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ORIGINAL RESEARCH PAPER

Enhanced photo-H_2 production by unsaturated flow condition in continuous culture

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Abstract A biofilm photobioreactor under unsaturated flow condition (BFPBR-U) is proposed using a polished optical fiber as the internal light source for photo-H_2 production in continuous culture. The main chamber was filled with spherical glass beads to create the reaction bed and the cells were immobilized to form a biofilm under unsaturated flow condition obtained by pumping substrate solution over a packing bed at a rate to create a thin fluid film and injecting the argon to maintain the gas phase space. The effects of operational conditions, including flow rate and influent substrate concentration, on the photo-H_2 production performance were investigated. The unsaturated flow conditions eliminated the inhibition caused by high organic loading rate and enhanced light transmission efficiency, leading to an improvement in the photo-H_2 production performance.

Keywords Biofilm photobioreactor · H_2 production · Optical fiber · Photo–H_2 production · Photosynthetic bacteria · Unsaturated flow conditions

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Introduction

Photo-H_2 production by photosynthetic bacteria is environmentally friendly and is a sustainable technology. It is recognized as a most promising candidate for large-scale H_2 production because of the advantages of high theoretical conversion yield and capability of utilizing a wide range of the solar spectrum (Hallenbeck and Benemann 2002). Photosynthetic bacteria can avoid inactivation of biological systems by O_2 caused by the oxygen-evolving activity as well as use a variety of organic substrates for waste treatment (Miyake et al. 1999). Thus, extensive efforts have been devoted to the studies of photosynthetic bacteria based on photo-H_2 production (Chen et al. 2006; Redwood et al. 2012; Zhu et al. 2011). Although promising, photo-H_2 production in a photobioreactor with photosynthetic bacteria is still relatively poor due to the low cell concentration caused by the cell washout in the continuous culture when the photo-H_2 production system is operated in the low hydraulic retention time (Guo et al. 2011).

One of the solutions to the problem for enhancing photo-H_2 production can be to use cell immobilization. In general, immobilization technology can be divided into two types: biofilm and cell entrapment techniques, both of which can not only improve the operation stability, but also increase the cell concentration, enhancing the volumetric productivity (Wang et al. 2010; Zhang et al. 2010). In particular, the biofilm technique is a more reasonable means because it can offer the advantages of strong mechanical strength, low mass transfer resistance and high stability for long-term operation over the entrapment technique (Bai et al. 2009; Tian et al. 2010). Currently, the biofilm technique is usually used under saturated flow conditions for photo-H_2 production (Guo et al. 2011; Liao et al. 2010; Tian et al. 2010). However, photo-H_2 production performance of biofilm photobioreactor suffers from two intrinsic characteristics: low energy conversion efficiency due to the shielding effects caused by the absorption of culture solution to light, and inhibition caused by high organic load rate which exceeds the maximum metabolism capacity of photosynthetic bacteria. To resolve these problems, therefore, the development of new strategy that can improve the utilization of energy and attenuate the inhibition of high organic load rate is significantly important.

In the present study, a biofilm photobioreactor under unsaturated flow conditions (BFPBR-U) is proposed for continuous photo-H_2 production using a polished optical fiber as the internal light source that achieved high light transmission efficiency and provided uniform light distribution obtained on the surface of optical fiber with a high relative ratio of illumination surface area to photobioreactor volume (Guo et al. 2011; Zhang et al. 2013). The objective of this study was to use unsaturated flow conditions created to avoid the shielding effects and eliminating the inhibition by a high organic load rate owing to the thin liquid film over the biofilm obtained by pumping the culture solution over the reaction bed and injecting argon to maintain the gas phase space. The effects of different operation variables, including flow rate and influent substrate concentration, on the photo-H_2 production performance of the BFPBR-U were also investigated in comparison with a biofilm photobioreactor under saturated flow condition (BFPBR-S).

Materials and methods

Microorganism and medium

Rhodopseudomonas palustris CQK 01, isolated from municipal sewage sludge by repetitious purification, was used as the producer for continuous photo-H_2 production. It was maintained in a synthetic medium (see Guo et al. 2011). For pre-culture, the pH value of culture medium was adjusted to seven by 0.1 M NaOH/HCl and glucose at 50 mM was used as the sole carbon source for the growth of the cells. Prior to incubation, the cells were cultivated at 30 °C for 72 h under an illumination intensity of approx. 4,000 lux and in an anaerobic atmosphere created by argon. The enriched cells were used as inocula for the start-up of the biofilm photobioreactor.

