



HAL
open science

Biohydrogen production using green microalgae as an approach to operate a small proton exchange membrane fuel cell

Samira Chader, Khaled Chetehouna, Bouziane Mahmah, Fethia Amrouche,
Kamel Abdeladim

► **To cite this version:**

Samira Chader, Khaled Chetehouna, Bouziane Mahmah, Fethia Amrouche, Kamel Abdeladim. Biohydrogen production using green microalgae as an approach to operate a small proton exchange membrane fuel cell. *International Journal of Hydrogen Energy*, 2011, 36, pp.4089-4093. hal-00652813

HAL Id: hal-00652813

<https://hal.science/hal-00652813>

Submitted on 16 Dec 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biohydrogen production using green microalgae as an approach to operate a small Proton Exchange Membrane Fuel Cell

**Samira Chader^{1,2,*}, Bouziane Mahmah¹, Khaled Chetehouna³, Fethia Amrouche¹,
Kamel Abdeladim¹**

¹ *Centre de Développement des Energies Renouvelables Bouzaréah, BP 62, Alger – Algérie.*

² *Centre National de Recherche & Développement Pêche et Aquaculture, 11 boulevard
Colonel Amirouche, Bou-Ismaïl, Tipaza – Algérie.*

³ *ENSI de Bourges, Institut PRISME UPRES EA 4229 EP-RES, 88 bd Lahitolle, 18020
Bourges cedex, France.*

*Corresponding author: Samira.chader@gmail.com

Abstract

In this paper the wild-type of *Chlorella sorokiniana*, a green microalga isolated from Algerian Sahara soil, is tested for its ability to produce hydrogen in a 500 ml photobioreactor coupled to a small Proton Exchange Membrane Fuel Cell (PEMFC). The strain grown in heterotrophically conditions (Tris-Acetate-Phosphate medium) under continuous light is transferred, in medium without sulfur to make anaerobiosis and activate the reversible hydrogenase, inducing the hydrogen production process. The evolution of hydrogen and oxygen concentrations in the photobioreactor during the sulfur-deprived conditions is measured. The difference of total carbohydrates amount and cell morphology at the beginning and the end of experience is also studied. The results of the

* Corresponding author. Tel./fax: + 213 21 90 15 60.
E-mail address: Samira.chader@gmail.com (S. Chader).

coupled system show that the produced biohydrogen can be used to operate a PEM Fuel Cell with good performances under standard conditions.

Keywords: Biohydrogen production; *Chlorella sorokiniana* strain Ce; PEM Fuel Cell (PEMFC).

1. Introduction

The growing preoccupation for environment and global warming in conjunction with the adherence of signatory countries to *Kyoto* Protocol including Algeria lead to use clean and renewable energy. Currently, several technologies developed energy systems based on the use of hydrogen as an optimal energy vector for the future. Except its biological production, all the other forms of generation (steam reforming, thermochemical cracking, gasification of coal ...) are consuming fossil energy, electricity and heat. However, biological production occurs under ambient temperature and pressure conditions does not require energy input. This production way is an environment-friendly process and it permits the valorisation of some natural resources, too often neglected.

The hydrogen gas photobiologically produced (i.e. biohydrogen) is considered as a secondary metabolite obtained by microorganism metabolism released under particular conditions. According to microorganism species and biochemical process, different biological ways of hydrogen production are reported in the literature [1]. Biological hydrogen generation can be classified into four categories: (i) direct biophotolysis using green microalgae [2-4] and some cyanobacteria [5], (ii) indirect biophotolysis with other cyanobacteria species and certain nitrogen-fixing bacteria [6,7], (iii) photofermentation of waste and effluents [8] and (iv) darkfermentation of rich sugar wastes [9,10].

Up to now the large utilization of hydrogen produced by biological way in industrial processes is difficult due to its low conversion rate [11]. However, the generation of electricity via small fuel cells using biohydrogen as fuel seems to be a promising application.

