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Hydrogen generation in a pressurized photobioreactor: unexpected enhancement of biohydrogen production by the phototrophic bacterium *Rhodobacter capsulatus*

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ABSTRACT: Environmental concerns are generating a growing interest of the hydrogen sources, however hydrogen exhibits critical storage barriers. The production of pressurized biohydrogen would facilitate the gas storage and make it economically viable. In this work, *Rhodobacter capsulatus* in a closed photosynthetic reactor exhibited the ability to produce hydrogen to a pressure of 8.25 bars. The amount of hydrogen produced from synthetic media (lactate (35 mmol L⁻¹) and glutamate (5 mmol L⁻¹)) in a closed vessel was 1.8 times that obtained in a vessel open to the atmosphere. Hydrogen purity surpassed 90 % with a lactate conversion rate of up to 70 %. Influences of buffer composition in synthetic media and the illumination process (white LED or Na-lamp) are discussed. Moreover, pressurized hydrogen was

successfully produced from a complex real effluent containing organic acids (lactate and acetate) generated by an initial dark biofermentation of hydrolyzed wheat straw. Therefore, under pressurized conditions, the stress increases the energetic demand and improves hydrogen production (survival vs growth). The energetic gain of the direct compression of biohydrogen is equal to 1.3 kWh / kg H₂.

Keywords: Hydrogen yield, pressurized photobioreactor, photo-fermentation, *Rhodobacter capsulatus*, stress

1. INTRODUCTION

The three major resource issues facing the world today are shortages of water, food, and energy. Human and economic growth have led to an increase in waste production and energy consumption which consequently led to climate change [1]. Hydrogen fuel and renewable hydrogen technology can substantially improve air quality and reduce climate change [2]. According to Ahmed F. Ghoniem [3] these clean technologies are not deployed at sufficiently large scale. Nowadays, most hydrogen produced has a fossil origin, therefore sustainable production of hydrogen that mankind strives to develop such as water photoelectrolysis is a well-studied option [4], but this process generally leads to low energy efficiency and/or low output power. The water photovoltaic-electrolysis also appears as a viable strategy [5], mainly the water electrolysis based on the PEM water electrolyzers (PEMWEs) well-matches with high energy efficiency with intermittent operation. The electrochemical reaction of water splitting requires a minimum applied voltage to decompose water, it is the standard reversible cell potential. Moreover, the water electrolysis process introduces several barriers, which are the reaction

kinetics and the transport of species such as ions and electrons. To decrease the magnitude of resistances due to electrochemical kinetics, it is necessary to develop new catalysts materials. These materials (Platinum-group metals) are scarce in the earth crust and have a high-cost: these features being clear hurdles to mass commercialization [6]. Nonetheless, alkaline water electrolyzers (AWE) use a long-lifetime technology based on non-PGM catalysts (e.g. Ni) [7], which conveys an indubitable economical interest, but conventional AWE cells operate at low current density and moderate energetic efficiency [8] and it is difficult to produce hydrogen with intermittent energy sources. Novel approach consists on the magnetic boost of electrochemical kinetics [9], nonetheless more work has to be done to make electrochemical magnetic heating industrially practical [10]. However, other methods such as the supercritical water gasification of biomass remains a cost effective process [11] with low environmental impact.

The conversion of wastewater to available energy, such as electricity or hydrogen, using bioprocessing has stimulated a keen interest as a strategy for sustainable development [312]. According to the work of McCarty [13], domestic wastewater exhibits great energy potential. This analysis could also be applied to agro-industrial wastewater or agro-waste. M. Yáñez *et al.* [14] have highlighted that the supplementary hydrogen sources can contribute to the penetration of renewable energies. Photobiohydrogen exhibits a positive global warming potential, low acidification potential, relevant social cost of carbon and a low potential production cost [15]. In the review of Da Silva Veras *et al.* [16], the technological routes for hydrogen production was described in the current energy landscape, then biomass processes appear as promising technologies. Furthermore, biohydrogen production operates under moderate conditions of temperature and pressure which minimizes the capital expense [17]

Then again, potentially energy saving and environmentally harmless processes are represented by biological methods. Pandey and Srivastava [18] have shown the current prospects and challenges in fermentative hydrogen production, biohydrogen production resulted of both dark and light fermentative processes. According to Ghimire et al. [19], dark and light fermentative biohydrogen production from food waste presents a relevant synergy of waste treatment and energy recovery. However, various toxic or inhibitory compounds can significantly limit dark fermentation routes [20] and also photobiohydrogen shows low efficiency. Moreover, biohydrogen routes requires further research studies to improve their production rates [17].

The photoconversion techniques are driven by intermittent resources (*i.e.*, daylight), and the produced biogas must be stored for later use. In this context, biohydrogen can be stored (compressed gas tank), and, at the request of the consumer, hydrogen can be converted into electrical energy via fuel cells. Consequently, photo-biohydrogen development must surpass current engineering limitations.

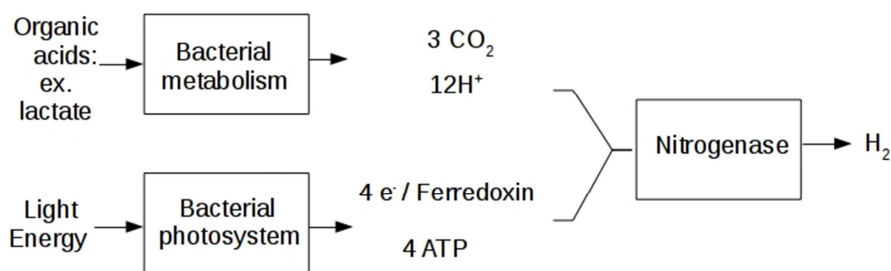
First, storing hydrogen requires a compressor. Compression of hydrogen from 1.02 bars to 8.0 bars consumes 1.37 kWh / kg H₂ [21]. DOE analysis shows that the cost of hydrogen distribution remains a major constraint, and the compression represents 30 % of the distribution cost. Mechanical gas compression has numerous disadvantages, such as energy costs and durability [22]. Therefore, G. Voitic *et al.* [23] have pointed out that the increase of hydrogen pressure in the production processes significantly improves the efficiency of the hydrogen economy.

Second, in a typical hydrogen production plant, after compression, residual oxygen is removed from the hydrogen gas by the deoxidizer unit, and the hydrogen gas is then dried [24], then the deoxidizer unit consumes a portion of the produced hydrogen [25]. According to P. Haug *et al.*

[26] another strategies consist on dynamic electrolyte cycling approach where the electrolyte was mixed and partly separated continuously, which makes the design of hydrogen plants more complex.

Third, the design of a photobiological reactor is a difficult endeavor. The productivity of the photobioreactor is light-limited, and the height-to-volume ratio is a relevant factor [27]. Irrespective of the biological and chemical systems at stake, numerous research efforts to achieve maximum productivity with minimum operating costs have been available in the literature [28].

