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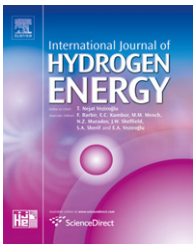

Bio-hydrogen production by mixed culture of photo- and dark-fermentation bacteria

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ABSTRACT

Clostridium butyricum and *Rhodopseudomonas faecalis* RLD-53 were employed to produce hydrogen in mixed culture with glucose as sole substrate. Due to the great difference on growth rate and acid-resistant capacity between photo-fermentative bacteria and dark-fermentative bacteria, directly mixed culture of the two kinds of bacteria in different ratio was studied in this work. Hydrogen yield, volatile acids, pH and biomass in different periods were evaluated. Acetic acid and butyric acid produced by *C. butyricum* were dominant terminal fermentation products, and they were effective substrates for photo-fermentative bacteria. The cooperation was formed in a way like food chain. But compared to the production rate of volatile acids produced by *C. butyricum*, the utilization rate by photo-fermentative bacteria was far slower. The results demonstrated that the growth of photo-fermentative bacteria was limited when pH decreased sharply. The best ratio of *C. butyricum* to *R. faecalis* RLD-53 was 1:600. The maximum yield of hydrogen reached 122.4 ml-H_2/vessel and hydrogen production rate was 0.5 ml-H_2/ml-culture/day.

1. Introduction

Hydrogen is acknowledged as an ideal clean energy carrier in 21st century. Dark-fermentation bacteria, photo-fermentation bacteria and algae are main functional microorganisms in hydrogen production. Volatile organic acids (VFAs) are generated from large molecular substrates by dark-fermentation, such as acetic acid, propionic acid and butyric acid. Those acids lead to a sharp decrease of pH and H_2 production was limited. However, photo-fermentation bacteria can further use VFAs to produce H_2. So mixed culture of photo- and dark-fermentation bacteria has been concerned by researchers for a high efficiency hydrogen production.

The approach of mixed-culture pattern has been proved to be feasible. And at the same time, it is found that the cooperation of the two kinds of functional bacteria can bring out a higher production yield and energy needed by photosynthetic bacteria is saved in the process. Weetall et al. [1] used agar immobilization of *Rhodospirillum rubrum* and *Klebsiella pneumoniae* in mixed way to produce hydrogen from cellulose and max hydrogen yield reached 6 mol-H_2/mol-glucose. Odom and Wall [2] mixed *Rhodopseudomonas capsulata* and *Cellulomonas*. sp to utilize cellulose for hydrogen and the result showed 4.6–6.2 mol-H_2/mol-glucose. Miyake et al. [3] increased hydrogen yield from 1.1 mol-H_2/mol-glucose to 7 mol-H_2/mol-glucose by combination of photosynthetic

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bacteria and *Clostridium butyricum*. Yokoi et al. [4] got the level of 6.6 mol-H_2/mol-glucose by mixture of *C. butyricum* and *Rhodobacter* sp. M-19, and it was concluded four times higher than single anaerobic bacteria. Because of the different properties between the two kinds of bacteria, it was hard to coexist and difficult to study further. In recent years, Asada et al. [5] immobilized *Lactobacillus* and *Rhodobacter sphaeroides* RV together for hydrogen from glucose and the results were 7.1 mol-H_2/mol-glucose. Herbert H.P. Fang et al. [6] mixed *C. butyricum* and *R. sphaeroides* to produce hydrogen and made quantitative analysis of two microbial communities in FISH technology. In a conclusion, the mixed culture of photo- and dark-fermentation bacteria can improve utilization rate of substrate and enhance hydrogen yield. But the further studies focus on how to make bacteria in good cooperation and how to control the substrate conversion in a food chain.

In this study, a mixed-culture pattern was investigated in different ratios of *C. butyricum* and *Rhodopseudomonas faecalis* RLD-53. Glucose was used as the sole substrate for hydrogen production. The hydrogen yield, volatile acids, pH of system and biomass were determined. It is expected that the results of pilot studies obtained from this study could provide useful information for further mixed-culture hydrogen production.

2. Material and methods

2.1. Bacterium and growth conditions

The photo-fermentation bacterium, *R. faecalis* RLD-53, was isolated from freshwater pond sludge [7]. The previously described medium [8] was used as the medium for pre-culture of photo-fermentation bacterium (PFBM).

