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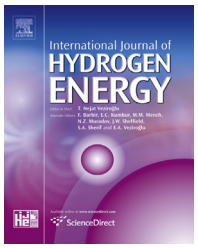

Photo-fermentative hydrogen production from mixed substrate by mixed bacteria

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ABSTRACT

Photo-fermentative H_2 production by mixed bacteria and pure bacterium *Rhodopseudomonas faecalis* RLD-53 using single and mixed substrate as carbon source was investigated in batch culture. Experimental results showed that 60 mmol/L acetate was the optimal concentration for mixed bacterial H_2 production and maximum cumulative H_2 volume was 2468 ± 123 mL H_2/L-culture. It was also found that propionate or butyrate was a key factor for enhancing H_2 production in mixed substrate system. Photo-H_2 production can be greatly promoted when proper concentration of propionate and butyrate were added into acetate medium as mixed substrate and a higher H_2 yield of 2931 ± 146 mL H_2/L-culture was obtained. In addition, it was worth noting that when the strain RLD-53 was added into mixed bacteria with different concentration ratios, H_2 yield did not yet increase. Interestingly, H_2 production capacity gradually decreased with ratio of strain RLD-53 to mixed bacteria from 8:0 to 4:4, and then gradually increased from 4:4 to 0:8. This implied that the competition relationship between strain RLD-53 and mixed bacteria in substrate utilization strongly influenced their H_2 production.
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Introduction

Hydrogen is a potential alternative, clean and renewable energy carrier, which is considered as the substitute of fossil fuel in the future. Bio-hydrogen production technology, especially photo-fermentation hydrogen production has attracted much attention in recent years because of its high conversion efficiency and wide utilization range of substrate. It is well known that photo-fermentation bacteria can convert various organic acids, such as acetate, lactate and butyrate, into hydrogen through catalysis of nitrogenase under light energy. Usually, these short chain organic acids are produced by dark fermentation. Thus, many researchers combined dark with photo-fermentation for hydrogen production and expected to obtain high hydrogen yield. But only few successful cases of high substrate conversion efficiency were reported [1–3], because the performance of photo-fermentative hydrogen production was highly dependent on the composition and concentration of organic acids from dark fermentation, which

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was a key determined the hydrogen production efficiency of photo-fermentation process. However, up to now, the information about the effect of composition of mixed acids on photo-hydrogen production is still lacking. And most studies focus on using one substrate for hydrogen production [4–6] and only few reports are about utilizing two substrate for hydrogen production [7,8]. To date, a large number of studies on photo-hydrogen production have been carried out by bacterial pure culture [9–12], but mixed bacteria are more potential for practical applications. So, the photo-fermentation hydrogen production by mixed bacteria using mixed acids as substrate is more likely to further enhance substrate conversion efficiency in industrial scales.

Therefore, this work investigated the photo-hydrogen production by mixed bacteria using single and mixed substrate. In addition, *Rhodopseudomonas faecalis* RLD-53, which was isolated from freshwater pond sludge and showed excellent ability for hydrogen production, was re-added into mixed bacteria system to enhance hydrogen production by bioaugmentaion.

Material and methods

H_2 producer and medium

The photo-fermentative bacterium *R. faecalis* RLD-53 was used in this study [13]. Mixed bacteria (belong to *Rhodopseudomonas* genus) were provided by the Research Center for Environmental Biotechnology, HIT of China. Growth medium (1 L) contained 1.0 g CH_3COONa, 1.0 g $C_4H_4Na_2O_4$, 1.0 g NH_4Cl, 1 g beef extract, 0.5 g peptone, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.1 g NaCl, 0.2 g $MgCl_2$, 0.08 g $CaCl_2$, 1.0 g $NaHCO_3$, 12 mg $FeSO_4{\cdot}7H_2O$, 0.1 g EDTA-Na, 0.5 g L-cysteine, trace element 1 mL, Vitamin 1 mL, pH 7.0; medium was sterilized at 121 °C for 15 min. The strain RLD-53 was pre-cultured at 35 °C for 24 h under light intensity of 2000 lux with incandescent lamps (60 W) and argon was used to maintain anaerobic condition. The composition of trace element (1 L) was the same as described by previous literature [8]. The pH of the medium was adjusted to 7.0 by using 1 M HCl or NaOH solution.

