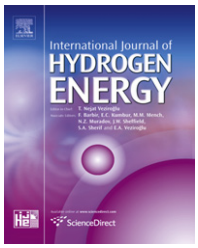
Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he



Technical Communication

Effects of nutrient deprivation on biochemical compositions and photo-hydrogen production of *Tetraselmis subcordiformis*

Chao-Fan Ji [a,b,c], *Xing-Ju Yu* [a], *Zhao-An Chen* [a], *Song Xue* [a], *Jack Legrand* [c], *Wei Zhang* [a,d,*]

[a] *Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*
[c] *GEPEA, University of Nantes, CNRS, Saint-Nazaire 44600, France*
[d] *Flinders Centre for Marine Bioprocessing and Bioproducts and Department of Medical Biotechnology, School of Medicine, Flinders University, Adelaide, SA 5042, Australia*

ARTICLE INFO

Article history:
Received 14 October 2010
Received in revised form
27 December 2010
Accepted 31 December 2010
Available online 26 March 2011

Keywords:
Tetraselmis subcordiformis
Green algae
Photo-hydrogen production
Nutrient starvation

ABSTRACT

Tetraselmis subcordiformis (formerly called *Platymonas subcordiformis*), a marine green alga, was previously demonstrated to photo-biologically produce hydrogen when treated with CCCP (Carbonyl Cyanide m-Chlorophenylhydrazone). The current results of our studies showed that there was a peak yield of hydrogen by *T. subcordiformis* at the stationary stage of its algal growth curve, suggesting that starvation of an essential element induced biochemical changes that enhanced hydrogen production by *T. subcordiformis*. Further investigation indicated that among nitrogen, sulphur and phosphorus (which are major components in the cultivation medium), nitrogen deprivation in the medium reduced the protein content inside the cells of *T. subcordiformis*, but increased the carbohydrate content over 4 times, resulting in 5.5 times increase in the hydrogen yield.

1. Introduction

Unicellular eukaryotic algae can photo-produce hydrogen in a hydrogenase-catalyzed reaction; however, this reaction only happens transiently due to severe inactivation of hydrogenase by photo-evolved O_2 [1,2]. To date, researchers have found methods to reversibly and partially inhibit the activity of oxygenic photosynthesis for sustained photobiological hydrogen production. Melis et al. [3] demonstrated a sulphur-deprivation protocol to force the rate of photosynthetically-produced oxygen to drop below that consumed by respiration; the sealed *Chlamydomonas reinhardtii* cultures would produce hydrogen for up to several days. Surzycki et al. [4] used inducible promoters to switch on/off the activity of PSII in the *nac2-26* mutant strain of *C. reinhardtii*. Their mutant strain was demonstrated to produce hydrogen simply by adding copper in the medium while the cells remained healthy. Guan et al. [5] in our laboratory made use of CCCP to increase the yield of hydrogen photoproduction by *T. subcordiformis*. Studies on the metabolic effects of CCCP indicate that [6,7] CCCP as an ADRY agent (agent accelerating the deactivation reactions of water-splitting enzyme system Y) rapidly inhibited the photosystem II (PSII) efficiency of *T. subcordiformis* cells. This resulted in a large decline in the oxygen evolution. Mitochondrial oxidative respiration which depleted O_2 in the light was only slightly inactivated by CCCP.

* *Corresponding author*. Flinders Centre for Marine Bioprocessing and Bioproducts and Department of Medical Biotechnology, School of Medicine, Flinders University, Adelaide, SA 5042, Australia. Tel./fax: +86 411 84379069.
E-mail address: Wei.Zhang@flinders.edu.au (W. Zhang).
0360-3199/$ – see front matter
doi:10.1016/j.ijhydene.2010.12.138

In the present study, we found that the yield of hydrogen production was much higher in the presence of CCCP when algae were harvested from the stationary stage. This is indicates that the deprivation of certain essential elements induced biochemical changes that enhanced hydrogen production yields in *T. subcordiformis* cells. It is known that nitrogen, phosphorus and sulphur are essential elements for algae physiology because they are constituents of all structural and functional proteins. Our study investigated what happened to *T. subcordiformis* under the deprivation of these three elements (to simulate the medium at the stationary phase), with respect to protein and carbohydrate content and hydrogen production capability.

