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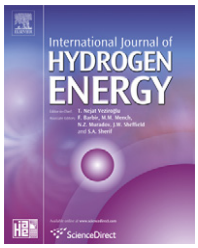

Biohydrogen production using sequential two-stage dark and photo fermentation processes

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ABSTRACT

A two-stage process combining dark/photo fermentation was used to increase the overall hydrogen yield from sucrose and also to reduce the chemical oxygen demand (COD) in the effluent. Dark-H_2 fermentation was conducted using *Clostridium pasteurianum* CH_4, giving a maximum H_2 production yield of 3.80 mol H_2/mol sucrose. The soluble metabolites resulting from dark fermentation, consisting of butyric and acetic acid, were further used for H_2 production in the subsequent photo fermentation. Using soluble products from dark fermentation as substrate, *Rhodopseudomonas palustris* WP3-5 could produce H_2 phototrophically, elevating the total hydrogen yield from 3.80 (dark fermentation) to 10.02 mol H_2/mol sucrose (dark/photo fermentation). Meanwhile, a 72.0% COD removal was also achieved. When the photobioreactor was illuminated with side-light optical fibers and was supplemented with 2.0% (w/v) of clay carriers, the overall H_2 yield of the two-stage process was further enhanced to 14.2 mol H_2/mol sucrose with a nearly 90% COD removal. Continuous photo fermentation was also carried out at 96 h HRT using effluent from dark fermentation as the feed. The continuous culture maintained stable for nearly 10 days with an average H_2 yield of 10.21 mol H_2/mol sucrose. This demonstrates the feasibility of using the two-stage process combining dark and photo fermentation for simultaneous hydrogen production and COD removal.

1. Introduction

Our economy and lifestyle heavily rely on the use of fossil fuels. However, the excessive dependence on fossil fuels has caused severe problems to human beings due to a continual rising of their cost, insecurity in their sustainability, as well as their impacts on global warming and environmental pollution [1–3]. As a result, technologies that could generate reliable and effective energy alternatives to fossil fuels have received increasing attention in recent years [4,5]. In particular, hydrogen is recognized as a promising future energy carrier because of its clean, recyclable and high efficient nature [6–10]. Conventional physicochemical methods for hydrogen production are usually costly and energy intensive. Thus, the innovation of technologies leading to safe, sustainable, economically feasible H_2 production is in urgent demand. Biological H_2 production (including photolysis of water, light-dependent or independent fermentative pathways) is considered as the most environmentally friendly route of producing H_2 [11–13]. In particular, hydrogen production

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through dark or photo fermentative conversion of organic substrates is of great interest due to its dual function of waste reduction and clean energy production, thereby acting as a promising option for biohydrogen production. [7,14–16].

Dark fermentation with mainly acidogenic bacteria (*Clostridium* sp. and *Enterobacter* sp.) has the ability to produce H_2 while converting organic substrates into volatile fatty acids and alcohols [17–19]. These soluble metabolites (e.g., acetic acid, butyric acid) can be further utilized via photo fermentation (with photosynthetic bacteria, such as purple nonsulfur bacteria) resulting in more H_2 production at the expense of light energy [15,20–24]. The possibility of using various industrial and agricultural wastewater containing acetic and butyric acid for photo-H_2 fermentation has been widely investigated [25]. In fact, combination of the dark and photo fermentation could achieve a theoretically maximum yield of 12 mol H_2/mol hexose [26,27]. Therefore, a two-stage process combining dark and photo-H_2 fermentation has been considered as an effective and efficient system to increase H_2 production yield, enhance energy recovery from organic wastewaters, and result in a lower chemical oxygen demand (COD) in the effluent [26,27].

In this study, engineering approaches were applied to combine dark–photo-H_2 fermentation for enhancing the overall H_2 yield. First, the feasibility of phototrophic hydrogen production was explored by direct utilization of soluble metabolites (mainly HAc and HBu) from dark-H_2 fermentation. Next, a constructed overlay contour plot method was developed to predict the performance of photo-H_2 fermentation at various HAc to HBu ratio in terms of hydrogen yield and maximum hydrogen production rate. Furthermore, based on our recent findings, the effect of using internal optical fiber illumination [28,29] and of adding porous carrier on the photo-H_2 production performance was determined. Finally, the stability of long-time operation of the photo-H_2 production system using effluent from dark-H_2 fermentation process was assessed.

