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Hydrogen production by indigenous photosynthetic bacterium *Rhodopseudomonas palustris* WP3–5 using optical fiber-illuminating photobioreactors

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Abstract

A novel optical fiber-based photobioreactor was utilized to produce H_2 by indigenous purple nonsulfur bacterium *Rhodopseudomonas palustris* WP3–5 using acetate as the sole carbon source. Plastic cladding of conventional end-light optical fibers was removed to obtain side-light optical fibers (SLOF), which was inserted into photobioreactors as the internal light source. External irradiation by conventional lamps may also be provided for the bioreactor as supplemental light sources. The H_2 production performance and light conversion efficiency of the photobioreactor were assessed when various illumination systems were used. The light sources examined included SLOF excited by halogen lamp (OF-HL), SLOF excited by metal–halide lamp (OF-MH), tungsten filament lamp (TL), halogen lamp (HL), and binary combinations of the above. Compared with bioreactors illuminated by external lamps, the OF-HL system produced more H_2 (625 ml), had higher light conversion efficiency (1.80%), and achieved higher H_2 yield (1.19 mol H_2/mol acetic acid). However, among the single light sources examined, HL gave the highest overall (ν_{H_2}) and specific (ν_{s,H_2}) H_2 production rate of 8.68 ml/l h and 3.01 ml/h g cell, respectively, primarily due to enabling better cell growth. Using OF-MH system resulted in poor H_2 production, indicating that emission spectrum of light sources was critical to photo-H_2 production. Combination of two different light sources appeared to further enhance photo-H_2 production, especially when optical fibers and external lamps were combined. Combination of OF-HL and TL exhibited the highest H_2 yield, ν_{H_2}, and ν_{s,H_2} of 2.64 mol H_2/mol acetic acid, 17.06 ml/h l, and 9.47 ml/h g cell, respectively. However, the highest total H_2 production (944 ml) and light conversion efficiency (1.42%) were attained when two types of optical fibers were incorporated (i.e., the OF-HL/OF-MH system).

Keywords: Photohydrogen production; Photobioreactor; Optical fiber; *Rhodopseudomonas palustris*

1. Introduction

Biological production of H_2 is considered the most environment-friendly route of producing H_2 [1–3], which is the most promising alternative to fossil fuels because it is clean, efficient, and recyclable. Microbial conversion of organic substrates into H_2 by light-dependent (e.g., photosynthetic bacteria) or light-independent (e.g., acidogenic bacteria) metabolic pathways [4] is of great interest due to the potential of producing clean energy (H_2) from renewable resources (e.g., organic wastes). In particular, photosynthetic bacteria have been frequently used to produce H_2 because they have high theoretical substrate conversion efficiency and produce a relatively small quantity of by-products (such as CO_2) [5,6]. In addition, photosynthetic bacteria can produce H_2 from mineralization of organic acids (e.g., acetic acid, butyric acid), which are predominant soluble metabolites from dark hydrogen fermentation [3]. This raises the possibility of using photosynthetic bacteria to degrade effluents from dark H_2 fermentation stage for further H_2 production and more complete biodegradation [7,8]. Therefore, dark and photo-H_2 production systems have been combined in series to produce H_2, achieving a higher yield and a lower chemical oxygen demand (COD) in the effluent [5,7,9].

Hydrogen production by photosynthetic bacteria is mainly catalyzed by nitrogenase [10], allowing evolution of H_2 in the absence of molecular nitrogen and oxygen, but with the consumption of ATP and free electrons originating from the

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reducing power [1]. Thus, sufficient supply of ATP becomes one of the major concerns for efficient photo-H_2 production. Since ATP synthesis in photosynthetic bacteria is a light-dependent event, requiring light energy primarily at the wavelength of 522 and 860 nm [11], it would be critical for a H_2-producing photobioreactor to use proper light sources that provide sufficient light energy with needed wavelengths. However, in conventional photobioreactors using external illumination systems (e.g., tungsten filament lamp or halogen lamp) [5,12–14], the light intensity tends to decrease rapidly due to the shielding effects arising from increases in the concentration of cells and products or from formation of biofilm on the surface of reactor vessels [15]. Furthermore, although a short light path is theoretically favorable for achieving high light efficiency, conventional light sources cannot be in close contact with the bacterial culture because they usually generate a considerable amount of heat. Consequently, the light conversion efficiency of conventional photobioreactors has been limited to less than 10% [16,17].

