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Technical Communication

Hydrogen production by *Chlamydomonas reinhardtii* in a two-stage process with and without illumination at alkaline pH

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ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form
16 December 2011
Accepted 19 December 2011
Available online 9 January 2012

Keywords:
Photosynthesis
Biological hydrogen production
Chlamydomonas reinhardtii
Microalgae

ABSTRACT

This work presents the results of a two-stage (carbon fixation and hydrogen production) experimental study for hydrogen production from microalgae using optical fiber as an internal light source. Effect of absence and presence of light on *Chlamydomonas reinhardtii* culture's pH shift is also evaluated. The culture pH value is a function of light intensity; the pH in the alkaline range changes from 7.5 to 9.5 in the presence and absence of optical fiber respectively. The maximum rate of hydrogen production in the presence of exogenic glucose and optical fiber is 6 mL/L_{cult}/hour, which is higher than other reported values. This study has also revealed that the presence of light reduces the lag time for hydrogen production from 12 to 5 h.

1. Introduction

Hydrogen is believed to be an ideal fuel for the future which does not contribute to greenhouse gas emissions [1]. Hydrogen can be used to produce electricity and contribute to power generation and future transportation. Hydrogen may be produced via pyrolysis, steam reforming and biological processes [2–4]. Among all these methods, the latter has attracted considerable attention due to its high energy production capacity in an economic and green manner [5,6].

The best producer of hydrogen is eukaryotes algae which contain the enzyme hydrogenase. The enzyme produces hydrogen as a waste product during metabolism. The goal of researchers working with algae is to take part of the enzyme which produces hydrogen and insert it into the plant's photosynthesis framework, thereby coaxing the algae to produce significant amounts of hydrogen by-product [7]. Hydrogenase occurs as a transient (which may last from several seconds to a few minutes) because, in addition to electrons and protons, the light-dependent oxidation of water entails the release of molecular oxygen. Oxygen is a key inhibitor to hydrogen production [8]. Current technological developments in this field have not yet succeeded in overcoming this mutually exclusive nature of the O_2 and H_2 photoproduction reactions [5].

Once the technique is developed, algae can provide immediate advantages over other biofuels. Algae can be grown nearly anywhere without the need for large areas of

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0360-3199/$ – see front matter
doi:10.1016/j.ijhydene.2011.12.115

land. It also doesn't compete with food crops for space like corn and other crops do [9].

Hydrogen production via a two-staged cyclic process (carbon fixation and hydrogen production) can get rid of the inhibitory effects of oxygen. Culture pH and light source has significant influence on process efficiency. Microalgae cells become active at a specific pH value and produce hydrogen, while light is an extra energy source.

Gaffron and co-workers first reported the ability of unicellular green algae to produce H_2 in the presence of light. This discovery initiated the curiosity of researchers in the direction of using algae for hydrogen production [10–13].

Chlamydomonas reinhardtii, is a green algae that can use light energy to produce hydrogen from water under anaerobic conditions [14]. The hydrogen evolution in this unicellular green alga is naturally induced upon nutrient deprivation [15]. Especially in the absence of sulfur, the photosynthetic oxygen evolution rate drops below the respiratory rate leading to intracellular anaerobiosis [16]. As sulfur is responsible for the synthesis of protein that produces oxygen, so in the absence of sulfur there is almost no chance of oxygen production. Some researchers reported that green algae can produce hydrogen with a conversion efficiency of more than 80% during photosynthesis [17].

