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Characteristics of Continuous Hydrogen Production within Groove-type Flat Panel Photo-biological Reactor

Zhang Chuan[1,2] Wang Baowen[1] Wang Weishu[1] Song Xiaoyong[1] Cheng Min[2] Zhang Quanguo[3]

(1. *Institute of Electric Power, North China University of Water Resources and Electric Power, Zhengzhou* 450011, *China*
2. *Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Ministry of Education, Chongqing* 400030, *China*
3. *Collaborative Innovation Center of Biomass Energy, Henan Province, Zhengzhou* 450002, *China*)

Abstract: Aiming for promoting cell-immobilization in the bioreactor and enhancing continuous hydrogen production, a novel groove-type flat panel photo-biological reactor (GFPR) was developed. Photosynthetic bacteria (PSB) strain of *Rhodopseudomonas palustris* was successfully attached on the groove-type surface of transparent material and generated PSB type biofilm. Strategies on improving continuous photo-biological hydrogen production within GFPR were comprehensively investigated. Experimental results revealed that hydrogen production rate, substrate degradation efficiency and light conversion efficiency of GFPR were obviously increased to 1.17 mmol/(L·h), 77.5% and 20.15% under the specific operating conditions of 590 nm of light wavelength, 9 W/m^2 of light intensity, 55 mmol/L of inlet substrate concentration and 960 mL/h of flow rate with glucose-based medium. Methods such as choosing proper light wavelength, light intensity to accelerate photophosphorylation for photosynthetic bacteria to accomplish hydrogen production metabolism and enhancing substrate transportation using convective mass transfer process were proved to be the effective way to promote performance of photobiological hydrogen production within the cell immobilized reactor operated under continuous flow mode.

Key words: groove-type surface; photo-biological hydrogen production; biofilm; convective mass transfer; light conversion efficiency

0 Introduction

With the highest energy intensity of 122 kJ/g and free of unfriendly environmental products during various energy conversion processes, hydrogen was regarded as the most appropriate alternative energy that met future demand. While deficiencies of traditional methods on hydrogen production lay on fossil fuels consumption and accompanied with pollution emission. Recently, more attractions have been drawn on photo-biological hydrogen production using photosynthetic bacteria (PSB) due to its merits of coupling green energy production with environmental improvement and easy operating under normal pressure and temperature, and it is considered to be a feasible technology to realize massive hydrogen production[1-2]. Investigations on efficient photo-biological bioreactors for hydrogen production are essential steps to attain photo-biological hydrogen production in large scale. Reactors of panel, tank and tube types have been employed in previous researches. Methods on biomass increasing and operation optimization were used to improve performance of these various bioreactors[3]. A novel panel-type reactor with higher rate of illuminating area to volume was developed for photo-biological hydrogen production by Gibert[4]. Due to its special natures of tiny, light and slow growth, it is difficult for PSB cells to self-immobilize and separate from liquid. Thus, many reactors used for photo-biological hydrogen have to be operated in batch and suspended culture manner, which not only lead to the unsteady hydrogen production, but also are

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Corresponding author: Zhang Chuan, Doctor. E-mail: zhangchuan@ncwu.edu.cn. Tel: +86-13838572060.

unavoidable of losing PSB cells. Coupling appropriate cell-immobilization approaches with hydrogen production under continuous flow manner was the efficient solution to keep adequate biomass with sufficient metabolic activity and stability in the PSB-based reactors for steady and sufficient operation in long term. Researches on professional procedures for cell-immobilization of biofilm and gel embedding revealed that higher performance was attained for immobilized PSB cells in hydrogen production than that in suspended culture way. But deficits of poor light supply, higher mass transfer resistance and mechanical strength shortage within gel embedding particle were indicated by some researches[5-6]. While biofilm technology can enhance hydrogen production of bioreactor for its particulate advantages of intensify biomass, eliminate poor light transmit and additional mass transfer resistance caused by gel embedding. But few packed or fluid beds using cell-immobilization technology can provide homogeneous sufficient light distribution within bioreactor for satisfying the PSB biofilm requirement on hydrogen production. Nevertheless, the mass transfer resistance aroused from biofilm itself restricted substrate transport and thus suppressed performance of bioreactors operated in continuous flow mode[7-10].

A novel groove-type flat panel bioreactor (GFPR) was developed to solve particular problems mentioned above. More illuminating surfaces manufactured in the GFPR can provide sufficient uniform and inhibit light attenuation. More grooves can also increase the area of surface for biofilm formation and realize high intensity cell immobilization within bioreactor. Moreover, substrate transport can be enhanced by fluctuant groove surfaces under continuous flow operating conditions which facilitate to accelerate substrate convers to hydrogen.

1 Materials and methods

1.1 Bioreactor and experimental system

A novel groove-type flat panel bioreactor (GFPR), as shown in Fig. 1, was developed in the present study[11]. It was made of polymethyl methacrylate (PMMA), which was transparent light material with excellent mechanical property. Regular rectangular grooves with 10 mm in depth and 10 mm in width were mechanically fabricated on the panel wall at intervals of 10 mm. The GFPR was a sealed vessel with working volume of $350(H) \times 40(D) \times 200(W)$ mm^3 that got 136.6 m^{-1} in specific surface.

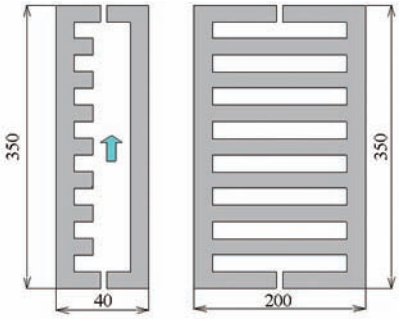

Fig. 1 Schematic of groove-type flat panel photobiological reactor

A schematic description of the experimental system was shown in Fig. 2, which consisted of GFPR, LED lamps, a peristaltic pump, a gas-liquid separator and a hydrogen collector.

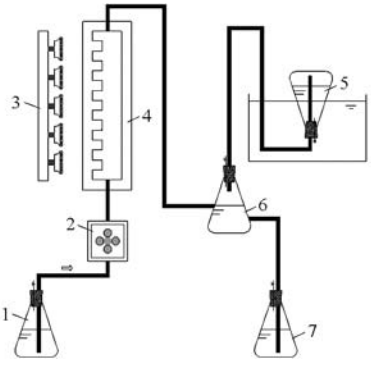

Fig. 2 Scheme of experimental apparatus

1. Glucose-based substrate container 2. Peristaltical pump 3. LED light 4. GFPR 5. H_2 collector 6. Liquid-gas separator 7. Liquid effluent container

1.2 Microorganism and medium

An indigenous photosynthetic bacterium cell identified as *Rhodopseudomonas palustris* was used for continuous photoheterotrophic hydrogen production. Glucose was used as the sole carbon resource for cell growth and hydrogen production. Other elements in the medium for experiments were (g/L): KH_2PO_4 1.006, K_3PO_4 0.544, $MgSO_4$ 0.2, $FeSO_4$ 0.041 7, $(NH_4)_6Mo_7$ 0.001, $ZnSO_4$ 0.001, NaCl 0.2, $CaCl_2$ 0.01, sodium glutamate 0.5 and urea 1.667.

1.3 Analysis methods

The composition of the produced biogas was analyzed using a gas chromatograph (SC-2000) equipped with a thermal conductivity detector (TCD) and a 2 m porous styrene polymer beads packed column. Argon gas was

used as the carrier for the gas chromatograph. The temperatures of gas chromatograph oven and TCD were maintained at 55 ℃ and 100 ℃, respectively. The electric current of TCD was adjusted to 70 mA. The concentration of substrate was measured by the 3,5-dinitrosalicylic acid method using UV spectrophotometer (756-MC). The pH value was measured using a pH meter. The light wavelength and intensity were measured using spectrometer (WSB-2000) and digital luxmeter (SA-190), respectively. An electronic analytical balance (BP-114) was used to determine the quantity of elements in the substrate. The maximum relative errors in hydrogen and glucose measurements were ±3.5% and ±5.1%, respectively.

