Enhanced Hydrogen Production by Various Type of Co-cultures of Facultative Anaerobes and Photosynthetic Bacteria

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Summary

In order to produce H_2 gas as clean energy source using biomass conversion, we studied on how the carbon source was converted efficiently by use of our co-culture system of facultative anaerobes and photosynthetic bacteria. For the first stage, *Enterobacter aerogenes* HU-101, was chosen for its rapid hydrogen production from glucose with by_products of organic acids and alcohols, some of which could be further converted into hydrogen by a photosynthetic bacteria, *Rhodobacter sphaeroides* RV. Based on that assumption, the strains HU-101 and RV were co-immobilized in agar gel, which was pre-cultured in liquid media. Following the pre-culture, the co-cultured gel was irradiated in 10klux and glucose was converted hydrogen gas with the yield of 3.15 moles of hydrogen per glucose. The reason for the low yield attributed to the production of alcohols which were not used by RV strain. By changing the anaerobe to lactic fermenter, we were further improving the yield. It was founded that changing to *Lactobacillus* was a significant improvement. Finally, we succesfully achieved at most 7.08moles H_2 gas production from one mole of glucose.

Keyword : Bio hydrogen, Organic waste decomposition, Photosynthetic bacteria, Co-culture

1. Introduction

Nowadays, the discharged organic waste is serious problem in enviroment. The 50 million tons of garbage a year is being discharged from the household and industry in Japan, and about its 30% is a organic garbage[1)]. Composting as fertilizer by using the raw garbage are one of the recycling ways. However, because of salt consent and transport charges are concerning situation. Therefore, new technology for resource recycling are enthusiastically required. So we must consider the alternative way of technology.

One of the prominent tool is bioconversion of discarded organic materials and industrial waste using function of organism.

The bio-generated hydrogen is clean energy source of primary energy, fuel battery and electricity[1)].

Our present study is focused on the attempt to obtain high yield production of hydrogen by use of combination of facultative anaerobes and photosynthetic bacteria. These anaerobes are able to utilize wide range of substance from various sugar source, starch, cellulose and plant materials such as molasses. We attempeted to produce hydrogen in both ways of hydrogenase of *Enterobacter aerogenes* HU-101 [2) 3) 4)], and by use of nitrogenase of photosynthetic bacteria

Rhodobacter sphaeroides RV. In this paper, we like to report on our unique technology of mixed fermentation using *E. aerogenes* HU-101, or *Lb. delbluechii*, as lactic acid fermenter and *Rh. sphaeroides* RV, or *Rh.shaeroides* No.7 as photosynthetic bacteria to produce hydrogen.

2. Material and Methods

2.1 Culture condition of *Rhodobacter sphaeroides* RV and *Rhodobacter sphaeroides* No.7

Rhodobacter sphaeroides RV and *Rhodobacter sphaeroides* No.7 were provided from Tissue Engineering Research Center in Tsukuba in Japan. The RV was cultured in a growth media (aSy), which consisted of a basal medium, ammonium salfate, sodium succinate, and yeast extract. The pH of the medium was adjusted to 6.8. The aSy medium was sterilized in an autoclave before being used for the culture. The cell were anaerobically cultivated at 30℃ for 48h under a light intensity of approximately 10 kLux.

2.2 Culture Condition of *E.aerogenes* HU-101

E.aerogenes HU-101 was cultivated in a growth medium with glucose, triptone and yeast extract (LB) at 30℃. The pH was adjusted to 6.3. These bacteria were facultative anaerobe having Gram-negative stained. There was no inhibition of growth by O_2, we agitated it in conical flask. The growth reached to plateau period in six hours and it was able to carry out anaerobic hydrogen generation immediately afterwards.

2.3 Culture Condition of *Lb. delbrueokii*

Lb.delbrueokii was cultivated in a growth medium (GYP) at 30℃ for about 24h, which consisted of glucose, yeast extract, bacto peptone and sodium acetate. The pH of the medium was adjusted to 6.8. These bacteria were facultative anaerobe without Gram-positive atrain. It is homolactic fermenter which can make lactic acid (2mol) from glucose (1mol) thoretically.

2.4 Hydrogen production in modified glucose media condition

First, all of the bacteria were grown to mid-log phase and were high viability in pre-culture.

The bacteria were harvested by centrifugation at 9000 rpm for 15min and they were washed once in basal medium. And then, they were suspened and their concentraion were set at 0.75 mg/ml dry weight cell.

And then, these bacteria were co-immobilized in agar gels. The immobilized gels were developed and cultured in the 200 ml flasks. The gel in flask was 30 ml which was consist of mixed of 15 ml bacterial innoculum and 15 ml autoclaved agar solution (4% w/w). In this culture, bacterial innoculum was set in co-culture. The immobilized bacteria were pre-cultured in the same flask in light-anaerobically in 180 ml LB or GYP medium. And then, second pre-culture, LB or GYP medium was carried out in 180 ml glutamate-lactate medium (GL medium) to induce hydrogen evolution with the activity of nitrogenase. After pre-culture, the medium was changed to Glucose-Glutamate medium (gG medium). The flask was sealed by silicon with two needles for collection of H_2 gas from sampling medium.