Increased bioreactor efficiency requires accurate control of the system, especially for opened systems (continuous production). It is well known, in chemical reactor design that irreversible reactions make the modeling, control and design of reactors easier [29]. In summary, an ideal photobioreactor for photobiohydrogen should operate continuously without oxygen trace and in pressurized vessels to avoid gas compressors, and the chemical route should be an irreversible addition. Moreover, because photobioreactors are light-limited, the geometry of the design is critical. During photoconversion, electrons and protons are delivered from photosynthesis. The nitrogenase route is possible for H₂ production because it has the advantage of catalyzing the unidirectional production of H₂, although the nitrogenase route has a theoretical maximum energy conversion efficiency lower than that of hydrogenase [30]. Photo-decomposition of organic compounds by photosynthetic bacteria is a promising microbial system for the bioproduction of hydrogen [31]. The major benefits are as follows: i) high theoretical conversion yields, ii) lack of O₂-evolving activity, iii) the ability to use a wide spectrum of light and iv) the ability to consume organic substrates derivable from wastes. The overall biochemical pathways for the photofermentation process of organic acids are depicted in Scheme 1.



Scheme 1. Main pathways of hydrogen production by photofermentation of organic acids by using photosynthetic bacteria.

The pathway used by nitrogenase of photosynthetic bacteria requires the largest numbers of photons, resulting in lower efficiency in comparison with other pathways, but energetic efficiency must be scrutinized in the whole system. The technical specification of “energy” hydrogen (fuel cell) involves hydrogen purity up to 90 %, oxygen-free conditions, and an operating pressure of up to 10 bars to make bio hydrogen economically practical.

In the present work, a hydrogen production process was achieved via anaerobic photosynthesis using the purple non-sulfur bacterium *Rhodobacter capsulatus*. The bacterial culture was carried out in a photobioreactor operated in a closed vessel or in an open vessel; lactate was used as a carbon source, and LED illumination was provided. In the closed vessel, the gas pressure increased with bacterial growth, and, surprisingly, pressurized hydrogen production was markedly increased (80 %) compared to atmospheric hydrogen production. In addition, hydrogen production from a complex effluent derived from the bio-treatment of hydrolyzed wheat straw has been successfully achieved.

2. MATERIALS AND METHODS

2.1. Bacterial strain and growth medium

The bacterial strain was the photosynthetic bacterium *Rhodobacter capsulatus*, wild-type strain B10 [32]. B10 strain was provided by Dr. JC Willison (CEA-DRF-BIGCBM, France). *Rhodobacter sphaeroides* DSM5864 was purchased from DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures). Hydrogen-producing growth was carried out under photosynthetic anaerobic conditions (30,000 lx) at 30°C and nitrogen-limited conditions in two synthetic media and one complex medium. The synthetic media contained Na lactate (35 mmol L⁻¹) as the carbon source and Na glutamate (5 mmol L⁻¹) as the nitrogen source. Two lactate-glutamate-based (LG) media were studied, with different types of buffers: phosphate buffer and borax buffer (Kolthoff buffer) [33], designated LGK and LGB, respectively [34-35]. A real effluent, resulting from a thermophilic bioprocess, was used as the complex medium. It originated from the bioconversion of a wheat bran hydrolyzed solution by the thermophilic bacterium *Thermotoga maritima* under dark fermentative conditions. The culture medium was centrifuged to remove biomass (MIO, Marseille, France). The supernatant, containing organic acids, was diluted four-fold with distilled water and completed with buffer before adding *R. capsulatus*. The lactate and acetate concentrations were 9 and 23 mmol L⁻¹, respectively. In addition, a second strain culture of the photosynthetic bacterium (*Rhodobacter sphaeroides*, DSM5864 (O.U. 001 [36]) was reported in supplementary materials.

2.2. Inert laboratory pressurized vessel system

The Miniclave reactor was used as a pressurized bioreactor (Büchiglasuster®). It consisted of a 350-ml cylindrical glass vessel with a 10-bar pressure limit. The glass vessel was shielded with a steel mesh, and a cover plate was fixed on top. The cover plate had four available holdings: one

bursting disc, one manometer, one in/outlet gas valve, and one in/outlet liquid valve. The bioreactor was autoclaved before use (120°C, 20 min) and then filled with 0.3 L of growth medium.

The bacterial culture was stirred using a magnetic stirrer. The volume of the free space above the liquid was 55 ml, and the air was replaced with sterile argon gas over the course of five minutes. The bioreactor was inoculated with 20 ml of B10 anaerobic preculture. Two experimental conditions were tested based on whether or not the gas produced was exhausted: a closed vessel (gas valve closed) and an open vessel (gas valve opened). In both cases, exhaust gas was led towards a gas measurement system: continuously for the open-vessel condition and at the end of batch culture for the closed-vessel condition. A strip of LEDs (60 LED/m, type 3014, IP 65) was fixed on a removable cylindrical steel mesh support. This support surrounded the steel mesh shield of the glass vessel at a distance of 5 cm. Total illumination was 30,000 lx at the surface of the steel mesh shield. The photosynthetic bacterial culture was carried out in a lab-made dark climatic enclosure. The light spectra of LED and Na lamps (Plantastar, 150 W, Osram) were measured by a spectrophotometer (USB2000, Ocean Optics, USA) coupled with a cosine receptor (3,900 µm). Light energy was measured via the Parspec module integrated into SpectraSuite software (32-bit version: 2009).

2.3. Analytical methods

Bacterial growth was monitored using optical density (OD) measurements thanks to a double-beam molecular adsorption spectrophotometer (Shimadzu UV-2501PC, software Jasco V-530). The pH of the media was measured with a pH meter (Crison GPL21), fitted with an Electrode Inlab Micro pH (Mettler Toledo), allowing pH to be measured in a small volume (0.2 ml). Lactate and acetate concentrations were quantified in an HPLC apparatus (Agilent Technologies,

1260 Infinity, Refractive Index detector) using an analysis column (Repromer H 9 μm , 250 x 8 mm, Ref RM9H0S2508) and a pre-guard column (Repromer H 9 μm , 20 x 8 mm, Ref RM9H0S0208). The eluent was constituted by a sulfuric acid solution (10 mmol L⁻¹). The analyses were carried out at 60°C with an eluent flow of 0.4 ml min⁻¹ for the filtered and diluted bacterial medium. A sample of bacterial culture was first filtered (nitrocellulose filter, 0.45 μm) and then diluted 10-fold with distilled water before HPLC analysis. Gas pressure (mainly H₂ and CO₂ species) inside the biological reactor was directly monitored. Gas production during open-vessel growth conditions was measured by an inverted-probe-based system and it was established that an experimental error in the range 8-15% [35]. Gas evolution was automatically monitored by a computer. Under the closed-vessel growth conditions, a similar procedure was employed only at the end of growth (off-line measure). CO₂ concentration in the biological gas was measured off-line by an infrared sensor (C20 miniature sensor, range 0-20 %, Euro-gas) at the end of the cultures.

RESULTS AND DISCUSSION

2.4. Feasibility of pressurized hydrogen bioproduction

Gas pressure increased in the closed vessel under LED illumination during the bacterial growth of *R. capsulatus* (Fig. 1A). Indeed, gas production resulting from biological pathways (Scheme 1), was gathered in the top of the vessel when it was closed (closed-vessel condition), and the pressure was raised. Surprisingly, the growth of *R. capsulatus* was not hampered by these conditions (Fig. 1).

