic depolarization reactions by using the hydrogen produced or forming caustic products. A substantial hydrogen production was also observed in a sulfate reductive microbial corrosion system [18]. It is highly possible that the zero-valent iron can be used as a novel supplementary reducing power (electron donor) to drive the dark fermentative hydrogen production and improve the hydrogen yield.

A series of batch cultivations using a mixed culture have been, thus, conducted to examine this concept. A zero-valent iron material (cast iron shavings) was tested in this study. Two other zero-valent metal shavings (aluminum and copper) were also tested as comparison. We here for the first time report that the zero-valent iron shavings is a sound material which can significantly enhance the dark fermentative hydrogen yield to above 4 mol hydrogen per hexose.

Materials and methods

Culture media

A pH buffered sucrose medium [19] which contained 10 g/L sucrose, 1.5 g/L KH_2PO_4, 3.2 g/L $Na_2HPO_4{\cdot}7H_2O$, 0.5 g/L NH_4Cl, 0.18 g/L $MgCl_4{\cdot}6H_2O$, 1.0 g/L yeast extract, 0.5 g/L meat extract and 0.5 g/L peptone was used as a standard medium for hydrogen production. A modified sucrose medium (low buffered) in which the phosphate buffer concentrations were reduced to 0.25 g/L of KH_2PO_4 and 0.65 g/L of $Na_2HPO_4{\cdot}7H_2O$ was also used to study the joint effect of pH buffering and iron shavings on the hydrogen production. The remainder of the phosphate buffer in the modified and lower buffered sucrose media was determined by a COD/TP of 100:1.5 for the minimum physiological requirement of phosphate for the bacterial growth. Except in the preliminary test for pH effect, all the tests used low buffered medium.

Zero-valent metal materials

Three zero-valent metals were used in the experiments: an iron shavings scribed from a spare cast iron pipe part using a file, copper shavings and aluminum shavings scribed from a spare copper pipe and an aluminum sheet respectively. The sizes of the shaving particles were diversified with the length ranged 0.0025–1.5 mm and the width ranged 0.0025–0.5 mm. All the metal shavings used in the experiment were freshly made. There was no any surface treatment for all these materials before use.

Cultivations

A microbial flora cultivated from a digested sludge sampled from local municipal wastewater treatment plant was used as inoculum for all the batch experiments. The method for the cultivation of the hydrogen producing microbial flora was