Unfortunately, few studies report the practicability of coupling the biohydrogen production to the operation of fuel cell. Electricity generated by the use of biohydrogen in fuel cell varies depending on the microorganism involved, carbon source used, experimental and physiological conditions. Recently, Wünschiers and Lindblad [12] used green algae *Scenedesmus obliquus* to produce hydrogen gas and developed a software interface to read several parameters such as power generation by the bioreactor connected to Proton Exchange Membrane Fuel Cell (PEMFC). Dante [13] investigated the possibility to exploit directly in a fuel cells stack, the hydrogen produced by *Chlamydomonas reinhardtii*. He and al. [14] showed that several strains of *Rhodobacter capsulatus* generate biogas containing hydrogen which is successfully used as feed for a small PEM Fuel Cell system. More recently, García-Peña and al. [15] established a semi-continuous biological system to produce hydrogen and generate electricity by coupling the bioreactor to a fuel cell using a seed sludge as inoculum. In the same context the present study attempts to investigate the feasibility of electricity generation from green microalgae and to assess the effectiveness of a photobioreactor coupling to a fuel cell. The main purpose of this paper is to estimate and measure the response of a small PEM Fuel Cell by injection of unpurified hydrogen gas produced by *Chlorella sorokiniana* strain Ce locally isolated from the soil in the Algerian Sahara and incubated under sulphur-deprivation conditions. The next section is dedicated to the description of the experimental setup, materials and methods. The photobioreactor coupled with a small PEM Fuel Cell is also illustrated in this section. The last section is devoted to the presentation of some obtained results and associated discussions. The comparison of the generated electricity by the proposed system with those given in the literature will be done.

2. Experimental methodology

In this section, the growth of the microalgae used and biohydrogen production process are described with the main analyse methods employed to evaluate the production rate of biohydrogen and the degradation rate of carbohydrate during sulfur-deprived conditions. The physiological cell behaviour and the system of photobioreactor coupled with small PEM fuel cell are also detailed.

Chlorella sorokiniana strain Ce (wild-type) isolated from microflora sampled from soil of Algerian Sahara was used (Fig. 1). It firstly grew in a Tris-Acetate-Phosphate (TAP) solid medium (pH 7.20) under continuous cool white fluorescence lamps ($\approx 100 \text{ photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Secondly, to produce hydrogen, the culture with an initial cells concentration of $5 \times 10^6 \text{ cell} \cdot \text{ml}^{-1}$ has been transferred in TAP liquid medium without Sulfur at incubation temperature of $23 \pm 2^\circ\text{C}$ with stirring under the same light intensity as indicated by Chader et al. [4] and Melis et al. [16,17].



Figure 1. Photography of a sampling area in Algerian Sahara.

Hydrogen and oxygen concentrations were measured in the gas phase by a single polarographic probe. This probe operated at a voltage, which is sequentially inverted by means of an electronic switch ($+0.7 \text{ V}$ for H_2 , -0.7 V for O_2). The amount of total

carbohydrates was determined by the anthrone-sulfuric acid method suitably adapted for microalgal biomass calculated by means of a calibration curve using D+ glucose dissolved in distilled water [4,18].

Cell morphology of *Chlorella sorokiniana* strain Ce used in this study as a function of sulfur-deprived conditions associated to biohydrogen production was observed at the beginning and the end of experiments under light microscope (Zeiss, Germany) operated at a magnification of 200×.

Experimental setup consists on a photobioreactor coupled with a small PEM Fuel Cell is illustrated in Fig. 2. A glass bioreactor of 500 ml volume, containing the liquid culture described above, is used for hydrogen production, surmounted by syringe to sample the gas from this photobioreactor and to inject it directly through a pipe system to a Proton Exchange Membrane Fuel Cell (H-TEC GmbH. Lübeck, Germany). It's characterised by an electrode surface of 16 cm² with a size of 105×200×130 mm³ (height×width×depth) producing a power of 1.2 W and maximum operating voltage of 0.9 V. A resistance of 10 Ω is used as the load of the PEM Fuel Cell. Voltage and current values are measured with a multimeter TUV 3275.

