Hydrogen Production by Biological Processes

Jong-Hwan Shin and Tai Hyun Park*
School of Chemical Engineering, Seoul National University

Abstract

Among biological hydrogen production processes, fermentative processes have some advantages. In this research, the hydrogen producing bacterium was isolated from domestic landfill area and identified as *Enterobacter sp.* The strain was named *Enterobacter sp.* SNU-1453. Important parameters for the hydrogen process include pH, temperature, concentration of initial glucose, and kind of sugars. The pH of the culture medium significantly decreased as fermentation proceeded due to the accumulation of various organic acids, and this inhibited the H_2 production seriously. When pH was controlled at pH 7.0, hydrogen production was 2614.5 ml/l in 17 hours. The increase of glucose concentration resulted in higher H_2 production. The productivity of this strain was 6.87 mmol H_2/l per hr on concentration of 25g glucose/l. *Enterobacter sp.* SNU-1453 could utilize various sugars. These results indicate that *Enterobacter sp.* SNU-1453 has a high potential as a fermentative H_2 producer.

1. Introduction

Today global energy requirements are mostly dependent on fossil fuels. This will eventually lead to the depletion of limited fossil energy resources. In order to remedy the depletion of fossil fuels and their environmental pollution hydrogen has been suggested as the energy carrier of the future. Hydrogen is the fuel of the future mainly due to its high conversion efficiency, recyclability and nonpolluting nature. Hydrogen is the most plentiful element in the universe. In combustion, water is the main product. Therefore hydrogen is regarded as a clean non-polluting fuel. Hydrogen is harmless to human and the environment. Biological hydrogen production processes are found to be more environment friendly and less energy intensive as compared to thermochemical and electrochemical processes [1-3]. Biological hydrogen production processes can be classified into four categories [1]:

(1) biophotolysis of water using algae and cyanobacteria
(2) photodecomposition of organic compounds by photosynthetic bacteria
(3) fermentative hydrogen production from organic compounds
(4) hybrid systems using photosynthetic and fermentative bacteria

In this article, biological hydrogen production processes were reviewed, and the fermentative hydrogen process using the strain isolated from soil was described.

2. Biological Hydrogen Production

2.1 Biophotolysis of Water

Cyanobacteria or blue-green algae are called nitrogen-fixing bacteria and they are capable of biophotolysis, the light-driven splitting of water into hydrogen and oxygen, in reactions which involve the nitrogenase and hydrogenase. Hydrogen production through nitrogenase or hydrogenase has been suggested as a good biological system for photoharvesting hydrogen from water [4].

2.2 Photo-Decomposition of Organic Compounds

Photosynthetic bacteria are indicated in the current literature as the most promising microbial system for the biological hydrogen production. Photosynthetic bacteria use nitrogenase for the hydrogen production. The overall biochemical pathways for the photo fermentation process can be expressed as follows [5]:

$$(CH_2O)_n \longrightarrow \text{Ferredoxin} \longrightarrow \text{Nitrogenase} \longrightarrow H_2$$

And photosynthetic bacteria can also use carbon monoxide for the hydrogen production using microbial shift reaction as follows [6]:

$$CO + H_2O \longrightarrow CO_2 + H_2$$

2.3 Fermentative hydrogen production

Hydrogen production by fermentation has been treated with little attention. But fermentative hydrogen production processes have some advantage:

(1) Fermentative bacteria have very high hydrogen production rate.

(2) Fermentative bacteria can produce hydrogen without light.

(3) Fermentative bacteria have very high growth rate.

There are two major metabolic pathways for the hydrogen production. One is the mixed acid fermentation or 2,3-butandial fermentation pathway, which evolves hydrogen through formate decomposition with formate hydrogen lyase [7].

$$\text{Glucose} \longrightarrow \text{Pyruvate} \longrightarrow \text{Formate} \longrightarrow H_2 + CO_2$$

The other is NADH pathway, which is made during the fermentative conversion of glucose to pyruvate called glycolysis [8].

$$NADH + H^+ \underset{\text{hydrogenase}}{\longrightarrow} H_2 + NAD^+$$

2.4 Hybrid System

Hybrid system comprises of non-photosynthetic and photosynthetic bacteria. Fermentative bacteria produce hydrogen and organic acids with the degradation of carbohydrates without using light. Resulting organic acids could be sources for photosynthetic bacteria to produce hydrogen. The combination of the both kinds of bacteria not only reduces the light energy demand of photosynthetic bacteria but also increases hydrogen production [9].

