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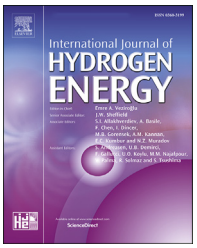

Hydrogen production from high slat medium by co-culture of *Rhodovulum sulfidophilum* and dark fermentative microflora

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ARTICLE INFO

Article history:
Received 24 August 2017
Received in revised form
13 April 2018
Accepted 1 May 2018
Available online 24 May 2018

Keywords:
Biological hydrogen production
Dark-fermentation
Photo-fermentation
Co-culture

ABSTRACT

Potential of pH decrease is one of the major obstacle in stable operation in coculture of dark- and photo-fermentative bacteria for hydrogen production. In this study, a dark fermentative bacterial consort and acid-tolerant marine photo-fermentative bacterium, *Rhodovulum sulfidophilum* TH-102, were individual or co-cultured in high salt medium for hydrogen production. All co-cultures produced more hydrogen than the individual culture of photo or dark fermentation. The dark/photo bacterial ratios were 1:5, 1:10, 1:15 and 1:20, respectively. Among the coculture ratios, bacterial ratio 1:10 produced the highest hydrogen yield (1694 ± 21 mL/L). The addition of the photo-fermentative bacterium to the dark fermentation consort stabilized the pH value and decreased the oxidation-reduction potential of the co-culture system and extended the hydrogen production period. Carbon fixation by the photo-fermentative bacterium may play some role in improving the hydrogen yield of the co-culture system.

Introduction

Biological hydrogen production is a promising method for the long-term development of a hydrogen-based economy because it occurs at ambient temperature and pressure, which will greatly decrease production costs. Algae, cyanobacteria, photo-fermentative bacteria, and dark-fermentative bacteria are used independently or in combination to produce biological hydrogen [1–4]. When bacteria are used independently, the key problems are by-product (e.g. oxygen and volatile fatty acids (VFAs)) inhibition and the utilization of organic substrates. Each microbial strain has its limitations when used to produce hydrogen [1–4]. For algae and cyanobacteria, the main limitation is oxygen inhibition; for photo-fermentative bacteria, the main limitation is their weak ability to degrade complex organic substrates; and for dark-fermentative bacteria, the main limitation is the incomplete

Abbreviations: ORP, oxidation-reduction potential; VFAs, volatile fatty acids.

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https://doi.org/10.1016/j.ijhydene.2018.05.014
0360-3199/

degradation of organic substrates and low hydrogen yield. A better option is to complement the hydrogen-producing abilities of different strains, which has been proved to be better than using single strains and which could greatly decrease costs [2].

Dark-fermentative bacteria produce hydrogen at high rates from various organic wastes, but they cannot degrade organic wastes completely due to thermodynamic limitations. The theoretical hydrogen yield in dark fermentation is 4 mol H_2/mol glucose or 2 mol H_2/mol glucose, when acetic acid or butyric acid is the only product. Experimental hydrogen yields range from 2 to 3 mol H_2/mol glucose due to microbial growth and the formation of VFAs, which is far from the theoretical yield of 12 mol H_2/mol glucose [2]. However, the VFA produced by dark-fermentative bacteria could be easily used by photo-fermentative bacteria as substrates for hydrogen production. According to their biological characteristics and hydrogen production mechanisms, an admixture of dark-fermentative bacteria with photo-fermentative bacteria can markedly enhance the hydrogen yield and make full use of organic wastes [2].

Dark- and photo-fermentative bacteria have been used in sequential or co-culture modes for hydrogen production. High hydrogen yields have been obtained in sequential fermentation mode when dark- and photo-fermentative bacteria are cultured separately under their optimal conditions [5–13]. However, complex pretreatment must be used for the dark fermentative effluent, such as diluting the fermentative liquor to the desired VFAs concentrations, regulating the pH and oxidation-reduction potentials (ORPs), adjusting the carbon to nitrogen ratio, etc [5,8,11,12]. These pretreatments greatly increase the operating costs. In addition, the sequential mode is uneconomical because it requires more reactors and supplements and it occupies more space. The co-culture mode may have advantages over sequential fermentation by reducing the fermentation time and operating costs [2,14].