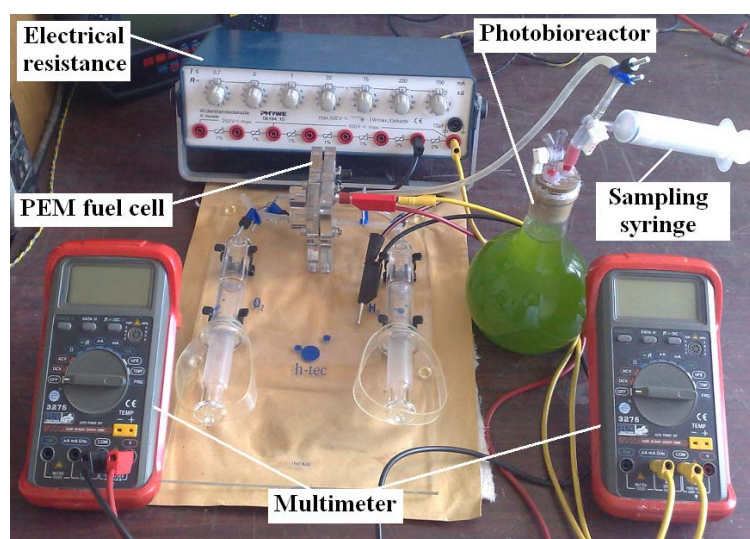


Figure 2. A system of photobioreactor coupled with a small PEM Fuel Cell.

3. Results and discussion

3.1 Hydrogen production

Under sulfur-deprived conditions sealed *Chlorella sorokiniana* culture exposed to a continuous light, becomes rapidly anaerobic medium after 12 to 50 h due to the significantly increased of respiration illustrated by oxygen consumption as shown in Fig. 3. After a short time of about 24 h, the hydrogen production begins due to the induction of hydrogenase activity. Indeed, compared to others green microalgae [16,19], the study strain starts earlier the hydrogen accumulation and can sustain production for high O₂ partial pressures.

The obtained results show that *Chlorella sorokiniana* strain Ce accumulates hydrogen gas under anaerobic conditions such as reported in the literature for several green microalgae [2,4,16]. Indeed, anaerobiosis (under continuous light) is necessary and sufficient for induction of the reversible hydrogenase and for photobiological hydrogen production [20,21]. As indicated by Melis et al. [16], the photobiological hydrogen production is based on the principle to circumvent the severe oxygen sensitivity of the reversible hydrogenase by separating photosynthetic oxygen evolution and carbon accumulation (photosynthesis and growth phase) of the consumption of cellular metabolites (oxidative respiration and hydrogen production phase).

