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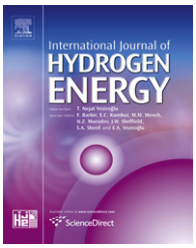

Engineering strategies for the enhanced photo-H_2 production using effluents of dark fermentation processes as substrate

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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_2 production performance of an indigenous purple non-sulfur bacterium *Rhodopseudomonas palustris* WP3-5. The best photo-H_2 production performance occurred at a dilution ratio of 1:2, giving a highest overall H_2 production rate of 10.72 ml/l/h and a higher overall H_2 yield of 6.14 mol H_2/mol sucrose. The maximum H_2 content was about 88.1% during the dilution ratio of 1:2. The photo-H_2 production performance was further improved by supplying yeast extract and glutamic acid as the nutrient. The results indicate that the overall H_2 production rate and H_2 yield increased to 17.02 ml/l/h and 10.25 mol H_2/mol sucrose, respectively. Using a novel solar-energy-excited optical fiber photobioreactor (SEEOFP) with supplementing tungsten filament lamp (TL) irradiation, the overall H_2 production rate was improved to 17.86 ml/l/h. Meanwhile, the power consumption by combining SEEOFP 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_2 production can be markedly enhanced along with a reduction of power consumption.

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 alterative 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_2 production methods need to be developed. Biological H_2 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_2

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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. 5.7×10^{24} J $year^{-1}$ [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 *Rhodopseudomonas 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^2).

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^2. 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.