In co-culture mode, VFAs produced by dark fermentation were in situ converted into hydrogen by photo-fermentation. This is also a way of alleviating the end product and low pH inhibition of dark fermentation. Many papers reported hydrogen production by co-culture of dark- and photo-fermentative bacteria [14–28]. However, in most co-cultures, the production of VFAs by dark-fermentative bacteria was faster than the utilization rate by photo-fermentative bacteria, resulting in acidification of the fermentation broth and a decrease in hydrogen production by both types of bacteria [29]. The potential of pH decrease is the major barrel in stable operation in the co-culture process [16]. Dark fermentative bacteria prefer acid pH for hydrogen production. And during dark fermentative process, pH fell to about 4.5–5.5 [16]. While photosynthetic bacteria prefer neutral or alkali pH for hydrogen production. To overcome this difficulty, the initial pH and ratio of bacterial dark- and photo-fermentative bacteria were optimized [16,29–31]. However, it is difficult to maintain stable against time-fluctuating between this two group of bacteria, because most photosynthetic bacteria have difficulty to adapt to the acid environment of dark fermentative broth. Using acid tolerant strains maybe have some advantages. Thus, developing a more efficient processing system and using tolerant bacterial strains may overcome these obstacles in the co-culture mode.

The high salt wastes are increasing. Much work should be done to treat these wastes. In this study, an acid-tolerant marine photo-fermentative bacterium, *Rhodovulum sulfidophilum* TH-102, was used in the co-culture hydrogen production technique. The acid-tolerant strain is more compatible to the optimum culture condition of dark hydrogen production process than most photosynthetic strains, for example acid and high temperature [32]. To ensure that the dark- and photo-fermentative bacteria complemented each other efficiently, different dark-to photo-fermentative bacterial inoculation ratios were evaluated using the method as described below. During hydrogen production, the hydrogen yield, glucose utilization, pH, and ORP variation were monitored.

Materials and methods

Inoculum and culture medium

A previously obtained acid-tolerant photo-fermentative bacterium, *Rhodovulum sulfidophilum* TH-102, was used in this study [32]. A dark-fermentative bacterial consort was acquired from the anaerobic sludge collected from a marine shrimp pond in Tianjin, China. The sludge was baked at 90 °C for 30 min to inactivate hydrogen-consuming bacteria and enrich hydrogen-producing bacteria. The ratio of pretreated sludge to LM medium was 1:10 by volume. The reactors were purged with argon gas and sealed with a rubber septum and an aluminum cap to produce an anaerobic environment. Bacteria were cultivated at 35 ± 1 °C at an initial pH of 8.0 in a gyratory incubator at 150 rpm. After one day of incubation, 10 mL of culture broth was inoculated into 90 mL of LM medium. The incubation procedure was repeated two times to obtain an enriched mixed dark microbial culture. The acquired bacteria consort was used as the dark-fermentative inoculum.

The LM medium was used for individual- and co-culture hydrogen production, and it was prepared according to a previous study [33], with some modifications. The LM medium consisted of (in g/L): glucose 9, beef extract 2, tryptone 4, yeast extract 1, K_2HPO_4 1.5, MgCl 0.1, $FeSO4•7H_2O$ 0.1, L-cysteine 0.5, NaCl 30, vitamin solution 10 mL, and trace element solution 10 mL. The pH of the medium was adjusted to 8.0. Cells were cultivated at 35 ± 1 °C and irradiated with 100 μmol photons/m^2 s.

Hydrogen production

To investigate the enhancing effect of photo-fermentative bacteria on fermentative hydrogen production, different dark-to photo-fermentative bacterial inoculation ratios were evaluated. In the dark-to photo-fermentative bacterial ratio test, 3.3, 1.8, 1.3, and 1.0 mL of the pre-cultured, dark-fermentative bacterial consort (OD_{600nm} = 1.6), along with 16.7, 18.2, 18.7, and 19.0 mL of pre-cultured strain TH-102 (OD_{660nm} = 1.6) were harvested and inoculated into the LM medium, respectively. Thus, the dark/photo-fermentative bacterial ratios were about 1:5, 1:10, 1:15 and 1:20, respectively.

A two-stage test was conducted to ascertain the changes in pH, ORP, and glucose resulting from the photo-fermentative