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Feasibility study on bioreactor strategies for enhanced photohydrogen production from *Rhodopseudomonas palustris* WP3-5 using optical-fiber-assisted illumination systems

Chun-Yen Chen[a], Chi-Mei Lee[b], Jo-Shu Chang[a,*]

[a]*Department of Chemical Engineering, National Cheng Kung University, Tainan 710, Taiwan*
[b]*Department of Environmental Engineering, National Chung Hsing University, Taichung, Taiwan*

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Abstract

A novel photobioreactor (PBR) was utilized to produce H_2 by indigenous purple nonsulfur bacterium *Rhodopseudomonas palustris* WP3-5 using acetate as the sole carbon source. The PBR was illuminated by combinative light sources including side-light optical fibers (internal light source) as well as external irradiation of halogen lamp and/or tungsten filament lamp. A fill and draw (F/D) operation of PBR was shown to improve the performance of photoH_2 production over the performance of batch and continuous cultures under similar operation conditions. For medium improvement, the PBR was conducted under different concentrations of carbon source (acetate) and nitrogen source (glutamic acid). The results show that the highest overall H_2 production rate (v_{H_2}) and H_2 yield (Y_{H_2}) occurred when the acetate concentration was 32.5 mmol/l and the glutamic acid concentration was 400 mg/l. The optimal acetate and glutamic acid concentration led to a v_{H_2} and Y_{H_2} of 20.9 ml/h l and 2.47 mol H_2/mol acetate, respectively. The H_2 production rate and yield was further enhanced to as high as 38.2 ml/h l and 3.15 mol H_2/mol acetate, respectively, while using a ternary-light-source (TLS) system, combining optical fiber, halogen lamp, and tungsten filament lamp (i.e., the OF/HL/TL system). Meanwhile, the high H_2 production efficiency with TLS system was stably maintained for nearly 30 day under the F/D operations.

Keywords: Photohydrogen production; Photosynthetic bacteria; *Rhodopseudomonas palustris*; Fill and draw; Photobioreactor; Optical fiber

1. Introduction

As the petroleum price keeps rising [1], the demand for reliable and effective energy alternatives is increasingly urgent. Among the candidates of alternative energy resources, H_2 has been considered an ideal energy carrier because it is clean, recyclable, and efficient. However, before the concept of hydrogen economy [2] could become a reality, a safe, economical, and sustainable way of producing H_2 needs to be developed. Conventional H_2 production methods, such as thermochemical conversion of fossil fuels, are usually expensive and energy-intensive; they also result in production of environmental pollutants and green-house gases. Therefore, alternative methods for H_2 production are of great demand.

In fact, biological production of H_2 is considered the most environment-friendly route of producing H_2. Hydrogen can be produced biologically from photolysis of water, and through light-dependent or independent

* Corresponding author. Tel.: +886 6 2757575x62651; fax: +886 6 2357146.
E-mail address: changjs@mail.ncku.edu.tw (J.-S. Chang).

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fermentative pathways using organic materials as the substrate [3,4]. Dark fermentation with mainly acidogenic bacteria (such as *Clostridium* sp.) produces H_2 while converting organic substrates into volatile fatty acids and alcohols. The soluble metabolites from dark fermentation could be mineralized by photosynthetic bacteria (e.g., purple nonsulfur bacteria) to produce more H_2. Thus, combination of dark and photoH_2 fermentation has been proposed to achieve the highest theoretical H_2 yield [5,6]. Compared to dark fermentation, photofermentative production is relatively slow mainly due to the low growth rate of photosynthetic bacteria as well as the poor efficiency of light energy conversion. Thus, upgrading the production rate of photoH_2 fermentation becomes a pivotal step towards a successful integrated fermentative H_2 production process.

Various strategies were applied to enhance the reaction rate of photohydrogen production. Fißler et al. [7] reported that using immobilized *Rhodopseudomonas palustris* cells with polymeric materials could improve photohydrogen production. El-Shishtawy et al. [8] proposed a novel photobioreactor design by combining light receiving face and reflection sheet to transfer light sources for better light energy conversion efficiency. Meanwhile, to decrease light energy loss, Tsygankov et al. [9,10] developed a novel photobioreactor made of three concentric glass cylinders and the incandescent lamps were directly placed into the photobioreactor. There are also reports describing improvement of photohydrogen production by using continuous-flow operations [11]. In another case, combining a continuous-flow photobioreactor with hollow fiber displayed an increase in photohydrogen production [12]. Nakada et al. [13] trapped the cells of *Rhodobacter sphaeroides* in a two-layer gel to investigate the distribution of pigment, cell growth and light penetration in the gels. Miyake et al. [14] constructed a sunlight-collecting system and simulated sunlight illumination pattern by using halogen lamp as the light source. Their main concept for bioreactor design was trying to enhance the irradiation area, cell concentration and surface-to-volume ratio in the bioreactor.

In this study, an indigenous photosynthetic bacterium *Rhodopseudomonas palustris* WP3-5 [15] was used as a model H_2 producer to investigate bioreactor strategies for a novel H_2-producing photobioreactor illuminated by combination of optical fiber and other conventional light sources. Optical fiber could be an ideal light source for photobioreactors because it provides uniform light distribution and proximal contact with light-demanding microorganisms to achieve high light transmission efficiency. However, to date there are still very few studies describing the use of optical fibers for photohydrogen production [16,17]. The goal of this work was to develop better bioreactor operation strategies with a novel photobioreactor to improve its H_2 production performance as well as operational stability. The knowledge obtained from this study could be used to assess the feasibility of utilizing the photobioreactor system in practical applications.

2. Materials and methods

2.1. Microorganism and medium

Rhodopseudomonas palustris WP3-5 isolated from a wastewater treatment plant in central Taiwan [15] was used for photo-heterotrophic H_2 production. The bacterium was grown with Rhodospirillaceae medium [15] 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. The cells were cultivated anaerobically at 32 °C for 48 h under illumination with tungsten filament lamp at a light intensity of approximately 50 W/m^2. The anaerobic condition was created by sparging the medium with argon gas and the initial pH of the medium was adjusted to 7.1 by 0.1 M NaOH.

2.2. Fabrication and operation of photobioreactor

The photobioreactor (PBR) was a sealed glass vessel with a working volume of 800 ml (Fig. 1). A side-light optical fiber (SLOF) was inserted into the photobioreactor from the top of the reactor. The SLOF was made from plastic-clad optical fibers (core material: polymethyl methacrylate, diameter: 11 mm, length: 25 cm; Baycom Optic-Electronic Co., Hsin-Chu, Taiwan) by mechanical polishing to remove the protective cladding. The SLOF was excited to achieve a light intensity of ca. 95 W/m^2 by a halogen light engine (150 W; Gorich Co., Hsin-Chu, Taiwan). External light sources were also mounted on both sides of the PBR (Fig. 1) using a conventional tungsten filament lamp (100 W) or a halogen lamp (100 W). The PBR was illuminated with single-light-source or multiple-light-source systems and the total light intensity for each illumination system was adjusted to ca. 95 W/m^2. After the reactor apparatus was sterilized by autoclave, cells of *Rhodopseudomonas*