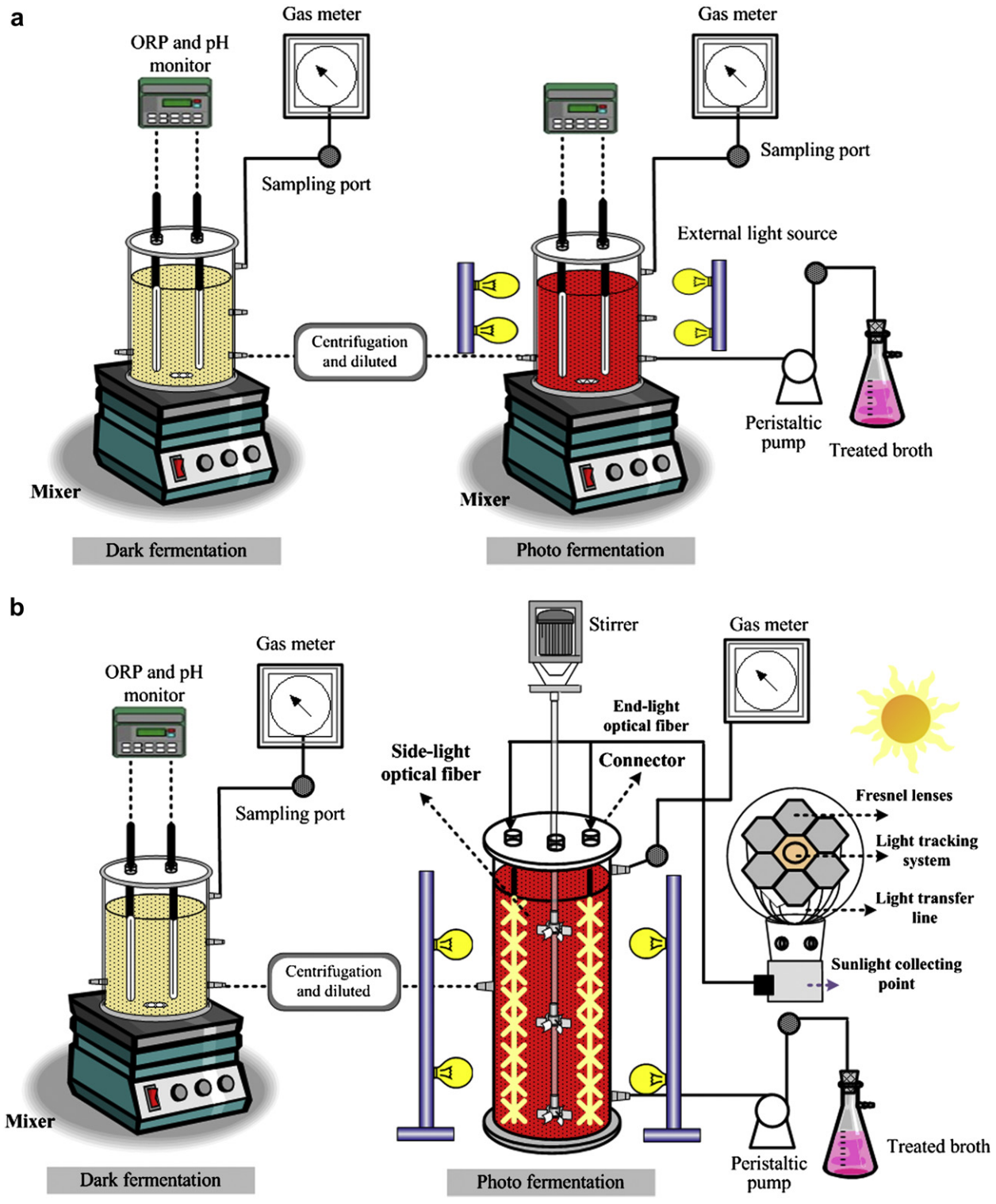

Fig. 1 – Schematic description of the two-stage process combining dark and photo fermentation equipped with (a) only external light sources and (b) combinative internal (optical fiber excited by sunlight collecting system) and external (four tungsten filament lamp) light sources.

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 made of quartz glass (diameter: 11 mm, length: 5 and 50 cm) obtained from Laforet Engineering Co. Ltd. (Tokyo, Japan). The side light optical fiber was excited by sunlight collecting system (Laforet Engineering Co. Ltd. Tokyo, Japan). The sunlight was collected by using fresnel lenses and then transmitted through an end-light optical fiber toward side-light optical fibers [3,37,40,41]. The light tracking system makes each fresnel lenses rotated toward the position of sun

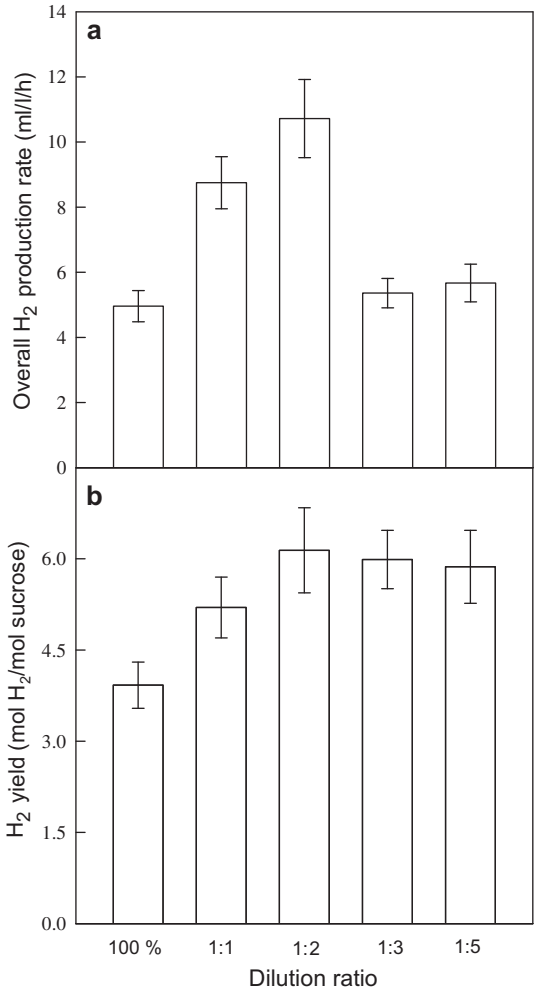

Fig. 2 – Effect of dilution ratio on the performance of phototrophic H_2 production under batch operations (a) overall H_2 production rate and (b) H_2 yield. (Light source: halogen and tungsten lamp (HL/TL); total light intensity = 95 W/m^2).

so that the SLOF could gain maximum light energy from sunlight during the daytime. After autoclave sterilization of the bioreactor, *R. palustris* WP3-5 cells were inoculated (10% inoculum) into the reactor. The batch photo-fermentation reactor was carried out at a light intensity of (95 W/m^2) with the illumination of tungsten filament lamps as the external light sources. The internal light source was solar-energy-excited side light optical fiber (SEEOF) whose light intensity varied within the range of 0–350 W/m^2 depending on the weather and time. 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 200 rpm (Fig. 2).

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 by gas syringe at desired time intervals to measure the gas composition. The liquid sample was also collected from the reactor as a function of time to determine cell concentration, pH and residual acetate concentration. For batch cultures, time-course data of cumulative H_2 production were simulated by modified Gompertz equation (Eq. (1)) [42] to determine the kinetic parameters of photo-H_2 production.

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

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 culture time (h), and λ denotes the lag time required for the onset of H_2 evolution (h).

2.3. Analytical methods

Cell concentration of the culture was regularly monitored by measuring optical density at 660 nm (*i.e.*, OD_{660}), which was converted to dry cell weight (DCW) concentration via proper calibration (1.0 OD_{660} approximately equals 4.47 g dry cell/l). The NH_3–N concentration was measured by using ammonia photometer (Model HI 93715, Hanna Instruments, Ann Arbor, Michigan, USA). Acetate (HAc) concentration was determined by gas chromatography (GC-14B, Shimadzu, Tokyo, Japan) equipped with flame ionization detector (FID). The detailed procedures for GC analysis were described in our recent work [40]. The gas products (H_2 and CO_2) were also analyzed by gas chromatography (Model 9800, China Chromatography, Taipei, Taiwan) using a thermal conductivity detector following the same procedures given in our recent reports [43,44].

