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Short communication

Prolongation of H_2 photoproduction by immobilized, sulfur-limited *Chlamydomonas reinhardtii* cultures

Tatyana V. Laurinavichene [a], Sergey N. Kosourov [a,b], Maria L. Ghirardi [b], Michael Seibert [b,*], Anatoly A. Tsygankov [a]

[a] *Institute of Basic Biological Problems RAS, Pushchino, Moscow Region 142290, Russia*
[b] *National Renewable Energy Laboratory Golden, CO 80401 USA*

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Abstract

Two approaches to prolong the duration of hydrogen production by immobilized, sulfur-limited *Chlamydomonas reinhardtii* cells are examined. The results demonstrate that continuous H_2 photoproduction can occur for at least 90 days under constant flow of TAP medium containing micromolar sulfate concentrations. Furthermore, it is also possible to prolong the duration of H_2 production by cycling immobilized cells between minus and plus sulfate conditions.

Keywords: Hydrogen production; Algae; *Chlamydomonas reinhardtii*; Immobilized cells; Sulfur deprivation; Limiting sulfur concentrations; Cyclic sulfur additions

Hydrogen production can be achieved by a number of different renewable technologies, including photobiological water-splitting processes (Prince and Kheshgi, 2005; Esper et al., 2006). The current major limitation on the use of microalgae for commercial H_2 production is the high sensitivity of algal hydrogenases to O_2 (Ghirardi et al., 1997), an obligatory by-product of water splitting. Progress was reported by Melis et al. (2000), who partially inactivated the photosynthetic O_2 evolution process by depriving the algal growth medium of sulfate nutrients. This method allowed for volumetric amounts of H_2 production and has been further optimized in suspension cultures (Kosourov et al., 2002; Laurinavichene et al., 2004; Kruse et al., 2005). A recent, promising optimization approach has involved algal cell immobilization on a glass fiber matrix, which resulted in increased productivity per reactor volume and prolongation of the H_2-production phase (Laurinavichene et al., 2006); however, the cultures were exposed to only one cycle of sulfur deprivation. We report here that H_2 production in immobilized cells under sulfur-deprived conditions can be extended by (a) continuously flowing medium containing limiting amounts of sulfate or (b) cycling the cells between minus and plus sulfate conditions.

The non-motile *Chlamydomonas reinhardtii* mutant, CC-1036 pf18 mt+, was grown, immobilized on fiber glass, and placed in a rectangular PhBR (160 ml volume) as described before (Laurinavichene et al., 2006). Hydrogen was produced by continuously flowing (10 ml h^{-1}) TAP medium containing 10–20 μM sulfate through the PhBR or by periodic sulfate re-additions (argon was also flowed through the PhBR). The latter was accomplished either by 1–2 days of medium flow with 60–100 μM sulfate at 10–30 ml h^{-1} or by rapid medium exchanges.

Decreasing the sulfate concentration to 20 μM in the flow medium 2 days after the start of the experiment resulted in an initial increase in the rate of H_2 photoproduction to a maximum of 11 ml d^{-1} per PhBR up to days 8–9, when the rate decreased to about 4–6 ml d^{-1} (Fig. 1). On day 16, the sulfate concentration in the flow medium was decreased to 10 μM. This change did not further affect the rate of H_2 production, which maintained a fairly constant level of around 6 ml d^{-1} before gradually decreasing toward the end of the experiment. Thus, the continuous presence

Abbreviations: Chl, total chlorophyll (*a* and *b*); PhBR, photobioreactor; PSII, photosystem II; TAP, Tris–acetate–phosphate medium.

* Corresponding author. Tel.: +1 303 384 6279; fax: +1 303 384 6150.
E-mail address: mike_seibert@nrel.gov (M. Seibert).

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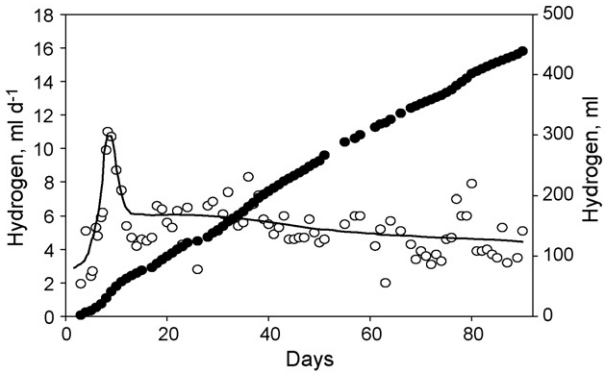

Fig. 1. Continuous H_2 photoproduction by immobilized *Chlamydomonas reinhardtii* cells. At the start of the experiment (day 0), the PhBR was operated under continuous flow of TAP + 200 μM sulfate medium. On the 2nd day, the concentration of sulfate was decreased to 20 μM, and the argon flow rate was set at 500 ml h^{-1}. From the 16th day on, the sulfate concentration was further decreased to 10 μM. The experiment shown in the figure was replicated three times. The two experiments not presented also exhibited an initial peak in the rate data at 10 ± 4 days but were only kept running for a total of 42 and 60 days (the 60-day experiment produced over two times the H_2 compared to that shown in the figure). H_2 production rates (◯); accumulated H_2 gas produced in the photobioreactor (●).

of small amounts of sulfate increased the duration of continuous H_2 production from 30 (Laurinavichene et al., 2006) to at least 90 days.

