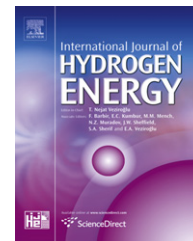


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# Engineering strategies for the enhanced photo-H<sub>2</sub> production using effluents of dark fermentation processes as substrate

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## ABSTRACT

The major obstacle of combining dark and photo fermentation for high-yield biohydrogen production is substrate inhibition while using dark fermentation effluent as the sole substrate. To solve this problem, the dark fermentation broth was diluted with different dilution ratio to improve photo-H<sub>2</sub> production performance of an indigenous purple non-sulfur bacterium *Rhodospseudomonas palustris* WP3-5. The best photo-H<sub>2</sub> production performance occurred at a dilution ratio of 1:2, giving a highest overall H<sub>2</sub> production rate of 10.72 ml/l/h and a higher overall H<sub>2</sub> yield of 6.14 mol H<sub>2</sub>/mol sucrose. The maximum H<sub>2</sub> content was about 88.1% during the dilution ratio of 1:2. The photo-H<sub>2</sub> production performance was further improved by supplying yeast extract and glutamic acid as the nutrient. The results indicate that the overall H<sub>2</sub> production rate and H<sub>2</sub> yield increased to 17.02 ml/l/h and 10.25 mol H<sub>2</sub>/mol sucrose, respectively. Using a novel solar-energy-excited optical fiber photobioreactor (SEEOF) with supplementing tungsten filament lamp (TL) irradiation, the overall H<sub>2</sub> production rate was improved to 17.86 ml/l/h. Meanwhile, the power consumption by combining SEEOF and TL was about 37.1% lower than using TL alone. This study demonstrates that using optimal light sources and proper dilution of dark fermentation effluent, the performance of photo-H<sub>2</sub> production can be markedly enhanced along with a reduction of power consumption.

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## 1. Introduction

As the petroleum price keeps rising and the global warming becomes a major threat to sustainability of eco-environment, the demand for reliable and effective energy alternatives is increasingly urgent [1]. Among the developing alternative energy resources, hydrogen is recognized as the most promising alternative to fossil fuels and is expected to play a major role in future energy supply because it is clean, recyclable, and efficient [2]. To achieve a “hydrogen economy”, non-polluting and sustainable H<sub>2</sub> production methods need to be developed.

Biological H<sub>2</sub> production by using light-dependent fermentative pathways appears to be a good candidate, as it is environment-friendly, less energy intensive and inherits theoretically high substrate conversion efficiency [3–5]. In particular, hydrogen production 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 [6,7], thereby acting as a promising option for biohydrogen production. [8–10].

Dark fermentation with mainly acidogenic bacteria (*Enterobacter*, *Bacillus*, and *Clostridium*) are well known to produce H<sub>2</sub>

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from converting various organic substrates (e.g., glucose and sucrose) into soluble metabolites (i.e., volatile fatty acids (VFAs) and alcohols, [11–14], which can be further utilized by photosynthetic bacteria (PSB) (such as purple nonsulfur bacteria) to produce more  $H_2$  at the expense of light energy [15–18]. Therefore, using anaerobic fermentative bacteria and PSB for sequential dark and photo fermentation has been regarded as an efficient system for further enhancement of the energy recovery, biological  $H_2$  production yield and total COD removal efficiency [19–24]. In particular, a theoretically maximum yield of 12 mol  $H_2$  per mol hexose could be achieved with an integrated dark/photo fermentation system [25], while a  $H_2$  yield of 8 mol/mol glucose is considered sufficient for economic applications [26]. Therefore, integration of dark and photo fermentation for high-yield  $H_2$  production should be done to ensure commercial viability of fermentative  $H_2$  production processes.