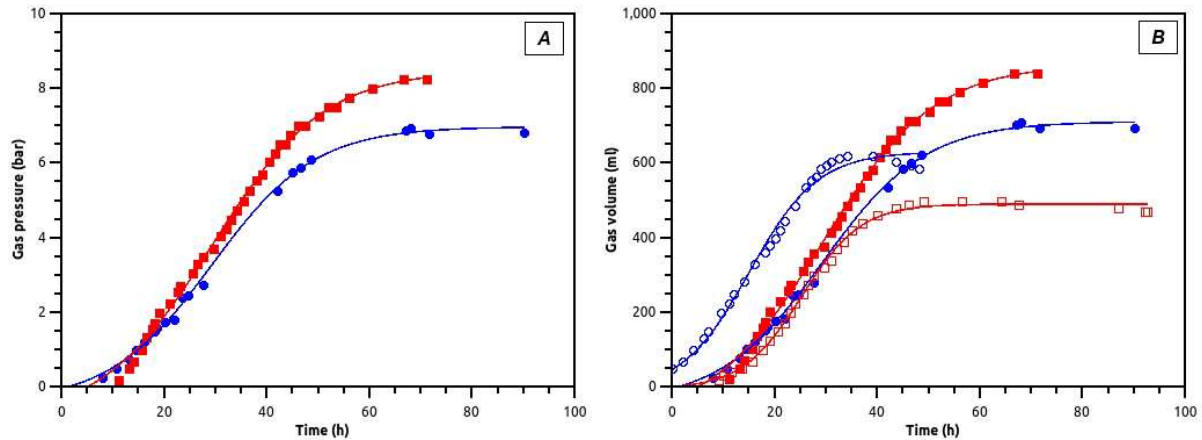


Figure 1. Evolution of gas pressure (A) and equivalent gas volume (B) during photosynthetic growth of *Rhodospirillum rubrum* strain B10 in a closed vessel (filled symbols) and an open vessel (open symbols) with the synthetic media LGK (rectangles) and LGB (circles).

Closing pressures reached 8.25 and 6.81 bars with LGK and LGB media, respectively. The gas was mainly hydrogen with a small amount of CO₂, between 2 and 7 % v:v, with LGK and LGB media, respectively. These closing pressures of 8.25 and 6.81 bars corresponded to gas volumes of 841 and 700 ml. The lactate conversion rates were 69.6 and 69 % for LGK and LGB media, respectively. H₂ production under open-vessel conditions (Fig. 1B) was also recorded. Surprisingly, this well-studied hydrogen production (atmospheric conditions) remained lower in terms of hydrogen production than in closed-vessel (pressurized) conditions, regardless of the studied medium: LGK or LGB. In opened atmospheric conditions (open-vessel), volumes of 470 ml (LGK) and 583 ml (LGB) were recorded for total gas produced. According to Castillo et al., borax buffer is a component of LGB medium that leads to better H₂ production. This effect was confirmed in open vessels under LED illumination (Fig. 1B). We observed enhancements of hydrogen production of 79 % in LGK medium and 20 % in LGB medium in closed-vessel

reactors versus open vessels. Therefore, LGK medium exhibits a higher hydrogen production than LGB medium under pressurized conditions. Hence, H₂ production resulted from bacterial growth, as observed in Fig. 2.

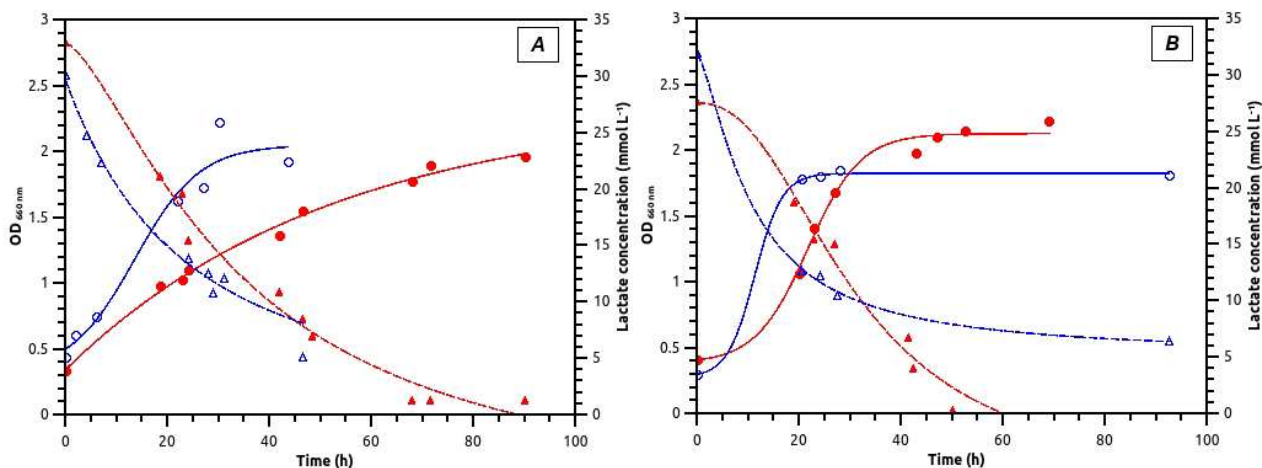


Figure 2. Kinetics of biomass production (circle) and lactate (triangle) consumption during photosynthetic growth of *Rhodobacter capsulatus* strain B10 in closed vessel (dark symbol) or in open vessel (open symbol) with the synthetic media LGK (A) and LGB (B).

The final OD values of the culture media were similar, within the range of 1.8-2.2, regardless of the experimental conditions: closed or open vessel. However, bacterial growth was faster in the open vessel than in the closed vessel. The time necessary for OD stabilization is approximately 20-30 h for LGK medium and approximately 50-70 h for LGB. The final pH values of LGK medium were similar in open and closed vessel conditions (pH: 7.06-7.09). In contrast, in LGB medium, the final pH increased from 7.16 (open vessel) to 8.25 (closed vessel). For both media, the initial pH was close to 6.8-6.9. This pH increase resulted from the lactate metabolism of *R. capsulatus* and, more particularly, from the formation of carbonate (i.e. dissolved CO₂) from the oxidation of lactate as described in Scheme 1.

Figure 2 shows that lactate was completely metabolized during H₂ production and bacterial growth in closed-vessel conditions. In contrast, approximately 5 % of the initial lactate was found in the final culture in the open-vessel condition. The conversion rates of lactate in hydrogen are unlike those in both experimental conditions. Accordingly, lactate conversion yields are equal to 43.3 and 56.3 % in the open vessels, and these yields increase to 69.6 and 69 % in the closed vessel. Lactate is degraded more rapidly in open vessels than in closed vessels, regardless of the medium composition: 0.809 mmol h⁻¹ (open vessel/LGK) – 0.558 mmol h⁻¹ (closed vessel/LGB) compared to 0.486 mmol h⁻¹ (open vessel/LGB) and 0.535 mmol h⁻¹ (closed vessel/LGB). The differences in lactate used were correlated with bacterial growth: bacterial growth under atmospheric conditions is faster than bacterial growth in closed vessels.

Similar experiments have been achieved with another strain (*Rhodobacter sphaeroides* DSM5864), in these experiments lactate to H₂ conversion rate did not increase with increasing hydrogen pressure. The enhancement of hydrogen conversion was only observed with the strain *R. capsulatus* (Table S1 and Figure S1).