Design and operation of biofilm photobioreactor

The biofilm photobioreactor developed in this work was a sealed vessel, ht 200 mm and inside diam. 45 mm, fabricated from polymethyl methacrylate (PMMA) and packed with spherical glass beads (4 ± 0.5 mm diam.). A side-light, plastic-clad optical fiber with the working length of 200 mm and diam. 18 mm was polished to give a desired light intensity of ca. 30 W/m^2. The optical fiber was first protected by a PMMA tube and then immersed into the biofilm photobioreactor as an internal light source to provide the light energy for photosynthetic bacteria immobilized in the biofilm.

The experiments were divided into the start-up stage and the performance testing stage. In the start-up stage, the medium with inoculum (250 ml) was pumped into the experimental system under unsaturated flow conditions. The medium was kept at 500 ml h^{-1} and recycled by a peristaltic pump in the experimental system until a stable biofilm with photosynthetic bacteria was obtained on the surface of spherical glass beads. In the process of biofilm formation, 50 ml medium was discharged daily and 50 ml fresh medium with glucose at 30 mM was fed into the experimental system. The start-up stage lasted about 30 days after which the photo-H_2 production performance of the biofilm photobioreactor became stable and a biofilm was visually formed on the surface of spherical glass beads,

indicating that the cells had been successfully immobilized to form the stable biofilm and the performance testing could be performed. In the performance testing stage, the experimental system was an open-loop system. Unsaturated flow conditions were obtained by bubbling argon to maintain the gas phase space and pumping the fresh medium over the reaction bed at a rate to create a thin fluid film. In addition, fresh medium was filled into the biofilm photobioreactor to create a saturated flow condition. The flow rate of the medium was controlled by a calibrated peristaltic pump and the water displacement method was used to collect the evolved biogas for determining the amount of H_2 produced.

which shows the economic efficiency of the biofilm photobioreactor for photo-H_2 production using glucose as the substrate, and is presented as (Liao et al. 2010)

$$\mathrm{HY} = \frac{\text{Amount of } H_2 \text{ produced (mmol)}}{\text{Amount of substrate degraded (mmol)}} \quad (2)$$

The efficiency of energy recovery of the biofilm photobioreactor can be explained by the ECE, which is defined as the percent ratio of H_2 energy produced to the sum of input energy, including the input light energy and degraded substrate energy. The ECE reflects the energy accumulation in the process of continuous photo-H_2 production, and is shown as (Su et al. 2009)

$$\begin{aligned}\mathrm{ECE}\,(\%) &= \frac{\text{produced } H_2 \text{ energy (J)}}{\text{input light energy (J)} + \text{degraded substrate energy (J)}} \times 100 \\ &= \frac{\Delta H_H \times HPR(\text{mmol}\,l^{-1}\,h^{-1}) \times \text{photobioreactor volume (l)}}{3600 \times I(\text{W}\,m^{-2}) \times A(m^2) + \Delta H_S \times SDR(\text{mmol}\ l^{-1}\,h^{-1}) \times \text{photobioreactor volume (l)}} \times 100\end{aligned} \quad (3)$$

Estimation of photobioreactor performance

In this study, the photo-H_2 production performance of biofilm photobioreactor was mainly assessed by three variables, including H_2 production rate (HPR), H_2 yield (HY) and energy conversion efficiency (ECE).

As an evaluation criterion of the continuous photo-H_2 production capacity, the HPR refers to the amount of H_2 produced (mmol) per unit time (h) and per unit volume of photobioreactor (l), and is calculated by Guo et al. (2011)

$$\mathrm{HPR}\ (\text{mmol}\,l^{-1}h^{-1}) = \frac{\text{Amount of } H_2 \text{ produced (mmol)}}{t\ (\text{h}) \times \text{photobioreactor volume (l)}} \quad (1)$$

where t represents the time interval of photo-H_2 production process from the testing beginning to the sampling time.