Due to the problems and limitations associated with conventional light sources, this work aimed to develop a novel photobioreactor using optical fibers as the internal illumination system. Meanwhile, conventional lamps were also installed externally as the supplemental light sources. Optical fiber is expected to markedly enhance light conversion efficiency of the photobioreactor because it provides uniform light distribution [18,19] with a high surface-to-volume ratio [20] and can be directly immersed in the bacterial culture to achieve efficient light energy transfer without heat generation. Although optical fibers have been applied in TiO_2-based photo-catalytic reactors [21], the idea of using optical fiber in biological systems is relatively novel. Most of the optical fiber-based photobioreactors were used for microbial desulfurization [15,19,20,22]. In contrast, to date only two reports describing using end-light [23] or light-diffusing [18] optical fibers for photo-H_2 production. In both cases, cells were immobilized on the optical fibers by natural gels (e.g., alginate, agar, and gellan gum).

In this study, a purple nonsulfur photosynthetic bacterium *Rhodopseudomonas palustris* WP3–5 isolated from central Taiwan [5] was used to produce H_2 in an optical fiber-implemented photobioreactor. The optical fibers were excited by different light engines (halogen or metal–halide lamps) to investigate the effect of light emission spectra on photo-H_2 production. Performance of the H_2-producing photobioreactors was also examined by using various single light sources and binary combinations of internal (optical fibers) and external (tungsten filament lamp or halogen lamp) light sources. The goal of this study was to develop a photobioreactor capable of producing H_2 at a fast production rate, a high H_2 yield, and enhanced light conversion efficiency.

2. Materials and methods

2.1. Organism and medium

The bacterial H_2 producer used in this study was an indigenous photosynthetic bacterium, *R. palustris* WP3–5, which was isolated from a swine wastewater treatment system located in central Taiwan [5]. The bacterium was grown with *Rhodospirillaceae* medium [5], consisting of (in g/l) K_2HPO_4, 1.5; 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 at 32 °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 anaerobic conditions.

2.2. Preparation of optical fiber

Plastic-clad optical fibers (11 mm in diameter, 25 cm in length) purchased from Baycom Optic-Electronic Co. (Hsin-Chu, Taiwan) were used in this study. The optical fiber was composed of a polymethyl methacrylate (PMMA) core coated with fluorinated alkyl methacrylate copolymer. The protective cladding was removed by mechanical polishing to allow direct light emission from the PMMA core (i.e., a side-light optical fiber). One of the two fiber-ends on which the light is incident was also polished to attain maximum light emission. Prior to being installed inside the photobioreactor, the side-light optical fiber was physically polished until the desired light intensity and uniform light distribution were obtained.

2.3. Fabrication and operation of photobioreactor

The photobioreactor was a sealed glass vessel with a working volume of 500 ml (Fig. 1). The side-light optical fiber was inserted into the photobioreactor from the top. The optical fiber was excited to achieve a light intensity of ca. 95 W/m^2 by a metal–halide lamp (150 W; Gorich Co., Hsin-Chu, Taiwan) or a halogen lamp (150 W; Gorich Co., Hsin-Chu, Taiwan). In some experiments, external light sources were also mounted in both sides of the bioreactor (Fig. 1b) using a conventional tungsten filament lamp (100 W) or a halogen lamp (100 W), resulting in a light intensity of ca. 95 W/m^2. Cells of *R. palustris* WP3–5 were inoculated into the reactor with a 10% inoculum. The reactor was operated at 32 °C, pH 7.1, and an agitation rate of 100 rpm. The initial acetate concentration was maintained at 2667 mg/l in all tests. A gas meter (Type TG1; Ritter Inc., Germany) was used to measure the amount of gas products generated and the gas volumes were calibrated to 25 °C and 760 mmHg. Gas samples were taken from sampling port by gas syringe at desired time intervals to measure the gas composition. The liquid sample was also collected from the sealed glass vessel with respect to time to determine cell concentration, pH and residual acetate concentration. Time-course data of cumulative H_2 production were simulated by modified Gompertz equation (Eq. (1)) [24,25] to determine the kinetic parameters of photo-H_2