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Characterization of hydrogen production by *Platymonas Subcordiformis* in torus photobioreactor

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Platymonas subcordiformis, a marine green alga, was demonstrated to photo-biologically produce hydrogen when regulated by a kind of proton uncoupler CCCP (Carbonyl Cyanide *m*-Chlorophenylhydrazone). In this paper, hydrogen production experiments by *P. sub-cordiformis* were carried out in a torus photobioreactor equipped with a mass spectrometer and other necessary sensors so that instantaneous gas components could be measured and other successive physiological states could be well recorded. Hydrogen production performances at different cell densities were compared. We demonstrated that with six folds biomass increasing, hydrogen yield increased almost thirty times. Along with the increase of total hydrogen production yield, rate and duration of mass hydrogen production yield were also much higher. This meant that each alga cell was more efficient in hydrogen production experiments at high cell density. The energy utilization efficiency of hydrogen production from *P. subcordiformis* was further calculated, average energetic yield of light conversion to hydrogen energy was about 0.3%. Here, characteristics of hydrogen metabolism were also studied. By comparing hydrogen production kinetics, photosystem II activity and change trends of endogenous substrates, we gained the knowledge that photosystem II activity and substrates consumption played an important role in *P. subcordiformis* hydrogen production at different phases.

1. Introduction

Nowadays one of man's endeavors is to cope with the energy crisis for the shortage of fossil fuels. Hydrogen, which is a clean, renewable and effective energy carrier, has attracted people's attention [1]. About sixty years ago, researchers [2,3] found green algae can produce hydrogen under anaerobic conditions. This hydrogen metabolism is catalyzed by an enzyme coded by the

algae nuclear genome, named [Fe]-hydrogenase. Fundamental studies [4] on hydrogen metabolism indicated that electrons from water photolysis were driven to [Fe]-hydrogenase active site via reduced ferredoxin through electron transport chain. Meanwhile, catabolism of endogenous substrates also fed electrons to plastoquinone pool between the two photosystems. Thereafter, electrons were driven by light absorbed by PSI to [Fe]-hydrogenase [5]. Ultimately, [Fe]-hydrogenase in the

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stroma of the algal chloroplast accepted electrons to protons to produce hydrogen.

It has been reported that oxygen inhibits the hydrogenase activity with a complete inactivation at partial pressures above 2% [6]. As a result, [Fe]-hydrogenase cannot be induced under natural physiological condition. Therefore, hydrogen production by green algae had practical meaning and commercial purposes only when methods were found to solve the oxygen-inhibition problem.

Melis et al. [7] found that deprivation of sulfur-nutrients in *Chlamydomonas reinhardtii* caused a reversible inhibition in the activity of oxygenic photosynthesis. Under this circumstance, oxygen evolved by photosynthesis dropped below oxygen consumed by respiration; the sealed cultures became anaerobic under illumination and then hydrogen production could last for several days.

At present, most of the work about hydrogen production by green microalgae has been performed intensively on the model organism *C. reinhardtii* [8–11]. However, topics such as whether other green algae could have better hydrogen production capacity or perform different metabolic pathways are also worth studying. Winkler et al. [12] studied performances of *Scenedesmus obliquus* under S-deficiency condition and hydrogenase activity among several green algae. Skjånes et al. [13] investigated hydrogen production capacity of 21 species of green algae they isolated under anaerobic environment.

In China, Guan et al. [14] employed a proton uncoupler CCCP to increase the yield of hydrogen photo-production by *Platymonas subcordiformis*. Hydrogen production yield (50 ml hydrogen/l culture) was achieved by Guo et al. [15].

Studies about metabolism effect of CCCP [16] indicated that similar with sulfur-deprivation effects, CCCP could also reduce the activity of oxygenic photosynthesis reversibly; the uncoupling effect of CCCP caused pH gradient across the thylakoid membrane and thus enhanced the accessibility of electron and proton to hydrogenase.