The HY is given by the ratio of the amount of H_2produced to the amount of substrate degraded, where ΔH_H is the heat value of H_2 (242 J mmol^{-1}), I the light intensity, A the irradiated area, and ΔH_s the heat value of substrate (2,888 J mmol^{-1}), SDR the substrate degradation rate, which can be calculated by

$$\mathrm{SDR}(\text{mmol}\ l^{-1}h^{-1}) = \frac{\text{Amount of substrate degraded (mmol)}}{t(\text{h}) \times \text{photobioreactor volume (l)}} \quad (4)$$

Analytical methods

Illumination intensity was measured by a digital luxmeter (ST-85, Beijing, China) and the irradiation intensity was determined by a digital radiometer (FZ-A, Beijing, China). The H_2 content in the produced biogas was analyzed by GC using a thermal conductivity detector (TCD) and a 2 m stainless-steel column packed with porous styrene particles. Argon was the carrier gas at 25 ml min^{-1}. The gas chromatograph oven and TCD were maintained at 55 and 100 °C, respectively. The electric current of the TCD was

80 mA. Glucose was determined spectrophotometrically using the 3,5-dinitrosalicylic acid method and the pH value of culture medium was adjusted by a pH meter calibrated using buffers (pH of 4 and 7). The flow rate of culture medium was matched to the rotation speed of peristaltic pump via calibration.

Results and discussion

The organic load rate, depending on flow rate and influent substrate concentration, is a key variable affecting the photo-H_2 production performance (Guo et al. 2011; Liao et al. 2010). Therefore, in this study, the effect of these operational conditions, including flow rate and influent substrate concentration, on the photo-H_2 production performance of the BFPBR-U was extensively investigated in compared with the BFPBR-S and the details are shown in the following subsections.

Effect of flow rate

The photo-H_2 production performances of the BFPBR-S and the BFPBR-U were studied at flow rates of 20, 50, 80 and 120 ml h^{-1} while the operational temperature, the pH value of culture medium and substrate concentration were maintained at 30 °C, 7 and 60 mM, respectively. The experimental results are shown in Figs. 1, 2 and 3.

As shown in Fig. 1, the flow rate ranging from 20 to 120 ml h^{-1} significantly influenced the HPRs of the BFPBR-S and the BFPBR-U. At a flow rate at 20 ml h^{-1}, due to the insufficient organic substrate provided, the HPRs of the BFPBR-S and the BFPBR-U were only 1.13 and 2.09 mM, respectively. With the increase in flow rate from 20 to 80 ml h^{-1}, the HPRs increased correspondingly owing to the improvement in organic loading rate caused by the increase of flow rate significantly enhanced the quantity of substrate transferred from the bulk fluid into the biofilm, meeting the metabolic capacity of photosynthetic bacteria and then improving the HPRs of the BFPBR-S and the BFPBR-U. A flow rate of 120 ml h^{-1} resulted in a sharp decrease of the HPR of the BFPBR-S duo to the inhibitory effect on the activity of photosynthetic bacteria obtained at too high substrate concentration and a large amount of intermediate

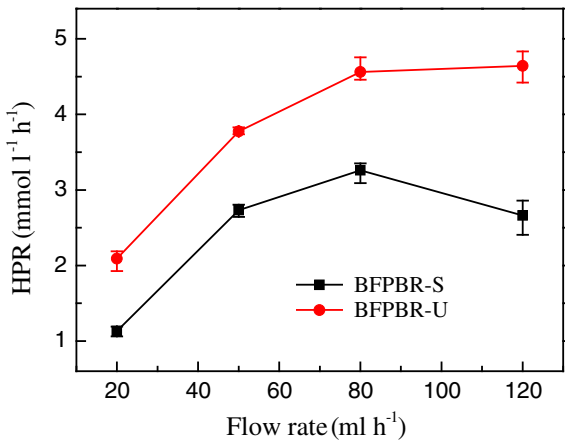

Fig. 1 Effect of flow rate on H_2 production rate (HPR)

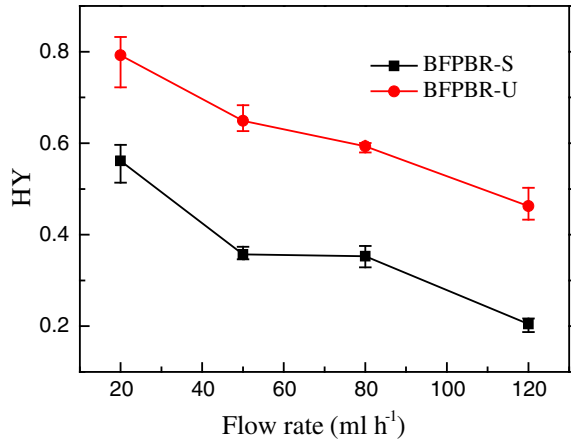

Fig. 2 Effect of flow rate on H_2 yield (HY)