The dark-fermentation bacterium, *C. butyricum*, was purchased from China General Microbiological Culture Collection Center, AS 1.209. The medium for pre-culture for Dark-fermentation bacterium (DFBM) consists of per l.0 L, glucose 9 g, $(NH_4)_2SO_4$ 2 g, yeast extract 1 g, K_2HPO_4 3.4 g, KH_2PO_4 1.3 g, $MgCl_2{\cdot}6H_2O$ 0.2 g, $CaCl_2$ 0.1 g, NaCl 0.1 g, L-cysteine·HCl·H_2O 0.5 g, trace element 1 ml, Vitamin 1 ml. The pH of the medium should be at 7.0. Trace element was same with PFBM.

2.2. H_2 production from glucose by the mixed culture

The hydrogen production experiment was carried out in triplicate with 50 ml of the medium in 100 ml serum bottles, which were sealed by rubber plugs and filled with argon to maintain anaerobic conditions. The OD_{660} came to 2.49 after pre-culture of *R. faecalis* RLD-53 for 24 h, and The OD_{660} came to 2.68 after pre-culture of *C. butyricum* for 24 h. *C. butyricum* and RLD-53 were mixed in ratio of 1:100, 1:200, 1:300, 1:400, 1:500 and 1:600, respectively. Inoculants (5 ml) of mixed culture were put into sterile fresh mixed-culture medium. The bottles were shaken at 120 rpm/min at constant temperature of 35 °C; the light intensity on the outside surface of the bottles was maintained at 4000 lx by incandescent lamps (60 W).

The hydrogen production medium for mixed culture consists of per l.0 L, glucose 9.0 g, sodium glutamate 1.0 g, yeast extract 1 g, K_2HPO_4 3.4 g, KH_2PO_4 1.3 g, $MgCl_2{\cdot}6H_2O$ 0.2 g, $CaCl_2$ 0.1 g, $FeSO_4{\cdot}7H_2O$ 0.012 g, NaCl 0.1 g, EDTA–Na 0.1 g, L-cysteine.HCl·H_2O 0.5 g, trace element 1 ml, Vitamin 1 ml. The pH of the medium should be at 7.0. Trace element was same with PFBM.

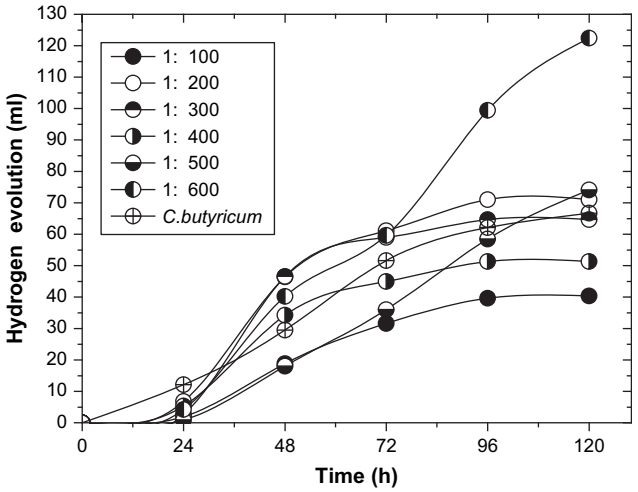

Fig. 1 – Hydrogen production by mixed culture of *C. butyricum* and RLD-53 in different ratio.

2.3. Analytical methods

Concentrations of glucose in the supernatants of culture broth were determined by the oxidase method. The volatile fatty acids in supernatant of the culture broth, and H_2 analysis in evolved gas were determined according to the method of Xing et al. [9]. The light intensity was measured by using a digital luxmeter (TES1330A, Junkai Co.). Cell concentration was determined by an Amersham pharmacia biotech ultrospec 34300 UV/Vis spectrophotometer.

3. Results and discussion

3.1. Hydrogen production by mixed culture

Fig. 1 showed that hydrogen yield increased with the ratio of dark-photo bacteria from 1:100 to 1:200. However, it decreased

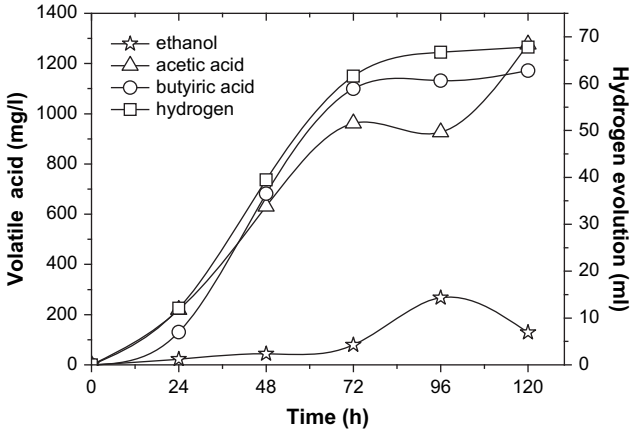

Fig. 2 – VFAs production by pure culture *C. butyricum*.