Biomass and Bioenergy 111 (2018) 88–95

Contents lists available at ScienceDirect

Biomass and Bioenergy

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

Research paper

Enhanced hydrogen production by optimization of immobilized cells of the green alga *Tetraspora* sp. CU2551 grown under anaerobic condition

Thanaporn Maswanna[a], Saranya Phunpruch[b,c], Peter Lindblad[d,*], Cherdsak Maneeruttanarungroj[b,c,**]

[a] Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand
[b] Bioenergy Research Unit, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[c] Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[d] Microbial chemistry, Department of Chemistry – Ångström Laboratory, Uppsala University, Box 523, SE-75120, Sweden

ARTICLE INFO

Keywords:
Alginate
Immobilization
Biohydrogen production
Green algae
Tetraspora sp. CU2551

ABSTRACT

The green alga *Tetraspora* sp. CU2551 has previously been identified and characterized as a photosynthetic microorganism with high potential for H_2 production. In the present study, cells of *Tetraspora* CU2551 were entrapped and immobilized in an alginate matrix with the aim to analyze the effect of cell stacking and a reduced exposure of O_2 to the cells. The results showed that the most favorable immobilization conditions were 4% (w/v) of final alginate concentration and a cell concentration of 0.125 mg cell dry wt/mL alginate with a bead diameter of 2.80–3.35 mm. The H_2 production yields increased when the immobilized cells were incubated in S-deprived medium and this could be repeated at least for 3 production times. Maximal total H_2 production reached 7.68 ± 0.88 mL H_2/25 mL medium, corresponding to a rate of 1182.45 ± 24.40 nmol H_2/h/mg DW. This production is about 6 times higher compared to by cells in suspension, 2–10 times higher when compared to by other green algae, and 10–50 times higher when comparing with cyanobacteria. Based on our observations, immobilized cells of *Tetraspora* CU2551 is considered a very promising biological system for significant photobiological H_2 production by a photosynthetic microorganism.

1. Introduction

H_2 is considered as a promising clean fuel in the future because the H_2 combustion reaction releases high energy of 142 kJ/g or 61,000 Btu/lb [1] and it generates only water as a by-product, with no greenhouse gas emission allowance. The variation of technologies for the development of H_2 production is being conducted to improve H_2 yield. H_2 production by green algae has recently drawn attention as an alternative energy source. Moreover, green algae can fix their own carbon through the photosynthetic CO_2 fixation process and take up needed nutrients from e.g. wastewater [2–4].

Generally, green algae have been known to produce H_2 via a direct biophotolysis process, which generate molecular H_2 and O_2 from water and light energy through the photosynthesis process [5,6]. H_2 production by green algae is catalyzed by a Fe-Fe hydrogenase, which combines protons and electrons to form the molecular H_2. Unfortunately, the hydrogenase can be inhibited by O_2. As a result, H_2 production is rapidly discontinued due to the accumulation of O_2 released from the water oxidation in photosystem II [7]. One of the approaches to overcome this inhibition is to use a two-stage process [8,9]. In the photosynthesis stage (stage I), the algal cells are grown in the normal medium containing all necessary nutrients under aerobic condition. During the H_2 production stage (stage II), the cell culture is made anaerobic (in the laboratory usually by purging the cell cultures with an inert gas) to remove the dissolved and atmospheric oxygen from the system in order to create anaerobic condition. Moreover, to prevent further O_2 evolution in this stage during H_2 incubation, a sulfur-deprived medium can be used instead of normal medium to down regulate the photosystem II activity [3,7,10–13]. Nevertheless, transferring cells from one growth medium to another needs some techniques such as centrifugation or dialysis. These techniques are time-consumption processes which need instruments. However, those obstacles can be overcome by using immobilized cells.

The immobilization of microalgae can be attained by entrapping the cells within stationary gel lattices, constructed either from natural matrices such as carragenan [14], agar [2,15,16], and alginate [16–20] or even from synthetic polymers such as glass fiber matrix [9] and fumed-silica particles [21]. Among all methods and techniques

* Corresponding author.
** Corresponding author. Bioenergy Research Unit, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
E-mail addresses: peter.lindblad@kemi.uu.se (P. Lindblad), cherdsak.ma@kmitl.ac.th (C. Maneeruttanarungroj).

https://doi.org/10.1016/j.biombioe.2018.01.005
Received 21 September 2017; Received in revised form 26 November 2017; Accepted 17 January 2018
0961-9534/

developed, the alginate gel matrix is the most employed to immobilize algal cells since it is a soft matrix where a transparent and permeable material effectively separates the cells from the medium. Any change of medium can be carried out with no need for a centrifugation step. As a consequence, the transferring process of immobilized cells might become quicker, simpler and cheaper than that of the suspension cultures. Furthermore, the immobilization matrix can also support the cells and allow more efficient light utilization per unit area basis [16]. In addition, immobilized cell also reduces the possibility of cell contamination [22]. This is particularly important for any long-term H_2 production. Moreover, the alginate polymer not only has the capability to effectively separate the entrapped cells from O_2 in the system, but also restricts O_2 diffusion into the matrix [17], resulting in a decreased possibility of O_2 penetration from the culture to the hydrogenase enzyme. As a result, the enzyme shows a relatively high activity resulting and thereby an increased H_2 evolution [16,17,23].

From a previous study using the green alga *Tetraspora* sp. CU2551, a suspension culture of this alga showed efficient H_2 production [24]. The present study experimentally addresses the possibility to enhance H_2 production by immobilizing the cells and demonstrates that immobilized *Tetraspora* cells can be used to further enhance the production of H_2.