Wykoff and co-workers in their study showed that upon limiting sulfur from the growth medium of *C. reinhardtii* the rate of oxygenic photosynthesis was reduced [18]. Melis and co-workers reported that, in closed cultures, imbalance in the photosynthesis–respiration relationship by sulfur deprivation resulted in a net consumption of oxygen by the cells causing anaerobiosis in the growth medium, a condition that automatically elicited H_2 production [19,20]. Thus, progress was achieved by circumventing the sensitivity of hydrogenase to O_2 through a temporal separation of the reactions of O_2 and H_2 photo-production, i.e. by the "two-stage photosynthesis and H_2 production" process [19]. In the first stage algal cells were allowed to grow in the presence of oxygen and carbon source while in second stage sulfur deprivation was imposed upon the cells in the growth medium, either by carefully limiting sulfur supply in the medium so that it was consumed entirely, or by permitting cells to concentrate in the growth chamber prior to medium replacement with one that lacked sulfur nutrients. Cells responded to this sulfur deprivation by fundamentally altering photosynthesis and cellular metabolism to survive [21].

The application of the two-stage process revealed the occurrence of hitherto unknown metabolic, regulatory, and electron transport pathways in *C. reinhardtii* [22], leading to a significant and sustainable light-dependent release of H_2 by the cells. In addition sulfur deprivation applications of nitrogen in the medium for 5–10 min may also have eliminated any residual oxygen from the medium [23]. The efficiency of biohydrogen production could also be increased by using exogenic sources of carbon like glucose [24].

In the present study glucose was used to increase hydrogen productivity significantly. In addition simultaneous induction of nitrogen gas and use of optical fiber light source created anaerobic conditions by photo-inhibition [25], as higher intensity of light suppressed photosynthesis. Despite the potential of the *C. reinhardtii* for hydrogen production, literature is scarce on evaluating this reaction in the absence of light. The advantages of producing hydrogen in the dark under anaerobic conditions involve less expensive accessories and low maintenance requirements. Furthermore, as water bodies are mostly alkaline, the present work investigated the relationship between the light intensity and medium pH.

2. Material and methods

The culture of *C. reinhardtii* (ATCC 824) was obtained from Qingdao Yijia Huuyi Co., China. Fiber optic high intensity light source (150 W/m^2) was obtained from Halance Technology Co. Limited, Guangdong, China.

2.1. Culture and growth of algae in aerobic environment

In the first step the cell cultures were inoculated photo-heterotrophically in sulfur medium at aerobic conditions (induced by vigorous aeration) having an initial pH of 8.30 at 25 °C. Culture was kept under fluorescent light during the growth phase. Cell culture was allowed to grow in the medium from 2 to 5 days.

2.2. Sulfur deprivation and photo-inhibition

After a suitable growth of cells, the cells were collected by centrifugation at 3000 rpm (C-5, Centrifuge, Lab Essentials) for 10–15 min. Cells were washed twice and re-suspended in 10 mM K_3PO_4, 20 mM KCl and 2.5 mM $MgCl_2$ solution. Nitrogen was introduced for 5–10 min in order to purge out any oxygen present in the culture medium [25,26].

2.3. Hydrogen production

In order to initiate hydrogen metabolism, after cells were incubated for 2–3 h in the absence of light and purged with nitrogen, a sulfur-deprived medium was added. A high intensity fluorescent optical fiber was used to create photo-inhibition conditions to stop photosynthesis [26,27].

Hydrogen collected in each set of experimental run was measured by means of gas chromatography (GC Model 9000, PerkinElmer, USA) having a Molecular Sieve 5A column (RESTEK) that used helium as a carrier gas at a rate of 30 mL/min. The packed column was then maintained at 80 °C and the thermal conductivity detector was set to 120 °C. Sometimes, the peaks corresponding to nitrogen and oxygen also appeared with those of hydrogen, since they were already present in the system.

2.4. Hydrogen cycling and use of optical fiber

Present work accentuated on "cyclic hydrogen production". The cells were shifted from stage 2 to stage 1 in a continuous manner, so that the cells could recover their cellular activities and produce hydrogen. A variable light high intensity fluorescent optical fiber was used during stage 2 as an internal light source. The optical fiber was introduced into the culture vessel in a way that it could distribute light to the cell culture without exposing it to the walls of the bottle (Fig. 1).