The performances on hydrogen production within GFPB were evaluated by hydrogen production rate, substrate consumption rate, light conversion efficiency and substrate degradation efficiency. Definitions of these parameters are[12-13]

$$H_{PR} = \frac{\Delta H_{H_2}}{tV}$$

$$S_{CR} = \frac{\Delta G}{tV}$$

$$L_{CE} = \frac{33.61\rho_{H_2}V_{H_2}}{IAt} \times 100\%$$

$$S_{DE} = \frac{C_{in} - C_{out}}{C_{in}} \times 100\%$$

where H_{PR} is hydrogen production rate, mmol/(L·h); S_{CR} is substrate consumption rate, mmol/(L·h); L_{CE} is light conversion efficiency, %; S_{DE} is substrate degradation efficiency, %; ΔH_{H_2} is the total hydrogen evolved, mmol; ΔG is total substrate consumed, mmol; t is the time that the experiment lasted, h; V is the volume of GFPR, L; ρ_{H_2} is the density of hydrogen, g/L; V_{H_2} is the volume of evolved hydrogen, L; I is the intensity of light, W/m^2; A is the illuminating area within GFPR, m^2; C_{in} is the inlet substrate concentration, mmol/L; C_{out} is outlet substrate concentration, mmol/L.

All experiments were repeated three times to eliminate random error in measurement. Then, the mean standard deviation and analysis of variance were calculated and the results can be expressed as the mean ± standard deviation.

1.4 Start-up of GFPB

Prior to inoculation, the photosynthetic bacteria were pre-cultured to harvest at the actively exponential growth phase. Then, they were inoculated in the GFPR by the proportion of 1∶10 in volume. A low circulate flow rate was employed at the start-up stage of bioreactor to help these suspended cells to attach repetitively on groove walls to form the stable PSB-type biofilm. To ensure the nutrient elements within substrate were utilized by the attached PSB cells and avoid nutrition-competition by suspended cells, cell concentration in the liquid phase of the bioreactor was regularly detected by the OD_{600} value. The anaerobic atmosphere within bioreactor was maintained by the pumped argon gas. Hydraulic resistance ability of biofilm was strengthened by a higher circulate flow rate employed at the later start-up stage. The evolution of H_2 production and glucose consumption rate in GFPR were examined at all start-up stage. Both the hydrogen production rate and glucose consumption rate achieved nearly constant after about 30 d. It was indicated that stable biofilm was formatted on groove-type surface within GFPR. Then, the PSB-biofilm morphology was monitored by scanning electronic microscopy (SEM) as shown in Fig. 3.

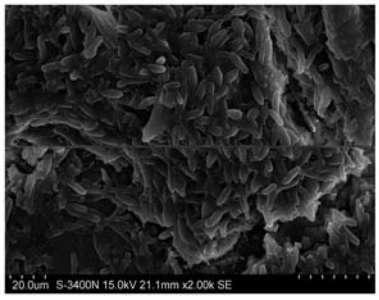

Fig. 3 SEM image of PSB-biofilm

1.5 Continuous hydrogen production stage

Continuous hydrogen production can be carried out within GFPR after accomplishment of cell-immobilization. Investigations on mechanism of photo-biological hydrogen production revealed that efficient molecular hydrogen production required large amount of reducing power (protons) and ATP. While protons were derived from substrate degradation by PSB cells and ATP was converted through the photophosphorylation process[14-15]. Therefore, the performance of continuous photo-biological hydrogen production within the GFPR was a comprehensive response of PSB biofilm to the coupled light conversion process with substrate transfer and utilization process. Operating

parameters associated closely with these processes should be investigated in detail. To keep the PSB biofilm stable, every operating parameter was adjusted back to start-up state for 24 h before another experiment was carried out.

2 Results and discussion

2.1 Light wavelength

Energy of photo-protons were absorbed by antenna and transmitted to light reaction centers of PSB cells to generate energetic electronics. Then ATPs produced these generate electronics by the phosphorylation processes provided energy needed for hydrogen production. It was indicated that particulate antenna with different pigments just absorbed the photo-protons with specific light wavelength to the photosystem reaction center[16]. Since major energy of sunlight was focused within visible light zone, which is suitable for PSB cells. LED lights with four light wavelengths of 460 nm, 530 nm, 590 nm and 620 nm were examined. Results were shown in Fig. 4.

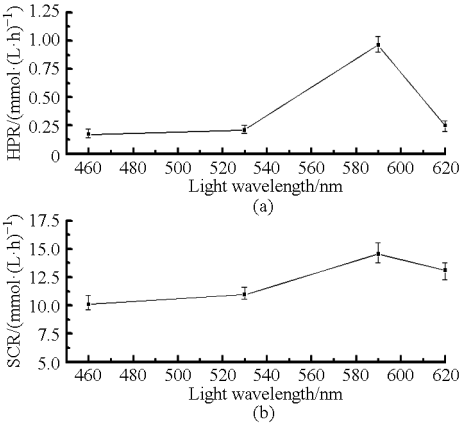

Fig. 4 Effect of light wavelength on performance of GFPR

It was shown in Fig. 4a that hydrogen production rate was not increased obviously in the range of 460 nm to 530 nm, while it was increased obviously when the light wavelength attained to 590 nm. But further increase of light wavelength to 620 nm resulted in decrease of hydrogen production rate. The same trend of substrate consumption rate was presented in Fig. 4b. The distinct absorption peak of 590 nm within visible light zone was found by full length scan on PSB cells absorbance.

As the incident light wavelength varied from 460 nm to 590 nm, light energy within longer light wavelength coincided with the requirement of light absorption compound within light antenna, which led a distinct stimulate on photosystem reaction center, and more ATPs were produced for hydrogen generation. While further increase of incident light wavelength to 630 nm led to insufficient ATPs produced and failed to stimulate photosystem reaction center.

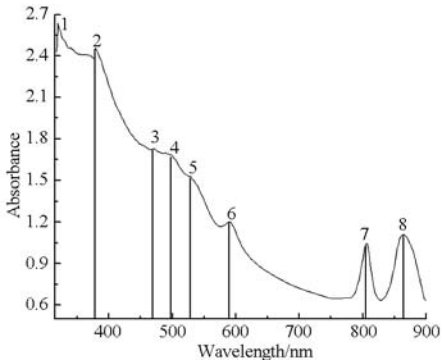

Fig. 5 Absorption spectrum of intact cells of *Rhodoseudomonas palustris*

2.2 Light intensity

Light intensity was another key factor affecting metabolism of hydrogen production within PSB cells. Enough photo protons and ATPs were essential for hydrogen production in photosystem reaction center[17]. Moreover, light intensity was the required parameter for bioreactor operating. Thus six light intensities (2 W/m^2, 3.5 W/m^2, 5 W/m^2, 7 W/m^2, 9 W/m^2 and 11 W/m^2) with the same light wavelength of 590 nm were selected to be investigated, results were shown in Fig. 6.

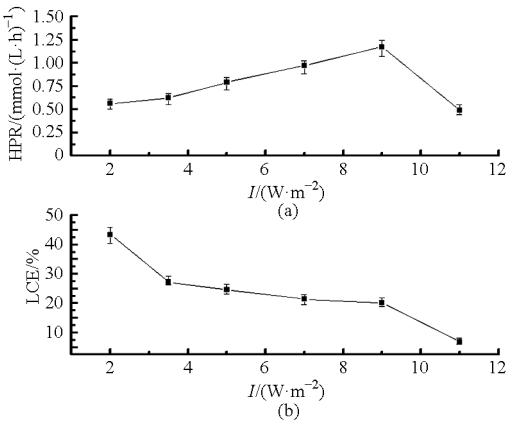

Fig. 6 Effect of light intensity on performance of GFPB

Hydrogen production rate was improved from 0.56 mmol/(L·h) to 1.17 mmol/(L·h) as the light intensity was added from 2 W/m^2 to 9 W/m^2 (illustrated in Fig. 6a). But hydrogen production rate was significantly decreased by 0.49 mmol/(L·h) when light intensity was further increased to 11 W/m^2, which was coincided with previous studies on light saturation phenomenon[18].

The reason that light conversion efficiency was decreased with the increase of light intensity (as shown in Fig. 6b) was part of light energy within the increased incident illumination can ultimately be transformed to hydrogen energy. It can also be shown in Fig. 6b that light conversion efficiency remained a low value between 24.5 % and 20.15% when the light intensity varied from 5 W/m^2 to 9 W/m^2. The result indicated that GFPR can keep high activity on hydrogen production with low light intensity. It was the uniform light distribution within biofilm zone caused by reflection and scattering effects occurred among these groove surfaces that promoted the activity of hydrogen production within the whole cell-immobilization region in GFPR.

