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STUDY OF HYDROGEN PRODUCTION IN A SEMI-CLOSED PHOTOBIOREACTOR BY THE STRAINS OF *CHLORELLA* ISOLATED FROM THE SOIL IN THE ALGERIAN SAHARA

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RESUME

Dans cette étude, la production photosynthétique d'hydrogène par quelques souches de microalgues vertes a été évaluée. Trois souches de *Chlorella* isolées du sol aride et des foggaras du Sahara Algérien ont été utilisées (*Chlorella, Ce* Mt et PT6). En absence du soufre dans le milieu de culture, les cultures se mettent à produire de l'hydrogène gazeux. Le dispositif expérimental mis en place comprend quatre réacteurs tubulaires parallèles, dans lesquelles les algues sont incubées en suspension et qui sont constamment agités. Les concentrations d'oxygène et d'hydrogène sont mesurées dans un gazomètre à la pression atmosphérique. Dans cette communication, la relation entre le comportement physiologique, biochimique caractéristique et les taux de production de gaz a été évalué et comparé par rapport à la production d'hydrogène par *Chlamydomonas reinhardtii,* souche de référence.

Mots clés : Production photobiologique de l'hydrogen; *Chlorella*; photobioreacteur , Sahara Algerien.

ABSTRACT

In this study, the photosynthetic hydrogen production rates by some locally strains of green microalgae were evaluated. Three strains of *Chlorella* isolated from arid soil and foggaras's water in the Algerian Sahara were used. *Chlorella sorokiniana* strain Ce, *Chlorella salina* strain Mt and *Chlorella sp* strain Pt6 produce, under sulfur deprived conditions, hydrogen gas, but its rate is dependent on strain type and oxygen partial pressure in medium. The experimental set-up includes four parallel tubular reactors, in which algal suspensions are continuously stirred. Oxygen and hydrogen concentrations are monitored continuously and the volume of evolved gas is measured in a gasometer at atmospheric pressure. In this communication, the relationship between physiological behaviour, biochemical characteristic and rates gas production was reported and compared with hydrogen production by *Chlamydomonas reinhardtii*.

Keywords: Photobiological hydrogen production; *Chlorella*; photobioreactor; Algerian Sahara.

1. INTRODUCTION

The virtues of hydrogen as vector of an electric and thermal power or as a fuel in transport with no emission of greenhouse gas are sufficiently describe. More than 95% of hydrogen currently used, is produced primarily starting from fossils fuels (oil and natural gas) by heavy, expensive processes (reforming, vaporeforming …) and especially very harmful with the environment. These last years scientists a paramount interest with the development of new technologies of hydrogen production starting from renewable energies, among which, the biological way of production which constitutes a privileged axis.

Hydrogen can be produced by photobiological water splitting, based on the high light conversion efficiency of these photosynthetic micro-organisms [1, 2 and 3]. Photo-oxidation of water (water splitting) by the photosynthetic organelles of green microalgae elevates the potential energy of the resulting electrons by passing them through the electron-transport chain in the thylakoid membrane to ferredoxin, and release H2 via Fe-hydrogenase pathway according to the reactions:

$$2H_2O \xrightarrow{\text{Water split}} 4H^+ + 4e^- + O_2 \qquad (1)$$

$$2H^+ + 2e^- \xrightarrow{\text{Hydrogenase}} H_2 \qquad (2)$$

The research tasks referring to this phenomenon are in perpetual progression to understand, determine and control all the intervening parameters with knowing; the nature of the species used the reactional enclosure and biotic factors (nutrients, temperature, pH, agitation…).

The photobioreactor designed for the hydrogen production by *Chlamydomonas sp* is toric geometry which promises according to the hydrodynamic study carried out in the agro alimentary field, a satisfying mixture and a rigorous control of the various physical parameters within the engine [4].