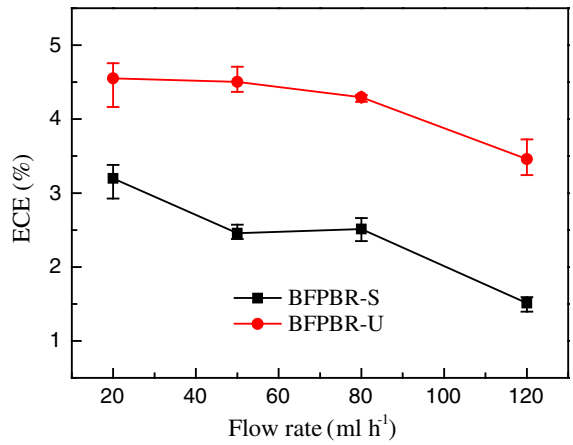

Fig. 3 Effect of flow rate on energy conversion efficiency (ECE)

species accumulated in the biofilm caused by the enhanced substrate transfer (Liao et al. 2010). However, for the BFPBR-U, the continuous increase of flow rate from 80 to 120 ml h^{-1} led to the slight enhancement of the HPR from 4.56 to 4.64 mmol l^{-1} h^{-1} because the limited liquid space of the rather thin liquid film formed on the biofilm caused the lower substrate and products load, avoiding the inhibitory effect on the activity of photosynthetic bacteria. Moreover, the upward trend of the HPR of the BFPBR-U slowed down with the variation of flow rate from 20 to 120 ml h^{-1}. This trend is reasonable because the mass transport is the key step for the BFPBR-U. With the continuous enhancement of flow rate, the substrate load of the BFPBR-U was gradually improved and then the metabolic capacity of photosynthetic bacteria was gradually sufficient for biodegrading substrate to H_2, weakening the restrictive effect of substrate on the HPR.

Figure 2 shows that the HYs of the BFPBR-S and the BFPBR-U decreased monotonically when the flow rate increased in the whole flow rate range studied. At a rate flow of 20 ml h^{-1}, the highest HYs of the BFPBR-S and the BFPBR-U were 0.56 and 0.79, respectively. The diminution trends of the HYs can be mainly attributed to an increase in flow rate improving the mass transfer of substrate and shortening the hydraulic retention time of medium culture, causing inadequate substrate biodegradation and thus lowering the HYs.

With respect to the ECEs, shown in Fig. 3, the ECEs decreased monotonically with increasing flow rate and the highest ECEs of 3.20 and 4.55 % were achieved at the flow rate of 20 ml h^{-1} for the BFPBR-S and the BFPBR-U, respectively. This can be explained when the hydraulic retention time of culture medium in the biofilm photobioreactor was shortened by increasing the flow rate, biodegradation of substrate was incomplete. At a special illumination condition, therefore, insufficient substrate biodegradation led to less energy to be used for photo-H_2 production. As a consequence, the reduction in the ECEs with increasing flow rate was obtained.

From Figs. 1, 2, and 3, the HPR, HY and ECE achieved in the BFPBR-U were higher than those obtained in the BFPBR-S. The reason is that the saturated flow condition led to a higher transport resistance of substrates and products as well as more loss of light energy due to the shielding effects of culture solution in the bulk fluid, which resulted in comparatively lower photo-H_2 production performance. By contrast, the BFPBR-U possessed a thin liquid film over the biofilm on the reaction bed which caused mass transfer enhancement of substrates and products as well as weakening the negative effects of light transmission (Zhang et al. 2006). Thus, higher photo-H_2 production can be obtained in the BFPBR-U.

Effect of influent substrate concentration

The effects of influent substrate concentration on the photo-H_2 production performances of the BFPBR-S and the BFPBR-U were investigated, where the flow rate was maintained at 80 ml h^{-1}, and the pH of culture medium and operation temperature were maintained at 7 and 30 °C, respectively. The influent substrate concentrations ranged from 30 to 120 mM and the experimental results are shown in Figs. 4, 5 and 6.