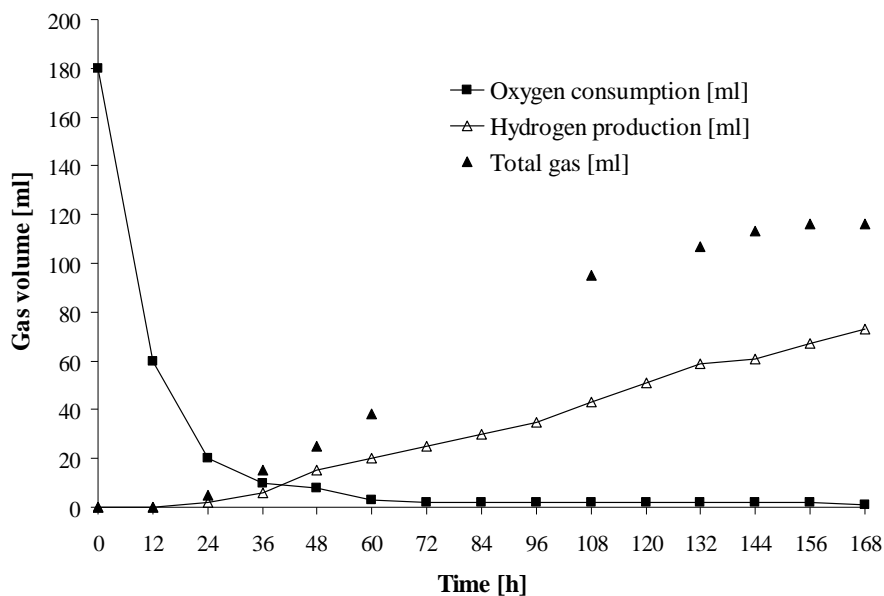


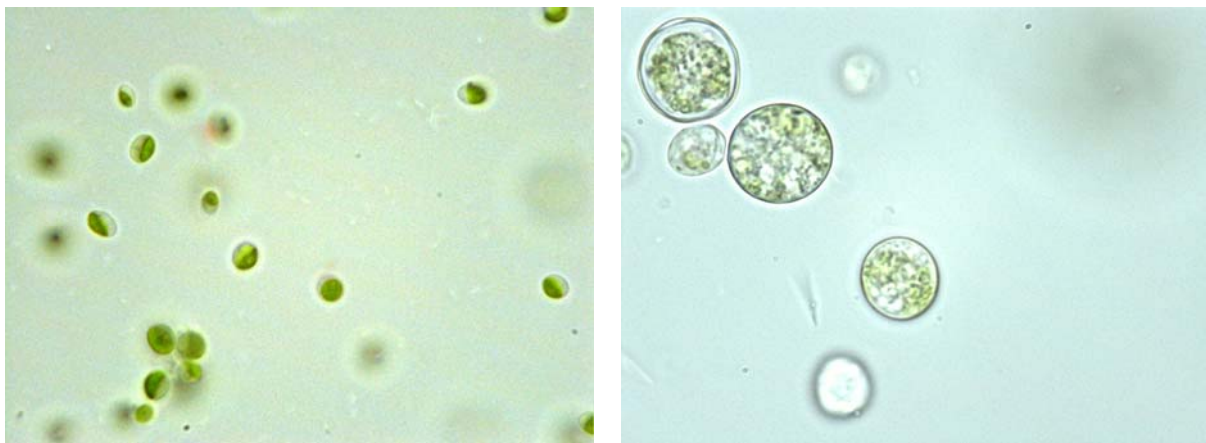
Figure 3. Hydrogen production and oxygen consumption by *Chlorella sorokiniana* cells under sulfur deprivation.

The total gas volume as well as the hydrogen one increase until to reach maximum values respectively 116 ml and 73 ml after 168 h of sulfur deprivation. At this time the concentration

of the hydrogen in the mixture containing N₂, O₂, CO₂ and H₂ [4] is about 63%. The total gaseous mixture produced is divided into five volumes which will be separately injected in a PEM Fuel Cell in order to generate electricity as will be detailed in the third subsection below.

3.2 Physiological cell behaviour

Chlorella sorokiniana in the log-linear of growth has small ellipsoid form or slightly ovate with parietal chloroplast and distinctive cell wall. This cellular morphology change severely under sulfur-deprived conditions (Fig. 4). As shown in this figure, cell volume increased and its form becomes spherical due to a large metabolism and degradation of endogenous reserves and carbohydrates (i.e. oxidative respiration) associated to water molecules production. Indeed, before sulfur deprivation this strain accumulates substantial starch in the cells (growth period) degraded during anaerobic period (hydrogen production period). The carbohydrates content decreased down to 54% of the highest starch accumulated level. Similar results concerning cell morphology changes were observed by Zhang et al. [19].



a. Small ellipsoid cells forms at 0 hours after sulfur deprivation.

b. Large spherical cells forms at 168 hours after sulfur deprivation.

Figure 4. Morphology of *Chlorella sorokiniana* cells under sulfur deprivation.

3.3 Electricity generation

In order to know if the biohydrogen produced by *Chlorella sorokiniana* strain Ce is able to generate electricity and to estimate the response of the Proton Exchange Membrane Fuel Cell, the unpurified gas produced is injected into five volumes: 8 ml, 10 ml, 15 ml, 25 ml and 43 ml. A typical voltage response of fuel cell to this gas injection (8 ml containing 5.04 ml of hydrogen) is illustrated in Fig. 5 below.