Ran and Guo's experiments [15,16] were carried out in a cylindrical photobioreactor with a long light path, and therefore hydrogen production experiments were performed at low cell density. In this study, experiments took place in a fully-controlled torus photobioreactor [17] (see Fig. 1.), equipped with a mass spectrometer to analyze gas composition online. This photobioreactor had shorter light path and performed superior in homogeneous mixing of algal cells compared to the reactor mentioned above.

Here, we have reported performance assessment of hydrogen production with the marine green alga *P. subcordiformis* in the torus photobioreactor, including hydrogen production at different cell densities, light energy conversion efficiency of hydrogen production by *P. subcordiformis* and characteristics of hydrogen metabolism.

2. Materials and methods

2.1. Cultivation condition

P. subcordiformis, a marine unicellular alga and wild-type strain, was obtained from Marine Bio-products engineering group in Dalian Institute of Chemical Physics. Medium

components' information is following: 27 g NaCl, 6.6 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5.6 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.5 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 g KNO_3 , 0.05 g KH_2PO_4 , 0.04 g NaHCO_3 , 1 ml of modified Walne medium, 1000 ml of demineralized water. Modified Walne medium: 0.8 g FeCl_3 , 0.4 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 33.6 g H_3BO_3 , 45.0 g $\text{EDTA} \cdot 2\text{Na}$, 20.0 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 100.0 g NaNO_3 , 0.021 g ZnCl_2 , 0.02 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.009 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.002 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.81 g Tris, 0.33 ml acetic acid, 1000 ml of demineralized water.

The algal cells were cultivated in a torus photobioreactor as described below. Light intensity for cultivation was from 100–250 $\mu\text{mol photon/m}^2 \text{s}$. pH of the culture maintained 7.5 by an auto-valve to control CO_2 injection. Temperature was 25 °C.

2.2. General description of hydrogen production system

The photobioreactor was designed to be a torus one (Fig. 1). The main body of the reactor was made of stainless steel (type 316L); the transparent optic surface was polymethyl methacrylate. It was sterilized by vapor stream (105 °C) for 20 min. The reactor was equipped with a mixing propeller, pH, and temperature and dissolve oxygen sensors. Working volume of the reactor was 1.5 l.

Light source (400–700 nm visible light) was placed in front of the reactor to provide light energy (0–1000 $\mu\text{mol photon/m}^2 \text{s}$). Mass flow controllers were hired to regulate nitrogen gas and carbon dioxide injection (The software was Flow Bus). Mass spectrometer (PFEIFFER VACUUM) was connected to the outlet to analyze the composition of gases online (The software was Quadstar 32).

2.3. Hydrogen production procedure

The algal culture was harvested and sealed in a torus photobioreactor as shown in Fig. 1. To induce H_2 production, the algal cells were subjected to a two-phase incubation. In the first phase, the algal cells were under dark anaerobic conditions for hydrogenase induction. Anaerobiosis was achieved by continuous flushing of pure nitrogen through the culture suspension to remove oxygen from the system. After 24 h of dark incubation, CCCP (final concentration was 15 μM .) was added and incubated in darkness for 10 min. The second phase of photobiological hydrogen production was then initiated by placing the cultures under continuous light illumination.

2.4. Biomass assay

10 ml of microalgae was filtered from the medium on a filter paper. Then, the filter paper was dried at 105 °C for 24 h. The extra weight of the paper was the weight of the algae.