Table 1 – Photo-H_2 production performance of *Rhodopseudomonas palustris* WP3-5 using dark fermentation effluent as substrate under different dilution ratio.

Dilution ratio	Cumulative H_2 production (ml)	Max. H_2 production rate (ml/l/h)	Model simulation[a]			
			H_{max} (ml)	R_{max} (ml/h)	λ(h)	r^2
No dilution	15.6	1.883	19.59	0.1883	4.56	0.997
1:1	89.2	14.15	8.9	1.415	3.926	0.992
1:2	99.5	19.07	100.4	1.907	9.17	0.999
1:3	70.1	17.34	65.0	1.734	1.182	0.989
1:5	44.1	18.40	42.5	1.84	16.34	0.997
1:19	19.7	6.52	19.23	0.652	3.18	0.993

a Simulation of time-course data by modified Gompertz equation.

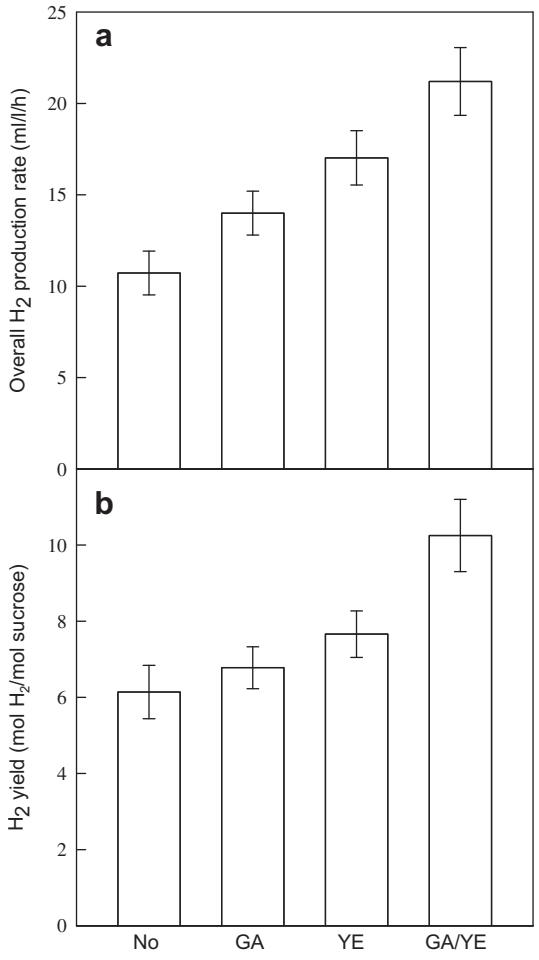

Fig. 3 – Effect of nutrient addition on the performance of phototrophic H_2 production under batch operations (a) overall H_2 production rate and (b) H_2 yield. (Added concentration of glutamic acid = 400 mg/l; added concentration of yeast extract = 200 mg/l; dilution ratio for dark fermentation broth = 1:2; Light source: halogen and tungsten lamp (HL/TL); total light intensity = 95 W/m^2).

3. Results and discussion

3.1. *Effect of dilution ratio of dark-H_2 fermentation broth on phototrophic H_2 production*

The major metabolic products from dark hydrogen fermentation process, mainly volatile fatty acids (VFA), could be further mineralized to produce more H_2 by photosynthetic bacteria via photo fermentation [37,45]. Improving phototrophic H_2 production rate is a substantial step towards development of a successful dark/photo-H_2 fermentation process. Many reports showed that the photosynthetic bacterium (*R. palustris*) could convert VFA into hydrogen with a high conversion efficiency [30,46,47]. Thus, in this study, different dilution ratio of dark fermentation broth was utilized as the substrate for photo-H_2 production with *R. palustris* WP 3–5. The halogen and tungsten filament lamps were used as the external light sources with a similar light intensity of ca. 95 W/m^2. The photo-H_2 production performance of using different dilution ratio with dark fermentation broth as substrate was shown in Table 1. The dilution ratio of 1:2 appeared to result in much better cumulative H_2 production and maximum H_2 production rate over those obtained from others dilution ratios. Meanwhile, using dilution ratio of 1:2 also gave the highest overall H_2 production rate of 10.72 ml/l/h (Fig. 2a). Also, according to the modified Gompertz equation (Eq. (1)), the R_{max} (representing the kinetic characteristics of H_2 production) was higher for the dilution ratio of 1:2 (1.907 ml/h), slightly higher than 1.734 and 1.84 ml/h obtained from the dilution ratio of 1:3 and 1:5, respectively, but much higher than 1.415 and 0.1883 ml/h for dilution ratio of 1:1 and original dark fermentation broth, respectively (Table 1). The significantly decrease in bioH2 production performance with lower dilution ratio or no dilution could be attributed to an excessive VFA concentration, resulting in inhibition to photo-H_2 production. This possible substrate inhibition effect is consistent with reports indicating that the photo-H_2 production was inhibited when initial substrate (VFA) concentration was higher than a threshold level (HAc concentration = 2250–2750 mg COD/l and HBu concentration = 2000–3800 mg COD/l) [11,42,48–50]. During the course of batch fermentation, the pH did not vary significantly as it maintained within the range of