The present study was initiated to examine the potentiality of some green algae isolated from arid soil in Algeria to produce hydrogen gas by splitting water and assess their degree of sensitivity to oxygen compared with known strains (e.g Chlamydomonas reinhardtii). The experiments were done to establish the relationship between the growth of microalgae and hydrogen production in batch cultures within photobioreactors. Measurements of starch and protein mobilisation in concomitant hydrogen production were also reported and compared with recent data.

2. MATERIALS AND METHODS

2.1. microalgae Strain :

Wild type strains Chlorella were used; they were isolated from soil and foggaras's water of Touat located in Adrar in the Algerian Sahara on March 2004. The strains were identified as *Chlorella sorokiniana* strain Ce, *Chlorella salina* strain Mt and *Chlorella sp* strain Pt6.

2.2. Photobioreactor and culture medium:

The experimental set-up includes four parallel tubular reactors, in which algal suspensions (640 ml per reactor) are continuously stirred by a moving glass ball. For this purpose, the reactors are fixed on an oscillating table. Oxygen and hydrogen concentrations are monitored continuously and the volume of evolved gas is measured in a gasometer at atmospheric pressure. Oxygen and

hydrogen concentrations are measured in the gas phase by a single polarographic probe: the probe is operated at a voltage, which is sequentially inverted by means of an electronic switch (+0.7 V for H_2, -0.7 V for O_2). The follow-up of the culture was ensured by a series of taking away and measurements carried out through pipes envisaged to this end (Fig.1).

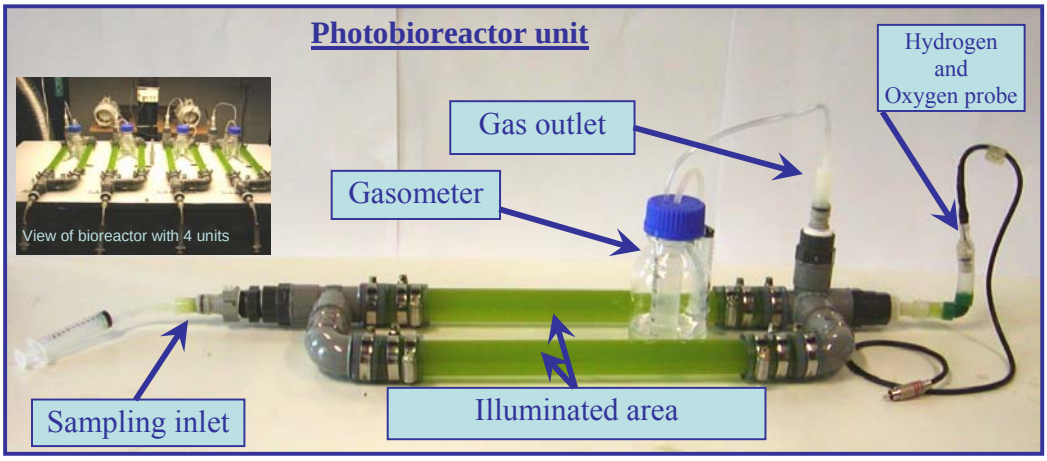

Figure 1. Schematic diagram of the photo hydrogen production

The algae strains were isolated on BG-11 medium, a specific environment for microalgae based on minerals, consisting of (g/l): ($NaNO_3$ 1.5, $K_2HPO_4.3H_2O$ 0.04, $MgSO_4.7H2O$ 0.75, $CaCl_2$. $2H_2O$ 0,036, Citric acid 0.006, ferric ammonium citrate 0.006, EDTA 0.001, Na_2CO_3 and Trace metal A5 0.02 ml/l) at pH 7.2 at 30°C. Then, they were grown photoheterotrophically in a Tris-Acetate-Phosphate (TAP) medium by stirring and under continuous cool white fluorescence lamps (≈120 photons m^{-2} s^{-1}). Cells grown as above were harvested by centrifugation, washed three times in sulfur-free TAP medium (TAP-S) and resuspended in the same medium and under the same incubation conditions of cell growth, at an optical density of 5.10^6cells ml^{-1}. For sulfur deprivation, sulfate salts were substituted with an equivalent amount of chloride salts in sulfur-free medium.