The major problems associated with photo fermentation systems are the high power consumption and high operation cost of the artificial light sources (e.g., tungsten filament lamp, halogen lamp or metal-halide lamp). To improve the light efficiency for phototrophic  $H_2$  production with a lower operation cost is a substantial step towards the development of a successful  $H_2$  production process. Among all the light sources available, solar light energy is the most abundant natural light source on earth. Its radiation provides the biggest energy flow of ca.  $5.7 \times 10^{24}$  J year<sup>-1</sup> [19,27,28]. This data is about 10 000 times higher than the total energy consumed by human beings [24]. Therefore, sunlight is commonly used as the energy source for photosynthetic bacteria during the period of cultivation [29]. Many advantages have been found for using sunlight, as it is free and contains full spectrum of light energy [6,7,20,30,31]. Therefore, efficient utilization of solar light energy can simultaneously solve the problems with using artificial light source, higher operation cost, energy problems and environmental pollution. Most of the commercial cultivation of photosynthetic bacteria is carried out in open ponds, as solar light energy is directly utilized. Kim et al. [32] presented the first report on a semi-continuous outdoor photobioreactor using sunlight as the light sources to produce  $H_2$  during the operation time of 45 days. However, the performance of those outdoor open ponds are usually poor due to being difficult to control the culture conditions, contamination, low light intensity or uneven light distribution [33], day-night cycles, diurnal variation and requirement for large area of land [34,35]. There might be desirable to develop a novel photobioreactor which can be efficiently illuminated by solar light. Miyake et al. [36] constructed a sunlight collecting system and simulated sunlight illumination pattern by using halogen lamp as the light source. The foregoing problems can be solved by using indoor photobioreactors [37], which are usually more efficient and easier to control.

In this study, engineering strategy was 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 using dark- $H_2$  fermentation broth with different dilution ratio. Furthermore, our previous findings [37] showed that phototrophic hydrogen production performance is closely related with the nutrient composition in the medium. Therefore, different

nutrient supplement, such as yeast extract and glutamic acid, was added into dark-fermented broth to explore their effects on phototrophic hydrogen production performance. Furthermore, based on our recent findings [33–35], a novel and more cost-effective illumination system, in which optical fibers excited by sunlight were used as the internal illumination system, was assessed for its effect on photo- $H_2$  production performance.

## 2. Experimental section

### 2.1. Microorganism and medium

The phototrophic  $H_2$  producer used in this study was *Rhodospseudomonas palustris* WP3-5, which was isolated from a swine wastewater treatment plant in central Taiwan [37]. The culture medium for the strain consisted of (in g/l) HAC, 2000 (mg COD/l; COD denotes chemical oxygen demand); glutamic acid, 0.4;  $K_2HPO_4$ , 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 grown anaerobically (with argon gas sparging the medium to create an anaerobic condition for cultivation) at pH 7.0, 32 °C for 48 h under illumination with tungsten filament lamps (light intensity = 50 W/m<sup>2</sup>).

### 2.2. Bioreactor setup and operation

Schematic description of the two-stage process combining dark and photo fermentation is shown in Fig. 1a. First, a pure strain of *Clostridium pasteurianum* CH4 isolated from effluent sludge of anaerobic  $H_2$ -producing bioreactors [38] was used to produce  $H_2$  via batch dark fermentation in 2 l serum vials with a culture temperature and pH of 37 °C and 7.0, respectively. 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 [25,38,39]. The dark- $H_2$  fermentation broth was centrifuged at 13 000 rpm. The collected supernatant was diluted with distilled water at different dilution ratio and then its pH was adjusted to 7.1. The dark fermentation gave an  $H_2$  yield of 3.80 mol  $H_2$ /mol sucrose, and meanwhile forming 2632 and 8530 mg COD/l of HAC and HBU, respectively [25]. This pretreated supernatant was used as the substrate for phototrophic  $H_2$  production with *R. palustris* WP3-5. The photobioreactor (PBR) was a 260 ml 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<sup>2</sup>. The external light source was mounted on both sides of the PBR as indicated in Fig. 1a. In some cases, the photobioreactor was a 2.7-liter glass-made vessel illuminated with solar-energy-excited side light optical fiber and external light sources (100 W tungsten filament lamp) (Fig. 1b). Two pieces of side-light optical fiber (SLOF) protected in a glass tube immersed into the liquid medium inside the photobioreactor.

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