Table 2 – Effect of photo-H_2 production performance of *Rhodopseudomonas palustris* WP3-5 under different illumination settings using dark fermentation effluent as substrate (Initial HBu and HAc concentration was 877 and 2843 mg COD/l, respectively).

Nutrient addition	Cumulative H_2 production (ml)	Max. H_2 production rate (ml/l/h)	Model simulation[d]			
			H_{max} (ml)	R_{max} (ml/h)	λ(h)	r^2
No	96.0	19.07	100.4	1.907	9.17	0.999
Glutamic acid (GA)[a]	126.5	20.8	127.7	2.08	8.46	0.992
Yeast extract (YE)[b]	164.2	21.0	169.3	2.1	3.40	0.988
GA/YE[c]	242	25.2	276	2.53	7.11	0.991

a Concentration = 400 mg/l.
b Concentration = 200 mg/l.
c Addition of GA (400 mg/l) and YE (200 mg/l).
d Simulation of time-course data by modified Gompertz equation.

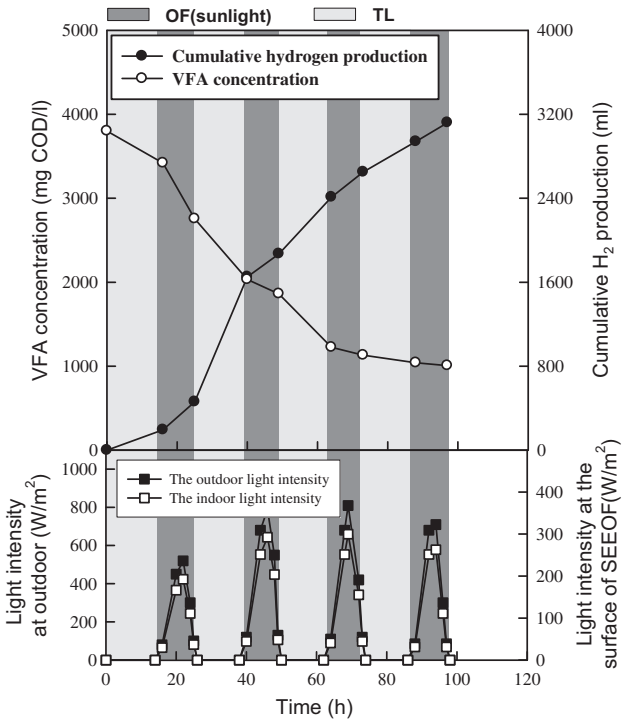

Fig. 4 – Effect ofsolar-energy-excited optical fiber photobioreactor (SEEOFP) on the performance of phototrophic H_2 production under batch operations (Dilution ratio from dark-fermented broth:1:2; Light source: OF(sunlight)/TL, where TL represents tungsten filament lamp and OF(sunlight) represents side light optical fiber excited by sunlight; added glutamic acid and yeast extract concentration was 400 and 200 mg/l, respectively).

7.0–7.8 for the dilution ratio of 1:1, 1:3 and 1:5 (data not shown). Nevertheless, higher H_2 yield was obtained from a dilution ratio of 1:2. The total hydrogen yield with dilution ratio of 1:2 increased from 3.80 mol H_2/mol sucrose for dark fermentation alone to 6.14 mol H_2/mol sucrose for the integrated dark/photo fermentation process with dilution ratio of 1:2. These results indicate that a dilution ratio of 1:2 could markedly enhance photo-H_2 production performance. This information is useful in practical applications, especially when dark-fermented broth or industrial wastewater was directly utilized as the substrate for photo-H_2 fermentation. However, there might still be some unknown photo fermentation inhibitors or insufficient nutrients in the dark fermentation broth, while it was used as the photo fermentation substrate. Therefore, further investigation is still needed to improve phototrophic hydrogen production performance from dark fermentation broth.