2.3 Substrate concentration

The process of substrate degradated and metabolized to hydrogen within GFPR was coupled with substrate transferred from fluid flow zone to biofilm zone under the continuous flow operating condition. Thus, performance of substrate transport within bioreactor was a key factor related to hydrogen production performance of GFPR. From the view of mass transfer, it was the primary photo-bioreaction within the PSB biofilm that led to a driven difference for mass transfer and decided the substrate transportation performance of GFPR. Inlet substrate concentration was one of key factor related to mass transfer difference of GFPR.

Effect of inlet substrate concentration (45 mmol/L, 50 mmol/L, 55 mmol/L, 60 mmol/L and 65 mmol/L) was examined, experimental results was shown in Fig. 7. As inlet substrate concentration was increased from 45 mmol/L to 55 mmol/L, hydrogen production rate was increased from 0.4 mmol/(L·h) to 1.17 mmol/(L·h). While further increment of inlet substrate concentration to 60 mmol/L resulted in decrease of hydrogen production rate by 0.44 mmol/(L·h). When the inlet substrate concentration was increased to 65 mmol/L, the hydrogen production rate of GFPB was decreased further to 0.2 mmol/(L·h). From the view of substrate transportation, when inlet substrate concentration was increased from 45 mmol/L to 55 mmol/L, the mass transfer difference between fluid flow zone and biofilm zone within GFPB was increased simultaneously. More substrate for hydrogen production was transferred to biofilm zone, which led to the increase of hydrogen production rate. While further increment of inlet substrate concentration to 60 mmol/L caused more substrate to transfer to biofim. But capacity of hydrogen production within biofilm was decided by activity of enzyme associated with hydrogen production. As mass transfer rate was greater than primary photo-bioreaction rate for hydrogen production, redundant substrate would be accumulated in biofilm zone, which would cause dehydration in PSB cell wall. Thus, substrate inhibition occurred and depressed activity of hydrogen production ultimately.

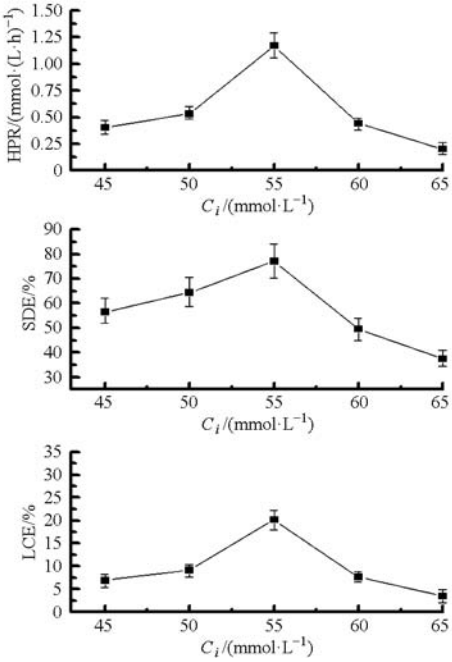

Fig. 7 Effect of inlet substrate concentration on performance of GFPR

When the inlet substrate concentration was increased from 45 mmol/L to 55 mmol/L, as illustrated in Fig. 7, substrate degradation efficiency of GFPR was increased from 56.5% to 77.1%. But further increment of inlet substrate concentration to 65 mmol/L led the decrease of substrate degradation efficiency to 37.5%. This was similar to substrate inhibition caused by high substrate concentration, which indicated the close relationship between hydrogen production process and mass transfer and degradation processes. The reason for light conversion efficiency of GFPR varied with the same trend as hydrogen production was the fixed light operating condition. So the maximum light conversion efficiency of 20.15% was achieved in GFPR.

2.4 Flow rate

A necessary pathway to realize photo-biological hydrogen production in large scale was to operate the bioreactor in continuous flow mode. Therefore, flow rate was one of the key operating parameters that should be carefully determined.

Flow rate was also one of key factors related to substrate transportation in the continuous operating bioreactor. From the view of convective mass transfer, flow rate determined the mass transfer coefficient between fluid flow zone and biofilm zone within GFPR. It can affect the convective mass transfer resistance outside of PSB biofilm. Various flow rates of 320 mL/h, 640 mL/h, 960 mL/h, 1 080 mL/h and 1 600 mL/h (corresponding to 5 h, 2.5 h, 1.67 h, 1.48 h and 1 h in hydraulic retention time) were selected. Results were shown in Fig. 8. Hydrogen production rate of GFPR was increased gradually when flow rate was increased from 320 mL/h to 960 mL/h, as illustrated in Fig. 8. While further increment of flow rate to 1 600 mL/h led to decrease of hydrogen production rate. Moreover, substrate consumption rate of GFPR varied with the same trend as hydrogen production rate, as shown in Fig. 8. Investigation on relationship between hydrogen production process and substrate transportation process within GFPR revealed that high flow rate within certain range was necessary to maintain appreciate micro-circumstance for immobilized PSB cells within GFPR to produce hydrogen. Because high flow rate could enhance both the substrate transportation from fluid flow zone to biofilm zone and products transported reversely.

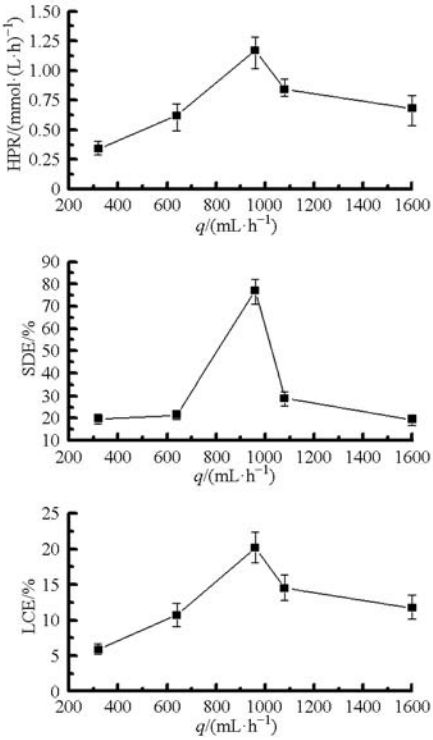

Fig. 8 Effect of flow rate on performance of GFPR

2.5 Comparison with other photo-biological reactors used for hydrogen production

Experimental results were compared with other photo-biological hydrogen production reactors using PSB strains, as shown in Tab. 1. It showed that various factors such as substrate, light source and operating mode had remarkable effects on performance of hydrogen production within different reactors. Compared with reactors using carbon sources of acetate or malate, glucose was the appreciated one for PSB strain used in this study that attained high hydrogen production rate. Compared with other light sources that emitted hybrid light wavelength, combined effects of using the appropriate light wavelength with diffusion

Tab. 1 Comparison of hydrogen production performance with other photo-biological reactors

Strains	Substrate	Light source	Operation/Cell state	H_{RT}/(mmol·(L·h)$^{-1}$)	L_{CE}/%	Reference
R. sphaeroides	malate	tungsten	Continuous/immobilized	0.49	0.60	[19]
R. palustris	acetate	sunlight	Continuous/immobilized	0.53	–	[20]
R. palustris	butyrate	incandescent	Batch/suspended	0.20	0.30	[21]
Rhodopseudomonas sp.	lactate	halogen	Batch/suspended	0.48	0.40	[22]
Rhodopseudomonas palustris WP3-5	acetate	tungsten	Continuous/immobilized	0.62	1.16	[23]
R. palustris	acetate	halogen	Continuous/immobilized	0.11	0.10	[24]
Rhodopseudomonas palustris	glucose	LED	Continuous/immobilized	1.17	20.15	This study

and scatter effects caused among grooved surfaces to promote uniform light distribution within PSB biofilm were proved to be sufficient in improvement of the light conversion efficiency. Moreover, cell-immobilization and continuous flow operating way within GFPR were proved to be effective in maintaining proper micro-environment for hydrogen production due to enhancement of both substrate and products transportation.

3 Conclusions

(1) Both photosynthetic bacteria cell immobilization and uniform light distribution were realized on novelty groove surfaces, which combined biofilm carrier with light transmit medium.

(2) With appropriate light spectrum and groove surfaces used for light intensity enhancement, performance of hydrogen production within GFPR was obviously improved to get hydrogen production rate and light conversion efficiency of 1.17 mmol/(L·h) and 20.15%, respectively.

