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Photoautotrophic hydrogen production by *Chlorella pyrenoidosa* without sulfur-deprivation

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ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form
27 March 2016
Accepted 29 March 2016
Available online 20 April 2016

Keywords:
Photoautotrophic
Hydrogen production
Chlorella pyrenoidosa
Sulfur-deprivation
Photosystem II

ABSTRACT

In this study, a photoautotrophic hydrogen production of *Chlorella pyrenoidosa* was presented, with $NaHCO_3$ (10 mM) instead of organic carbon and DCMU addition (10 μM) instead of sulfur-deprivation. The results showed that the algal O_2 evolution was significantly inhibited by DCMU, and the earlier it was added the more serious the inhibition was. Overall, when DCMU was added within 9 h after illumination, the relative O_2 concentrations could be regarded as anaerobic (<6%). Comparatively, the algal H_2 photoproduction was also influenced by DCMU addition time, but with opposite trend to the O_2 evolution. In summary, the highest H_2 production occurred when DCMU was injected at 9 h, being fourfold of that in sulfur-deprived TAP medium (93.86 mL L^{-1} vs 23.12 mL L^{-1}). Further study suggested that the higher H_2 productivity might be mainly due to a relatively higher PSII photochemical activity.

Introduction

H_2 photoproduction by microalgae using sunlight and water presents tremendous potential for obtaining an alternate source of clean and sustainable energy [1–3]. In green algae, H_2 is generated by hydrogenase which obtains electrons mainly from water photolysis by photosystem II (PSII) [4]. However, during the water photolysis, O_2 is also generated to which, as commonly known, the algal hydrogenase is highly sensitive [5]. For instance, the hydrogenase of *Chlamydomonas reinhardtii* was reported to be irreversibly deactivated when environmental O_2 partial pressure was higher than 2.8% [6,7]. To inhibit O_2 evolution in water photolysis and obtain an anoxic condition for hydrogenase reaction, a sulfur-deprivation method was firstly reported by Melis et al. [8] in which significant volumes of H_2 was accumulated. Thereafter, the sulfur-deprivation method was widely used in the following researches on algal H_2 photoproduction [1,5].

Besides sulfur-deprivation, exogenic organic carbon sources (e.g. acetate, glucose) was also commonly used for algal H_2 production, which were supposed to be benefit for hydrogenase action by consuming O_2 and/or providing electrons [9–12]. However, when using exogenic organic carbon for microalgal H_2 production, there exist two problems: rapid contamination of heterotrophic microorganisms and expensive cost for obtaining such organic carbon [10,13,14]. Notably, Fouchard et al. [10] found that supplement with HCO_3^- instead of acetate in sulfur-deprived Tris-acetate-phosphate (TAP) medium stimulated starch accumulation and subsequent H_2 production of *C. reinhardtii* 137c. However, when using

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http://dx.doi.org/10.1016/j.ijhydene.2016.03.191
0360-3199/

inorganic carbon instead of organic carbon, PSII should be more strongly inhibited to maintain anoxia so as to produce microalgal H_2.

3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) has been often used for O_2 evolution inhibition [4,7,10]. Therefore, it has been found to be able to improve microalgal H_2 production in several reports [14,15]. However, those reports were mostly using organic carbon and sulfur-deprived medium. Little is known for whether it could work in algal photoautotrophic H_2 production without sulfur deprivation.

In this study, photoautotrophic H_2 production of *Chlorella pyrenoidosa* using inorganic carbon was investigated, in which O_2 evolution was inhibited by DCMU addition instead of sulfur deprivation. To obtain a proper anoxic condition for algal hydrogenase reaction of *C. pyrenoidosa*, DCMU was added at different illumination time. Besides algal H_2 productivity and O_2 evolution, chlorophyll and starch content in the H_2 photoproduction system was also monitored and discussed by comparing the photoautotrophic H_2 production system with normal sulfur-deprived TAP system. Finally, the algal PSII activity was observed to further understand the possible mechanism.

Materials and methods

Alga strain and pre-culture

The alga strain of *C. pyrenoidosa* was obtained from the Culture Collection of Algae, Institute of Hydrobiology, Chinese Academy of Sciences. And the algal cells were pre-incubated in TAP medium containing 2.0 g L^{-1} sodium acetate (28 °C, continuous light of 180 μE m^{-2} s^{-1}). Medium pH was adjusted to 7.0 before autoclaving.