Medium for photo-hydrogen production contained sodium glutamate 10 mmol/L, K_2HPO_4 0.5 g/L, KH_2PO_4 0.5 g/L, NaCl 0.1 g/L, $CaCl_2$ 0.08 g/L, $FeSO_4.7H_2O$ 0.012 g/L, EDTA-Na 0.1 g/L, L-cysteine 0.5 g/L. The kind and concentration of carbon source in the medium were determined according to different experimental tests.

The acetate, at different concentrations of 10, 10, 20, 40, 60 and 80 mmol/L was used as sole carbon source for hydrogen production when experiments explored the effect of single substrate on photo-hydrogen production by mixed bacteria (Fig. 1).

The acetate, propionate and butyrate were used as mixed carbon sources for hydrogen production when experiments explored the effect of mixed substrate on photo-hydrogen production by mixed bacteria, and their concentrations were listed in Table 1.

60 mmol/L acetate was used as sole carbon source for hydrogen production when experiments explored the hydrogen production ability of strain RLD-53 added in mixed bacteria.

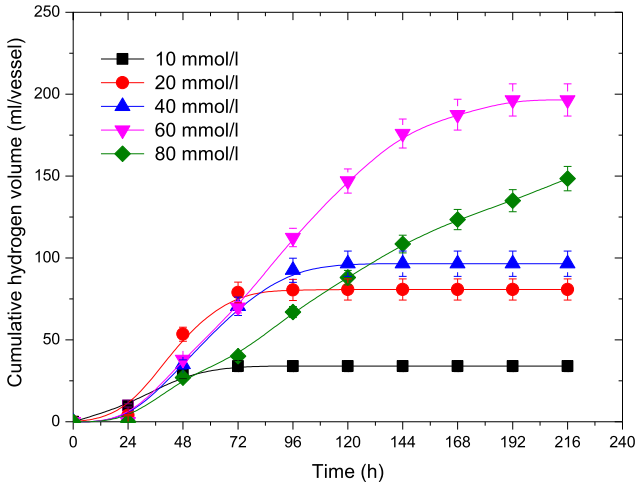

Fig. 1 – The effect of acetate concentration on hydrogen production of mixed bacteria.

H_2 production experiments in batch culture reactors

The photo-fermentative hydrogen production experiment was carried out in 100 mL serum bottles with a working volume of 80 mL in batch culture, and these bottles were sealed by rubber plugs and top area of bottles filled with argon to maintain anaerobic conditions. The bottles with supernatant were autoclaved at 121 °C for 15 min. After 24 h of culture, the bacterial cells entered mid-exponential growth phase, the inoculants of 10% (V/V, volume ratio of inoculums volume to medium volume) were added into serum bottles. The bottles were shaken at 120 rpm with constant temperature of 35 °C; the light intensity on the outside surface of the bottles was maintained at 4000 lux by incandescent lamps (60 W). All tests were run in triplicate. Gases were continuously released to the outside of reaction bottles. The drainage method was adopted to collect the gases. Firstly, the measuring cylinder was filled

Table 1 – The composition of mixed acids during photohydrogen production by mixed bacteria.

Run	Acetate mmol/L	Propionate mmol/L	Butyrate mmol/L
1	10	15	0
2	10	15	5
3	10	15	10
4	10	0	10
5	10	5	10
6	10	10	10
7	20	5	0
8	20	5	5
9	20	5	10
10	20	0	5
11	20	10	5
12	20	15	5
13	30	5	5
14	30	10	5
15	30	25	5
16	30	15	5
17	30	15	10
18	30	15	0

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