2.5. Application of real effluents containing organic acid

We have successfully carried out biohydrogen production in synthetic medium with lactate as the carbon source and glutamate as the nitrogen source under pressurized conditions. Next, a complex effluent, supplied from the bio-treatment of hydrolyzed wheat straw, was used as the carbon source for photosynthetic bacterial growth in both vessel conditions (Fig. 3).

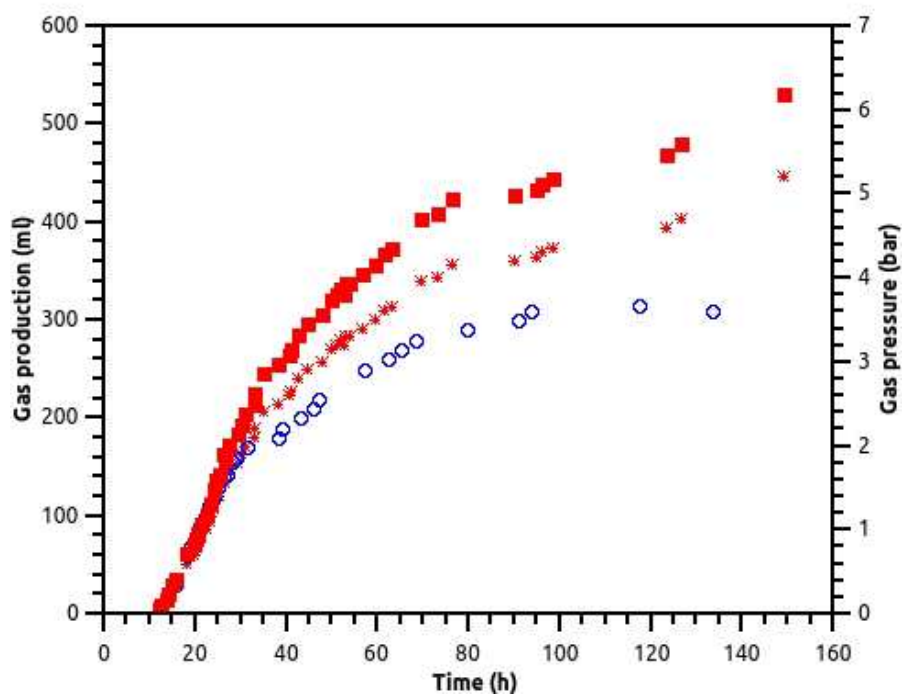


Figure 3. Evolution of gas production expressed as pressure (asterisks) and volume (dark rectangles and open circles) during photosynthetic growth of *Rhodobacter capsulatus* strain B10: in a closed vessel (dark rectangle) and in an open vessel (circle) with complex medium proceed from dark biofermentation processing of hydrolyzed wheat straw.

In the closed-vessel condition, a closing gas pressure equal to 5.2 bars was observed, corresponding to a 530-ml volume of hydrogen with only 3.3 % CO₂. This total volume was greater than that obtained in the open-vessel condition: 315 ml of hydrogen with CO₂ at 5.2 % v:v. In this complex medium, as well as in the synthetic medium, the hydrogen bioproduction resulted from bacterial growth. Figure 4 shows, as expected, increasing optical density correlating to biomass concentration and decreasing concentrations of lactate and acetate in the medium.

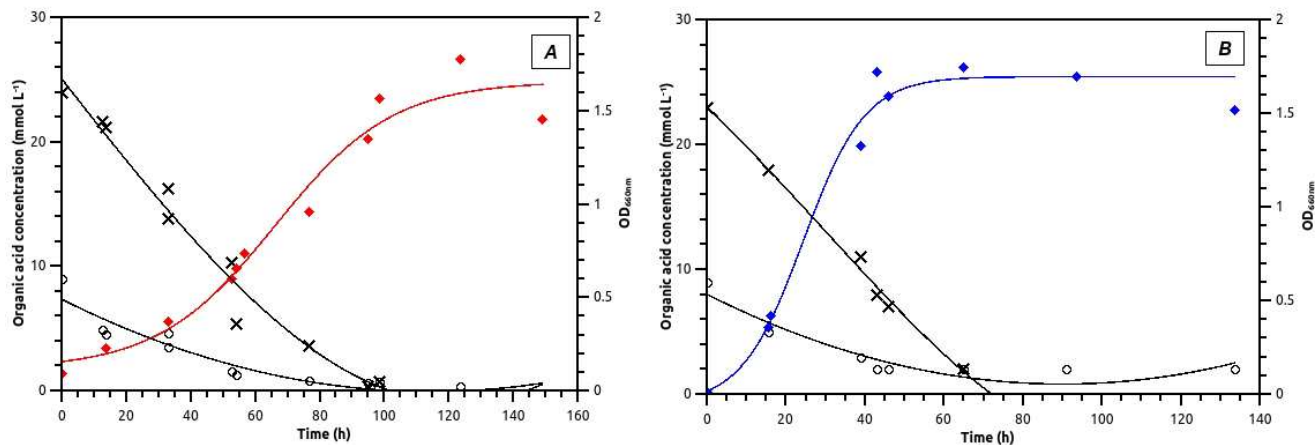


Figure 4. Kinetics of bacterial production (diamonds) and lactate (circles) and acetate (crosses) consumption during photosynthetic growth of *Rhodospirillum rubrum* strain B10 in a closed vessel (A) or an open vessel (B) with complex medium derived from dark biofermentation of hydrolyzed wheat straw.

Lactate and acetate were completely consumed by *R. capsulatus* under either vessel condition. However, the degradation kinetics of organic acids differed according to the type of reactor. Lactate was degraded more quickly during closed culture of *R. capsulatus* than in open culture: $0.327 \text{ mmol lactate L}^{-1} \text{ h}^{-1}$ (closed vessel) and $0.148 \text{ mmol lactate L}^{-1} \text{ h}^{-1}$ (open vessel) (Fig. 4A). In contrast, acetate was consumed at comparable rates: $0.291 \text{ mmol acetate L}^{-1} \text{ h}^{-1}$ and $0.330 \text{ mmol acetate L}^{-1} \text{ h}^{-1}$ in the closed and open vessels, respectively (Fig. 4B). Bacterial growth in open vessels was faster than in closed vessels, as previously observed in synthetic media (LGK and LGB). The time until OD stabilization was equal to 60 h (open vessel) and 140 h (closed vessel). The bioconversion of organic acids in hydrogen was approximately 69.3 % for the

closed vessel and 41.2 % for the open vessel. Thus, hydrogen production was enhanced by approximately 68 % in the closed vessel compared to the open vessel.

2.6. Illumination technique effects

Artifacts could be imagined, such as the effects of LED illumination, but LED illumination is similar between closed and open vessels. Of course, H₂ bioproduction on lactate as the carbon source with *R. capsulatus* is dependent on energy, specifically on light energy. The effects of illumination were simply investigated using three light sources: two based on LEDs and the other on a Na lamp. The light sources were tested in pressurized conditions (closed vessel). Figure 5 shows that H₂ pressure and the correlated H₂ production were greater under white LED illumination than under Na-lamp illumination: 8.25 bars (841 ml, 2 % CO₂) with the LED and 4.25 bars (433 ml, 6 % CO₂) with the Na lamp. This difference was mainly due to the lighting.