2.3. Total carbohydrates:

The method used to determine the rate of the carbohydrates is inspired of that of the sulphuric acid reaction + anthrone adapted to the algal biomass [5]. 100 Mg of biomass was added to 8 ml of perchloric acid. The mixture is strongly agitated and left for hydrolysis during 12 h. 5 ml of the reagent to the coldly prepared anthrone are added to 1 ml of the filtrate previously obtained then heated with 100°c during 12 minutes. A green colour develops when the complex glucose – anthrone was formed, to which one determines the optical density with 630 nm after cooling of the mixture. A calibration curve is carried out using D + glucose dissolved in distilled water.

2.4. Hydrogen and oxygen analysis

Hydrogen and oxygen production were measured using a chromatograph (GC Trace, Thermo Finnigan) equipped with two columns (Carbowax and Molecular seive 5A) using a Thermal Conductivity Detector (TCD). To separate H_2, O_2, CO_2, and N_2, argon was used as the carrier gas under 5 bars of pressure (23.5 ml/min) and at oven temperature of 60°C. The temperature of TCD was set at 210°C for the detector and 30°C for the injector. The total H_2 and O_2 volumes

produced were calculated on the basis of their concentration in percent (%) measured by chromatography.

3. RESULTS AND DISCUSSIONS

3.1. Produced gases (O_2, H_2 and CO_2)

The oxygen rate dissolved in the medium increased considerably during the first hours, it was the photosynthesis period (O_2 release), then decreases slightly during breathing (absorption of O_2). At the same moment, a gas production was noted by a displacement of the liquid level inside the tube emerged in the system of gases trapping. That could be allotted to the release of the hydrogen produced by the microalgae in this period of anaerobia (Fig.2 and 3).

Indeed, under the favourable conditions of culture and light, the cells manufacture their organic matter and achieve their life cycle. After exhaustion of the culture medium, the cells always exposed to the light, continue to photolysis the molecule of water, but instead of manufacturing its organic matter, it produces hydrogen gas. Recent work [6, 7 and 8] brings back similar observations.

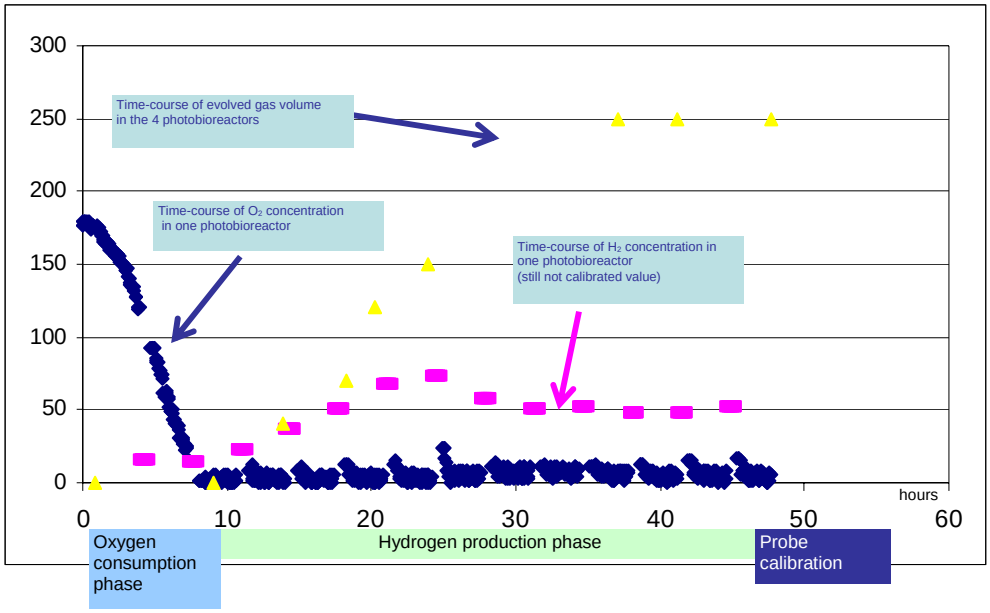

Figure 2. Time-course of a typical experiment using wild-type cells of *Chlorella.*