(3) Strategies of adjusting operating parameters aimed for enhancement on mass transfer were proved to be effective to maintain the suitable and stable micro-circumstance for hydrogen production by the PSB biofilm.

References

[1] Ren N Q, Guo W X, Liu B F. Development and application prospect of bio-hydrogen production technology [J]. Journal of Harbin Institute of Technology, 2010, 42(6): 855 – 863.

[2] Zhang Q G, Wang Y. Research progress of hydrogen production technology with photosynthetic bacteria [J]. Transaction of the Chinese Society for Agricultural Machinery, 2013, 44(6): 156 – 161.

[3] Eroglu E, Melis A. Photobiological hydrogen production: recent advances and state of the art [J]. Bioresource Technology, 2011, 102: 8403 – 8413.

[4] Keskin T, Hashesh M, Hallenbeck P C. Photofermentative hydrogen production from wastes [J]. Bioresource Technology, 2011, 102(18): 8557 – 8568.

[5] Zhu H, Suzuki T, Tsygankov A A, et al. Hydrogen production from tofu wastewater by *Rhodobacter sphaeroides* immobilized in agar gels [J]. International Journal of Hydrogen Energy, 1999, 24(4): 305 – 310.

[6] Zhang C, Liu X Y, Wang Y, et al. Performance of substrate transport and bio-degradation within annular optical-fiber-illuminating biofilm reactor during continuous photo-H2 production [J]. Transaction of the Chinese Society for Agricultural Machinery, 2015, 46(3): 174 – 179.

[7] Liao Q, Zhang C, Zhu X, et al. Advance in research of bioreactor for hydrogen production using photosynthetic bacteria [J]. Chinese Journal of Applied and Environmental Biology, 2008, 14(6): 871 – 876.

[8] Kim M S, Kim D H. Hydrogenases for biological hydrogen production [J]. Bioresource Technolgoy, 2011, 102: 8423 – 8431.

[9] Liao Q, Wang Y J, Wang Y Z, et al. Formation and hydrogen production of photosynthetic bacterial biofilm under various illumination conditions [J]. Bioresource Technology, 2010, 101: 5315 – 5324.

[10] Chang J S, Lee K S, Lin P J. Biohydrogen production with fixed-bed bioreactors [J]. International Journal of Hydrogen Energy, 2002, 27(11 – 12): 1167 – 1174.

[11] Liao Q, Zhang C, Zhu X, et al. Groove-type flat panel photo-biological reactor [P]. Chinese Patent, 200710078274.2, 2007 – 08 – 22.

[12] Androga D D, Sevinc P, Koku H, et al. Optimization of temperature and light intensity for improved photofermentative hydrogen production using Rhodobacter capsulatus DSM 1710 [J]. International Journal of Hydrogen Energy, 2014, 39(6): 2472 – 2480.

[13] Akkerman I, Janssen M, Rocha J, et al. Photobiological hydrogen production photochemical efficiency and bioreactor design [J]. International Journal of Hydrogen Energy, 2002, 27(11 – 12): 1195 – 1208.

[14] Koku H. Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides* [J]. International Journal of Hydrogen Energy, 2002, 27(11 – 12): 1315 – 1329.

[15] Zhang M, Shi J L. Mechanism of hydrogen photoproduction by photosynthetic bacteria [J]. Chinese Journal of Applied and Environmental Biology, 1999, 5(Supp.): 25 – 29.

[16] Lata D B, Chandra R, Kumar A, et al. Effect of light on generation of hydrogen by Halobacterium halobium NCIM 2852 [J]. International Journal of Hydrogen Energy, 2007, 32(15): 3293 – 3300.

[17] Laurinavichene T, Tolstygina I, Tsygankov A. The effect of light intensity on hydrogen production by sulfur-deprived *Chlamydomonas reinhardtii* [J]. Journal of Biotechnology, 2004, 114(1 – 2): 143 – 151.

[18] Liu J G, Bukatin V E, Tsygankov A A. Light energy conversion into H_2 by anabaena variabilis mutant PK84 dense cultures exposed to nitrogen limitations [J].

International Journal of Hydrogen Energy, 2006, 31(11): 1591 – 1596.

[19] Zabut B, Kahlout K. Hydrogen gas production by combined systems of *Rhodobacter sphaeroides* O. U. 001 and *Halobacterium salinarum* in a photobioreactor[J]. International Journal of Hydrogen Energy, 2006, 31(11): 1553 – 1562.

[20] Chen C Y, Saratale G D, Lee C M, et al. Phototrophic hydrogen production in photobioreactors coupled with solar-energy-excited optical fibers [J]. International Journal of Hydrogen Energy, 2008, 33 (23): 6886 – 6895.

[21] Lee J Z, Klaus D M, Maness P C, et al. The effect of butyrate concentration on hydrogen production via photofermentation for use in a Martian habitat resource recovery process[J]. International Journal of Hydrogen Energy, 2007, 32(15): 3301 – 3307.

[22] Guo C L, Zhu X, Liao Q, et al. Enhancement of photo-hydrogen production in a biofilm photobioreactor using optical fiber with additional rough surface [J]. Bioresouce Technology, 2011, 102(18): 8507 – 8513.

[23] Chen C Y, Chang J S. Enhancing phototropic hydrogen production by solid-carrier assisted fermentation and internal optical-fiber illumination [J]. Process Biochemistry, 2006, 41(9): 2041 – 2049.

[24] Chen C Y, Lee C M, Chang J S. Hydrogen production by indigenous photosynthetic bacterium *Rhodopseudomonas palustris* WP3-5 using optical-fiber illuminating photobioreactor [J]. Biochemical Engineering Journal, 2006, 32(1): 33 – 42.

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微槽透光板式光合制氢反应器连续产氢性能研究

张　川[1]　王保文[1]　王为术[1]　宋小勇[1]　程　旻[2]　张全国[3]

(1.华北水利水电大学电力学院,郑州 450011;2.重庆大学低品位能源利用技术及系统教育部重点实验室,重庆 400030;
3.生物质能源河南省协同创新中心,郑州 450002)

摘要:从增加反应器中光合细菌的细胞持有量并强化光能利用和底物传输的角度出发,构造了新型的微槽透光板式光合制氢反应器。通过沼泽红假单胞菌(*Rhodopseudomonaspalustris*)的连续流产氢实验研究表明:当以葡萄糖为碳源底物时,在反应器光波长为 590 nm、光照强度为 9 W/m^2、进口底物浓度为 55 mmol/L、流速为 960 mL/h 运行工况下,产氢速率、底物降解效率和光能转化效率均有显著增加,分别达到 1.17 mmol/(L·h)、77.5% 和 20.15%。研究表明,选择与光合细菌产氢代谢相适应的光波长和光照强度以强化光合磷酸化过程,并通过传质强化以促进底物的传输,是提高连续流光合制氢反应器产氢性能的有效方法。

关键词:微槽表面;光生物制氢;生物膜;对流传质;光能转化效率

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Characteristics of Continuous Hydrogen Production within Groove-type Flat Panel Photo-biological Reactor

Zhang Chuan[1]　Wang Baowen[1]　Wang Weishu[1]　Song Xiaoyong[1]　Cheng Min[2]　Zhang Quanguo[3]

(1. *Institue of Electric Power*, *North China University of Water Resources and Electric Power*, *Zhengzhou* 450011, *China*
2. *Key Laboratory of Low-grade Energy Utilization Technologies and Systems*, *Ministry of Education*, *Chongqing University*, *Chongqing* 400030, *China*
3. *Collaborative Innovation Center of Biomass Energy in Henan Province*, *Zhengzhou* 450002, *China*)

Abstract: Aiming for promoting cell-immobilization in the bioreactor and enhancing continuous hydrogen production, a novel groove-type flat panel photo-biological reactor (GFPR) was developed. Photosynthetic bacteria (PSB) strain of *Rhodopseudomonaspalustris* was successfully attached on the groove-type surface of transparent material and generated PSB type biofilm. Strategies on improving continuous photo-biological hydrogen production within GFPR were comprehensively investigated. Experimental results revealed that hydrogen production rate, substrate degradation efficiency and light conversion efficiency of GFPR were obviously increased to 1.17 mmol/(L·h), 77.5% and 20.15%, respectively, under specific operating conditions of 590 nm of light wavelength, 9 W/m^2 of light intensity, 55 mmol/L of inlet substrate concentration and 960 mL/h of flow rate with glucose-based medium. Methods such as choosing proper light wavelength, light intensity to accelerate photophosphorylation for photosynthetic bacteria to accomplish hydrogen production metabolism and enhancing substrate transportation using convective mass transfer process were proved to be the effective way for promoting performance of photo-biological hydrogen production within the cell immobilized reactor operated under continuous flow mode. The experiment results were introduced to further research photo-biological reactor for practical hydrogen production.