In Fig. 2 sulfate was added to the flow medium (downward arrows) at, respectively, days 9, 17, 23 and 26 of the process. The time at which sulfate was re-added was prompted by a decrease in the rate of H_2 photoproduction to about 60–80% of the preceding maximum. Sulfate was re-added at concentrations of 60–200 μM (medium flow rates of either 10 or 30 ml h^{-1} for periods of 2 days) or by quick (1–3 h) medium replacement procedures. The results demonstrate that sulfur re-additions do not immediately stop H_2-production activity, which in fact continues to decrease slowly for about another 2–3 days after the re-addition is completed (up arrows). At the end of this period, the rates started to increase again, and reached a maximum value after about two more days, which was somewhat lower than the preceding maximum. Thus, periodic additions of sulfate are able to restore H_2-production activity of the cultures, in a much simpler way than with suspension cultures. Additional experiments will be necessary to identify a protocol that will optimize the process for both methods presented here. Heterogeneity in cell concentration throughout the matrix, examined earlier by measuring light penetration in different parts of the matrix (Laurinavichene et al., 2006), is an important factor decreasing the rate of H_2 production. Variability of the photochemical activity of PSII (measured as described in Antal et al., 2003) is not only a function of sulfur re-addition but also a function of location along the photobioreactor where the measurements were taken (data not shown). The degree of physiological heterogeneity might depend on (a) the properties of the glass matrix itself, which is inevitably deformed during the course of preliminary handling, (b) non-equivalent conditions for green algal cell binding in different parts of matrix during pre-immobilization, and particularly, (c) inadequate mixing of the input medium inside the PhBR. Thus, methods for homogeneous immobilization must be examined.

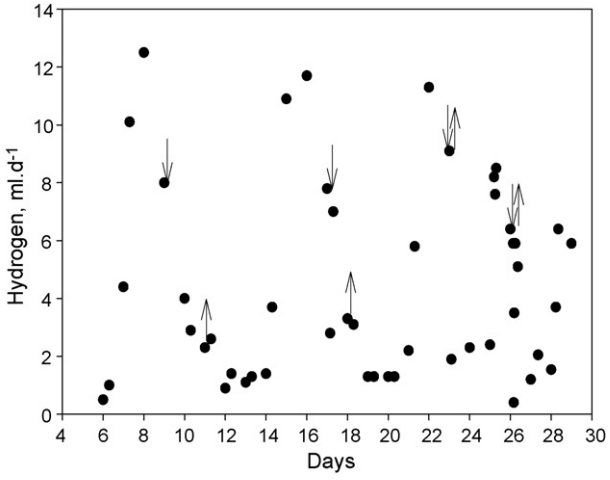

Fig. 2. Changes in the H_2-production rates measured during cycles of minus sulfate and plus sulfate. On days 1–4, the PhBR was operated under TAP + 60 μM sulfate medium flowing at 10 ml h^{-1}. On the 5th day, the medium was changed to TAP minus sulfate also at a flow rate of 10 ml h^{-1}. The argon flow rate was set at 540 ml h^{-1}. Times during which sulfate was included in the flow buffer are indicated in the figure as periods between the downward and upward arrows. Sulfate was added as follows—days 9–11: 60 μM at a flow rate of 10 ml h^{-1}; days 17–18: 100 μM at a flow rate of 30 ml h^{-1}; day 23: 200 μM for 1 h (rapid medium change); day 26: 200 μM for 3 h (rapid medium change). The entire experiment was replicated four other times, but different concentrations of sulfate were flowed for different amounts of time. In every case oscillations in the rates of H_2 production resulting from periodic additions of sulfate were observed similar to those reported in this figure.

The immobilization work reported here provides some cause for optimism. The duration of continuous H_2 production from algae can be prolonged with low sulfate concentration by a factor of at least three compared to the batch system described in our previous immobilization paper (Laurinavichene et al., 2006). It is also possible to recycle the immobilized system by sulfur re-additions as has been shown in suspension cultures (Ghirardi et al., 2000) with the potential for significant process cost reduction.

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