INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2017) 1–6

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Hydrogen production with the cyanobacterium *Spirulina platensis*

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ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form
5 December 2016
Accepted 13 December 2016
Available online xxx

Keywords:
H_2 bio-production
Cyanobacterium
Spirulina platensis
Bioreactors configuration

ABSTRACT

The non-nitrogen-fixing and filamentous cyanobacterium *Spirulina platensis* was examined under continuous illuminations of 0.8, 1.5, 2, 2.5, 3, 3.5 and 5 cloaks for a production of bio-hydrogen in three different photobioreactors (cylindrical, conical and conical with an excavated base). The bacterial cell was first grown on a Zarrouk culture medium under batch operational conditions in order to examine the effects of physicochemical parameters on photobiological hydrogen production at an incubation temperature of 34 °C. The photo-production of hydrogen was dependent on the $NaHCO_3$ and NaCl concentrations, pH, light intensity, and photobioreactors design. Indeed, the main result shows that the hydrogen evolution by the cyanobacterium *S. platensis* was improved by using a conical photobioreactor with an excavated base designed in our laboratory. The high bio-hydrogen volume produced, (220 mL, was achieved at 3.5 klx in this photobioreactor of a 200 mL culture volume. This photobioreactor provides an important illuminated surface of 255 cm^2 and limits the shade and photolysis phenomena in dense cell cultures.

Introduction

The production of hydrogen, a future fuel, which can be converted into heat and electricity with a minimal environmental impact, comes mainly from natural gas reforming and from naphtha-oil reforming in the chemical industry [1–5]. However, methane, naphtha-oil and their combustion products are both greenhouse gases and there are limited reserves of fossil fuels on earth. Environmental concerns about the climate changes and the limited availability in the future of fossil fuels force the transformation of the energy system from a scheme mainly based on the combustion of fossil fuels to another based on sustainable CO_2-free sources [6,7] or the development of renewable non-polluting energy sources, including photobiological hydrogen production [8–14]. It is expected that the development of renewable technologies could bring water electrolysis from wind and solar, or thermo-chemical solar to a competitive market. Only a low percentage of worldwide hydrogen production is based on water

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http://dx.doi.org/10.1016/j.ijhydene.2016.12.056
0360-3199/

electrolysis that can be adapted to a CO_2-free production when produced by renewable sources. This fact is the consequence of the cost competitiveness of the production methods [6].

Nevertheless, one of the alternatives, as an environmentally suitable fuel, is the production of bio-hydrogen by photosynthetic microorganisms like cyanobacteria species and green algae. With cyanobacteria, hydrogen is produced by a light-dependent reaction catalyzed by a nitrogenase or in dark anaerobic conditions by a hydrogenase [9,15,16], while in the green algae hydrogen is produced photo-synthetically [9,17]. Cyanobacteria are a morphologically diverse group with unicellular (*Chroococcidiopsis thermalis*), filamentous (*Spirulina platensis*, *Anabaena variabilis*), colonial forms and are unique in their capacity to use the CO_2 of the air as a carbon source and solar energy as an energy source for producing hydrogen.

Cyanobacteria are adapted to a wide range of habitats, including aquatic, terrestrial and extreme environments like cold lakes of the Antarctic [12] or alkaline soda lakes (pH 9.5 to 11) at a high concentration of sodium [11]. They are also cultivated in controlled closed systems called photobioreactors. A large variety of closed photobioreactors have been proposed to generate a biomass free of contaminants [18,19]. Developed bioreactor requires a detailed knowledge of light distribution, mass transfer, shear stress, scalability, and biology of algae cells [19]. Fernandez et al. Have provided a method for designing an airlift-driven tubular photobioreactor with continuous run solar loop tubing. This airlift-driven photobioreactor with a continuous run tubular solar receiver essentially consists of two parts, the airlift system, and the looped solar receiver. Its advantages include a better control over culture variables, enabling higher productivities and reducing power consumption [18]. Zijffers et al. have constructed a flat plate photobioreactor in which the sunlight is focused on the top of the bioreactor by a dual-axis positioning of linear Fresnel lenses, captured by vertical plastic light guides, reflected internally in these guides, and then eventually distributed into the photobioreactor compartment. With this design, the sunlight can be more evenly distributed in the bioreactor and a better light utilization is expected [20]. The purpose of the present work is to study the geometric configuration of three photobioreactors in photobiological hydrogen production by the cyanobacterium *S. platensis*. The relationship between the illuminated surface in various types of photobioreactors and hydrogen photo-activity is reported here.