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作者简介:张川(1974—),男,讲师,博士后,主要从事可再生能源利用中的工程热物理研究,E-mail: zhangchuan@ncwu.edu.cn

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引言

在现有能源中,氢能因单位热值高(122 kJ/g),且在能量转换过程中不释放危害环境的产物而备受关注。传统的规模制氢方法不仅依赖于化石燃料,且制备过程对环境造成二次破坏。光生物制氢技术能将氢能生产与太阳能利用和有机污染物的降解耦合起来,且制备条件仅需常温常压,被认为具备规模化制氢的前景[1-2]。开发高效的光合制氢反应器是将该技术引向规模化发展的必要前提。光合制氢反应器目前主要有板式、管式和罐式等类型。研究者力求通过增加反应器中的菌株持有量和优化运行条件等方法来提高产氢性能[3]。Keskin 等[4]利用板式反应器受光面积比大的特点构造了板式光合制氢反应器。光合细菌存在菌体小、自重轻、增殖慢等特点,不仅难以实现细胞自固定化也难以完成菌株和液态底物的分离操作,因此以往反应器多以序批次悬浮培养方式运行,这使得制氢过程处于非稳态,难以维持反应器长期、高效和稳态运行,且无法避免因菌种流失而造成制氢成本的增加。采取适合光合细菌的细胞固定化技术保证反应器中持有一定数量具有生理活性和代谢稳定性的菌株,并通过连续流的运行方式使光合制氢反应器长期维持在稳态、高效状态是解决上述问题的有效途径。生物膜法和包埋法是目前较成熟的细胞固定化技术,一些学者对此进行了研究[5-6]。结果表明,光合细菌的细胞固定化效果稳定,且产氢性能优于细胞悬浮培养条件。但也有学者指出,包埋法由于存在着材料透光性不足、包埋材料增加传质阻力和包埋颗粒机械强度不足等缺点,难以维持反应器长期高效运行。而生物膜法能使生物量更密集,且消除因包埋材料引起的透光性差和传质阻力问题,利于提高光合制氢反应器的性能。但广泛采用生物膜细胞固定化技术的固定床和流化床生物反应器中,缺乏保证反应器具备良好光分布特性,满足光合细菌生物膜产氢所需充足光照的技术手段,且生物膜组织所形成的传质阻力也将影响底物传输,进而制约连续流光生物制氢反应器的产氢性能[7-10]。

本文通过在高透光性材料表面开设微槽的方法构造微槽透光板式光合制氢反应器。

1　实验系统及方法

1.1　反应器和实验装置

微槽透光板式光合制氢反应器(Groove-type flat panel photo-biological reactor, GFPR)的结构如图 1 所示[11]。反应器材料选择导光性能良好、机械强度高、轻质、易加工的聚甲基丙烯酸酯(PMMA)。用机械加工方法开取规则微槽,微槽尺寸:10 mm × 10 mm × 10 mm(深 × 宽 × 间距)。微槽透光板式光合制氢反应器尺寸:350 mm × 200 mm(长 × 宽),容积 1 600 mL,比表面积 136.6 m^{-1}。

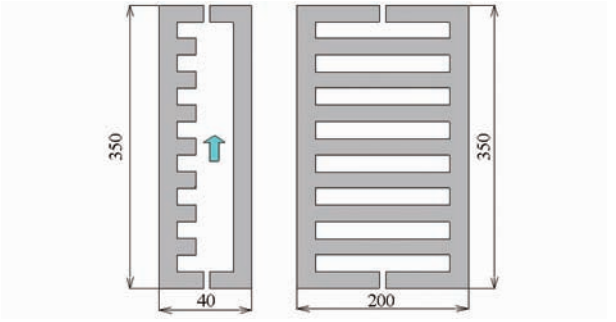

图 1　微槽透光板式光合制氢反应器

Fig. 1　Schematic of groove-type flat panel photo-biological reactor

连续流产氢实验装置由微槽透光板式光合制氢反应器、LED 光源、恒流泵、气液分离瓶、氢气收集瓶等构成,如图 2 所示。

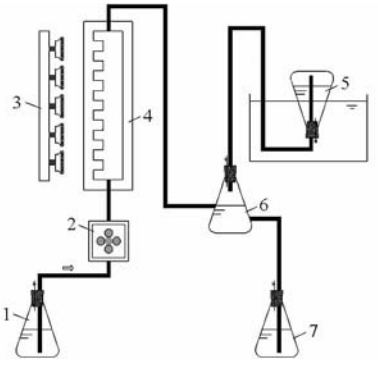

图 2　微槽透光板式光合制氢反应器实验装置

Fig. 2　Experimental apparatus of groove-type flat panel photo-biological reactor

1、7. 储液瓶　2. 恒流泵　3. LED 光源　4. 微槽透光板式光合制氢反应器　5. 氢气收集瓶　6. 气液分离瓶

1.2　光合细菌和培养基

菌种采用自行分离驯化得到的沼泽红假单胞菌(*Rhodopseudomonaspalustris*)。以葡萄糖作为培养基的碳源,除葡萄糖外的营养成分质量浓度为:磷酸氢二钾 1.006 g/L,磷酸钾 0.544 g/L, 硫酸镁 0.2 g/L, 硫酸亚铁 0.041 7 g/L,钼酸铵 0.001 g/L, 硫酸锌 0.001 g/L,氯化钠 0.2 g/L,氯化钙 0.01 g/L,谷氨酸钠 0.5 g/L,尿素 1.677 g/L,酵母膏 1.0 g/L。

1.3 检测方法

气体成分分析采用 SC－2000 型气相色谱仪。色谱柱填料为苯乙烯高分子多孔小球,柱长 2 m,载气为氩气。TCD 检测器,电流 70 mA,柱箱温度 55℃,气化室和检测室温度均为 100℃。底物浓度用 3,5-二硝基水杨酸试剂法测量,检测仪器为 756 MC 型紫外可见光分光光度计。pH 值采用 pH 酸度计测量。光波长检测采用 WSB 2000 型光谱仪。光照强度测量采用 SA－190 型辐照计。培养基成分采用 Sartorius BP114 型电子分析天平称量。氢气含量检测相对误差范围 ±3.5%;葡萄糖浓度测量相对误差范围 ±5.1%。

采用以下指标来评价反应器的产氢性能[12-13]:

产氢速率计算公式为

$$H_{PR}=\frac{\Delta H_{H_2}}{tV}$$

底物消耗速率计算公式

$$S_{CR}=\frac{\Delta G}{tV}$$

光能转化效率计算公式为

$$L_{CE}=\frac{33.61\rho_{H_2}V_{H_2}}{IAt}\times 100\%$$

底物降解效率计算公式为

$$S_{DE}=\frac{C_{in}-C_{out}}{C_{in}}\times 100\%$$

式中 H_{PR}——产氢速率, mmol/(L·h)

S_{CR}——底物消耗速率, mmol/(L·h)

L_{CE}——光能转化效率,%

S_{DE}——底物降解效率,%

ΔH_{H_2}——产氢量,mmol

ΔG——底物消耗量,mmol

t——产氢时间,h

V——反应器容积, L

ρ_{H_2}——氢气密度,g/L

V_{H_2}——氢气体积,L

I——光照强度,W/m^2

A——光照面积,m^2

C_{in}、C_{out}——反应器进、出口底物浓度,mmol/L

连续流产氢性能实验阶段为消除实验中的随机误差,每次实验重复 3 次,并将实验结果整理成平均值 ± 误差的形式。

1.4 反应器挂膜启动

先将光合细菌预培养至活性较高的指数生长期。然后将菌种与培养基按照体积比 1∶10 配成细胞悬浮液接种于微槽透光板式光合制氢反应器中。采用低速循环挂膜法,用氩气建立厌氧环境并密闭循环运行,使光合细菌与微槽载体表面反复接触,逐步完成吸附生长并在反应器微槽表面形成稳定的光合细菌生物膜。挂膜初期定期检查液相悬浮光合细菌的细胞浓度在 600 nm 处 OD 值,限制液相悬浮生长的生物量,保证循环底物用于已附着的光合细菌快速生长。挂膜后期逐步加大循环流速,提高生物膜的抗水力冲击能力。整个挂膜期间定期检测反应器的产氢速率和底物消耗速率,根据其值是否趋于稳定,并结合生物膜形态观察来判断是否获得稳定的生物膜。