2. Materials and methods

2.1. Algal strain and growth conditions

The unicellular green alga *Tetraspora* sp. CU2551 was obtained from the Laboratory of Cyanobacterial Biotechnology, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Thailand. The alga was cultivated in 125 mL Erlenmeyer flasks containing 50 mL of standard Tris-Acetate-Phosphate (TAP) medium at pH 7.2 [25]. The algal cells were cultivated with an initial OD_{730} of 0.1, determined by using a spectrophotometer. The flasks were placed on a shaking incubator with a rotary speed of 140 rpm and illuminated from the top with cool-white fluorescence lamps at light intensity of 29 $\mu E/m^2/s$ and a temperature of 36 °C for 24 h. These growth conditions have previously been found to be optimal for *Tetraspora* sp. CU2551 [24].

2.2. Initial OD_{730} optimization for H_2 production

The alga cells were cultivated in 125 mL Erlenmeyer flasks containing of 50 mL of standard TAP medium by pipette culture suspension into flasks to obtain an initial OD_{730} ranging from 0.005 to 0.10. The volume of the cultures suspension for pipette was obtained by calculation with rule of three in arithmetic. The Erlenmeyer flasks were kept on a shaking incubator with rotary speed of 140 rpm and the temperature of 36 °C under the light intensity of 29 $\mu E/m^2/s$ for 24 h (stage I). Then, the cell culture was transferred to gas-tight vial and purged with argon gas in order to eliminate the atmospheric oxygen. The vial was incubated for H_2 production (stage II) according to a procedure as described in section 2.3.

2.3. Measurement of H_2 production

For both free cells and immobilized cells experiments, an equivalent to 3.75 mg cell dry weight were transferred to a 100 mL gas-tight vial containing 25 mL of the nutrient-deprived or normal TAP medium (as a control). The vials were sealed with a rubber stopper and aluminum rim and later purged with argon gas at 0.2 psi for 15 min to eliminate dissolved oxygen in medium and atmospheric oxygen in head space. The vials were placed in an incubator shaker with a continuous shaking of 140 rpm at 36 °C under light intensity of 29 $\mu E/m^2/s$ prior to measure H_2 gas. 0.4 mL of headspace gas phase was withdrawn with a gas-tight syringe at 18 h (for optimization experiments) and at indicated time intervals (for nutrient deprivation experiments) and injected into a calibrated gas chromatograph (HP 5890 series II, Japan) with a column packed with molecular sieve 13X, using argon as a carrier gas. The packed column was maintained at 50 °C and thermal conductivity detector (TCD) was set at 120 °C for H_2 analysis.

2.4. Cell immobilization and optimization

After 24 h of growth (stage I), the cells were harvested by centrifugation at 3,000 × g for 2 min, washed and resuspended in TAP medium. The algal cell suspension was added to the sterile alginate solution (Alginic acid, sodium salt, ACROS Organics™) to make the final alginate concentration of 4% (w/v) and mixed homogeneously at room temperature. Then, the mixture was dropped into sterile 2% (w/v) $CaCl_2$ solution through a syringe connected with needle using various hypodermic needle sizes, 18G to 30G (NIPRO, Thailand). Formed alginate beads were left to harden for 1 h at room temperature. The beads were ready to use in parameters optimization including the cell bead size, cell concentration in bead, and alginate concentration. All selected conditions (stage II) were performed using normal TAP medium.

2.5. Nutrient deprivation

The successfully immobilized cells were transferred to vials containing 25 mL of nutrient-deprived TAP media including nitrogen-deprived (TAP-N), phosphorus-deprived (TAP-P), sulfur-deprived (TAP-S), combination of nitrogen-phosphorus-deprived (TAP-N-P), combination of nitrogen-sulfur-deprived (TAP-N-S), combination of phosphorus-sulfur-deprived (TAP-P-S), and combination of nitrogen-phosphorus-sulfur-deprived (TAP-N-P-S) media. The pH of all media (TAP and others) were set to 7.2 before being autoclaved. The normal TAP medium was used as the control with salt replacements for deprivation conditions as indicated in Table 1.

2.6. Hydrogenase activity measurements

Hydrogenase activity was determined by the rate of H_2 production from dithionite-reduced methyl viologen. One milliliter of culture samples were withdrawn anaerobically from vials at 24 h after nutrient deprivations of the first round in a stage of gas production, and injected into 13-ml vial under anaerobic condition, containing 250 µl of 40 mM methyl viologen and 750 µl 50 mM potassium-phosphate buffer (pH 6.9) with 0.2% w/v Triton X-100. The vials were then purged and refilled with argon gas at 0.1 psi for 5 min, and kept the temperature at 30 °C. The reaction was started by the addition of 100 µl of 100 mM sodium dithionite. The H_2 concentration was measured in the dark after 10 min of incubation using a gas chromatograph.

2.7. Repeated H_2 production

H_2 production was analyzed in repeated batches. After no further increased H_2 was observed, the vials were unsealed to remove the old medium. The 25 mL new fresh medium according to each condition was re-filled into the respective vials and further processed according to the method as described in section 2.3 to immediately re-establish the anaerobic condition.

2.8. Statistical testing

Obtained results, using biological and technical triplicates, on H_2 production under different conditions were tested for the significance of difference at 99% confidence level by One-way ANOVA and Duncan or post hoc tests using IBM SPSS statistics version 23, for Windows (IBM SPSS software, New York, USA).