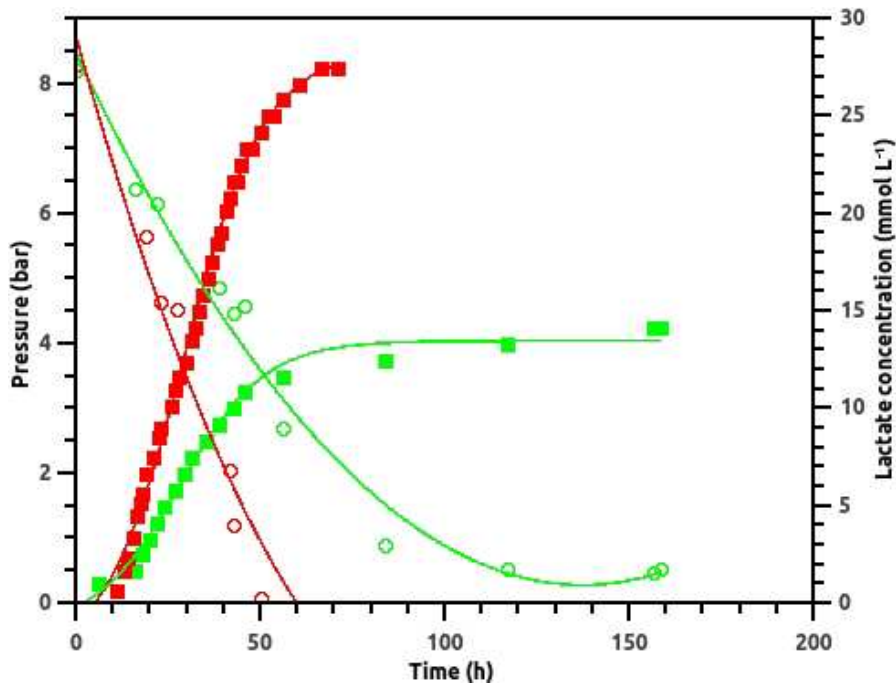


Figure 5. Kinetics of hydrogen production (squares) and lactate consumption (circles) during photosynthetic growth on LGK medium (lactate 35 mmol L⁻¹, glutamate 5 mmol L⁻¹) of

Rhodobacter capsulatus strain B10 in a closed-vessel. Comparison of illumination: white LED (red symbols) versus Na-Lamp (green symbols).

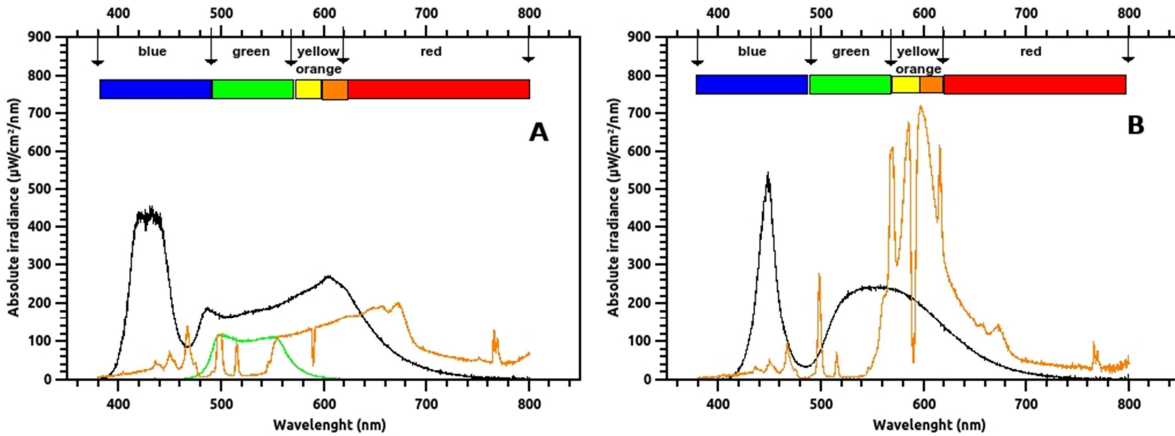


Figure 6. Light spectra of white LEDs (dark line), green LEDs (green line), and Na-lamp (discontinuous orange line) at 20,000 lx (A) and at 30,000 lx (B).

It is well known that photosynthetically active radiation (PAR) is a more representative characterization parameter of incident light. PAR represents the amount of light available for photosynthesis, a quantum process, expressed in $\mu\text{mol gamma m}^{-2} \text{s}^{-1}$. Consequently, the quantity of biochemical energy (ATP) produced by the chemical reactions of photosynthesis was more important. PAR measurements were 2,152 and 2,723 $\mu\text{mol gamma m}^{-2} \text{s}^{-1}$, respectively, for LEDs and Na-Lamp. Figure 6 shows spectrophotoradiograms of lighting systems. Light spectra of illumination sources differed according to the nature of the lamp, either Na lamp or LEDs.

The white LED spectrum (Fig. 6) was characterized by two peaks found in blue (25.4 % of total spectra) and a large peak from green-green (37.27 %), (orange (13.72 %), yellow color (8.19%) to the red area (16.6 %). The Na-lamp condition showed a more complex spectrum: orange to red colors were predominant, with 40.2 and 34.58 % of total spectra, respectively.

Although the illumination (30,000 lx) was similar with LED and Na-lamp, the incident energy issued from white LEDs (646 W m^{-2}) was 1.18 times more important than Na-Lamp (547 W m^{-2}), ratio similar at this observed at 20,000 lx. In addition green LEDs (only at 20,000 lx) mostly emitted in green color (80.2%) and minority in blue (12.8 %) and yellow-orange-red area (7 %).

LED lighting was well distributed thanks to the strip conformation, whereas the Na lamp provided directional irradiation. Therefore, bacterial metabolism was faster under LED lighting, as observed by the complete lactate consumption in a 50-h period. In contrast, under Na-lamp illumination, lactate metabolism required more than 150 h. Although the LEDs and Na lamp provided similar illumination (30,000 lx), the incident energy issued from the Na lamp (812 W m^{-2}) was 1.7 times greater than that from the LEDs (479 W m^{-2}).

Gas production is directly correlated with the light energy (W/m^2). As expected, lactate to H_2 conversion rate by *R. capsulatus* B10 increase with light energy until photo inhibition (Fig.7 and additional measurements with varying luminosity on figure S2). The influence of illumination or light energy on H_2 production by photosynthetic bacteria was previously described [35]. Obeid and al. [35] described an inhibitory effect on H_2 production by *R. capsulatus* B10 when luminosity was higher than 30000 lx with a stabilization in H_2 production in open-vessel with Na-lamp illumination. Figure 7 shows a sharp decrease in lactate to H_2 conversion rate, from 82

% to 48 %, with energy light superior to 646 W m^{-2} (30,000 lx). High light intensity causes a decrease in the amount of photosynthetic complexes in phototrophically grown *Rhodobacter* cultures [37]. Therefore, the unexpected enhancement of hydrogen production depends only on bacterial strain behaviors with pressure, light exposure affect the usual performances of biological systems. However, it should be noted that green spectrum exhibits higher efficiency than white spectrum and in both cases closed vessel experiments show higher conversion rates. Lactate to H_2 conversion rate as high as 49 % in closed-vessel was obtained with a light energy of 100 W m^2 (figure S3). This value is 2.45 time greater that the graph-extrapolated value of 20 % for white LEDs with the same light energy.

