Study on improvement of continuous hydrogen production by photosynthetic biofilm in interior illuminant reactor

Wenhui Liu1,2, Linjiang Yuan1* and Bo Wei3

1School of Environmental and Municipal Engineering, Xi’an University of Architecture and Technology, Xi’an-710055, China
2School of Material and Chemical Engineering, Xi’an Technological University, Xi’an-710021, China,
3School of Civil Engineering and Architecture, Xinjiang University, Urumqi-830047, China

*Corresponding Author E-mail: yuanlinjiang@xauat.edu.cn

Abstract

In the present study, a new type of interior optical fiber illuminating reactor was developed for H2 production to solve the problem of luminous intensity attenuation at the center portion of a reactor, and an immobilization technique was used to enhance the stability of a continuous hydrogen production process with attached photosynthetic bacteria, using glucose as a sole carbon substrate for the indigenous photosynthetic bacteria (PSB) Rhodopseudomonas palustris SP-6. Results of the experiments showed that the interior optical fiber illuminating reactor produces H2 more efficiently and productively than the exterior light source reactor, with the cumulative H2 production, the maximum H2 production rate and H2 yield increased by 813 ml, 11.3 ml l–1 h–1 and 22.3%, respectively. The stability of the product of continuous hydrogen was realized by immobilizing PSB on the surface of powder active carbon (PAC). After adding the dosage of 2.0 g l–1 PAC, the continuous steady operation of H2 production gave a high H2 yield of 1.398 mol H2 mol–1 glucose and an average H2 production rate of 35.1 ml l–1 h–1 illuminating with a single interior optical fiber light source. Meanwhile, a higher H2 yield of 1.495 mol H2 mol–1 glucose and an average H2 production rate of 38.7 ml l–1 h–1 were attained illuminating with a compound lamp in the continuous H2 production for 20 days.

Key words

Photosynthetic bacteria, Hydrogen production, Interior illuminant photobioreactor, Rhodopseudomonas palustris, Optical fiber

Introduction

Hydrogen is currently the focus of very active investigation as a promising future energy source due to its clean, recyclable, and highly eco-friendly nature in comparison to fossil fuels (Kalia and Purohit, 2008). Biological hydrogen production has attracted much recent attention because it is a process with little adverse environmental impact, and along with microbial hydrogen production has the potential to eliminate environmental deterioration. The process of biological hydrogen production has been widely studied under dark-fermentative and photo-fermentative conditions. The dark-fermentative bacteria, such as the acidogenic bacteria, ferment complex organic substances to H2 and volatile fatty acids (VFA), but cannot utilize organic acids as electron donors. The photosynthetic bacteria (PSB), in particular, purple nonsulfur bacteria, can use saccharide and small chain organic acids as electron donors for the production of H2 at the expense of light energy (Barbosa et al., 2001). In contrast to dark-fermentative process, photosynthetic hydrogen production process has the advantages of high efficiency of hydrogen evolution in terms of high theoretical conversion. A more efficient and complete process for production of H2 by combining acidogenic bacteria with PSB has been proposed (Chen et al., 2001). In
such a system, organic materials in waste products are fermented to H₂ and volatile fatty acids (VFAs) in a dark acidogenic reactor, while the VFAs in the effluent from the acidogenic reactor can be further converted to H₂ and CO₂ in a subsequent photosynthetic reactor. In spite of the advantages, as compared with dark fermentation, the main challenge during the photo fermentative hydrogen production is the lower rate of production of phototrophic hydrogen, mainly due to low growth rate of PSB and inefficient utilization of light energy in photobioreactors (Das and Veziroglu, 2001; Kondo et al., 2002; Chen et al., 2006).