Figure 4 shows that the HPR of the BFPBR-S increased as the influent substrate concentration increased from 30 to 60 mM. With influent substrate concentration further increasing to 120 mM, the trend reversed, indicating the optimal influent substrate concentration for the photo-H_2 production performance of the BFPBR-S is 60 mM. However, the HPR of the BFPBR-U increased monotonically when the influent substrate varied from 30 to 120 mM. The reasons leading to these phenomena are as follows. At the lower influent substrate concentration, the metabolic capacity of photosynthetic bacteria immobilized

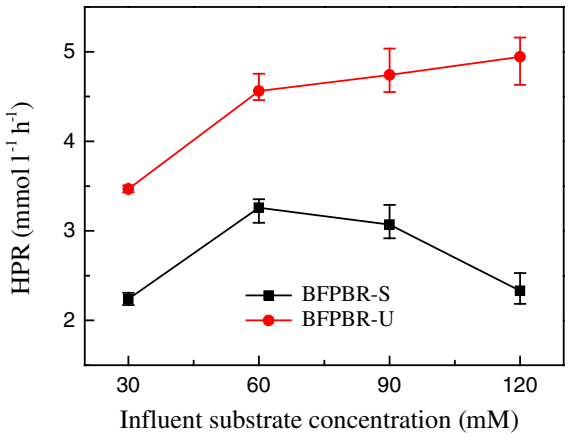

Fig. 4 Effect of influent substrate concentration on H_2 production rate (HPR)

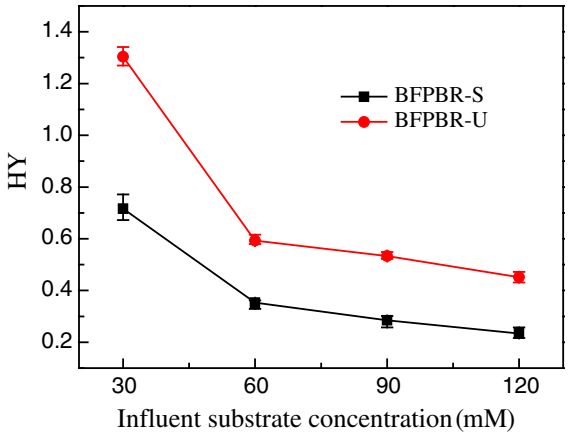

Fig. 5 Effect of influent substrate concentration on H_2 yield (HY)

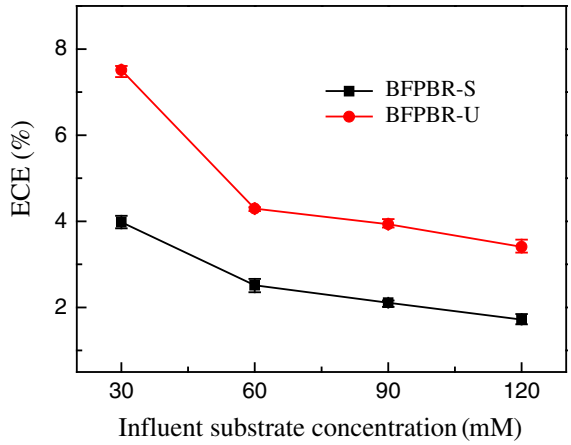

Fig. 6 Effect of influent substrate concentration on energy conversion efficiency (ECE)

in the biofilm is sufficient for converting substrate to H_2. Thus the HPRs of the BFPBR-S and the BFPBR-U are primarily dominated by the mass transfer rate of substrate implying that they increase with increases in the influent substrate concentration. By contrast, when the influent substrate concentration was increased to a higher value, the HPR of the BFPBR-S decreased significantly but the HPR of the BFPBR-U still increased slowly as the influent substrate concentration increased. This is because a high influent substrate concentration for the BFPBR-S presents a large amount of substrate to the cells and products are therefore accumulated inside the biofilm. This, in turn, inhibits the activity of photosynthetic bacteria thereby lowering their ability to further biodegrade the substrate to H_2 (Guo et al. 2011). However, for the BFPBR-U, the liquid film formed on the biofilm is very thin and thus the liquid space is limited. This eliminates the accumulation of products in the biofilm, avoiding the negative effect of higher influent substrate concentration on the HPR of the BFPBR-U (Zhang et al. 2006).