Dry weight (g/l) = extra weight (g) ÷ culture volume (l)

2.5. Other analytical procedures

Microalgae protein concentration was measured by the method of Lowry [18]. Total intracellular sugar in the algae

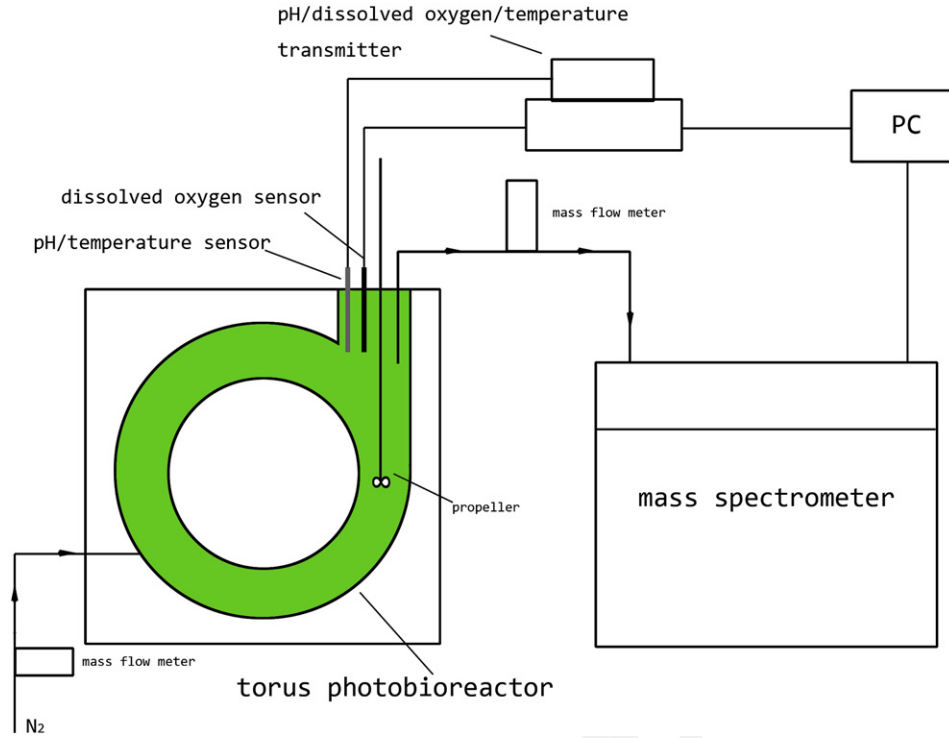


Fig. 1 – Sketch map of front view of the torus photobioreactor.

was detected by the phenol-sulfate method [19]. For starch assay, the determination was applied according to Fouchard's method [10]. Photo system II activity of algal cell was measured by a chlorophyll fluorometer (Water-PAM WALZ, Germany) with the pulse-amplitude-modulation (PAM) measuring principle [16].

3. Results and discussion

3.1. Light energy conversion efficiency of hydrogen production by *P. subcordiformis*

The accepted important variables [20,21] about photobiological hydrogen production process are the photochemical efficiency (PE) and the hydrogen production yield. Here, we try to determine these variables to evaluate hydrogen production by *P. subcordiformis*.

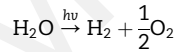
For photochemical efficiency, the most accepted equation [20] is following:

$$\text{efficiency (\%)} = \frac{\text{H}_2 \text{ production rate} \cdot \text{H}_2 \text{ energy content}}{\text{Absorbed light energy}}$$

$$\eta_{\text{hydrogen}} = \frac{\Delta G_{\text{hydrogen}} \cdot R_{\text{hydrogen}}}{E_s \cdot A} \times 100\%$$

R_{hydrogen} (mol/s): average hydrogen production rate, the hydrogen is supposed to be at standard condition: 273.15 K, 1 atm. 1 mol hydrogen is equal to 22.4 l.

$\Delta G_{\text{hydrogen}}$ (J/mol): Gibbs free energy of the following reaction:



$$\Delta G_{\text{hydrogen}}^{\theta} = 237.17 \text{ kJ/mol}$$

$$P = 100 \text{ kPa}, \quad T = 298.15 \text{ K}$$

$$\Delta G_{\text{hydrogen}} = \Delta G_{\text{hydrogen}}^{\theta} - RT \ln(P^{\theta}/P)$$

Here P is considered to be equal to P^{θ} for their little differences. So,

$$\Delta G_{\text{hydrogen}} = \Delta G_{\text{hydrogen}}^{\theta} = 237.17 \text{ kJ/mol}$$