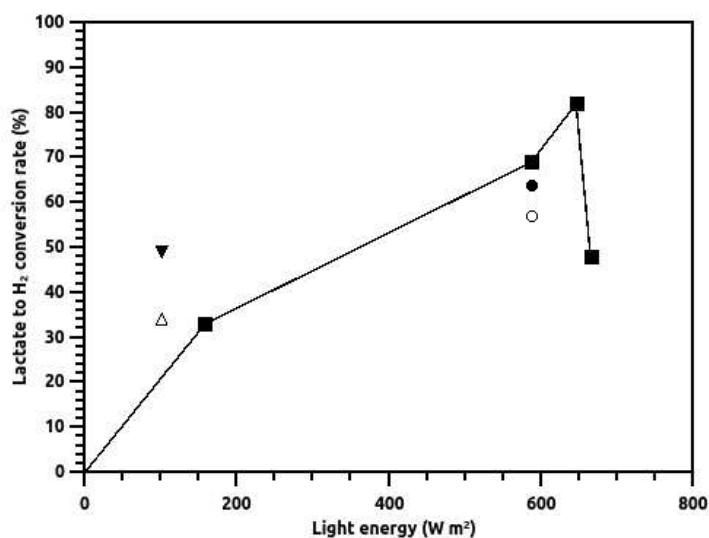


Figure 7 Influence of light energy (W m^{-2} , dark rectangle) on lactate to H_2 conversion rate by *R. capsulatus* B10 in a closed vessel (LGK Medium, lactate 20mM, glutamate 5 mM). Supplementary points were correlated to white LED (circle, 20,000 lx, LGK Medium, lactate 35mM, glutamate 5 mM) and green LED (triangle, 20,000 lx) in open vessel (clear symbol) and closed vessel (dark symbol).

2.7. Substrate limitations

Another possible artifact is the non-reliable yield of produced H_2 / lactate and substrate concentration. A pressure as high as 10 bars was attained with a hydrogen purity greater than 95.3 % v:v (carbon dioxide equal to 4.7 % v:v) with an initial lactate concentration of 57 mmol L^{-1} and an initial glutamate concentration of 5 mmol L^{-1} (Fig. 8).

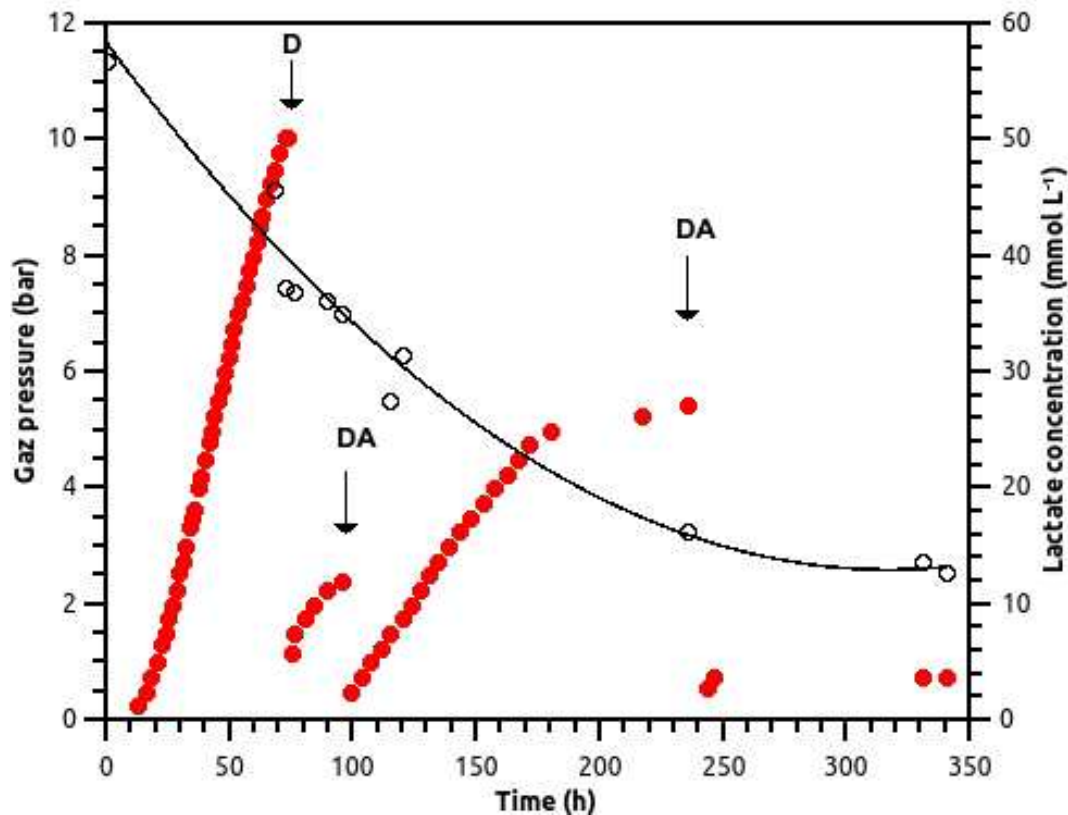


Figure 8. Kinetics of gas ($H_2 + CO_2$) production (closed circles) and lactate consumption (open circles) during photosynthetic growth of *Rhodobacter capsulatus* strain B10 in pressurized reactors with synthetic medium LGB containing 55 mmol L^{-1} lactate and 5 mmol L^{-1} glutamate.

(D) depressurization step, (DA) depressurization step followed by addition of 5 mmol L⁻¹ glutamate.

After a 10-h period, the pressure increased to 10 bars during a three-day culture (0.186 bars h⁻¹ in the linear phase). This value was close to the maximal admissible value for the closed vessel. Therefore, a slow depressurization step was achieved to attain 1.1 bars inside the bioreactor (5 min), and the collected gas was equal to 0.8 L. An additional 24 hours of bacterial growth led to a pressure increase of 2.4 bars. The kinetics of gas evolution was not quasi-linear as previously described but exhibited a curvature towards a stable value close to 2.4 bars, corresponding to a volume of 140 ml H₂ with 10.3 % CO₂ (v:v). The bacterial concentration reached an OD of 1.56. Only 19 mmol L⁻¹ of lactate was consumed during this first step, corresponding to 33.3 % of the total lactate consumption.

The trend of pressure evolution towards a stable value led us to ask about the limiting factor for bacterial growth. Various factors could be limiting, such as the concentrations of carbon, nitrogen, or inhibitory products or physical parameters such as light illumination. Illumination and lactate, evaluated as the carbon concentration, were, respectively, stable and present in sufficient concentrations (38 mmol L⁻¹) in the culture medium. Consequently, the nitrogen concentration, evaluated as the glutamate concentration, was tested for its effect on hydrogen bioproduction in the closed vessel. Na-glutamate was aseptically added to the closed vessel (arrow DA in Fig. 8) after a depressurization step. An increase in pressure was observed during 80 hours until stabilization close to 5.45 bars. Degassing of the reactor (duration 5 min) from 5.45 to 0.5 bars produced a gas volume of 0.58 L of hydrogen (purity 89.4 %). The bacterial concentration had reached an OD of 2.8. Lactate was not completely degraded, as confirmed by

leftover lactate concentration (16 mmol L^{-1}). Supplementary addition of Na glutamate (arrow DA on Fig. 8) led to a weak pressure increase, less than 0.3 bars (0.13 L H_2 containing 7.6 % CO_2), correlated to weak lactate consumption but without an increase in bacterial concentration. This freeze in growth was probably due to light distribution inside the bacterial culture; carbon and nitrogen concentrations were sufficient for effective growth, but the OD reached a level twice that from previous results (Figure 4).