Current considerable batch experiment was carried out to improve the rate of hydrogen production by optimizing operating conditions, screening efficient hydrogen-producing bacteria in laboratory scale experiments (Chen et al., 2007; Wakayama and Miyake, 2005). However, little research has been successful in continuous hydrogen production and in a large volume photobioreactor, mainly due to low growth rate of PSB resulting in a loss of photosynthetic bacteria in continuous hydrogen production, and the fast attenuation of light intensity resulting from the absorption of suspended solids leading to insufficient light intensity and inefficient utilization of light energy in the reactors. Thus, improving the rate of continuous phototrophic hydrogen production and the structure of the photobioreactor would be a substantial step towards development of a successful hydrogen production process.

In the present study, to solve the problem of luminous intensity attenuation the center portion of a large diameter reactor, a new type of interior optical fiber illuminating reactor was developed initially for H₂ production. In order to obtain uniform light intensity, a number of optical fibers were installed uniformly in the distribution plate of the reactor with small spacing, to lead the light beam from external light source or compound lamp in the continuous H₂ production for an extended period of time.

**Materials and Methods**

**Strain and culture medium**: Strain PS-6 of photosynthetic bacterium *Rhodopseudomonas palustris* was used in this phototrophic hydrogen production experiment, which was separated from the Fifth Wastewater Treatment Plant in Xi’an, China. The liquid medium was used as the strain growth medium, which included (g l⁻¹) the following: 3.0 sodium acetate; 0.6 NH₄Cl; 0.2 MgSO₄; 0.2 CaCl₂; 0.4 K₂HPO₄; 0.6 KH₂PO₄; 1.5 NaCO₃; 0.5 NaCl; 0.2 Yeast extract; 5.0ml ferrie citrate solution (1g l⁻¹); 1.0 ml trace element solution and 1.0ml growth factor. Trace element solution consisted (mg l⁻¹) 100 NiCl₂·6H₂O; 50 CuCl₂·2H₂O; 70 MnCl₂·4H₂O; 60 H₃BO₃; 50 NaMoO₄·2H₂O; 70 ZnCl₂; 200 CoCl₂·6H₂O and 1 ml l⁻¹ HCl (25%). The growth factor solution included (in mg l⁻¹) 1 Vitamin B1; 1 nicotinic acid; 0.1bifotin and 0.1 para aminobenzoic acid.

For continuous hydrogen production medium, the feeding liquid contained 8-10 g l⁻¹ glucose and 1.0 g l⁻¹ sodium glutamate. The culture of bacterial strain was carried out at 33±1°C and a light intensity was maintained at 4000 lux with the illumination of tungsten filament lamps. The pH of the medium was adjusted to 7.2 by using 1.0M HCl or NaOH solution prior to autoclaving.

**Setup and operation of photobioreactor**: The photobioreactor was made of Polymethyl methacrylate cylinder shaped container (10 cm diameter, 60 cm height), equipped with interior side glowing optical fiber (SGOF) (light machines, 150 W halogen lamp) and external light sources (60 W tungsten filament lamps) (Fig. 1).

![Fig. 1: Schematic diagram of photobioreactor system with internal and external light sources](image-url)
The SGOF composed of a polymethyl methacrylate core coated with fluorinated alkyl methacrylate copolymer. To attain maximum light emission from the polymethyl methacrylate core, covering layer was removed, so the SGOF could emit light from its clad surface to make the optical fiber glow fully.

In photofermentative hydrogen production, the suspending growth of photosynthetic bacteria needed the intense and uniform illumination. In order to obtain uniform light intensity, 28 optical fibers (6mm diameter, 50cm length) were installed uniformly in 10 cm diameter distribution plate spaced a centimeter apart both vertically and horizontally.

The PBR was illuminated with single or multiple light sources (e.g. interior optical fiber, exterior tungsten filament lamp), while the light intensity for each illumination system was kept at 4000lux (5.84W m²). In interior optical fiber illuminant bioreactor, the distribution of light intensity measured was uniform along the axial optical fiber surface. After sterilization of the bioreactor, *Rhodopseudomonas palustris* PS-6 cells were inoculated (10% inoculum) into the reactor containing 4 l of culture medium.