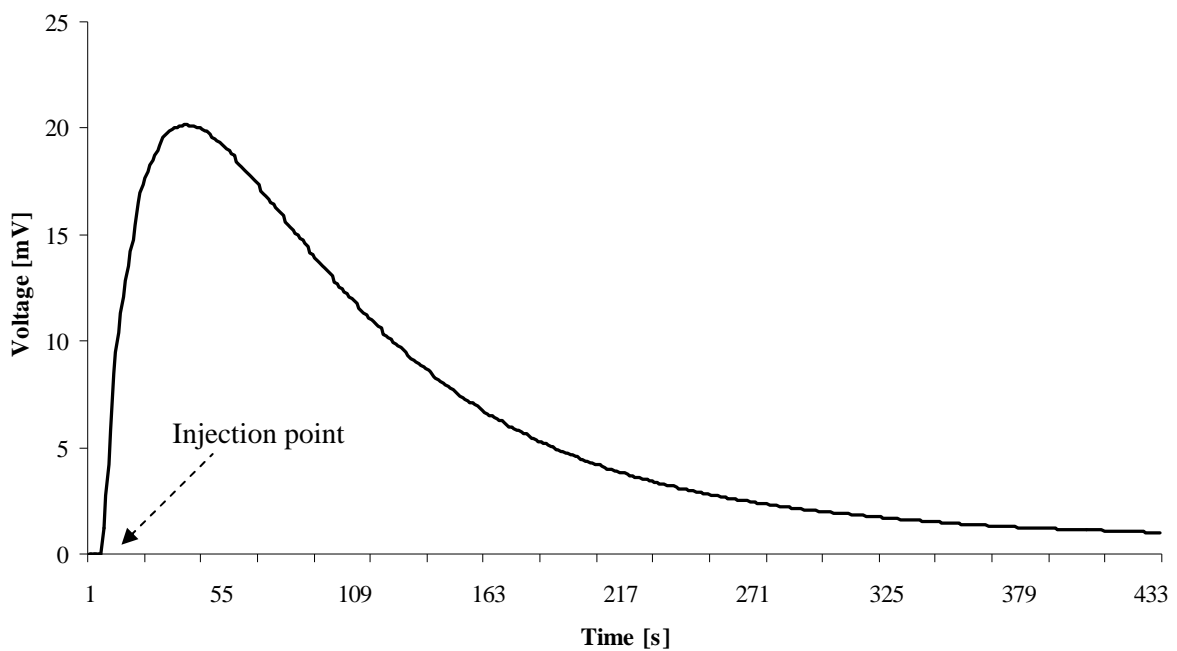


Figure 5. Voltage versus time response of PEM Fuel Cell obtained with 8 ml volume of total gas (5.04 ml of hydrogen) produced by *Chlorella sorokiniana* culture.

Once the gas is injected, the voltage increased rapidly due to the hydrogen conversion into the proton exchange membrane and tends to level off to reach a steady state. Indeed, the ionic equilibrium at the surface of the membrane [22] is obtained after about two minutes giving a maximum voltage of 20.15 mV during 19 seconds. Those values depend on the injected gas volume and the discontinuous injection mode used in this study. After the steady state phase, the voltage decreases slowly until a value of 0.1 mV at the end of experiment. The voltage

curve described above is similar to voltage distribution in PEMFC operated with pure hydrogen under standard conditions [23,24].

Furthermore, concerning the performance of the PEM Fuel Cell for different volumes of hydrogen, the results presented in Table 1 show that these hydrogen amounts generate maximum voltages between 20.1 and 89.1 mV. This interval of efficiencies conversion of biohydrogen is better compared to maximum voltages obtained by the same fuel cell coupled continuously with culture of *Scenedesmus obliquus* obtained by Wünschiers and Lindblad [12]. *Chlorella sorokiniana* generates a maximum current of 8.9 mA with 27.09 ml of hydrogen injected. Using a close hydrogen amount, this maximum current value is higher than the one reported by García-Peña et al. [15] with a dark fermentation process.

Table 1 - Response PEM Fuel Cell for different injected hydrogen volumes.

Total gas volume [ml]	Hydrogen volume [ml]	Maximum tension [mV]	Maximum current [mA]
8	5.04	20,1	2.0
10	6.30	54	5.4
15	9.45	68	6.8
25	15.75	80.9	8.1
43	27.09	89.1	8.9

The results of this study show that the hydrogen produced by *Chlorella sorokiniana* strain Ce and injected through a PEMFC was efficiently converted into electricity that has generated measurable voltage and current in agreement with the characteristics of the fuel cell used. We plan to investigate in a future work the permanent coupling of hydrogen bioreactor and a FEM Fuel Cell.

4. Conclusion

Chlorella sorokiniana strain Ce culture produced a high amount of biohydrogen under sulfur-deprived conditions. The hydrogen production starts early despite the high oxygen partial pressure in photobioreator. This photobiological process is accompanied by some morphological and physiological changes of cells. Hydrogen content in the mixture gas injected in Proton Exchange Membrane Fuel Cell has been converted in electricity. This fuel cell is successfully operated using the produced biohydrogen and has generate similar response that obtained with pure hydrogen under standard conditions.

Acknowledgements

The authors would like to acknowledge Youcef Mohammadi for his help in designing the experimental set-up.

