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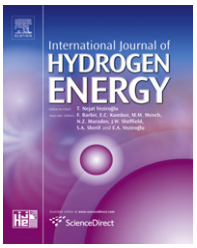

Comparative study of biohydrogen production by four dark fermentative bacteria

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ABSTRACT

Dark fermentation is a promising biological method for hydrogen production because of its high production rate in the absence of light source and variety of the substrates. In this study, hydrogen production potential of four dark fermentative bacteria (*Clostridium butyricum*, *Clostridium pasteurianum*, *Clostridium beijerinckii*, and *Enterobacter aerogenes*) using glucose as substrate was investigated under anaerobic conditions. Batch experiments were conducted to study the effects of initial glucose concentration on hydrogen yield, hydrogen production rate and concentration of volatile fatty acids (VFA) in the effluents. Among the four different fermentative bacteria, *C. butyricum* showed great performance at 10 g/L of glucose with hydrogen production rate of 18.29 mL-H_2/L-medium/hand specific hydrogen production rate of 3.90 mL-H_2/g-biomass/h. In addition, it was found that the distribution of volatile fatty acids was different among the fermentative bacteria. *C. butyricum* and *C. pasteurianum* had higher ratio of acetate to butyrate compared to the other two species, which favored hydrogen generation.

1. Introduction

The global energy need has demonstrated a great increase during last decades, together with fast depletion of fossil fuels and huge amount of green house gas emissions. As a result, exploration of alternative clean energy sources has been a hot topic for researchers all over the world. Hydrogen is considered a viable alternative fuel and "energy carrier" for the future due to its renewability as well as its clean end of usage (i.e. water is the only by-product) [1]. Hydrogen has high-energy yield (142.35 kJ/g), which is 2.75 times greater than hydrocarbon fuels [2,3]. Furthermore, hydrogen releases the energy explosively in heat engines or generates electricity quietly in fuel cells superior to CH_4 and alcohol. It can also be used as raw material for the synthesis of ammonia, alcohol and aldehydes, and for the hydrogenation of various petroleum products [4]. It has been reported that 50 million tonnes of hydrogen are traded annually worldwide with a growth rate of nearly 10% per year for the time being. Based on the National Hydrogen program of the United States, the contribution of hydrogen to total energy market will be 8–10% by 2025 [5]. Currently, the issue in utilization of hydrogen as a fuel is its expensive production methods [6].

Electrolysis of water, steam reforming of hydrocarbons and auto-thermal processes are well-known methods for hydrogen production, but they are not cost-effective.

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Biohydrogen production from renewable sources has received considerable attention in recent years [7]. Biohydrogen production through biological processes mainly involves biophotolysis by algae or cyanobacteria, dark fermentation and photo fermentation. Biophotolysis hydrogen production is quite slow, requires sunlight, and often inhibited by oxygen. Dark and photo fermentation processes are considered more environmental beneficial and feasible due to simultaneous waste treatment and hydrogen production. Dark fermentation is faster than photo fermentation and has advantages: a) hydrogen is produced at higher rate and b) it can use various organic waste and wastewater enriched with carbohydrates as the substrate.

Among the dark fermentative hydrogen producers, *Clostridium* sp. and *Enterobacter* sp. have attracted more attention due to their high growth rate [8,9]. For this purpose, *Clostridium butyricum*, *Clostridium pasteurianum*, *Clostridium beijerinckii* and *Enterobacter aerogenes* were investigated and compared at different initial glucose concentrations. Glucose utilization efficiency, cumulative hydrogen production, and hydrogen production rate for these four species were studied.

2. Materials and methods

2.1. Bacteria strains and medium

C. butyricum DSM 10702, *C. pasteurianum* DSM 525, *C. beijerinckii* DSM 791 and *E. aerogenes* DSM 30053 were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in lyophilized form. The medium used to cultivate the four species consisted of 3 g/L yeast extract, 10 g/L 'Lab-Lemco' powder (beef extract), 10 g/L peptone, 5 g/L glucose, 1 g/L soluble starch, 5 g/L sodium chloride, 3 g/L sodium acetate, 0.5 g/L cysteine hydrochloride. The medium was adjusted at pH 6.8 ± 0.2. *Clostridium* species and *E. aerogenes* were cultivated in incubators at 37 °C and 30 °C, respectively.