\bar{E}_s (W/m²): average radiant illumination (light within the wavelength range of 400–700 nm), the average light wavelength is 550 nm, assuming that the photons' distribution is uniform at different wavelength, every photon contains energy:

$$\bar{e} = h\nu = \frac{hc}{\lambda} = \frac{6.63 \times 10^{-34} \times 3 \times 10^8}{550 \times 10^{-9}} = 3.6 \times 10^{-19} \text{ J}$$

$\bar{E}_s = \bar{e} \cdot N_A \cdot \text{PFD}$, N_A is Avogadro constant; PFD is photon flux density of light source.

A (m²): irradiated area. For torus photobioreactor, it is 0.0375 m².

The results of efficiency of hydrogen production are presented in Table 1. We could get the knowledge that average energetic yield of light conversion to hydrogen energy was about 0.3% in high cell density hydrogen production experiment. Although it was higher than that in low cell density condition, it was still far away from 10%, which is the target for hydrogen production practical use [1]. For microalgae, cells

Table 1 – Comparison of hydrogen production by *Platymonas subcordiformis* at different cell density.

	Hydrogen production by <i>P. Subcordiformis</i> at low cell density	Hydrogen production by <i>P. Subcordiformis</i> at high cell density
Dry weight, g/l	0.5	3.2
Light intensity, $\mu\text{mol}/\text{m}^2 \text{ s}$	160	400
Culture volume, l	1.5	1.5
Hydrogen production, ml	8.0 ± 0.2	236.6 ± 7.0
Hydrogen production, ml/(h g culture)	10.7	49.2
Maximum hydrogen production rate, ml/h	0.75	7.20
Mass maximum hydrogen production rate, ml/(h g culture)	1.004	1.500
Hydrogen production period hours	24	72
Average hydrogen production rate, ml/h	0.33	3.29
Mass average hydrogen production rate, ml/(h g culture)	0.44	0.69
Efficiency of hydrogen production η_{hydrogen}	0.074%	0.296%

in the surface of algae culture absorb most light energy; as a result, algal cells in the depth of the culture cannot capture enough energy due to this self-shading phenomenon, which make the 10% aim difficult to realize. In future, more research about genetically modified *P. subcordiformis* is necessary. In that case, we may get strains that do not waste so many absorbed photons in antenna.

3.2. Hydrogen production by *P. subcordiformis* at different cell density

As is presented in Table 1, several key parameters of hydrogen production by *P. subcordiformis* at different cell densities were compared. At low cell density (0.5 g/l), hydrogen production yield was 8 ml; with about six folds biomass increase, hydrogen production yield increased almost 30 times that means the hydrogen productivity of each cell also increased. Maximum hydrogen production rate had also increased about 10 times with cell density, from 0.75 ml/h to 7.20 ml/h. Meanwhile, hydrogen production at low cell density continued 24 h, while hydrogen production at high cell density lasted for more than 72 h. The higher the cell density was, the higher the hydrogen production rate and the longer the duration of hydrogen production was.

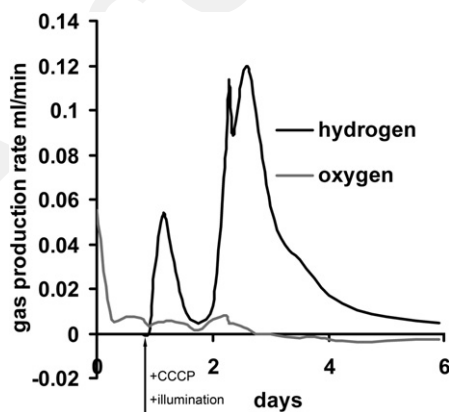


Fig. 2 – Hydrogen production kinetics. (The first 24 h is dark incubation stage; then CCCP was injected into the culture and illumination started.)