经过约 30 d 连续循环挂膜,反应器中的微槽表面得到了清晰的光合细菌生物膜(图 3),实现了细胞固定化。

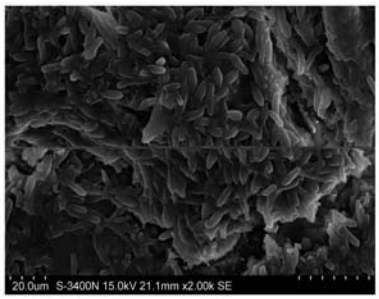

图 3 光合细菌生物膜扫描电子显微图片

Fig. 3 SEM image of PSB-biofilm

1.5 反应器连续流产氢

实现光合细菌细胞固定化后,进行微槽透光板式光合制氢反应器的连续流产氢实验。光合细菌产氢机理的研究表明:光合细菌利用光合磷酸化过程提供的 ATP,将底物降解提供的质子还原为氢气[14-15]。因此微槽透光板式光合制氢反应器的连续流产氢性能是光能利用与有机底物传递和转化过程的耦合。故需选择与上述过程密切相关的运行操作参数进行研究。实验中为保证生物膜始终处于稳定状态,每次性能实验后均将运行工况回调至挂膜工况,保持 24 h 后再进行下次实验。

2 结果与讨论

2.1 入射光波长

光合细菌通过光合色素天线系统捕获和吸收光子后将其能量传送到光合反应中心产生高能电子,该高能电子经环式磷酸化过程产生 ATP,为产氢提供能量。研究表明,色素天线总是选择吸收合适波长的光子提供给光反应中心[16]。由于太阳辐射主要集中在可见光部分(400 ~ 760 nm),光合细菌也适合吸收可见光区域的光谱,故本文在可见光范围内采用 4 种不同波长(460、530、590、620 nm)的单色 LED 光源分别进行实验研究。结果如图 4 所示。

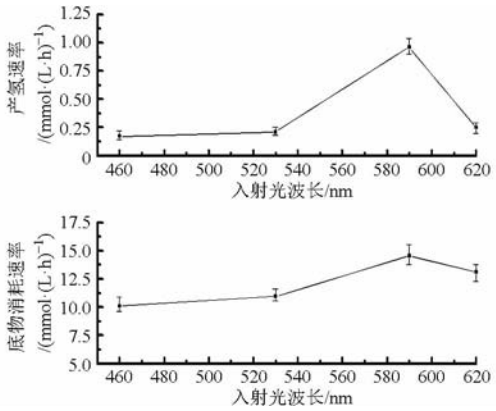

图 4　入射波长对反应器产氢性能的影响

Fig. 4　Effect of light wavelength on performance of GFPR

从图 4 可以看到当波长从 460 nm 增加到 530 nm 时，产氢速率略有上升，继续增加到 590 nm 时，反应器的产氢速率明显增加。但当波长增至 620 nm 时产氢速率急剧下降。说明适合反应器产氢的入射光波长为 590 nm。从图 4 还可看到，反应器中的底物消耗速率与产氢速率的变化趋势相似。对光合细菌进行全波长范围的吸收峰扫描，结果如图 5 所示。发现在可见光范围的 590 nm 附近存在一个明显的吸收峰。

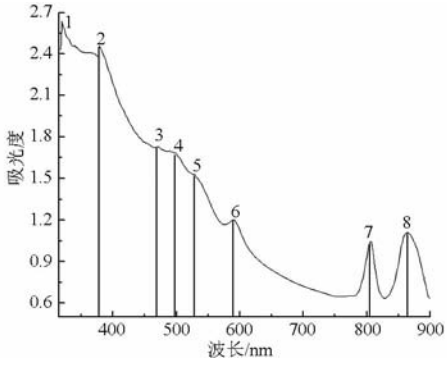

图 5　光合细菌 *Rhodoseudomonaspalustris* 的吸收光谱曲线

Fig. 5　Absorption spectrum of intact *Rhodoseudomonaspalustris* cell

对照反应器的产氢性能实验结果可知，当入射光波长从 460 nm 增加到 590 nm 时，较长光波波长的光能逐渐与光合色素天线系统捕光复合物能量相吻合，在光合细菌的光反应中心获得较大的有效激发，合成 ATP 的能量增加并使产氢活性提高。这必然加速光合细菌对底物的降解并强化产氢。但当光波长上升到 630 nm 时，光合色素天线系统无法获得较高的激发状态，因此合成光合产氢的 ATP 能量不足，不能维持产氢代谢所需的活跃状态，导致底物降解速率下降，并因此削弱了反应器的产氢性能。

2.2　光照强度

光合细菌的产氢代谢除了具有光波长选择性之外还依赖于光照强度。光反应中心只有接收到足够数量的光子数，才能为产氢代谢提供充足的电子数和必要的 ATP 能量水平[17]。对反应器而言，光照强度还是必需确定的主要运行技术参数。本文在给定光波长 590 nm 的条件下，选择 6 组光照强度（2、3.5、5、7、9、11 W/m^2）进行实验研究。结果如图 6 所示。

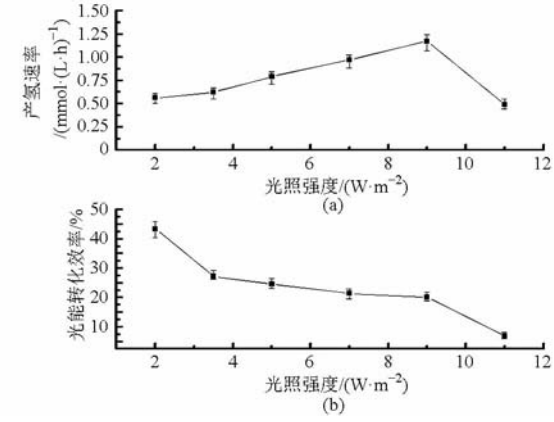

图 6　光照强度对反应器产氢性能的影响

Fig. 6　Effect of light intensity on performance of GFPR

从图 6a 可以看到，当光照强度从 2 W/m^2 增加到 9 W/m^2 时，产氢速率从 0.56 mmol/(L·h) 增加到 1.17 mmol/(L·h)。进一步增加光照强度至 11 W/m^2 时，产氢速率急剧降低至 0.49 mmol/(L·h)。这与文献报道的光饱和抑制效应相吻合[18]。

从图 6b 可知，反应器的光能转化效率随着光照强度的增加而减小。这是由于当光照强度增加时，光合产氢代谢只能将部分增加的光能转化为氢能。从图 6b 还可以发现，当光照强度在 5 ~ 9 W/m^2 之间时，光能转化效率的变化相对比较小，从 24.5% 降低到 20.15%，说明微槽透光板式光合制氢反应器能在相对低的光照条件下达到较高产氢活性。这是因为微槽表面能通过反射和散射作用增强生物膜区域光强分布的均匀性，避免反应器中出现局部光照过强而局部光照不足的现象，促进了生物膜产氢活性达到整体同步。

2.3　反应器进口底物浓度

在连续流产氢的方式下，底物在微槽透光板式光合制氢反应器内被还原分解并生成产物氢气的过程，是伴随底物从反应器的主流区传递至生物膜区的传输过程耦合发生的。因此底物的传输特性对反应器的产氢性能有重要影响。从传递原理的角度来看，正是因为微槽透光板式光合制氢反应器的生物膜区域存在消耗底物的原初生化反应，才使主流区与生物膜区之间存在必然的浓度差，这也构成了底物传递的传质驱动势，并影响着反应器的传输特性。而进口底物浓度是构成微槽透光板式光合制氢反应器中底物传输的传质驱动势的主要原因。