2.2. Batch fermentation experiments

The dark fermentation batch experiments were performed in 60 mL serum bottles that were purged with nitrogen and then filled with 30 mL medium. The bottles were sealed with both butyl rubber stoppers and aluminum crimp caps. The medium was heated until boiling, purged with nitrogen to maintain anaerobic conditions and sterilized at 121 °C for 15 min. When the cells entered mid-exponential growth phase, the inoculants were injected into serum bottles by 3 mL syringes. Afterward, the bottles were placed in two shaking incubators operating at 150 rpm at 37 °C for *Clostridium* species and 30 °C for *E. aerogenes*, respectively. The initial glucose concentrations were ranged from 5 to 20 g/L. The batch experiments were conducted in triplicate and lasted for 72 h.

2.3. Analytical methods

Gas products (H_2 and CO_2) were analyzed by gas chromatography equipped with a thermal conductivity detector, GC-TCD-TCD (Agilent, USA). Argon and helium were used as carrier gases. Columns used in the analysis were molecular sieve 13X and molecular sieve 5A. Oven and detector temperatures were 115 °C and 150 °C, respectively. Gas production was measured periodically by using a 100 mL glass syringe (SAMCO, UK). The cumulative H_2 volume was calculated from the H_2 percentage and total volume of the biogas produced.

Liquid samples (0.1 mL) were taken with 1 mL conventional plastic syringe to measure glucose concentration, which was quantified by the phenol–sulfuric acid method and detected by UV-spectrophotometer (Agilent Cary 50, USA). The volatile fatty acids (VFA) in the supernatant of the culture broth were determined using gas chromatography with flame ionization detector, GC-FID (Agilent 7890A, USA). The liquor samples were filtered through a 0.45 μm nylon membrane before free acids were analyzed. Nitrogen was used as carrier gas. Biomass concentrations in the experimental bottles were measured as cell dry weight (CDW) according to Standard Methods by filtering sample through a 0.45 μm Millipore filter paper, drying overnight at 105 °C, cool to room temperature in a desiccator jar, and determining the constant dry weight [10].

3. Results and discussion

3.1. Glucose consumption and utilization efficiency

Fig. 1 shows glucose consumption and utilization efficiencies by the four dark fermentative bacteria at different initial glucose concentration levels (5, 10, 15, 20 g/L). Glucose was the sole carbon source during the dark fermentation process. The glucose consumption efficiency was calculated according to the remaining amount of glucose in the solution. It was observed that glucose consumption and utilization efficiency increased with the increase of initial glucose concentration by *E. aerogenes* and *C. beijerinckii*. On the contrary, *C. butyricum* and *C. pasteurianum* presented different trends. Among the fermentative bacteria, *E. aerogenes* had the higher utilization efficiency (more than 85%). The *C. butyricum* and *C. pasteurianum* had limited consumption efficiency since above 10 g/L initial glucose concentration there was little change at glucose consumption. Therefore, the utilization efficiency was decreased by increasing glucose concentration. Several researchers have addressed similar results regarding the initial glucose concentration about the consumption efficiency [11].

3.2. Effect of initial glucose concentrations on hydrogen production

Effect of initial glucose concentration (5, 10, 15, 20 g/L) on biohydrogen production was also studied (Fig. 2). During the early stage, the initial glucose concentrations had less impact on hydrogen production (adaptation period). After that, the hydrogen production increased rapidly during the first 20 h. It was observed that at low glucose concentration (5 g/L), the cumulative hydrogen production was around 30 mL for *Clostridium* sp., while *E. aerogenes* produced less than 5 mL. After 30 h, the production practically ceased. At higher glucose concentration, the fermentation process could continue even after 45 h.

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