Hydrogen production catalyzed by hydrogenase is a reversible reaction which would be influenced by high hydrogen partial pressure due to product inhibition. In the torus photobioreactor, nitrogen was injected into the photobioreactor continuously, which would dilute the hydrogen concentration and maintain a relatively low hydrogen gas pressure in the photobioreactor. Low hydrogen pressure would facilitate hydrogen production and the duration of hydrogen production was prolonged; the yield was promoted accordingly.

In Guo's [15] works about hydrogen production with *P. subcordiformis*, maximum yield of hydrogen production was 50 ml hydrogen/l culture at about 1.5 g/l algal density, which was limited by the long light path photobioreactor they used. In this study, the torus photobioreactor had superior ability in homogeneous mixing of culture so that maximum yield of hydrogen production reached 157.7 ml hydrogen/l culture at 3.2 g/l algal density. In the next section, characteristics of hydrogen metabolism in this torus photobioreactor would be studied. We chose *P. subcordiformis* at 3.2 g/l cell density for further investigation.

3.3. Characteristics of hydrogen metabolism of *P. subcordiformis* in torus photobioreactor

Fig. 2 shows the kinetics of hydrogen production by *P. subcordiformis*. The first 20 h was dark incubation period, then CCCP was injected into the culture and light was provided

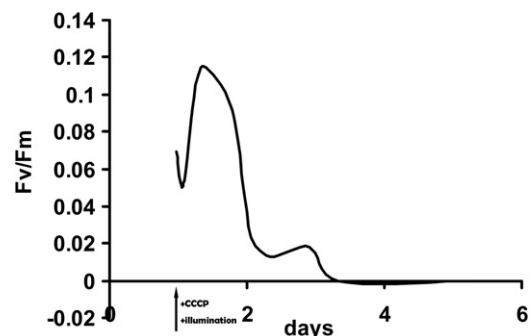


Fig. 3 – Photosynthetic system II activity variation trend during H_2 production phase.

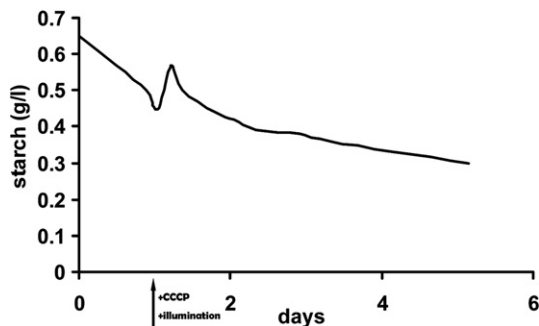


Fig. 4 – Starch content of algal cells in the culture during H₂ production phase.

continuously. Hydrogen started to release thereafter. In the first peak of hydrogen production stage (from 0.8 d to 1.8 d), microalgae released 30.0 ± 0.9 ml hydrogen, and in the second peak of hydrogen production phase (from 1.8 d to 5.2 d), the algae produced 206.0 ± 6.2 ml hydrogen gas. The Fv/Fm is a relative measure of the maximal PSII quantum yield. Here, we employed this value to show photosystem II activity. PSII activity (Fig. 3.) was always under 0.13 during this period, showing injection of CCCP reduced the activity of photosynthesis (without inhibition addition, PSII activity of *P. subcordiformis* is around 0.7 normally). As is shown in Figs. 4–6, at the beginning of this stage (1.0 d), carbohydrate, starch and protein contents were correspondingly 1.10 g/l, 0.46 g/l and 0.10 g/l; in the end of this stage (1.8 d), carbohydrate, starch and protein contents were correspondingly 0.97 g/l, 0.50 g/l and 0.11 g/l. If we didn't take procedure into consideration, there were very few changes of endogenous substrate contents in the culture during first hydrogen peak stage. We could draw the conclusion that in the first peak of hydrogen production, electrons for hydrogen production were mainly given by the photosystem II through electron transport chain. Here, we also noticed when first H₂ peak was highest, value of PSII activity was on its rise period (around 0.08); the variation trend of first hydrogen production peak was not accordant with PSII activity exactly. Thereafter, rate of hydrogen production decreased; meanwhile, carbohydrate and protein contents increased. This phenomenon indicated that electrons generated by PSII during this period were mostly donated to endogenous substrate synthetic. We presumed that PSII activity recovery caused oxygen release; though the concentration of oxygen in gas phase was not higher than