H_2 production is directly linked to substrate concentration. We have not recorded a limitation of hydrogen production due to the hydrogen pressure. However, hydrogen production depends on limiting factors such as the sodium glutamate concentration and undoubtedly the light distribution. The addition of Na glutamate twice during culture with a high lactate concentration (55 mmol L^{-1}) resulted in a global volume of pure hydrogen of 1.526 L, correlated to a lactate degradation rate of 89.1 %. On the other hand, pH stability is clearly a critical factor to ensure good conversion of lactate into H_2 under pressure. However, pH stability does not explain this unexpected enhancement.

2.8. Resource allocation model

The nitrogenase route is an irreversible chemical route of energetic conversion, and the nitrogenase enzyme is resistant to high pressures [38]. These features are advantageous for the pressurized process, but again, these advantages do not explain the enhancement of the acetate/hydrogen conversion rate. Li *et al.* [39] reported the opposite effect of pressure on hydrogen production during photosynthetic growth of *Rhodobacter sphaeroides* ZX-5. At the same culture time (76 h), a decrease in the total pressure in the photobioreactor from $1.08 \cdot 10^5$

(1.08 bar) to $0.944 \cdot 10^5$ Pa (0.944 bar) increased the malate (substrate) conversion efficiency from 86.07 % to 95.56 %. Of course, our experimental setup and the bacterial strain are inherently different, and our unexpected improvements are more reliably studied, but our observations do not correspond to the usual model of optimization of biohydrogen production. Nevertheless, it is well known that under stress, resources are reallocated. Acclimation requires redirecting energy and nutrients from resource acquisition: from growth pathways to producing protective molecules [40]. This mechanism makes substantial amounts of carbon and nitrogen vulnerable to loss [41]. In the present cultures, the partial changing pathway is possible from growth to survival; this mechanism could explain the lower growth in the closed vessel than in the open vessel. Moreover, lactate conversion yields are lower in the open vessel (48 %) than in the closed vessel (69.3 %), and the consumption rate of lactate is higher in the open vessel than in the closed vessel. Therefore, during culture under pressurized conditions, stress appears, and this stress increases the energetic demand and improves hydrogen production. However, we do not know the nature of this stress, and we do not have a hypothesis regarding its origin.

3. Perspectives

The comparison between photobiohydrogen production processes depends on multiple operating parameters such as reactor geometry, substrate source, illumination condition [42]. Therefore, only experiments carried out in the same operating conditions can be directly compared, in our experiments only one parameter moved: the pressure (closed or open reactor). In the present case, the mean production rate of hydrogen is equal to $46.7 \text{ ml H}_2 \text{ L}^{-1}\text{h}^{-1}$ in closed vessel and $31.3 \text{ ml H}_2 \text{ L}^{-1}\text{h}^{-1}$ in open vessel. According to the review of Eroglu and Melis [42], this rate depends

on many parameters and it is difficult to compare various bacterial strains and operating conditions, but our results appear in high range values of literature [32] without optimization. To scrutinize the hydrogen production we need to compare bacterial growth rate to hydrogen production rate. Mathematical tools can be provided to compare various experimental conditions [43]. The Luedeking-Piret model [44] describes well the fermentative process; this classical model considers the relationship of cell growth to product formation (i.e. P the product concentration) as follow:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (1)$$

with the parameters of fermentation: β the non-growth rate constant and α the growth rate constant. In addition, bacterial growth kinetics is given by:

$$\frac{dX}{dt} = \mu X \quad (2)$$

with μ is the specific growth rate of the microorganisms and X the biomass concentration. Therefore, combining equation (1) and equation (2) we obtain the following equation:

$$\frac{dP}{dt} = \left(\alpha + \frac{\beta}{\mu}\right) \frac{dX}{dt} \cong \gamma \frac{dX}{dt} \quad (3)$$

Except possibly for the lag phase and at the very end of the fermentation this yield γ is fixed by the organism, substrate, pH, lighting and temperature. To compare biohydrogen production we need mean values of bacterial growth and production rate. In the present study we observe an unexpected enhancement of biohydrogen production by the phototrophic bacterium *Rhodobacter capsulatus*, because the increase operating pressure substantially increases the γ yield. The model showed in previous subsection can explain this observation. Summing-up, the γ yield is better than the mean production rate of hydrogen to optimize solar biofuel plant.

Advantage of hydrogen over other biofuels is the high conversion efficiency (solar-to-product) from biomass. However, life cycle environmental impact assessment of biohydrogen of “well to pump” is also required to compare biofuels. A promising biofuel has ability to grow on non-arable land and weak waste streams. It is demonstrated that to generate value from wastewater and reduce energetic hydrogen production using bioprocess exhibits a high energetic interest [45]. The anaerobic photosynthesis using the purple non-sulfur bacteria can reduce agro-industrial wastewaters (lactate) or agricultural wastes (e.g. wheat straw) and produce hydrogen. Improving the γ yield contributes also to decrease the waste streams of the bioprocess. The use of renewable waste materials as substrate, like organic acid containing waste issued from food process [31] or from different biomass source [46], represents a great advantage in terms of eco-friendly process. The sustainability of current technology devoted to biohydrogen production and future perspectives were recently covered by Singh and Rathore [47]. Many techniques such as pretreatment, cell immobilization, sequential fermentation, combined fermentation have outlined in order to enhance hydrogen production. The objective of numerous current research projects is to increase the efficiency by optimizing strains, media, culture conditions. According to M A Rossen [49] exergy analysis can provide a more realistic view of a bioprocess by scrutinizing the local irreversibilities within the components of the system. This way, the most effective solutions can be distinguished to improve or retrofit a process under consideration by providing useful information on the influence of the thermodynamic variables on the process efficiency. Exergy analysis could be employed as an adaptable framework to determine and compare the renewability of biological hydrogen production using different routes in order to choose the most suitable approach and conditions. Exergy analysis was successfully applied to hydrogen production issued from water-gas shift by *Rhodospirillum rubrum* depending on various carbon

sources in a batch reactor [50] or depending on liquid flow rate and agitation speed in a continuous reactor [51]. Hydrogen production by *Rhodospseudomonas palustris* PT on acetate was studied with exergy analysis by Hosseini et al. 2016 [52]. In this work, short-chain fatty acids were identified as the most appropriate carbon source for biohydrogen production from the exergy point of view. Just to give some meaningfulness to biohydrogen from lactate or acetate sources, the life cycle assessment is the relevant tool in assessing process energetics and environmental impacts of this kind of bioproduction systems [53]. In the present work, we proposed to use LGB (boric buffer) as an alternative to phosphate buffer (LGK), and then this strategy shall be scrutinized thanks to life cycle assessment.