2. Materials and methods

2.1. Cultivation condition

The growth medium for *T. subcordiformis* was as follows: 1.00 g KNO_3, 0.05 g KH_2PO_4, 0.81 g Tris, 0.33 ml glacial acetic acid, 1 ml of modified Walne medium, 1000 ml artificial sea water. The artificial seawater contained 27 g/l NaCl, 6.6 g/l $MgSO_4 \cdot 7H_2O$, 5.6 g/l $MgCl_2 \cdot 6H_2O$, 1.5 g/l $CaCl_2 \cdot 2H_2O$, 0.04 g/l $NaHCO_3$. Modified Walne Medium contained 0.8 g $FeCl_3$, 0.4 g $MnCl_2 \cdot 4H_2O$, 33.6 g H_3BO_3, 45.0 g EDTA·2Na, 20.0 g $NaH_2PO_4 \cdot 2H_2O$, 100.0 g $NaNO_3$, 0.021 g $ZnCl_2$, 0.02 g $CoCl_2 \cdot 6H_2O$, 0.009 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.002 g $CuSO_4 \cdot 5H_2O$, 1000 ml of water (Synergy water purification system, Millipore). Chemicals we used for medium preparation were all analytical pure.

For the N, S and P deprivation media and artificial seawater, the corresponding elements were depleted or substituted. KNO_3 and KH_2PO_4 were depleted in the N and P deficiency media, respectively. $MgSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ were replaced by $MgCl_2 \cdot 6H_2O$ and $CuCl_2$ in the S deficiency medium and S free artificial seawater. Algal cells were harvested at logarithmic phase (cell density: $5–6 \times 10^6$ cells per ml), then washed three times and suspended in sterilized N, P or S free medium for further cultivation.

Cultivation was performed in a sterilized 600 ml airlift photobioreactor with a 500 ml working volume. The cultivation conditions in the bioreactor were controlled at an irradiance of 14 h of light (cool white fluorescence lamps; 85–110 μE/$m^2 \cdot s$) and 10 h of dark in one day and at a temperature of 25 °C.

2.2. Hydrogen production procedure

To induce H_2 production, the algal cells were harvested, washed and suspended in sterilized N-, P- and S-free artificial seawater for hydrogenase induction under dark anaerobic conditions; anaerobiosis was achieved by 10 min flushing with pure nitrogen through the sealed culture suspension to remove oxygen from the system. The experimental system for hydrogen photoproduction by the marine green alga *T. subcordiformis* was previously described [7]. After 12 h of dark incubation, 15 μM CCCP (final concentration in the culture) was added and the microalgae were then placed under continuous illumination at 100 μE/m^2 s. Hydrogen photoproduction reaction lasted for 30–48 h, and H_2 gas was analyzed by a gas chromatograph (model GC-960T, Haixin Instruments, China) equipped with a thermal conductivity detector and a 5 Å 2 mm × 2 m molecular sieve column with argon as the carrier gas.

2.3. Biomass assay

A 5–10 ml algal suspension sample was deposited on a piece of filter paper (Whatman GF/C) of known weight, dried at 105 °C for 24 h and weighted again to determine the amount of added biomass.

2.4. Other analytical procedures

The microalgae protein concentration was measured by the Lowry method [8]. The total intracellular sugar was detected by the phenol-sulphate method [9]. Fv/Fm of algal cells was measured by a chlorophyll fluorometer (Water-PAM WALZ, Germany) [10].

3. Results and discussion

3.1. Yield of hydrogen production by T. subcordiformis at different cultivation stages

T. subcordiformis photo-produces hydrogen when treated with CCCP, due to partial inhibition of photosynthetic oxygen evolution that results in intracellular anaerobiosis. In our study, we demonstrated in repeated experiments that the yield of hydrogen production at the same cell density (all concentrated or diluted to 6×10^6 cells/ml) peaked during the stationary phase (Fig. 1). It is known that this phase begins when algal cells start to consume their internal reservoirs; most species of microalgae experience a shift in their metabolic flux when deprived of essential elements. For instance, Brown and Hohmann [11] observed a reduction in the ascorbic acid concentration during stationary phases in *Isochrysis* sp.

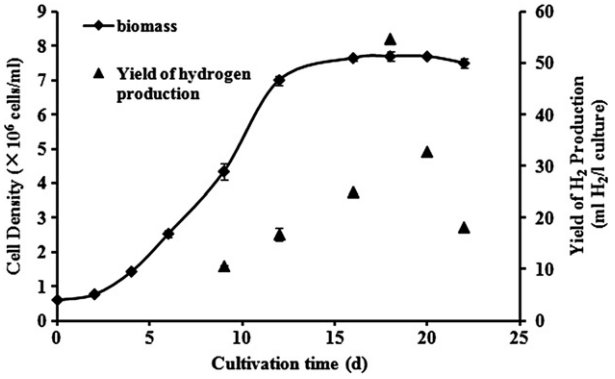


Fig. 1 – Cultivation of *T. subcordiformis* and hydrogen evolution. *T. subcordiformis* cells were harvested at different time points and resuspended at 6×10^6 cells/ml for hydrogen production experiments. The samples were then incubated in the dark for 12 h, treated with 15 μM CCCP and then exposed to illumination. This reaction would last for 30–48 h. In our study, the yield of hydrogen production was calculated at 48 h in each experiment.