3.2. *Effect of nitrogen source and nutrients on the phototrophic H_2 production*

It is well known that hydrogen production from anoxygenic phototrophic bacteria may be inhibited when an excess amount of NH_4^+ ions is present due to repression of the synthesis of nitrogenase responsible for photo-H_2 production [14,37,41,51]. The original ammonia nitrogen concentration in our dark fermentation broth was ca. 65 mg/l, which appears to be high enough to inhibit photo-H_2 production. Hence, a proper dilution of the original dark fermentation broth is necessary for effective photo fermentation, as indicated in the preceding section. With a dilution ratio of 1:2, the ammonia nitrogen concentration was simultaneously diluted to ca. 21.5 mg/l reaching a non-inhibitory level for photo-H_2 production according to the literature [30]. With this dilution strategy, the residual ammonia nitrogen concentration in the medium rapidly decreased from 21.5 to 0.4 mg/l during the 2-day operation (data not shown). It is also noticed that the concentration of nutrient was insufficient for photo-fermentation process while using the diluted dark fermentation broth (dilution ratio of 1:2). This insufficiency may be resolved by addition of nutrients into the diluted dark fermentation broth.

In this work, both glutamic acid (GA) and yeast extract (YE) were used as added nutrient source to investigate whether the photo-H_2 production performance could be further improved under the same total light intensity (ca. 95 W/m^2), light sources (HL/TL) and dilution ratio (1:2). As shown in Fig. 3, the overall H_2 production rate and H_2 yield obviously increased with addition of GA (400 mg/l) and YE (200 mg/l) when compared with the performance from using the original dark fermentation broth as substrate. Using GA and YE resulted in similar H_2 yield of 6.78 and 7.66 ml H_2/mol sucrose, respectively (Fig. 3b). However, addition of YE gave a higher overall H_2 production rate (17.02 ml/l/h) than using GA (14.0 ml/l/h) (Fig. 3a). The highest overall H_2 production rate (21.2 ml/l/h) and H_2 yield (10.25 mol H_2/mol sucrose) were obtained with adding both GA and YE together (Fig. 3). This performance represents a 152% increase in cumulative H_2 production, 97.8% increase in overall H_2 production rate, and 66.9% increase in H_2 yield when compared to the control experiments (i.e., without nitrogen source addition) (Table 2, Fig. 3). Moreover, the addition of both GA and YE attained the highest R_{max} of 2.53 ml/h at a dilution ratio of 1:2, followed by 2.1 ml/h for YE culture, 2.08 ml/h for GA culture and 1.907 ml/h for the culture without nitrogen source addition (Table 2). The foregoing results seem to suggest that YE and GA are both important nutrients for enhancing the phototrophic hydrogen production from *R. palustris* WP 3–5 either by accelerating nitrogenase activity or by improving cell growth.

By using the two-stage integrated dark/photo fermentation process, the total hydrogen yield increased from 6.14 mol H_2/mol sucrose (with dilution ratio of 1:2) to 10.25 mol H_2/mol sucrose (with dilution ratio of 1:2 and addition of GA and YE). The high hydrogen yield not only indicates a good efficiency of converting carbon substrate into the target product (H_2), but could also displays high economic feasibility of the biohydrogen conversion system [52–55].

3.3. *Phototrophic H_2 production from using solar-energy-excited optical fiber photobioreactor (SEEOFP)*

Our previous work showed that solar-energy-excited optical fiber photobioreactor (SEEOFP) could markedly stimulate cell growth and phototrphic H_2 production by *R. palustris* WP3-5 [42]. In the present study, the SEEOFP system was combined with optimal dark fermentation broth dilution ratio and

nitrogen source addition to further improve photo fermentation efficiency and to reduce the operation cost. In this study, the SEEOFP was illuminated by combinative light sources OF(sunlight)/TL composed of a daytime internal illumination with optical fiber excited by solar energy (OF(sunlight)) and a night-time external irradiation with tungsten filament lamp (TL). The light intensity from OF(sunlight) was within a range of 0–350 W/m^2 (depending on the weather, the season, and the time frame of the day; for instance, light intensity was 0 at night), while the light intensity of TL was fixed at ca. 95 W/m^2. Time–course profiles of cumulative H_2 production, VFA concentration and outdoor/indoor light intensity are shown in Fig. 4. The initial and final VFA concentration was 3800 and 1007 mg COD/l, respectively. This indicates that using OF(sunlight)/TL gave a cumulative H_2 production and total COD removal efficiency of 3120 ml and 73.5%, respectively (Fig. 4). The total hydrogen yield with a dilution ratio of 1:2 increased from 3.80 mol H_2/mol sucrose for dark- fermentation alone to 7.88 mol H_2/mol sucrose for combining dark fermentation and photo fermentation (using SEEOFP). This performance is much higher than a yield of 6.63 mol H_2/mol sucrose obtained from relevant studies reported in the literature [56].