本文分别选取进口底物浓度为 45、50、55、60、65 mmol/L 进行实验研究。结果如图 7 所示。当进口底物浓度从 45 mmol/L 增加到 55 mmol/L 时,反应器的产氢速率逐渐从 0.4 mmol/(L·h)增加到 1.17 mmol/(L·h)。但进口底物浓度继续增加到 60 mmol/L 时,产氢速率减小至 0.44 mmol/(L·h)。进一步增加到 65 mmol/L 时,产氢速率继续减小至 0.2 mmol/(L·h)。从底物传输的角度来分析,当反应器进口底物浓度从 45 mmol/L 增加到 55 mmol/L 时,反应器内主流区与生物膜区之间的传质驱动势逐渐增大,使更多的底物传输到生物膜区域用于产氢代谢,因此反应器产氢速率随之增大。但当进口底物浓度继续增加至 60 mmol/L 时,主流区与生物膜区域之间的传质驱动势也进一步增大,使得更多的底物被传递到生物膜区域。然而,生物膜区域的产氢速率取决于相关酶的代谢活性。当传质速率大于产氢生化反应的速率时,多余的底物在生物膜区域累积,使得生物膜区域的底物浓度增大。当底物浓度过高时,会使光合细菌的细胞壁在高渗透压力下产生脱水危险,此时发生破坏光合细菌生物活性的底物抑制现象,并导致反应器产氢速率的降低。

从图 7 还可以看到,当底物浓度从 45 mmol/L 增加到 55 mmol/L 时,反应器底物降解效率也从 56.5% 增加到 77.1%。而当底物浓度继续增加至 65 mmol/L 时,底物降解效率从 77.1% 降低至 37.5%,这与底物浓度过大所导致的底物抑制作用相一致。也进一步说明了光合细菌的产氢过程与底物传输与降解之间具有密切的关联性。从图 7c 还可看到,反应器中光能转化效率的变化趋势与产氢速率的趋势类似,这是由于实验过程中光工况不变的结果。反应器的光能转化效率最大可达 20.15%。

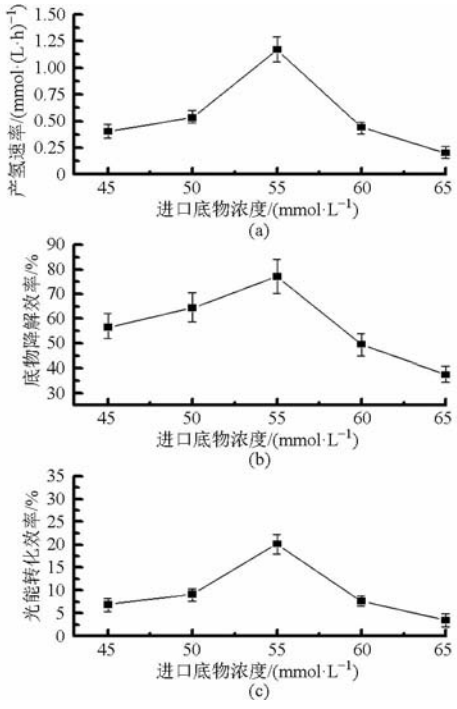

图 7 进口底物浓度对反应器产氢性能的影响

Fig. 7 Effect of inlet substrate concentration on performance of GFPR

2.4 流速

连续流产氢是光生物制氢走向规模化的有效途径。选定合适的流速成为确定微槽透光板式光合制氢反应器运行工况的重要内容。

流速也是影响反应器底物传输的重要因素。从传质原理角度来看,流速影响着生物膜与主流之间的对流传质系数,进而影响反应器中生物膜区域外侧对流传质阻力的大小。本文分别选择流速为 320、640、960、1 080、1 600 mL/h(即水力停留时间分别为 5、2.5、1.67、1.48、1 h)进行研究。实验结果如图 8 所示。从图中可见,当反应器流速从 320 mL/h 增加到 960 mL/h 时,反应器中的产氢速率逐渐增加,但流速继续增加至 1 600 mL/h 时,产氢速率不断降低。从图中还可以看到,反应器底物消耗速率的变化与产氢速率随流速变化的规律相同。通过分析反应器中产氢过程与底物传输过程之间的关系可以发现,在一定的流速变化区间内,当反应器主流区的底物浓度一定时,主流区与生物膜区之间的传质驱动势基本不变。但是流速提高增大了主流区与生物膜区域之间的对流传质系数,使底物从主区流向生物膜区域的传质过程得到强化,满足

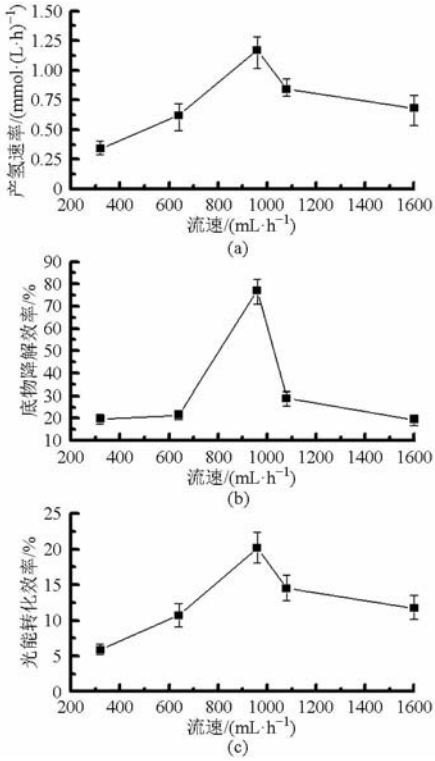

图 8 流速对反应器产氢性能的影响

Fig. 8 Effect of flow rate on performance of GFPR

了光合细菌生物膜产氢代谢对底物消耗的需要，使得产氢速率增加。然而继续增加流速，也会使传质速率大于生物膜的底物降解速率，造成剩余底物在生物膜区域的累积并产生底物抑制作用，致使反应器产氢速率降低。

从实验结果还可以看到，微槽透光板式光合制氢反应器的产氢性能在较小的水力停留时间变化范围内(2.5 ~ 1.67 h)得到明显的强化。这是由于在连续流的运行条件下，由于生物膜附着在起伏的微槽表面，增强了主流区与生物膜区之间扰动使得两区之间的对流传质过程得到强化，这样不仅能够强化底物从主流区向生物膜区域的传输，也可以促进代谢产物从生物膜区域向主流区的传输，从而有利于在生物膜区域形成宜于产氢的微环境而强化微槽透光板式光合制氢反应器的产氢性能。

2.5　与其他光生物制氢反应器性能的比较

将本文的实验结果与其他同样采用沼泽红假单胞菌的光生物制氢反应器的性能进行比较，结果如表 1 所示。发现不同的底物类型、光源和操作方式都对反应器的产氢性能有重要影响。与其他采用醋酸、苹果酸等为底物的反应器相比，本实验因采用了适合该菌种产氢的葡萄糖为碳源的培养基，而获得了较高的产氢速率。与采用混合波长的光源相比，本实验采用适合菌种产氢的特定波长，并因为透光性好的扩展表面对入射光的漫射和反射作用，促进了生物膜区域的光照均匀性，利于生物膜整体达到同步较高的代谢活性，因而可以提高反应器的光能转化效率。采用固定化细胞连续流的操作方式，既可以保持生物膜区域底物浓度处于适合产氢的水平，又能有效地排除代谢产物，防止抑制作用。扩展表面的结构既可以有效强化底物传输又能促进代谢产物从生物膜排除，具有双向强化的作用，有效地保证了生物膜区域的光合细菌处在稳定、适宜产氢的微环境，从而获得强化产氢的效果。

表 1　微槽透光板式光合制氢反应器与其他光生物制氢反应器产氢性能的比较

Tab. 1　Comparison of hydrogen production performance of GFPR with other photo-biological reactors

菌株	底物	光源	操作方式/细胞状态	产氢速率/(mmol·(L·h)$^{-1}$)	光能转化效率/%	数据来源
R. sphaeroides	苹果酸	钨灯	连续流/固定化	0.49	0.60	文献[19]
R. palustris	醋酸	太阳光	连续流/固定化	0.53		文献[20]
R. palustris	丁酸	白炽灯	序批次/悬浮	0.20	0.30	文献[21]
Rhodopseudomona ssp	乳酸	卤素灯	序批次/悬浮	0.48	0.40	文献[22]
Rhodopseudomonaspalustris WP3 - 5	醋酸	钨灯	连续流/固定化	0.62	1.16	文献[23]
R. palustris	醋酸	卤素灯	连续流/固定化	0.11	0.10	文献[24]
Rhodopseudomonaspalustris	葡萄糖	LED	连续流/固定化	1.17	20.15	本文