The hydrogen production was carried out in a polyacrylic water bath at 30℃. The incident

illumination for culture was maintained at 10 kLux.

2.5 Analysis

The hydrogen concentration was analyzed by a Shimadzu gas-chromatograph. Organic acid in medium were analyzed by Shimadzu LC10A HPLC which was equipped with Ultron PS-80H column at 60℃.

The glucose concentration of gG medium was determined by using Glucose C Ⅱ-Test (Wako, Japan) .

3. Results

Experiment of hydrogen production from co-cultivation of E.aerogenes HU-101 and Rh.sphaeroides RV

E.aerogenes HU-101 and *Rh.sphaeroides* RV were co-immobilized and pre-cultivatied in PGY medium and gLmedium, after they were grown in synchronized condition to mid-log phase. And then media were changed to gG media for more efficient production of hydrogen.

Effect of the OD ratio co-cultivated E.aerogenes HU-101 and Rh.sphaeroides RV

The OD ratio of co-immobilized *E.aerogenes* HU-101 to *Rh.sphaeroides* RV was changed from 1:5, 1:10, 1:15 and up to 1:20 and they were produce hydrogen from gL media. The ratio of mixed cells 1:5 was the optimum condition in the ration of co-culture. And then the yield of hydrogen per mol glucose was 3.15 mol/mol glucose at most.

Effect of the OD ratio co-cultivated E.aerogenes HU-101, Rh.sphaeroides RV and Rh.sphaeroides No.7

The OD ratio of co-immobilized *E.aerogenes* HU-101 to *Rh.sphaeroides* RV and *Rh.sphaeroides* No.7 were changed from 1:0:0, 1:0:5, 1:5:5, 1:5:3, 1:5:1, up to 1:5:0 and they were set to produce hydrogen from gG media. The optimal immobilizing ration was 1:5:3 respectively. And then, the yield of hydrogen per mol glucose was 3.01 mol / mol.

This experiment was carried out using roux flask sealed with a needle dispenser for colecting H_2 gas. When gas was sampled, a small volume of oxygen was go through needle into roux flask. Considering anaerobic condition, after gas was sampled, argon gas was introduced into roux flask for keeping anaerobic condition. The optimal immobilizing ration was 1:5:3 and the yield of hydrogen per mol glucose was 3.11 mol / mol glucose.

Experiment of growth phase hydrogen production by co-cultivate of Lb.delbrueckii and Rh.sphaeroides RV

Lb.delbrueckii was over growth in PGY medium and it produced more lactate in the medium. And the tact caused high pH condition. Therefore, the PGY concentration was diluted in 1/5 at the first pre-cultivation medium and the OD ratio of co-immobilized *Lb.delbrueckii* and *Rh.sphaeroides* RV was modified to 1:5 and 1.7:5 and they produced hydrogen from gG medium. The optimal immobilizing ratio was 1:5. And from these experiment, the yield of hydrogen per mol glucose was greatly increased to 7.08 mol / mol glucose. The yield was approximately 59 % of theoretical yield.

The production of hydrogen shown in respect to time course of glucose consumption in gG medium.

Glucose was consumed mostly in 4 to 5 days.

4. Discussion

Almost photosynthetic bacteria are not able to utilize sugar directly but it can utilize organic acid in preference. Miyake et al. established efficient production system of hydrogen from sugar by co-culture of photosynthetic bacteria and anaerobic bacteria. These anaerobic bacteria carried out exergonic reaction (Sugar → H_2 + Organic acid), On the other hand the photosynthetic bacteria carried out endergonic reaction (Organic acid → H_2 + CO_2) using light source as photo energy).

Conclusion

Our recently developed culturing two type of microbes are key-issue to reuse of organic waste into biohydrogen for the use of energy source for fuel cell batteries Our E.aerogenes HU-101 and Rh sphaeroides RV strain were imobilised in mixed culture system works as efficient tool to produce biohydrogen from ono industrial waste . We liked to investigate further option of selecting fermentatived microbes to provide more biohydrogen wastes from enviromentaly hazardous organic wastefrom industry.

Reffrence

1） Miyake, J., et al., J. Ferment. Technol., 62, 531-535 (1984).

2） Rachman, M., et al., J. Ferment. Bioeng. 83, 358-363 (1997).

3） Yokoi. H., et al., Biomass and Bioenergy, 22, 389-395 (2002)

4） Nakashimada.Y.,et al., International J. Hydrogen energy, 27, 1399-1405 (2002)