To explore possible influencing factors that could limit the total hydrogen yield from the integrated dark/photo fermentation process, the ammonia nitrogen concentration and VFA composition in the medium were monitored. It was found that the residual ammonia nitrogen concentration in medium was close to 0.6 mg/l, which appears to be too low to inhibit photo-H_2 production performance. On the other hand, analysis of the residual VFA composition shows a large quantity of butyric acid, which accounted for nearly 90% of total soluble metabolites. The high butyric acid concentration seemed to inhibit total H_2 yield and total COD removal efficiency, as recent studies showed that the photo-H_2 production performance was inhibited when butyric acid was in excess [30]. Meanwhile, from the aspect of electricity consumption, using OF(sunlight)/TL is a sustainable and economical illumination system for photo-H_2 production. The electricity consumption by combining SEEOFP (daytime) and TL (night-time) was about 37.1% lower than using TL (whole day). Therefore, combining medium improvement and photobioreactor design, the bioH_2 production performance could be much improved. In addition, to our best knowledge, this study appears to be the first report raising the idea of combining solar energy optical fiber system in integration of dark and photo fermentative H_2 producing process.

4. Conclusions

This work is a successful demonstration of combining dark and photo fermentation for high-yield bioH_2 production. Engineering strategies including medium pretreatment and improvement as well as innovative photobioreactor design were applied for a much improvement in bioH_2 production performance. Using the optimal medium conditions (1:2 dilution ratio for original dark fermentation broth and addition of dual nutrients) and the SEEOFP photobioreactor for photo-H_2 fermentation, the total hydrogen yield could be increased from 3.85 to 7.88 mol H_2/mol sucrose, while most of the soluble metabolites from dark fermentation (i.e., volatile fatty acids) was consumed, thereby reducing the COD level of the dark-fermented broth. Photo-H_2 production performance was significantly stimulated by the addition of the yeast extract and glutamic acid. The electricity consumption for phototrophic H_2 production was reduced 37.1% by using the SEEOFP system. The information provided from this work would be helpful in developing high-yield and low-cost bioH_2 production system using two-stage dark/photo fermentation process.

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REFERENCES

[1] Goldemberg J, Coehlo ST, Lucon O. How adequate policies can push renewables. Energy Policy 2004;32:1141–6.
[2] Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. Enzyme Microb Technol 2006;38(5):569–82.
[3] Chen CY, Lee CM, Chang JS. Hydrogen production by indigenous photosynthetic bacterium *Rhodopseudomonas palustris* WP3-5 using optical-fiber-illuminating photobioreactors. Biochem Eng J 2006;32:33–42.
[4] Ust'ak S, Havrland B, Muñoz JOJ, Fernández EC, Lachman J. Experimental verification of various methods for biological hydrogen production. Int J Hydrogen Energy 2007;32(12): 1736–41.
[5] Meher Kotay S, Das D. Biohydrogen as a renewable energy resource—prospects and potentials. Int J Hydrogen Energy 2008;33(1):258–63.
[6] Kataoka N, Miya A, Kiriyama K. Studies on hydrogen production by continuous culture system of hydrogen-producing anaerobic bacteria. Water Sci Technol 1997;36(6–7): 41–7.
[7] Miyake J, Mao XY, Kawamura S. Photoproduction of hydrogen from glucose by a co-culture of a photosynethic bacterium and *Clostridium butyricum*. J Ferment Technol 1984; 62:531–5.
[8] Tao Y, He Y, Wu Y, Liu F, Li X, Zong W, et al. Characteristics of a new photosynthetic bacterial strain for hydrogen production and its application in wastewater treatment. Int J Hydrogen Energy 2008;33(3):963–73.
[9] Lee JZ, Klaus DM, Maness PC, Spear JR. The effect of butyrate concentration on hydrogen production via photofermentation for use in a Martian habitat resource recovery process. Int J Hydrogen Energy 2007;32:3301–7.
[10] Lata DB, Chandra R, Kumar A, Misra A. Effect of light on generation of hydrogen by *Halobacterium halobium* NCIM 2852. Int J Hydrogen Energy 2007;32(15):3293–300.
[11] Lo YC, Chen SD, Chen CY, Huang TI, Lin CY, Chang JS. Combining enzymatic hydrolysis and dark-photo fermentation processes for hydrogen production from starch feedstock: a feasibility study. Int J Hydrogen Energy 2008; 33(19):5224–33.
[12] Lo YC, Chen WM, Hung CH, Chen SD, Chang JS. Dark H2 fermentation from sucrose and xylose using H2-producing

