INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 37 (2012) 16437–16445

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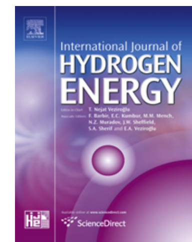

Biohydrogen production by *Rhodobacter capsulatus* Hup⁻ mutant in pilot solar tubular photobioreactor

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ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form
24 February 2012
Accepted 28 February 2012
Available online 24 March 2012

Keywords:
Biohydrogen
Pilot
Tubular photobioreactor
Rhodobacter capsulatus
Hup⁻ mutant
Photofermentation

ABSTRACT

In this study, a pilot solar tubular photobioreactor was successfully implemented for fed batch operation in outdoor conditions for photofermentative hydrogen production with *Rhodobacter capsulatus* (Hup⁻) mutant. The bacteria had a rapid growth with a specific growth rate of 0.052 h^{-1} in the batch exponential phase and cell dry weight remained in the range of 1–1.5 g/L throughout the fed batch operation. The feeding strategy was to keep acetic acid concentration in the photobioreactor at the range of 20 mM by adjusting feed acetate concentration. The maximum molar productivity obtained was 0.40 mol H_2/(m^3 h) and the yield obtained was 0.35 mol H_2 per mole of acetic acid fed. Evolved gas contained 95–99% hydrogen and the rest was carbon dioxide by volume.
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1. Introduction

Molecular hydrogen is considered as an important energy carrier of the future. Currently hydrogen production depends on fossil fuels. However, to make the hydrogen economy fully sustainable, renewable sources should be used [1,2]. Biological hydrogen production methods provide sustainability to the hydrogen production as they utilize various renewable sources like biomass and sunlight.

Photofermentative hydrogen production by purple non-sulfur (PNS) bacteria is advantageous in terms of its high conversion efficiency on various carbon sources and operation at ambient process conditions. PNS bacteria are versatile organisms capable of growing at different modes [3]. Hydrogen production by PNS bacteria *Rhodobacter capsulatus* occurs in photoheterotrophic growth mode under anaerobic and nitrogen limited conditions, and is catalyzed by the nitrogenase enzyme complex. *R. capsulatus* also possesses a membrane-bound uptake [NiFe]-hydrogenase, which catalyzes the oxidation of molecular hydrogen to protons and electrons. Therefore, mutants lacking genes coding for uptake hydrogenase producing genes (Hup⁻) showed better performance for hydrogen production [4,5].

As the ultimate goal of photofermentative hydrogen production is to operate the photobioreactors in outdoor conditions, the design of a solar photobioreactor (PBR) has to meet several conditions. The enclosed system should provide anaerobic conditions. As the reaction is light dependent,

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doi:10.1016/j.ijhydene.2012.02.171

surface to volume ratio of the reactor should be high in order to have better distribution of light over the reactor volume. Reactor design should allow an efficient mass transfer rate of produced hydrogen and the material used should have no hydrogen diffusivity. Moreover, the material thickness and reactor size should be selected according to high visible light and near infrared transmissions [6–8]. A homogeneous substrate distribution within the reactor should be provided through continuous circulation, which would also keep the cells suspended, thereby, providing a better light distribution and stripping of the hydrogen produced. The reactor design should allow easy temperature control in outdoor conditions. In terms of the design geometry of the reactor, they can be classified as panel or tubular reactors. Tubular PBRs consist of long transparent tubes and when compared with the panel, tubular reactors have high surface to volume ratio [8]. There are considerable data related to cyanobacteria cultivations [9–13] and reports on photofermentative hydrogen production in tubular PBRs [12,14–18]. Previously, a comparative operation of large-scale panel and tubular PBRs in outdoor conditions for photofermentative hydrogen production have been reported and their performance were evaluated in terms of ground area (GA) and illuminated reactor surface (IRS) [19]. The mean hydrogen productivity for the panel and the tubular reactors were 0.17 mmol $H_2/(m^2_{IRS} \cdot d)$ and 0.15 mmol $H_2/(m^2_{IRS} \cdot d)$, respectively. A winter period operation of tubular PBR with *R. capsulatus* produced hydrogen at an average productivity of 0.31 mol $H_2/m^3 \cdot h$ on artificial medium with continuous circulation of the reactor [20]. Similarly, 90 L pilot tubular PBR was operated with *R. capsulatus* by using thick juice dark fermenter effluent (DFE). The average molar productivity calculated according to daylight hour was 0.15 mol $H_2/(m^3\ h)$ with regard to the total reactor volume and the yield obtained was 0.5 mol H_2 per mole of acetic acid fed [21].

The main objective of this study is to develop a continuous process for a long-term, stable photofermentative hydrogen production in a pilot tubular PBR (90 L) in outdoor conditions using *R. capsulatus* Hup^-. Accordingly, a new strategy was followed in this study to hold both acetic acid and bacterial concentration at optimum ranges. Moreover, the effect of daily light energy on hydrogen production was investigated under temperature controlled (not to exceed 40 °C during daytime) conditions in tubular PBR.

2. Materials and methods

2.1. Bacterial strains and growth conditions

R. capsulatus YO3 (Hup^-), an uptake hydrogenase gene deleted mutant strain of *R. capsulatus* MT1131 [5], which was previously developed in our laboratory, was used in this study. Bacteria were activated in modified Biebl and Phennig (BP) [22] medium containing acetate (20 mM) as carbon source, glutamate (10 mM) as nitrogen source and 22 mM of potassium phosphate buffer (pH 6.4). The bacteria were activated in a temperature controlled (30 °C) incubator under 200 W/m^2 continuous illuminations. No antibiotics were added to the media. Freshly grown bacteria (OD_{660} = 0.5–1.0 g/L) were inoculated (20%) into the pilot tubular photobioreactor.

2.2. Pilot solar tubular photobioreactor

A modified version of pilot solar tubular photobioreactor (90 L), which was designed and constructed in our previous study [20], was used. To increase the durability of the tubes, two layers of tubes (pipe-in-pipe assembly) were used. In order to control the reactor temperature, PVC cooling coils were inserted in each transparent tube and connected to a process water cooler (Fig. 1). Cooling water temperature was constant in a range between 5 and 10 °C and the flow rate of the cooling water was 255 mL/s. Sterilization was carried out as previously discussed [20,23]. At the start-up, artificial BP medium containing 20 mM acetic acid and 10 mM glutamate was used. *R. capsulatus* YO3 (Hup^-) was grown in the pilot solar tubular photobioreactor under anaerobic conditions by flushing the tubular reactor with argon at the start-up. Until the exponential phase, the reactor was continuously illuminated with artificial halogen light source (2 × 500 W) and the culture was not circulated. At the start of the exponential phase, artificial illumination was turned off and the culture was circulated for 5 min at every hour at a rate of 255 mL/min for energy saving purposes. At the late exponential phase, feeding started. Feeding strategy was to hold acetic acid at certain concentration (15–20 mM) in the reactor throughout the operation by adjusting the feed's acetic acid concentration. Glutamate concentration in the feed was 2 mM. Feeding rate was 20 L/day to provide cell concentration between 1 and 1.5 g/L.

2.3. Analytical methods

The acetic acid concentration in the PBR effluents were analyzed by using gas chromatography (Agilent Technologies 6890N) equipped with FID detector and an HP-FFAP column (30 m × 320 μm × 0.25 μm). The pH of the samples was adjusted to 2.5–3.0 by *o*-phosphoric acid. Evolved gas was analyzed by gas chromatography (Agilent Technologies

Fig. 1 – The pilot tubular PBR. Integration and direction of cooling coils inside the tubular PBR are shown by arrows.

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