Experimental design

To study whether the algal strain could photoautotrophically grow, algal cells were firstly autotrophically cultivated using photobioreactor referred to Ge et al. [16]. Standard TAP medium containing 2.0 g L^{-1} sodium acetate was prepared as control. Then an autotrophic medium was prepared similar to the TAP medium but using $NaHCO_3$ (10 mM) to replace acetate and sodium acetate. This autotrophic medium was abbreviated as TCP medium. All media were adjusted to pH 7.0 and autoclaved at 121 °C for 15 min. After that, the media were respectively added into the photobioreactors and randomly placed in a room with constant temperature of 28 °C under continuous cool white florescent light (light intensity 180 ± 10 μE m^{-2} s^{-1}).

To produce H_2, algal cells of *C. pyrenoidosa* in logarithmic expected time were collected and washed twice with autoclaved sulfur-deprived TAP (TAP-S, control) or TCP medium, then resuspended in anaerobic bottles with 75 mL corresponding autoclaved TAP-S or TCP medium (20 mg Chl L^{-1}). The anaerobic bottle was purged with N_2 for 10 min, then sealed and placed under darkness for 24 h, followed by a continuous illumination (180 ± 10 μE m^{-2} s^{-1}) at 28 °C. To obtain anoxic condition for algal H_2 production, DCMU (10 μM) was injected at different time after illumination.

During the experiment, algal H_2 productivity and O_2 evolution were monitored. To better understand the possible mechanisms, other parameters which are closely related to algal H_2 production were also investigated, including chlorophyll content, starch content, as well as PSII photochemical activity.

Gas measurement

H_2 and O_2 levels in headspace of the culture were gas chromatographically measured. The gas chromatograph (SP6890, Lunan, China) was equipped with a thermal conductivity detector, and a 2 m × 2 mm (inside diameter) molecular sieve stainless-steel column while helium was used as the carrier gas. Injector, detector and column temperatures were kept at 60 °C, 90 °C and 90 °C, respectively. H_2 production was expressed as value of accumulated H_2 per liter of culture medium. O_2 concentration was expressed as percentage of total gas volume.

Starch and chlorophyll measurement

Algal cell pellets for starch quantification were obtained by centrifugation (5000 × g, 5 min, 4 °C) and stained with a modified iodine solution according to Jeffrey et al.'s [17] method. The cell pellets were resuspended in 500 μL of 80% calcium nitrate solution, and the mixture was boiled for 25 min to dissolve starch. Then 500 μL of double-distilled water was supplemented into the mixture, and the mixture was re-centrifuged (5000 × g, 5 min, 4 °C). Afterwards, 700 μL of the supernatant were mixed with 2 mL of double-distilled water and 300 μL of potassium iodine solution containing 0.35% (w/v) potassium iodine and 0.15% (w/v) iodine. Finally, algal starch content in the supernatant was spectrophotometrically measured at wavelengths of 580 nm.

For chlorophyll measurement, a known volume of the algal suspension was sonicated (1000 w, 10 min) in ice bath, and then centrifugally collected (5000 × g, 5 min, 4 °C). All the collected cells were extracted in 90% (v/v) acetone solution repeatedly till the cellular residue turned colorless, and the cellular residue was removed by centrifugation (12,000 × g, 5 min, 4 °C). Finally, chlorophyll content in the pooled extract was spectrophotometrically measured and the algal chlorophyll content was quantified according to Jeffrey et al.'s [17] method.

PSII photochemical activity measurement

PSII photochemical activity was indicated by the maximum PSII quantum yield (Fv/Fm) which was measured using a Dual-PAM-100 measuring system. The fluorescence parameters are defined as follows: F_0, the initial level of chlorophyll fluorescence, which was determined in the measuring cuvette after 5 min of incubation in the dark; Fm, maximum chlorophyll fluorescence, which was determined using pulses (0.5 s) of red light (5000 μmol m^{-2} s^{-1}); Fv, variable chlorophyll fluorescence, $Fv = Fm - F_0$.