Figure 5 shows that the HYs of the BFPBR-S and the BFPBR-U decreased monotonically with increases of influent substrate from 30 to 120 mM. The highest HYs were achieved at 30 mM for the BFPBR-S and the BFPBR-U, respectively. The reasons are that the organic loading rate was significantly affected by the influent substrate concentration which can change the metabolic pathway of photosynthetic bacteria immobilized in the biofilm. When the influent substrate concentration varied from 30 to 120 mM, the organic loading rate increased significantly resulting in the accumulation of organic acids generated in the photo-H_2 production process, and thus inducing the reduction of the HYs.

As shown in Fig. 6, with the variation of influent substrate concentration from 30 to 120 mM, the ECEs of the BFPBR-S and the BFPBR-U decreased monotonically from 3.98 and 7.5 % to 1.72 and 3.4 %, respectively. The trends are due to the lower influent substrate concentration decreasing the organic loading rate thereby ensuring sufficient substrate biodegradation. When illumination was remained at constant, the sufficient substrate biodegradation resulted in more substrate energy being converted to H_2. As such, the highest ECEs of the BFPBR-S and the BFPBR-U were obtained with the influent substrate at 30 mM.

Figures 4, 5 and 6 also show that the photo-H_2 production performance of the BFPBR-U was better than the BFPBR-S. The trends are due to the thinner liquid film over the biofilm within the BFPBR-U accelerating the mass transfer of substrates from the fluid phase to the active photosynthetic bacteria through the biofilm zone and the backward transport of end-products into the fluid phase zone as well as improving the light transmission by reducing the loss of light in fluid phase zone. Thus, the BFPBR-U can be employed to enhance photo-H_2 production using photosynthetic bacteria immobilized in a biofilm.

Table 1 Comparison of the photo-H_2 production performance obtained from this study and comparable researches

PBR[a]	Substrate	Concentration	HPR (mmol l^{-1} h^{-1})	HY	ECE (%)	References
PBR with fill and draw operation	Acetate	32.5 mM	1.71[b]	NA	NA	Chen et al. (2006)
Rectangular biofilm PBR	Glucose	120 mM	1.74[b]	0.2	NA	Tian et al. (2010)
Flat-panel PBR	Glucose	60 mM	1.12[b]	0.25	NA	Liao et al. (2010)
PBR with ultra-sonication pretreatment	Glucose	50 mM	0.54	1.2	9.03	Zhu et al. (2011)
Biofilm PBR with additional rough surface	Glucose	60 mM	1.75	NA	NA	Guo et al. (2011)
Entrapped-cell PBR	Glucose	4.2 mmol/h[c]	2.61	0.62	4.8	Wang et al. (2013)
BFPBR-S	Glucose	60 mM	3.26	0.35	2.51	This study
BFPBR-U	Glucose	60 mM	4.56	0.59	4.29	This study
BFPBR-U	Glucose	30 mM	3.47	1.3	7.51	This study

NA not available

[a] Photobioreactor

[b] Calculated by ourselves

[c] Substrate loading rate

Evaluation of photo-H_2 production

For commercial scale photo-H_2 production, good photobioreactor performance would benefit the follow-up application of H_2; therefore, the HPR, HY and ECE, which represent the most important performance evaluation criteria of the photo-H_2 production system, would be the key factors to be considered in bioreactor design. In this study, the HPR of the BFPBR-S was in the range of 1.13–3.26 mM h^{-1}. The highest HPR of the BFPBR-S was obtained with a flow rate of 80 ml l^{-1} and the influent substrate at 60 mM. In this situation, the HY and the ECE were 0.35 and 2.51 %, respectively. Thus conditions also gave a higher HPR of 4.56 mM h^{-1} in the BFPBR-U, which is superior to the data reported previously (Table 1). With the decrease of influent substrate from 60 to 30 mM, the HY and the ECE of the BFPBR-U were increased to 1.3 and 7.51 %, respectively, while the HPR was 3.47 mmol l^{-1} h^{-1}. These results are higher than most of the reported values shown in Table 1. The excellent experimental data obtained from this study indicates that the BFPBR-U is feasible for scale up practice.

Conclusions

A biofilm photobioreactor with an optical fiber as an internal light source and *R. palustris* CQK 01 as producer was developed for continuous photo-H_2 production under unsaturated flow conditions. Parametric studies, including flow rate and influent substrate concentration, on photo-H_2production by the biofilm photobioreactor showed that, compared with saturated flow condition, unsaturated flow conditions lead to an enhancement in photo-H_2 production due to the elimination of inhibition caused by a high organic load rate and an improvement in energy conversion efficiency by avoiding the shielding effects of culture solution.

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