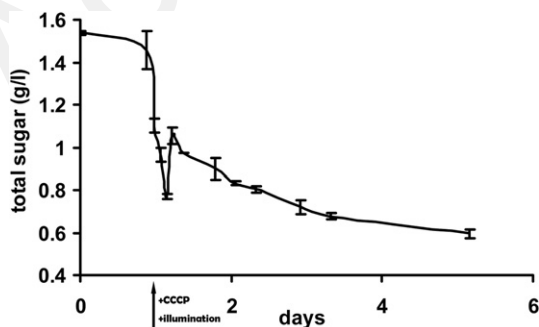


Fig. 5 – Total sugar content of algal cells in the culture during H₂ production phase.

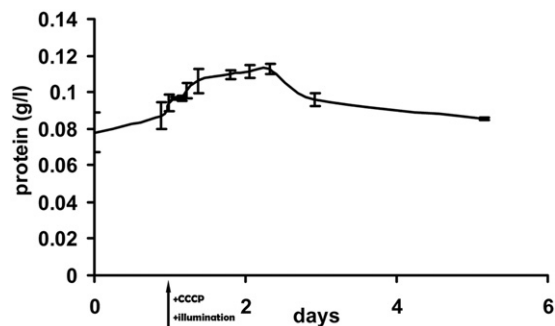


Fig. 6 – Protein content of algal cells in the culture during H₂ production phase.

1.5%, it accumulated shortly inside algal cells and thus influenced hydrogen production during this period.

During the second peak of hydrogen production stage (from 1.8 d to 5.2 d), PSII activity was very low (Fv/Fm was around 0.01). Endogenous substrate consumption was analyzed. For total sugar, the concentration declined from 0.88 g/l to 0.60 g/l at the stage of second peak of hydrogen production (from 1.8 d to 5.2 d). At the same time, starch reduced from 0.44 g/l to 0.30 g/l; protein decreased from 0.11 g/l to 0.09 g/l. As is known, catabolism of endogenous substrate may feed electrons to plastoquinone pool between the two photosystems. The results showed carbohydrate and protein catabolism generated electrons into the electron transport chain and thus contributing to hydrogen production at second peak of hydrogen production stage as PSII activity was quite low during this phase.

4. Conclusion

Here, we demonstrated hydrogen production by *P. subcordiformis* in the torus photobioreactor. Our results indicated that cells of *P. subcordiformis* were more efficient in photohydrogen production experiments at high cell density when the photobioreactor provided superior illumination condition. However, average energetic yield of light conversion to hydrogen energy was about 0.3%, which was far away from hydrogen production practical use (10%). Further studies are required about truncating chlorophyll antenna size at gene level. Investigation about characteristics of hydrogen production by *P. subcordiformis* showed the key factor that influenced hydrogen metabolism was photosystem II activity the beginning of hydrogen production; thereafter breakdown of endogenous substrates played an essential role for hydrogen photo-production when PS II activity was close to zero.

This paper is the beginning of a metabolism study about hydrogen production by *P. subcordiformis*; more intensive experiments about key rate-limiting enzymes and limiting steps are being carried out to give more comprehensive explanations.