3　结论

(1)采用将生物膜吸附载体和导光介质相结合的微槽表面来构造反应器，可以在实现光合细菌细胞固定化的同时，保证光生物制氢过程对生物膜区域具有良好光分布特性的要求。

(2)采用适合产氢的光谱频段并配合增加生物膜区域光照强度微槽表面的结构，可使微槽透光板式光合制氢反应器的产氢速率和光能转化效率分别达到 1.17 mmol/(L·h)和 20.15%，而使反应器产氢性能显著提高。

(3)从强化传质的角度出发，调整运行操作参数，可以使光合细菌的生物膜区域具有稳定、适宜的产氢微环境，从而强化反应器产氢性能。

参　考　文　献

1　任南琪，郭婉茜，刘冰峰. 生物制氢技术的发展及应用前景[J]. 哈尔滨工业大学学报，2010，42(6)：855 - 863.
Ren N Q, Guo W X, Liu B F. Development and application prospect of bio-hydrogen production technology[J]. Journal of Harbin Institute of Technology, 2010, 42(6): 855 - 863. (in Chinese)

2　张全国，王毅. 光合细菌生物制氢技术研究进展[J]. 农业机械学报，2013，44(6)：156 - 161.
Zhang Quanguo, Wang Yi. Research progress of hydrogen production technology with photosynthetic bacteria[J]. Transactions of the Chinese Society for Agricultural Machinery, 2013, 44(6): 156 - 161. (in Chinese)

3　Eroglu E, Melis A. Photobiological hydrogen production: recent advances and state of the art[J]. Bioresource Technology, 2011, 102: 8403 - 8413.

4　Keskin T, Hashesh M, Hallenbeck P C. Photofermentative hydrogen production from wastes[J]. Bioresource Technology, 2011, 102(18): 8557 - 8568.

5　Zhu H, Suzuki T, Tsygankov A A, et al. Hydrogen production from tofu wastewater by *Rhodobactersphaeroides* immobilized in agar gels[J]. International Journal of Hydrogen Energy, 1999, 24(4): 305 - 310.

6 张川,刘新阳,王毅,等. 环流型光纤生物膜制氢反应器的底物传输与降解特性[J]. 农业机械学报,2015,46(3):174 - 179.
Zhang Chuan, Liu Xinyang, Wang Yi, et al. Performance of substrate transport and bio-degradation within annular optical-fiber-illuminating biofilm reactor during continuous photo-H_2 production[J]. Transactions of the Chinese Society for Agricultural Machinery,2015,46(3):174 - 179. (in Chinese)

7 廖强,张川,朱恂,等. 光合细菌生物制氢反应器研究进展[J]. 应用与环境生物学报,2008,14(6):871 - 876.
Liao Q, Zhang C, Zhu X, et al. Advance in research of bioreactor for hydrogen production using photosynthetic bacteria[J]. Chinese Journal of Applied and Environmental Biology,2008,14(6):871 - 876. (in Chinese)

8 Kim M S, Kim D H. Hydrogenases for biological hydrogen production[J]. Bioresource Technolgoy, 2011,102: 8423 - 8431.

9 Liao Q, Wang Y J, Wang Y Z, et al. Formation and hydrogen production of photosynthetic bacterial biofilm under various illumination conditions[J]. Bioresource Technology,2010,101:5315 - 5324.

10 Chang J S, Lee K S, Lin P J. Biohydrogen production with fixed-bed bioreactors[J]. International Journal of Hydrogen Energy, 2002,27(11 - 12):1167 - 1174.

11 廖强,张川,朱恂,等. 微槽透光板式光生物制氢反应器: 中国,200710078274.2[P]. 2007 - 08 - 22.

12 Androga D D, Sevinc P, Koku H, et al. Optimization of temperature and light intensity for improved photofermentative hydrogen production using *Rhodobactercapsulatus* DSM 1710[J]. International Journal of Hydrogen Energy, 2014,39(6):2472 - 2480.

13 Akkerman I, Janssen M, Rocha J,et al. Photobiological hydrogen production photochemical efficiency and bioreactor design[J]. International Journal of Hydrogen Energy,2002,27(11 - 12): 1195 - 1208.

14 Koku H. Aspects of the metabolism of hydrogen production by *Rhodobactersphaeroides*[J]. International Journal of Hydrogen Energy,2002, 27(11 - 12):1315 - 1329.

15 张明,史家梁. 光合细菌光合产氢机理研究进展[J]. 应用与环境生物学报,1999,5(增刊):25 - 29.
Zhang M, Shi J L. Mechanism of hydrogen photoproduction by photosynthetic bacteria[J]. Chinese Journal of Applied and Environmental Biology,1999,5(Supp.):25 - 29. (in Chinese)

16 Lata D B, Chandra R, Kumar A, et al. Effect of light on generation of hydrogen by *Halobacteriumhalobium* NCIM 2852[J]. International Journal of Hydrogen Energy,2007,32(15): 3293 - 3300.

17 Laurinavichene T, Tolstygina I, Tsygankov A. The effect of light intensity on hydrogen production by sulfur-deprived *Chlamydomonasreinhardtii*[J]. Journal of Biotechnology,2004,114(1 - 2): 143 - 151.

18 Liu J G, Bukatin V E, Tsygankov A A. Light energy conversion into H_2 by *Anabaena variabilis* mutant PK84 dense cultures exposed to nitrogen limitations[J]. International Journal of Hydrogen Energy,2006,31(11):1591 - 1596.

19 Zabut B, Kahlout K. Hydrogen gas production by combined systems of *Rhodobactersphaeroides* O. U. 001 and *Halobacteriumsalinarum* in a photobioreactor[J]. International Journal of Hydrogen Energy,2006,31(11):1553 - 1562.

20 Chen C Y, Saratale G D, Lee C M, et al. Phototrophic hydrogen production in photobioreactors coupled with solar-energy-excited optical fibers[J]. International Journal of Hydrogen Energy,2008,33(23):6886 - 6895.

21 Lee J Z, Klaus D M, Maness P C, et al. The effect of butyrate concentration on hydrogen production via photofermentation for use in a Martian habitat resource recovery process[J]. International Journal of Hydrogen Energy,2007,32(15):3301 - 3307.

22 Guo C L, Zhu X, Liao Q, et al. Enhancement of photo-hydrogen production in a biofilm photobioreactor using optical fiber with additional rough surface[J]. Bioresouce Technology,2011,102(18):8507 - 8513.

23 Chen C Y,Chang J S. Enhancing phototropic hydrogen production by solid-carrier assisted fermentation and internal optical-fiber illumination[J]. Process Biochemistry,2006,41(9):2041 - 2049.

24 Chen C Y, Lee C M, Chang J S. Hydrogen production by indigenous photosynthetic bacterium *Rhodopseudomonaspalustris* WP3-5 using optical-fiber illuminating photobioreactor[J]. Biochemical Engineering Journal,2006,32(1):33 - 42.

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21 赵宇侠,祝春水. 生活废弃物生物质炭对 Cd(Ⅱ)污染废水处理的影响因素研究[J]. 淮海工学院学报:自然科学版,2014,23(1): 43 - 45.
Zhao Yuxia, Zhu Chunshui. Factors affecting domestic waste biomass charcoal for Cd(Ⅱ) adsorption[J]. Journal of Huaihai Institute of Technology:Natrual Science Edition,2014,23(1): 43 - 45. (in Chinese)

22 朱庆祥. 生物炭对 Pb、Cd 污染土壤的修复试验研究[D]. 重庆:重庆大学, 2011.
Zhu Qingxiang. Experimental study on lead and cadmium contaminated soil remediation with biochar[D]. Chongqing: Chongqing Univesity,2011. (in Chinese)

23 鲁如坤. 土壤农业化学分析方法[M]. 北京:中国农业科技出版社,2000.

24 刘莹莹. 不同原料生物质炭基本性质及其对溶液 Cd(Ⅱ)和 Pb(Ⅱ)吸附特性的研究[D]. 南京:南京农业大学,2012.
Liu Yingying. A study on characteristics and adsorption of Cd(Ⅱ)and Pb(Ⅱ)in aqueous solution by biochars produced from the pyrolysis of different crop feedstock[D]. Nanjing:Nanjing Agricultural University,2012. (in Chinese)