References

- [1] Das D and Veziroglu TN. Hydrogen production by biological processes: a survey of literature. *International Journal of Hydrogen Energy* 2001; 26(1): 13-28.
- [2] Melis A, Happe T. Hydrogen production: green algae as a source of energy. *Plant Physiology* 2001; 127(3):740-8.
- [3] Levin DB, Pitt L, Love M. Biohydrogen production: prospects and limitations to practical application. *International Journal of Hydrogen Energy* 2004; 29: 173–85.
- [4] Chader S, Hacene H, Agathos SN. Study of hydrogen production by three strains of *Chlorella* isolated from soil in the Algerian Sahara. *International Journal of Hydrogen Energy* 2009; 34: 4941–4946.
- [5] Smith GD, Ewart GD, Tucker W. Hydrogen production by cyanobacteria. *International Journal of Hydrogen Energy* 1992; 17: 695-698.
- [6] Vyas D, Kumar HD. Nitrogen fixation and hydrogen uptake in four cyanobacteria. *International Journal of Hydrogen Energy* 1995; 22:163- 168.
- [7] Mizuno O, Dinsdale R, Hawkes FR, Hawkes DL, Noike T. Enhancement of hydrogen production from glucose by nitrogen gas sparging. *Bioresource Technology* 2000; 73:59–65.
- [8] Aoyama K, Uemura I, Miyake J and Asada Y. Fermentative metabolism to produce hydrogen gas and organic compounds in a Cyanobacterium, *Spirulina platensis*. *Journal of Fermentation and Bioengineering* 1997; 83:17-20.
- [9] Mahyudin AR, Furutani Y, Nakashimada Y, Kakizono T, Nishio N. Enhanced hydrogen production in altered mixed acid fermentation of glucose by *Enterobacter aerogenes*. *Journal of Fermentation and Bioengineering* 1997; 83(4): 358–363.
- [10] Tanisho S, Kuromoto M, Kadokura N. Effect of CO₂ removal on hydrogen production by fermentation. *International Journal of Hydrogen Energy* 1998; 23:559–63.

- [11] Akkerman I. Photobiological hydrogen production: photochemical efficiency and bioreactor design. *International Journal of Hydrogen Energy* 2002; 27:1195-1208.
- [12] Wünschiers R, Lindblad P. Hydrogen in education – a biological approach. *International Journal of Hydrogen Energy* 2002; 27:1131-1240.
- [13] Dante RC. Hypotheses for direct PEM fuel cells applications of photobioproduced hydrogen by *Chlamydomonas reinhardtii*. *International Journal of Hydrogen Energy* 2005; 30:421 – 424.
- [14] He D, Bultel Y, Magnin JP, Roux C, Willison JC. Hydrogen photosynthesis by *Rhodobacter capsulatus* and its coupling to a PEM fuel cell. *Journal of Power Sources* 2005; 141:19-21.
- [15] García-Peña EI, Guerrero-Barajas C, Ramirez D, Arriaga-Hurtado LG. Semi-continuous biohydrogen production as an approach to generate electricity. *Bioresource Technology* 2009; 100:6369–6377.
- [16] Melis A, Zhang L, Forestier M, Ghirardi ML, Seibert M. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiology* 2000; 117: 127–36.
- [17] Melis A. Green alga hydrogen production: progress, challenges and prospects. *International Journal of Hydrogen Energy* 2002; 27:1217–28.
- [18] Sanchez Miron A, Ceron Garcia MC, Contreras Gomez A, Garcia Camacho F, Molina Grima E, Chisti Y. Shear stress tolerance and biochemical characterisation of *Phaeodactylum tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors. *Biochemical Engineering Journal* 2003; 16:287-297.
- [19] Zhang L, Happe T and Melis A. Biochemical and morphological characterisation of sulphur deprived and H₂-producing *Chlamydomonas reinhardtii* (green alga). *Planta* 2002; 214 (4): 552 - 561.

- [20] Happe T, Mosler B, Naber JD. Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*. European journal of Biochemistry 1994; 22:769-774.
- [21] Ghirardi ML, Togasaki RK, Seibert M. Oxygen sensitivity of algal H₂-production. Applied Biochemistry Biotechnology 1997; 63:141-151.
- [22] Didierjean S, Lottin O, Maranzana G, Geneston T. PEM fuel cell voltage transient response to a thermal perturbation. Electrochimica Acta 2008; 53:7313-7320.
- [23] Shon YJ, Park GG, Yang TH, Yoon YG, Lee WY, Yim SD, Kim CS. Operating characteristics of an air-cooling PEMFC for portable applications. Journal of Power Sources 2005; 145:604-609.
- [24] Shen Q, Hou M, Yan X, Liang D, Zang Z, Hao L, Shao Z, Hou Z, Ming P, Yi B. The voltage characteristics of proton exchange membrane fuel cell (PEMFC) under steady and transient states. Journal of Power Sources 2008; 179:292-296.