indigenous bacteria: feasibility and kinetic studies 42. Water Res 2008;42:827–42.
[13] Kumar N, Das D. Continuous hydrogen production by immobilized *Enterobacter cloacae* IIT-BT 08 using lignocellulosic materials as solid matrices. Enzyme Microb Technol 2001;29:280–7.
[14] Fang HHP, Zhu HG, Zhang T. Phototrophic hydrogen production from glucose by pure and co-cultures of *Clostridium butyricum* and *Rhodobacter sphaeroides*. Int J Hydrogen Energy 2006;31(15):2223–30.
[15] Singh SP, Srivastava SC, Pandey KD. Hydrogen production by *Rhodopseudomonas* at the expense of vegetatable starch, sugar cane juice and whey. Int J Hydrogen Energy 1994;19(4):437–40.
[16] Thangaraj A, Kulandaivelu G. Biological hydrogen photoproduction using dairy and sugar cane wastewater. Bioresour Technol 1994;48(1):9–12.
[17] Shi XY, Yu HQ. Conversion of individual and mixed volatile fatty acids to hydrogen by *Rhodopseudomonas capsulata*. Int Biodeterior Biodegradation 2006;58(2):82–8.
[18] Shi XY, Yu HQ. Continuous production of hydrogen from mixed volatile fatty acids with *Rhodopseudomonas capsulate*. Int J Hydrogen Energy 2006;31(12):1641–7.
[19] Das D, Veziroglu TN. Hydrogen production by biological processes:a survey of literature. Int J Hydrogen Energy 2001; 26:13–28.
[20] Oh YK, Seol EH, Kim MS, Park S. Photoproduction of hydrogen from acetate by a chemoheterotrophic bacterium *Rhodopseudomonas palustris* P4. Int J Hydrogen Energy 2004;29: 1115–21.
[21] Uyar B, Eroglu I, Yücel M, Gündüz U, Türker L. Effect of light intensity, wavelength and illumination protocol on hydrogen production in photobioreactors. Int J Hydrogen Energy 2008; 32(18):4670–7.
[22] Lee JZ, Klaus DM, Maness P-C, Spear JR. The effect of butyrate concentration on hydrogen production via photofermentation for use in a Martian habitat resource recovery process. Int J Hydrogen Energy 2007;32(15):3301–7.
[23] Bolliger R, Zürrer H, Bachofen R. Photoproduction of molecular hydrogen from wastewater of a sugar refinery by photosynthetic bacteria. Appl Microbiol Biotechnol 1985;23: 147–51.
[24] Miyake J, Miyake M, Asada Y. Biotechnological hydrogen production:research for efficient light energy conversion. J Biotechnol 1999;70(1–3):89–101.
[25] Chen CY, Yang MH, Yeh KL, Liu CH, Chang JS. Biohydrogen production using sequential dark and photo fermentation processes. Int J Hydrogen Energy 2008;33(18):4755–62.
[26] Keasling JD, Benemann JR, Pramanik J, Carrier TA, Jones KL, Van Dien SJ. A toolkit for metabolic engineering of bacteria: application to hydrogen production. In: Zaborski OR, Benemann JR, Matsunaga T, Miyake J, San Pietro A, editors. Biohydrogen. New York: Plenum Press; 1997. p. 87.
[27] Tsygankov AA, Laurinavichene TV. Influence of the degree and mode of light limitation on growth characteristics of the *Rhodobacter capsulatus* continuous cultures. Biotechnol Bioeng 1996;51(5):605–12.
[28] Tsygankov AA, Hirata Y, Miyake M, Asada Y, Miyake J. Photobioreactor with photosynthetic bacteria immobilized on porous glass for hydrogen photoproduction. J Ferment Bioeng 1994;77(5):575–8.
[29] Wakayama T, Miyake J. Light shade bands for the improvement of solar hydrogen production efficiency by *Rhodobacter sphaeroides* RV. Int J Hydrogen Energy 2002; 27(11–12):1495–500.
[30] Chen CY, Lu WB, Liu CH, Chang JS. Improved phototrophic H_2 production with *Rhodopseudomonas palustris* WP3-5 using acetate and butyrate as dual carbon substrates. Bioresour Technol 2008;99:3609–16.
[31] Ogbonna JC, Soejima T, Tanaka H. An integrated solar and artificial light system for internal illumination of photobioreactors. J Biotechnol 1999;70:289–97.
[32] Kim JS, Ito K, Izaki K, Takabatake H. Production of molecular hydrogen by a semi-continues outdoor culture of *Rhodopseudomonas sphaeroides*. Agric Biol Chem 1987;51(4): 1173–4.
[33] Kim BW, Chang KP, Chang HN. Effect of light source on the microbiological desulfurization in a photobioreactor. Bioprocess Eng 1997;17:343–8.
[34] Terry KL, Raymond LP. System design for the autotrophic production of microalgae. Enzyme Microb Technol 1985;7: 474–87.
[35] Laws EA, Taguchi S, Hirata J, Pang L. High algal production rates achieved in a shallow outdoor fume. Biotechnol Bioeng 1986;28(2):191–7.
[36] Miyake J, Wakayama T, Schnackenberg J, Arai T, Asada Y. Simulation of the daily sunlight illumination pattern for bacterial photo-hydrogen production. J Biosci Bioeng 1999; 88(6):659–63.
[37] Chen CY, Chang JS. Feasibility strategies for enhanced photohydrogen production from *Rhodopseudomonas palustris* WP3-5 using optical-fiber-assisted illumination systems. Int J Hydrogen Energy 2006;31(15):2345–55.
[38] Lee KS, Wu JF, Lin PJ, Chang JS. Anaerobic hydrogen production with an efficient carrier-induced granular sludge bed bioreactor. Biotechnol Bioeng 2004;87(5):648–57.
[39] Lee KS, Lo YS, Lo YC, Lin PJ, Chang JS. Hydrogen fermentation with anaerobic sludge using activated-carbon supported packed-bed bioreactors. Biotechnol Lett 2003;25: 133–8.
[40] Chen CY, Saratale GD, Lee CM, Chen PC, Chang JS. Phototrophic hydrogen production in photobioreactors coupled with solar-energy-excited optical fibers. Int J Hydrogen Energy 2008;33:6878–85.
[41] Chen CY, Chang JS. Enhancing phototropic hydrogen production by solid-carrier assisted fermentation and internal optical-fiber illumination. Process Biochem 2006; 41(9):2041–9.
[42] Lee CM, Chen PC, Wang CC, Tung YC. Photohydrogen production using purple nonsulfur bacteria with hydrogen fermentation reactor effluent. Int J Hydrogen Energy 2002; 27(11–12):1309–13.
[43] Ginkel SV, Sung S, Lay JJ. Biohydrogen production as a function of pH and substrate concentration. Environ Sci Technol 2001;35:4726–30.
[44] Lin CY, Lay CH. Effects of carbonate and phosphate concentrations on hydrogen production using anaerobic sewage sludge microflora. Int J Hydrogen Energy 2004;29:275–81.
[45] Chen CY, Lu WB, Wu JF, Chang JS. Enhancing phototrophic hydrogen production of *Rhodopseudomonas palustris* via statistical experimental design. Int J Hydrogen Energy 2007; 32:940–9.
[46] Asadaa Y, Ohsawa M, Nagai Y, Ishimi K, Fukatsu M, Hideno A, et al. Re-evaluation of hydrogen productivity from acetate by some photosynthetic bacteria. Int J Hydrogen Energy 2008;33(19):5147–50.
[47] Burrows EH, Chaplen FWR, Ely RL. Optimization of media nutrient composition for increased photofermentative hydrogen production by *Synechocystis* sp. PCC 6803. Int J Hydrogen Energy 2008;33(21):6092–9.
[48] Nath K, Muthukumar M, Kumar A, Das D. Kinetics of two-stage fermentation process for the production of hydrogen. Int J Hydrogen Energy 2008;33(4):1195–203.
[49] Laurinavichene TV, Tekucheva DN, Laurinavichius KS, Ghirardi ML, Seibert M, Tsygankov AA. Towards the integration of dark and photo fermentative waste treatment. 1. Hydrogen photoproduction by purple