The culture of bacterial strain was carried out at 33±1°C and light intensity was maintained at 4000 lux with illumination of tungsten filament lamps. The pH of the medium was adjusted to 7.2 by 1.0M HCl or NaOH solution prior to autoclaving. Both batch and continuous cultures were carried out at 33±1°C, pH 7.3, and 200 rpm magnetic stirrers with a working volume of 4.0 l. In the second phase of the experiment, the powdered activated carbon used as carriers was added into the bioreactors to promote phototrophic H₂ production.

The PAC obtained from Lantian activated carbon, Inc. (Xi’an, China) was of power particles with a diameter of 38-50μm (sieving mesh -300-+400mu). The carriers were sterilized prior to use. In order to realize the continuity and stability of hydrogen production and solve the problem of the loss of photosynthetic bacteria resulting from low growth rate of PSB, a water filter (pore diameter <5μm) was assembled with an interior optic fiber bioreactor to intercept photosynthetic bacteria adhered on the surface of PAC and return to the bioreactor.

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 interval to measure the gas composition. Liquid sample was also collected from the reactor as a function of time to determine cell concentration, pH and residual acetate concentration.

**Analytical methods**: Samples were collected from the reactor three to four times daily, depending on reactor performance. The amount of biogas produced from the reactor was collected by volumetric method by displacement of 10% NaOH solution, which stripped CO₂ from the gas stream. The functional gas in the gas was analyzed by using a gas chromatograph (GC, 6890N, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and a 2m stainless column packed with 5%Å molecular sieve. The operational temperature at the injection port, the column oven and detector were 100, 60 and 105 °C, respectively. Argon was used as a carrier gas at a flow rate of 30 ml min⁻¹.

Cell concentration was determined by measuring optical density at 660 nm (i.e., OD₆₆₀) using a UV-VIS double-beam spectrophotometer (model UV1902, Shimadzu, Japan). The dry weights of the cell were obtained by filtering 20 ml reaction solutions through a cellulose acetate membrane filter (0.45μm pore size). Each loaded filter was dried at 105 °C until the weight was constant, then the dry weight of the filter was subtracted to obtain cell dry weight (CDW). Correlation of the OD₆₆₀ value with dry cell weight (CDW) gave the following relationship by CDW (g dry cell l⁻¹) = 1.26 × OD₆₆₀²⁻¹.

Light intensity was measured by light intensity meter (UV-365A, Kuhnast, Germany). The morphology of microorganism carrier surface was monitored by scanning electron microscopy (SEM, 2570,Hitachis, Japan) The pH of the samples was measured by a pH meter (Sartorius, PB-10, Germany). For continuous operation, batch cell growth was initially performed until reaching a late exponential growth phase. The medium was then fed continuously at a hydraulic retention time (HRT) of 48 hr. After reaching steady state, the HRT of continuous culture was shortened to 36 hr. The HRT is calculated by the following equation:

\[
\text{HRT}=\frac{V}{F}
\]

where, V is the working volume of the culture (ml) and F is the volumetric feeding rate of the medium (ml hr⁻¹).

**Results and Discussion**

The conventional light sources, for instance, halogen lamp, incandescent lamp, tungsten filament lamp, were often used as external light sources for the photobioreactors (Yang Shi and QingYu,2002; Lee et al., 2002; Miyake et al., 1999, 1984, 1987). However, the light intensity of these light sources decreased rapidly due to the shading effects resulting from biomass retention and/or aggregation of photosynthetic bacterium on the walls of the container due to biological phototaxis. The studies of Katsuda T.etc. have shown that the
relations between the attenuation of light intensity and the thickness of bacteria cell suspension solution was the rule of exponential decrease in photo-bioreactors (Katsuda et al., 2000). Thus, photosynthetic bacteria would have low activity if the diameter of the reactor was large in size.