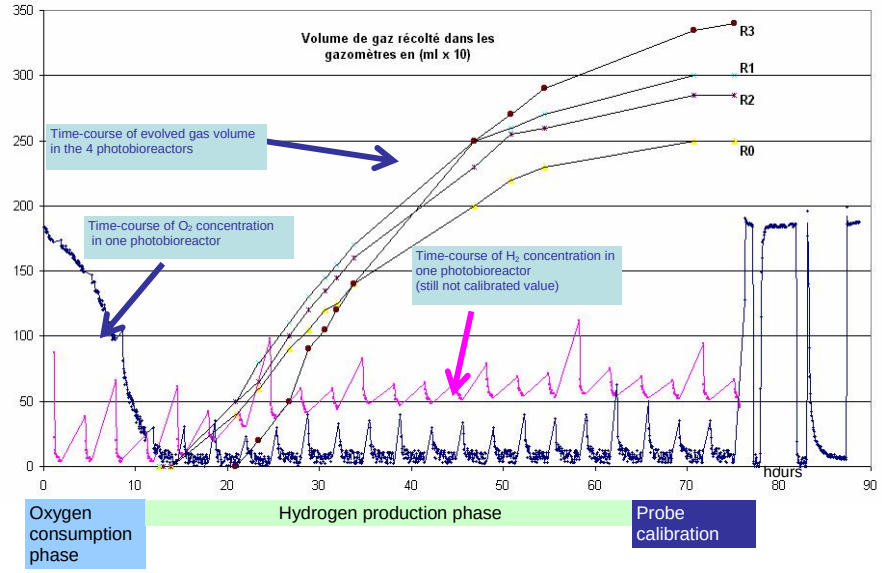

Figure 3. Time-course of a typical experiment using wild-type cells of *Chlamydomonas reinhardtii*

The green alga, *Chlamydomonas reinhardtii,* is capable of sustained H_2 photoproduction when grown under sulfur-deprived conditions. This effect is a result of the partial deactivation of photosynthetic O_2-evolution activity in response to sulfur deprivation. At these reduced rates of water-oxidation, oxidative respiration under continuous illumination can establish an anaerobic environment in the culture. After 10-15 hours of anaerobiosis, sulfur-deprived algal cells induce a reversible hydrogenase and start to evolve H_2 gas in the light.

3.2. Biochemical analysis

Considering the quality of the taken samples, the biochemical analysis, resulted in microscopic observations. The signs of a satisfactory growth in term of biomass, chlorophylls and viability are definitely apparent. Indeed, dice the first days. One attends regular divisions and a cellular viability reaching the 100%, in the same way for the production of the pigments and the accumulation of the reserves.

4. CONCLUSION

The study presented made it possible to characterize, initially, the physiological behaviour of the microalgal cells within the toric photobioreactor. This last, in spite of the conclusive hydrodynamic study in term of mixture and the facility of the physical factors control, shows adjustment for the regular follow-up of biometric measurements. A progressive evolution of the algal biomass as well as cellular viability made it possible to account for the effectiveness of the engine as an enclosure of culture.

In addition, the hydrogen production by *Cholorella* locally isolated is similar to the production in *Chlamydomonas reinhardtii.* The displacement of the water level inside the emerged tube would be with the hydrogen produced at the time of the anaerobia period of the medium (reduction in the dissolved oxygen rate) which has occurred 03 days after the active period of photosynthesis and the building up of reserves. Beyond this period, the cells always subjected to the continuous

light photolysis the water molecule, but instead of manufacturing its organic matter, they produce hydrogen gas, since the nutritive elements essential was decreased, the preferential way of the protons becomes the hydrogenase.

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