Materials and methods

Batch cultures

The nonnitrogen-fixing and filamentous cyanobacterium *Spirulina* was collected, on a filter paper, from a *sebkha* in the Sahara region of Tamanrasset (South of Algeria) and transported in the state of a fresh paste. The cyanobacterium was cultivated at 34 °C in the Zarrouk culture medium: 1 L of this culture medium contained 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 40 mg $CaCl_2$, 10 mg $FeSO_4 \cdot 7H_2O$, 80 mg EDTA and 1 mL trace metals solution A5 (2.9 g H_3BO_3, 1.8 g $MnCl_2 \cdot 4H_2O$, 0.2 g $ZnSO_4 \cdot 7H_2O$, 80 mg $CuSO_4 \cdot 7H_2O$, 10 mg MoO_3) and B6 (44 mg $Co(NO_3)_2 \cdot 6H_2O$, …) under alternative 12 h illumination by a white fluorescent light (3.4 μmol m^{-2} s^{-1}) 241.4 klx and 12 h in darkness cycles. Cultures were bubbled with a moderate air flow using a *Champion CX-0088* air pump. The cyanobacterium concentration, pH = 9.1, was adjusted through the optical density at 618 nm using a spectrophotometer *Shimadzu 1240 UV mini*. Dry cell weights were determined by centrifuging, washing and drying the cell suspension at a temperature of 90 °C to constant weight.

The fermentative H2 production under various $NaHCO_3$ concentrations ranging from 200 to 350 mM were achieved with Zarrouk culture medium free $NaHCO_3$. The $NaHCO_3$ concentrations 200; 250; 300 and 350 mM were obtained by adding the $NaHCO_3$ salt to the solution.

The pH of the fermentative H2 solution (6.5; 7.5; 8; 8 .5; 9; 9.5; 10 and 12) were adjusted by addition of acid and basis (HCl and NaOH).

The effect of NaCl on the H2 production was determined with concentration salt ranging from 17 mM to 90 mM.

Photobioreactor design

To provide maximum yields of hydrogen production, during photosynthesis with a minimal oxygen evolution, different types of photobioreactors can be used. The schematic diagrams of our three types of photobioreactors used in this study are shown in Fig. 1. The Cylindrical (A) and the conical (B) photobioreactors consisted of 200 mL culture rubber-stopper vessels of an 8 cm diameter and a surface of 100 cm^2. The photobioreactor (C), designed in our laboratory, consisted of a 200 mL culture conical vessel with an excavated base which permits an important illuminated surface (255 cm^2) and limits the shade and photolysis phenomena. The main advantage of the new photobioreactor compared to the two others classic ones is the easy access of the light to the culture induced by its narrow canal (15 mm). The cylindrical and conical canal attained values of about 85 mm and 120 mm, respectively [21]. Photobioreactors were first sterilized and supplied with a young cyanobacterium culture harvested in the late logarithmic growth phase obtained after 20 days. This culture was before extracted and purified by filtration and then included in the photobioreactors with a quantity of 1 g/250 mL of $Na_2S_2O_2$. The bioreactors were then

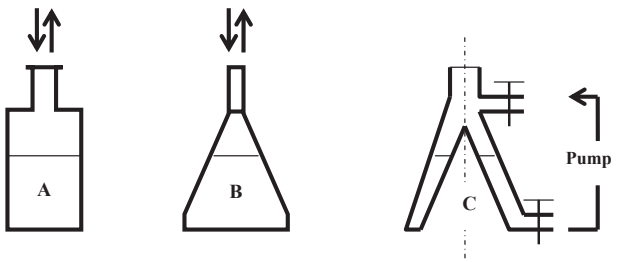

Fig. 1 – Photobioreactor diagrams: (A) Cylindrical photobioreactor, (B) Conical photobioreactor and (C) Conical photobioreactor with an excavated base.

Please cite this article in press as: Ainas M, et al., Hydrogen production with the cyanobacterium *Spirulina platensis*, International Journal of Hydrogen Energy (2017), http://dx.doi.org/10.1016/j.ijhydene.2016.12.056