To raise the phototrophic H₂ productivity, a new-type of bioreactor was developed with better optical fibers as light sources and internal irradiation, which offered more uniform illumination and a larger volume. In order to evaluate the performance of the new-type bioreactor, comparative batch experiment was performed in the photobioreactor (4 litre) illuminated with a single interior optical fiber light source or exterior tungsten filament lamp respectively. With same light intensity (4000 lux, 5.84 W m⁻²), initial glucose concentration (55mmol, 10200mg COD l⁻¹), value of liquid strain addition (on logarithmic phase, 20%v/v), phosphate buffer dosage and time of argon blowing (30min, 35ml min⁻¹), the H₂ producing performance was much enhanced with a single interior optical fiber light sources (Fig.2).

The data showed that the process of hydrogen production was influenced by pH when a large number of volatile fatty acids were present resulting from the fermentation of organic matter by photosynthetic bacteria. After addition of the phosphate buffer in the experiment, the value of pH was maintained between 6.2 to 6.6, which was a suitable pH range for hydrogen production.

Results of the experiment showed that using a new internal illumination system (with optical fibers), the cumulative H₂ production, maximum H₂ production rate and H₂ yield increased by 813 ml, 11.3 ml h⁻¹ and 22.3%, respectively, for exterior illumination system (with tungsten filament lamp) (Fig.2). However, the cell concentrations (OD₆60) slightly increased 6.3–8.2%.

The results indicate that inadequate light intensity not suitable for production of hydrogen was available for the growth of photosynthetic bacteria. The enhancing effect of the internal optical fiber illumination provided more uniform and efficient light energy distribution and utilization, as well as provided more irradiation area. In continuous hydrogen production, loss of photosynthetic bacteria resulting from low growth rate of PSB reduced or event inhibited the rate of hydrogen production, which was substantially influenced by biomass retention in the photobioreactor.

Recently, immobilization techniques have been used for cell stabilization (Mohan et al., 2008; Ivanova et al., 2008; Tsygankov et al., 1993; Kim et al., 2005) showing that immobilization is an effective approach to elevate biomass retention in bioreactors.

This knowledge led to examining whether adding carriers could also promote phototrophic H₂ production by Rhodopseudomonas palustris PS-6. For this purpose, a different dosage of PAC (0.0, 1.0, 2.0, 3.0, 4.0 g l⁻¹) was added to the bioreactors (1.0 l) containing initial glucose concentration of 55 m mol l⁻¹, liquid strain addition of 20% and light intensity of 5.84 W m⁻². The H₂ production performance of the carrier supplemented cultures is shown in Table 1. Results of the experiment showed that the highest production rate (21.2ml l⁻¹ h⁻¹) and cumulative H₂ production (1287ml) were obtained for cultures supplemented with the dosage of 2.0g l⁻¹ PAC, respectively. A dosage of 2.0 g l⁻¹ PAC appeared to result in much better H₂ production, H₂ production rate, OD₆60 and cell concentration than those obtained from the other dosages culture. At the same time, a large dosage of PAC (3.0g l⁻¹ and 4.0g l⁻¹) showed no improvement in the performance of H₂ production over the dosage of 1g l⁻¹ PAC and carrier-free culture.

Hence, among the suitable dosage of inorganic carriers in combination with an adequate porosity are a promising family of materials used for photosynthetic bacterial entrapment, which can provide a higher density of biological loading and biogas production rate. However, it should be noted that a large dosage of carriers can lower cell concentration and H₂ production rate, a drawback due to difficulties in light penetration and inadequate light intensity.

In order to explore a possible mechanism for H₂ production enhanced by immobilization of photosynthetic bacteria, scanning electron microscopy was carried out to assess the morphology of bacterial cell and microstructure of carrier granules surface. Results show that cells attached to the surface of PAC form biofilms (Fig. 3). It can be seen in Fig. 3(a), magnification factor was 10k, and dosage of PAC was 2.0g l⁻¹, there were number of cells firmly attached to the surface of PAC. Nevertheless, there were small number of cells attached to PAC surface when the dosage of PAC was 4g l⁻¹ (Fig. 3b). This suggests that huge porous microstructure of solid carriers might provide extra surface area for attached cell growth, and leading to an increase in H₂ production. Meanwhile, photosynthetic bacteria was intercepted and filtered easily due to enlarged size by adhering to the particle of PAC, making it easy to immobilize microorganism in photobioreactors.