bacterium *Rhodobacter capsulatus* using potential products of starch fermentation. Int J Hydrogen Energy 2008;33:7020–6.
[50] Eroğlu E, Eroğlu I, Gündüz U, Yücel M. Effect of clay pretreatment on photofermentative hydrogen production from olive mill wastewater. Bioresour Technol 2008;99(15):6799–808.
[51] Fang HHP, Liu H, Zhang T. Phototrophic hydrogen production from acetate and butyrate in wastewater. Int J Hydrogen Energy 2005;30:785–93.
[52] Koku H, Eroğlu İ, Gündüz U, Yücel M, Türker L. Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides*. Int J Hydrogen Energy 2002;27(11–12):1315–29.
[53] Zhu HG, Wakayama T, Asada Y, Miyake J. Hydrogen production by four cultures with participation by anoxygenic phototrophic bacterium and anaerobic bacterium in the presence of NH^{4+}. Int J Hydrogen Energy 2001;26:1149–54.
[54] Hillmer P, Gest H. H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*: production and utilization of H_2 by resting cells. J Bacteriol 1977;129:732–9.
[55] Hillmer P, Gest H. H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*: H_2 production by growing cultures. J Bacteriol 1977;129:724–31.
[56] Tao Y, Chen Y, Wu Y, He Y, Zhou Z. High hydrogen yield from a two-step process of dark- and photo-fermentation of sucrose. Int J Hydrogen Energy 2006;32(2):200–6.