3. Results and Discussion

3.1 Screening and Identification

Soil samples collected from several domestic landfill areas were incubated into 50 mL of LB medium for several hours. After the incubation, 0.5 mL of supernatant was transferred into 50 mL of minimal medium composed of 50 mM L-HPA as a sole nitrogen source, 50 mM potassium phosphate buffer (pH 7.2), 100 mM glycerol, 1 g/L $MgSO_4 \cdot 7H_2O$, 0.2 mM $CaCl_2$, 0.1 mg/L $ZnCl_2$, 0.1 mg/L $MnSO_4 \cdot 4H_2O$, 0.02 mg/L H_3BO_3, 0.1 mg/L $CuSO_4 \cdot 5H_2O$, 0.05 mg/L $CoCl_2$, 0.1 mg/L $NiSO_4 \cdot 6H_2O$, 2.0 mg/L $NaMoO_4$, and 4.0 mg/L $FeSO_4 \cdot 7H_2O$. After 2 days of cultivation at 37°C, 0.5 mL of the culture broth was diluted with 50 mL of the same minimal medium (~100-fold dilutions). In order to simulate enrichment culture, these procedures were repeated three times. After the enrichment steps the culture broth was spread out on the agar plate with the same composition as the minimal media. Each colony on the plate was cultivated in the same kind of minimal or LB media. This strain stored in 25% glycerol solution at -70℃ for further study [10].

After 16S rRNA gene analysis of this strain, the results compared with Ribosomal Database Project(RDP) for the identification of this strain. As a result of identification, this strain was named *Enterobacter sp.* SNU-1453.

3.2 Effect of pH on Productivity

The pH of the culture medium significantly decreased as fermentation proceeded due to the accumulation of various organic acids, and this inhibited the H_2 production seriously. When pH was controlled at pH 7.0, hydrogen production and productivity were 632.8 ml/L and 4.0 mmol/L/hr with 10 g glucose/L in 7 hours, respectively. Fig. 1 shows the effect of pH on the productivity. *Enterobacter sp.* SNU-1453 could produce H_2 from glucose under wide ranges of pH (4 ~ 7.5). The optimal pH of *Enterobacter sp.* SNU-1453 was 7.

3.3 Batch Fermentation Profiles of Glucose as a Substrate

As the initial glucose concentration increased, both the final cell concentration and H_2 production increased. Fig. 2 shows batch fermentation profile of *Enterobacter sp.* SNU-1453 for H_2 production on 25g glucose/L. The maximal H_2 production rate and productivity were 398 ml/L/hr and 6.87 mmol/L/hr, respectively.

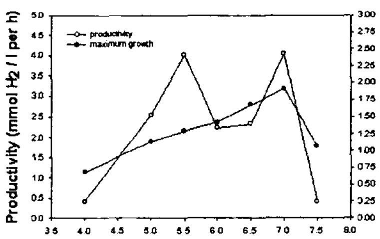

Fig. 1. Effect of pH on Productivity at 37℃

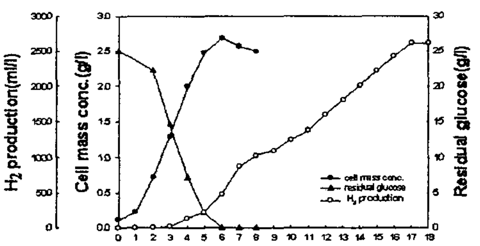

Fig. 2. Batch Fermentation of *Enterobacter sp.* SNU-1453 for H_2 Production on 25 g/L of Glucose Concentration

4. References

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