To realize the continuity and stability of hydrogen production and solve the problem of loss of photosynthetic bacteria resulting from low growth rate of PSB, a water filter (pore diameter < 5μm) was assembled with an interior optic fiber bioreactor to intercept photosynthetic bacteria adhered on the surface of PAC and return it to the bioreactor. A comparative continuous experiment was performed in the interior optic fiber photobio-reactor (4.0 litre) illuminating...
Production of hydrogen by photosynthetic biofilm

Fig. 2: Performance comparisons of H₂ production in photobioreactor lighting with interior optical fiber and exterior tungsten filament lamp

- **a** pH comparison
- **b** OD (660 nm) comparison
- **c** H₂ production rate (ml g⁻¹ h⁻¹) comparison
- **d** Cumulative H₂ production (L) comparison
The experiment was divided into two stages including batch culture stage of photosynthetic bacteria and continuous H₂ production stage. The batch culture was set at a initial stage within the first 60 hrs with a value of the liquid strain addition (on logarithmic phase, 20%v/v), in order to elevate the concentration of bacteria, prolonging the biomass retention in bioreactors, and accelerating immobilization of photosynthetic bacteria. The continuous H₂ production was started at 60 hour, reaching a steady state within two days in terms of H₂ production rate and H₂ yield (Fig.4). Both of the H₂ producing performance irradiating with different light source were much enhanced because bacteria was intercepted and returned to the bioreactor under same initial glucose concentration (55mmol), dosage of PAC(2.0g l⁻¹), phosphate buffer dosage and time of argon blowing (30min, 35ml min⁻¹) (Fig.4).

Results of the experiment showed that a continuous H₂ production gave high H₂ yield of 1.398 mol H₂ mol⁻¹ glucose and an average H₂ production rate of 35.1ml l⁻¹ h⁻¹, illuminated with a single interior optical fiber light sources.

Meanwhile a continuous H₂ production illuminated with a compound lamp attained a higher steady-state H₂ yield of 1.495 mol H₂ mol⁻¹ glucose and an average H₂ production rate of 38.7ml l⁻¹ hr⁻¹ for 20 day steady operation.

The value of pH was maintained between 6.7 to 6.9 which was better than the pH value of bath hydrogen production without adding PAC. The cell concentrations (OD₆₆₀) were up to 3.544 and 3.325, increased by 31 and 23% relative to the cell concentrations of bath hydrogen production without adding PAC, separately.

The mechanism of carrier-induced promoting effects on continuous phototrophic H₂ production was cell growth rate increased when solid carriers were added (Chen et al., 2007). Similarly, recent studies have shown that immobilized bacteria play some role in enhancing biosurfactant production from Bacillus subtilis (Yeh M-S et al., 2005).

This study demonstrated that a new type of interior optical fiber illuminating reactor showed more efficient and productive performance of H₂ production than the conventional reactor to solve the problem of luminous intensity attenuation at the center portion of reactor.
Fig. 4: Performance of the continuous $H_2$ production in photobioreactors illuminated with only inner light source and multiple light sources after adding solid carriers.
Meanwhile, application of immobilization technique with attached photosynthetic bacteria on the surface of PAC could enhance the stability of continuous hydrogen production process, and improve H₂ production rate and yield when compared to the carrier-free culture.

**Acknowledgments**

This research was supported by the Major Science and Technology Projects of the Ministry of Environmental protection of China (ZX07212-002). Thanks to Prof. Hui Wu for technical assistance construction of a hydrogen fermentation reactor.

**References**