4. CONCLUSIONS

We observed only a positive effect of pressure on hydrogen production by the photosynthetic bacterium *Rhodobacter capsulatus*. Produced hydrogen purity exceeded 90 % with a lactate conversion rate of up to 70 % and in closed vessel, this production was 1.8 times that obtained in a vessel open to the atmosphere (841 ml versus 470 ml). Optimal H₂ conversion rate (82 %) was recorded with energy light close to 646 W m⁻² (30,000 lx). It is suspected that the energetic demand of *Rhodobacter capsulatus* increases during culture under pressurized condition and improves hydrogen production. Moreover, the energetic gain due to the hydrogen self-compression from 1.0135 bars to 10 bars is significant (above 1.3 kWh / kg H₂). This unexpected enhancement of hydrogen production makes it more relevant as a proof of concept for pressurized production using a photosynthetic bacterium (*Rhodobacter capsulatus*), which exhibits numerous advantages:

- i) self-compression of produced hydrogen,

- ii) easier control of batch feeding or continuous operation,
- iii) lower risk of contamination,
- iv) easier storage of hydrogen.

The latter is of critical importance in the economics of this renewable energy generation. The main disadvantage of hydrogen is the difficulty of inexpensive easy storing and dispensing of the hydrogen gas. Direct compression of biohydrogen is a benefit to reach the widespread commercial applications.

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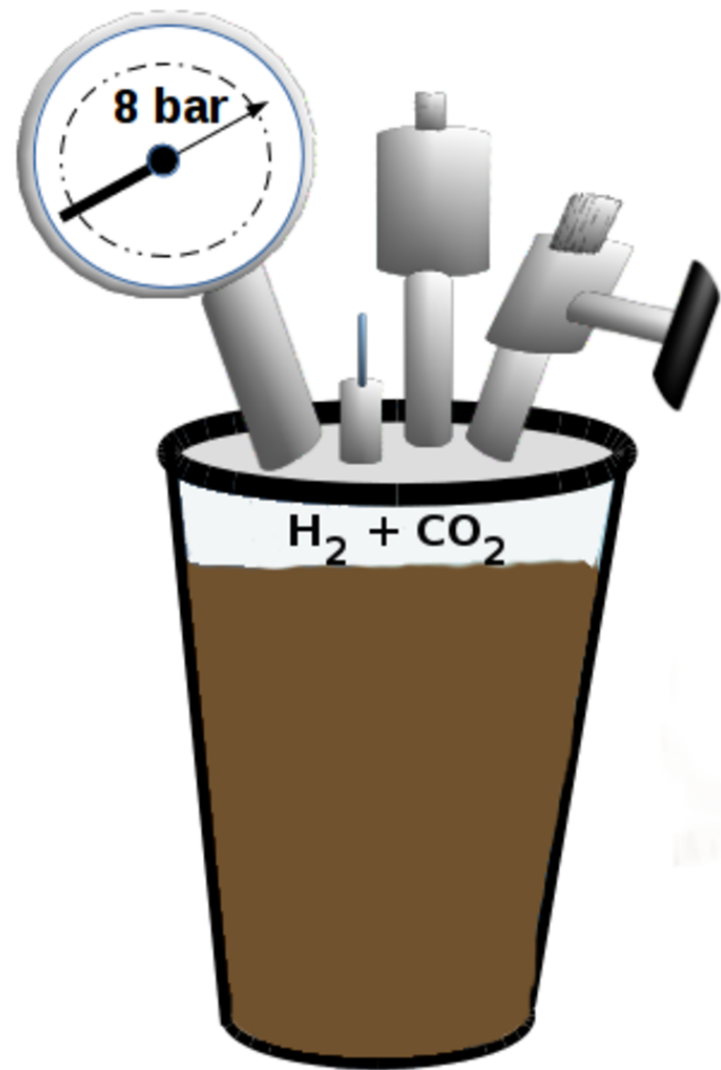
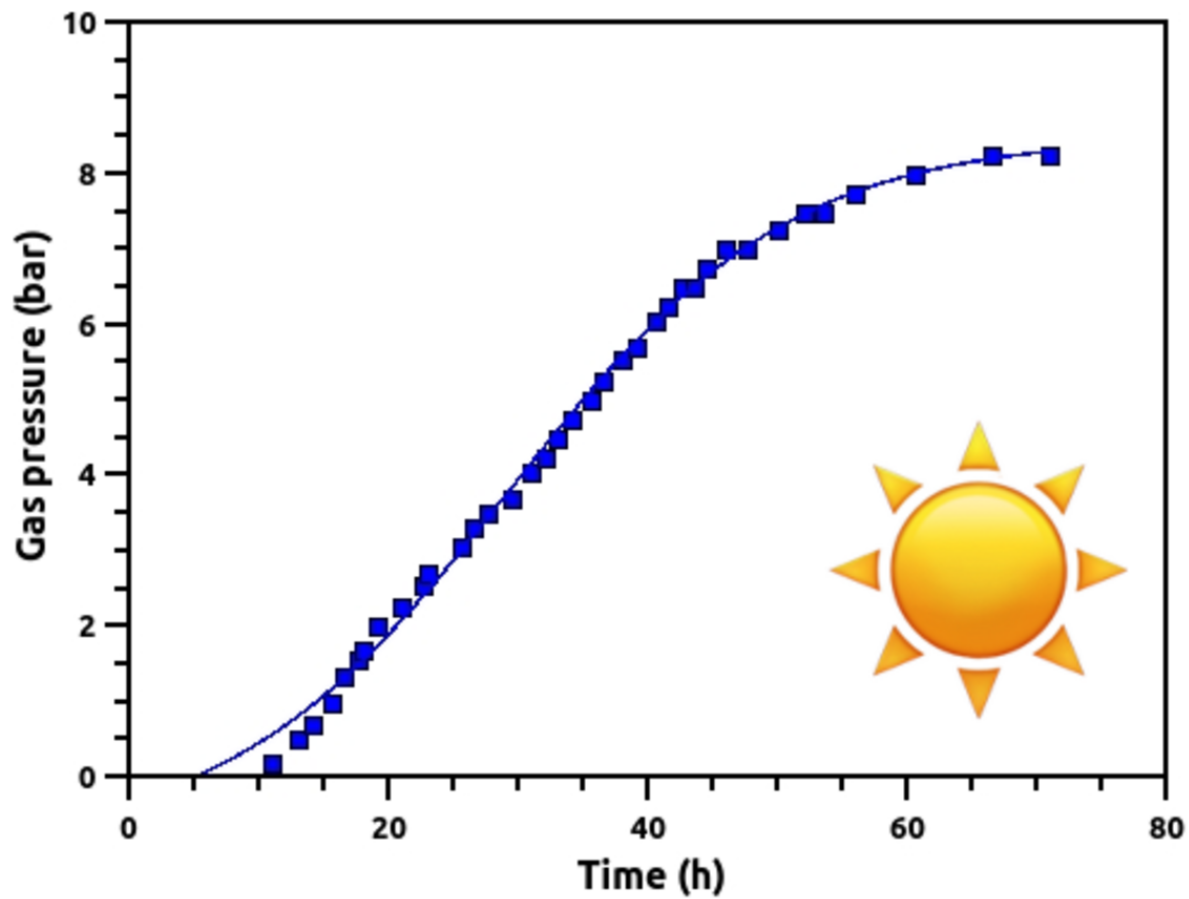
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