A Pilot Study of Nitrogen Composition and Effect on Biohydrogen Production

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Biohydrogen Production from Combined Dark-photo Fermentation under a high Ammonia Content in the Dark Fermentation Effluent

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Abstract

Integrated dark and photo (two-stage) fermentation was employed to enhance the performance of H_2 production. First, the continuous dark fermentation using indigenous *Clostridium butyricum* CGS5 was carried out at 12 h HRT and fed with sucrose at a concentration of 18750 mg/l. The overall H_2 production rate and H_2 yield were fairly stable with a mean value of 87.5 ml/l/h and 1.015 mol H_2/mol sucrose, respectively. In addition, a relatively high ammonia nitrogen content (574 mg/l) in the dark fermentation effluent was observed. The soluble metabolites from dark fermentation, consisting mainly of butyric, lactic and acetic acids, were directly used as the influent of continuous photo-H_2 production process inoculated with *Rhodopseudomonas palutris* WP 3-5 under the condition of 35^{o}C, 10000 lux irradiation, pH 7.0 and 48 h HRT. The maximum overall hydrogen production rate from photo fermentation was 16.4 ml H_2/l/h, and the utilization of the soluble metabolites could reach 90%. The maximum H_2 yield dramatically increased from 1.015 mol H_2/mol sucrose (in dark fermentation only) to 6.04 mol H_2/mol sucrose in the combined dark and photo fermentation. Surprisingly, the operation strategy applied in this work was able to attain an average NH_3-N removal efficiency of 92%, implying that our photo-H_2 production system has a higher NH_3-N tolerance, demonstrating its high applicability in an integrated dark-photo fermentation system.

1 Introduction

Hydrogen is a clean energy since combustion of H_2 produces only water without greenhouse gases. Hydrogen can also be directly utilized by hydrogen fuel cell to generate electricity at very high efficiency [1], thereby being considered a promising alternative energy carrier of the future [2,3]. To this end, developing hydrogen production technology leading to a sufficient and sustainable H_2 supply is highly demanded. Biological H_2 production considered as the most environmentally friendly route of producing H_2 [4], thereby fulfilling the goals of recycling of renewable resources and clean energy production [5]. Hydrogen can be produced biologically through dark fermentation and photo fermentation [6]. These routes all possess advantages and drawbacks, but they seem to interact complementarily. Thus, effective integration of the three pathways may lead to optimal performance of biohydrogen

production [7]. In this study, sucrose was used in a dark-fermentation batch bioreactor with *Clostridium butyricum* CGS5 to produce hydrogen and volatile fatty acids. The effluent from dark fermentation broth (containing mainly volatile fatty acids) was continuously introduced to photo fermentation culture inoculated by *Rhodopseudomonas palustris* WP3-5. The stability of continuous operation of the integrated dark/photo H_2 fermentation system was evaluated.

2 Materials and Methods

2.1 Bacterial strain and cultivation medium

The strain used for dark fermentation was *Clostridium butyricum* CGS5 isolated from municipal sewage sludge in central Taiwan [8]. The *Rhodopseudomonas palustris* WP3-5 isolated from a swine wastewater treatment plant located in central Taiwan [9] was used for phototrophic H_2 production. The culture medium was using 2000 mg/l sodium acetate as a sole carbon substrate. The cells were cultivated at 32^{o}C anaerobically for 48 h under a light intensity of approximately 50 W/m^2 (illuminated by tungsten filament lamp). The initial pH value of medium prior to incubation was adjusted to 7.0-7.1. Argon gas was used to create an anaerobic condition.

2.2 Setup of the bioreactor

The batch dark H_2 fermentation conducted by inoculating 3 ml of *C. butyricum* CGS5 into 200 ml flasks containing 150 ml of dark-fermentation medium, which was incubated at 37^{o}C and an initial pH of 7.5. A continuously stirred tank reactor (CSTR) was also conducted to produce H_2 from sucrose via dark fermentation using *C. butyricum* CGS5 as the H_2 producer. The continuous culture was operated at 37^{o}C, pH 6.5. The dark H_2 fermentation broth was centrifuged (9000 × g, 10 min) and the collected supernatant was diluted and then the pH was adjusted to 7.1. This pretreated supernatant was used as the substrate for phototrophic H_2 production with *R. palustris* WP3-5. The photobioreactor (PBR) was a 1-liter glass-made vessel equipped with external light sources (100 W tungsten filament lamps) adjusted to a light intensity of ca. 95 W/m^2.

3 Results and Discussion

3.1 Continuous dark-fermentation H_2 production using sucrose as substrate

In this work, a pure strain of *Clostridium pasteurianum* CGS5 was used to produce H_2 via continuous dark fermentation using sucrose as the carbon source. The soluble products generated from dark fermentation were then used for phototrophic H_2 production in the following stage. The dark fermentation operated at 32 ^{o}C, 12 h HRT and fed with a sucrose concentration of 18750 mg/l gave an H_2 yield of 1.105 mol H_2/mol sucrose, and overall H_2 production rate of 87.5 ml/l/h, respectively. Moreover, a relatively high ammonia nitrogen content of 574 mg/l was observed in the effluent of dark fermentation system. Meanwhile, the nearly 70-90% of soluble metabolites in the dark fermentation broth (mainly lactate, acetate, and butyrate) was produced to serve as substrates for photo fermentation, which

seems to be feasible substrates for H_2 production in the photo fermentation system as will be discussed next.

3.2 Continuous bioH_2 production with integrated photo fermentation processes

The dark fermentation metabolites mentioned above were further utilized as the influent of continuous photo-H_2 fermentation process inoculated with *R. palutris* WP3-5 under the condition of 32^{o}C, 100 W/m^2 irradiation, pH 7.0 and 96 h HRT. The overall H_2 production rate in photo fermentation was fairly stable with a mean value of 16.4±1.31 ml H_2/l/h. The cell concentration also reached a steady-state value of nearly 5.18±0.51 g/l over a 5-day operation. The H_2 content in biogas was essentially constant at 91.3±1.75% for 5-fold soluble metabolites. The total hydrogen yield calculated from integration of the yield from dark and photo fermentation reached a high value of 6.04 mol H_2/mol sucrose, which is nearly 5.95-fold of that obtained from using dark fermentation alone (1.015 mol H_2/mol sucrose). Nearly 92.0±1.5% for 5-fold soluble metabolites of the ammonia nitrogen entering the photo fermentation process was consumed, implying that our photo-H_2 production system has a higher NH_3-N tolerance, demonstrating its high applicability in an integrated dark-photo fermentation system. This suggests the feasibility and advantage of using the two-stage process combining dark and photo fermentation for high-yield bioH_2 production with an excellent stability.

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