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references

- [1] IEA agreement on the production and utilization of hydrogen. Annual report, <http://www.ieahia.org/page.php?s=d&p=annual;2007>.
- [2] Gaffron H. The oxyhydrogen reaction in green algae and the reduction of carbon dioxide in the dark. *Science* 1940;91:529–30.
- [3] Gaffron H, Rubin J. Fermentative and photochemical production of hydrogen in algae. *J Gen Physiol* 1942;26:219–40.
- [4] Florin L, Tsokoglou A, Happe T. A novel type of [Fe]-hydrogenase in the green alga *Scenedesmus obliquus* is linked to the photosynthetic electron transport chain. *J Biol Chem* 2001;276:6125–32.
- [5] Stuart TS, Gaffron H. The mechanism of hydrogen photoproduction by several algae. II. The contribution of photosystem II. *Planta* (Berlin) 1972;106:101.
- [6] Ghirardi ML, Togasaki RK, Seibert M. Oxygen sensitivity of algal H₂-production. *Appl Biochem Biotechnol* 1997;63:141–51.
- [7] Melis A, Zhang LP, Forestier M, Maria L, Ghirardi ML, Seibert M. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol* 2000;12:127–35.
- [8] Tsygankov A, Kosourov S, Seibert M, Ghirardi ML. Hydrogen photoproduction under continuous illumination by sulfur-deprived, synchronous *Chlamydomonas reinhardtii* cultures. *Int J Hydrogen Energy* 2002;27:1239–44.
- [9] White AL, Melis A. Biochemistry of hydrogen metabolism in *Chlamydomonas reinhardtii* wild type and a Rubisco-less mutant. *Int J Hydrogen Energy* 2006;31:455–64.
- [10] Fouchard S, Hemschemeier A, Caruana A, Pruvost J, Legrand J, Happe T, et al. Autotrophic and mixotrophic hydrogen photoproduction in sulfur-deprived *Chlamydomonas* cells. *Appl Environ Microbiol* 2005;71:6199–205.
- [11] Kruse O, Rupprecht J, Bader KP, Thomas-Hall S, Schenk PM, Finazzi G, et al. Improved photobiological H₂ production in engineered green algal cells. *J Biol Chem* 2005;280:34170–7.
- [12] Winkler M, Hemschemeier A, Gotor C, Melis A, Happe T. [Fe]-hydrogenases in green algae: photo-fermentation and hydrogen evolution under sulfur deprivation. *Int J Hydrogen Energy* 2002;27:1431–9.
- [13] Skjånes K, Knutsen G, Källqvist T, Lindblad P. H₂ production from marine and freshwater species of green algae during sulfur deprivation and considerations for bioreactor design. *Int J Hydrogen Energy* 2008;33:511–21.
- [14] Guan YF, Deng MC, Yu XJ, Zhang W. Two-stage photobiological production of hydrogen by marine green algae *Platymonas subcordiformis*. *Biochem Eng J* 2004;19:69–73.
- [15] Guo Z, Chen ZA, Zhang W, Yu XJ, Jin MF. Improved hydrogen photoproduction regulated by carbonylcyanide m-chlorophenylhydrazone from marine green alga *Platymonas subcordiformis* grown in CO₂-supplemented air bubble column bioreactor. *Biotechnol Lett* 2008;30:877–83.
- [16] Ran CQ, Yu XJ, Jin MF, Zhang W. Role of carbonyl cyanide m-chlorophenylhydrazone in enhancing photobiological hydrogen production by marine green alga *Platymonas subcordiformis*. *Biotechnol Prog* 2006;22:438–43.
- [17] Fouchard S, Pruvost J, Degrenne B, Legrand J. Investigation of H₂ production using the green microalga *Chlamydomonas reinhardtii* in a fully controlled photobioreactor fitted with on-line gas analysis. *Int J Hydrogen Energy* 2008;33:3302–10.
- [18] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;194:265–75.
- [19] Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956;28(3):350–6.
- [20] Akkerman I, Janssen M, Rocha J, Wijffels RH. Photobiological hydrogen production: photochemical efficiency and bioreactor design. *Int J Hydrogen Energy* 2002;27:1195–208.
- [21] Levin DB, Pitt L, Love M. Biohydrogen production: prospect and limitations to practical application. *Int J Hydrogen Energy* 2004;29:173–85.