2. Materials and methods

2.1. Bacterial strain and medium for dark-H_2 fermentation

Clostridium pasteurianum CH_4 isolated from effluent sludge of anaerobic H_2-producing bioreactors [30] was used as the H_2 producer for dark-H_2 fermentation. The medium composition for the pure-culture dark fermentation was (g/l): sucrose, 17.81; $NaHCO_3$, 15; NH_4Cl, 0.717; K_2HPO_4, 0.125; $MgCl_2{\cdot}6H_2O$, 0.1; $MnSO_4{\cdot}6H_2O$, 0.015; $FeSO_4{\cdot}7H_2O$, 0.025; $CuSO_4{\cdot}5H_2O$, 0.005; $CoCl_2{\cdot}5H_2O$, 0.000125; $CaCl_2{\cdot}2H_2O$, 0.1 [30,31].

2.2. Bacterial strain and medium for photo-H_2 fermentation

Rhodopseudomonas palustris WP3-5 isolated from a swine wastewater treatment plant located in central Taiwan [32] was used for phototrophic H_2 production. The bacterium was grown with *Rhodospirillaceae* medium [32] consisting of (in g/l) K_2HPO_4, 0.5; KH_2PO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; NaCl, 0.4; $CaCl_2{\cdot}2H_2O$, 0.05; yeast extract, 0.2; iron citrate solution (1.0 g/l), 5 ml/l; trace element solution, 1 ml. The trace element solution contained (in mg/l) $ZnCl_2$, 70; $MnCl_2{\cdot}4H_2O$, 100; H_3BO_3, 60; $CoCl_2{\cdot}6H_2O$, 200; $CuCl_2{\cdot}2H_2O$, 20; $NiCl_2{\cdot}6H_2O$, 20; $NaMoO_4{\cdot}2H_2O$, 40; HCl (25%), 1 ml/l. For preculture, *Rhodospirillaceae* medium was supplemented with butyric acid (2900 mg COD/l) and acetic acid (900 mg COD/l) as the carbon source. The WP3-5 strain was grown anaerobically at 32 °C for 48 h under illumination with tungsten filament lamps (light intensity = ca. 70 W/m^2). The anaerobic condition was created by sparging the medium with argon gas. The initial pH value of the medium was adjusted to 7.0 by 0.1 N NaOH.

2.3. Operation of bioreactor

Schematic description of the two-stage process combining dark and photo fermentation is shown in Fig. 1. The batch dark-H_2 fermentation was performed in 2 l serum vials with a culture temperature and pH of 32 °C and 7.0, respectively. The dark-H_2-fermentation broth was centrifuged at 13 000 rpm. 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 *Rhodopseudomonas palustris* WP3-5.

The photobioreactor (PBR) was a 1-liter glass-made vessel equipped with external light sources (100 W tungsten filament lamps and 100 W halogen lamps) adjusted to a light intensity of ca. 95 W/m^2. The external light source was mounted on both sides of the PBR as indicated in Fig. 1. Moreover, the side-light optical fiber (SLOF) protected in a glass tube was inserted into fermentation broth from the top of the photobioreactor. Since the SLOF did not have direct contact with the medium, there was no need to autoclave the optical fibers prior to use. The SLOF was excited by a halogen light engine (150 W; Gorich Co., Hsin-Chu, Taiwan), achieving a light intensity of ca. 95 W/m^2. Detailed procedures for preparing SLOF were described elsewhere [28]. The initial cell concentration of *R. palustris* WP3-5 was 0.875 g/l. The batch reactors were controlled at 32 °C, pH 7.1, and an agitation rate of 100 rpm. In some cases, expanded clay (spherical particles with an average diameter of 5 mm; obtained from Taihort Inc., Taipei, Taiwan) was added to the fermentation broth at a weight to volume ratio (w/v) of 2.0% to enhance photo-H_2 production performance [33]. A gas collecting device was used to monitor gas production (Fig. 1) and the gas volume was calibrated to 25 °C and 760 mmHg. The compositions of gas products were measured with respect to time. The culture samples were also collected from the sealed glass vessel at designated time intervals to determine cell concentration, pH and residual concentration of acetic acid and butyric acid. The cumulative H_2 production was simulated by modified Gompertz equation (Eq. (1)) [34,35] and the kinetic parameters for photo-H_2 production were estimated via Sigma Plot 10.0 (SPSS Inc., Point Richmond, CA, USA).

$$H = H_{\max}\exp\left\{-\exp\left[\frac{R_{\max,H_2}\times e}{H_{\max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where, H denotes cumulative H_2 production (ml), $H_{\max}$ denotes maximum cumulative H_2 production (ml), $R_{\max}$ denotes maximum H_2 production rate (ml/h), t denotes