Available online at www.sciencedirect.com
ScienceDirect
International Journal of Hydrogen Energy 32 (2007) 4670–4677
International Journal of HYDROGEN ENERGY
www.elsevier.com/locate/ijhydene

Effect of light intensity, wavelength and illumination protocol on hydrogen production in photobioreactors

Basar Uyar[a], Inci Eroglu[b,*], Meral Yücel[c], Ufuk Gündüz[c], Lemi Türker[d]

[a]*Department of Biotechnology, Middle East Technical University, 06531 Ankara, Turkey*
[b]*Department of Chemical Engineering, Middle East Technical University, 06531 Ankara, Turkey*
[c]*Department of Biology , Middle East Technical University, 06531 Ankara, Turkey*
[d]*Department of Chemistry, Middle East Technical University, 06531 Ankara, Turkey*

Received 19 February 2007; received in revised form 28 April 2007; accepted 2 July 2007
Available online 16 August 2007

Abstract

Rhodobacter sphaeroides O.U. 001 is a purple non-sulfur bacterium which evolves hydrogen from the breakdown of organic acids under illumination and anaerobic conditions. In this study, the effect of light intensity, light wavelength and illumination protocol on the growth and hydrogen production of *R. sphaeroides* O.U. 001 was investigated in gas-tight glass photobioreactors with defined medium. The results showed that the rate of hydrogen production increased with increasing light intensity and reached saturation at around 270 W/m^2. Also it was found that lack of infrared light (750–950 nm wavelength) decreased photoproduction of hydrogen by 39%. Another factor evaluated was the effect of different illumination protocols on the growth and hydrogen production. It was observed that illumination after inoculation stimulates hydrogen production, increases substrate conversion efficiency and hydrogen production rate; no hydrogen was produced during the dark periods.

Keywords: Biological hydrogen production; *Rhodobacter sphaeroides*; Photobioreactors; Photofermentation

1. Introduction

Rhodobacter sphaeroides O.U. 001 is a purple non-sulfur bacterium which is capable of producing molecular hydrogen under anaerobic conditions by photofermentation of organic acids. The investigations of the effect of light intensity, wavelength of the light and illumination protocols are important to set a basis for further trials of hydrogen production in outdoor photobioreactors under natural sunlight. Photoproduction of hydrogen has been reported to be saturated around 5000 lx [1]. It has also been suggested that absence of light during dark periods of light–dark cycle is a problem but is not insuperable; bacteria can survive and maintain their hydrogen production activity, the hydrogen production recovers more or less the same rate once illumination is resumed [2]. Wakayama and Miyake [3] showed that light conversion efficiency decreased with high light intensity. In order to overcome this decline, they developed a new bioreactor with light shade bands set on the surface. Nakada et al. [4] analyzed the penetration of light into a photobioreactor and its relation to hydrogen production in a four compartment bioreactor, the cells in the deep parts showed higher light energy conversion efficiencies although the light intensity was low. Kitajima et al. [5] used plane-type photobioreactors of different depths to examine the effect of reactor depth on hydrogen production and found that the rate of hydrogen production decreased and organic acid concentration increased as reactor depth increased. They stated that the penetration of light into the cell culture and absence of dark zone in the bioreactor is an important factor to be considered for scaling-up of the photobioreactors. Wakayama et al. [6] also studied the production of hydrogen under sunlight by photosynthetic bacteria and found that the hydrogen levels produced depended on the irradiation intensity of the sunlight; the hydrogen production rate varied with light intensity with a time lag of 4 h. They stated that the distribution of light in the reactor influences bacterial activity and when the irradiation exceeded a threshold level, the hydrogen production rate by the photosynthetic bacteria reached a saturation level.

* Corresponding author. Tel.: +90 312 2102609; fax: +90 312 2102600.
E-mail address: ieroglu@metu.edu.tr (I. Eroglu).

0360-3199/$ - see front matter © 2007 International Association for Hydrogen Energy. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijhydene.2007.07.002

Recent studies showed that light intensity and light–dark cycles are two key parameters which affect hydrogen production by photofermentative bacteria. The effect of the wavelength of the incident light on hydrogen production, however, was not shown although it is known that bacteria absorbs red-infrared region of the spectrum by its bacteriochloropyll *a* complex. The design and scale up of more efficient photobioreactors, especially for those that will be operated under natural sunlight at outdoor conditions, require knowledge and models that relate the hydrogen production to the light intensity and wavelength, and to illumination patterns. Therefore, the effects of changes in these parameters on bacterial growth and hydrogen production should be further investigated.

In the present study, we report the effect of light intensity, wavelength of the light, different light–dark cycle protocols and different initial dark periods on growth and hydrogen production of *R. sphaeroides* O.U. 001. The presented results set a solid base for operation of photobioreactors which would be illuminated by sunlight at outdoor conditions and are exposed to the diurnal cycle.

2. Experimental

2.1. Bacterium and culture

R. sphaeroides O.U. 001 (DSM 5864) strain was used in this study. The inoculum was prepared by growing cells in the modified medium of Biebl and Pfennig [7]. In hydrogen production experiments, the nitrogen source was sodium glutamate (2 mM) and the carbon source was malate (15 mM). The sterilization of the photobioreactors and media were accomplished by autoclaving. Ten percent inoculation by volume of the fresh medium was made into the bioreactors [8].

2.2. Photobioreactors

Most of the hydrogen photobioreactors have a head space and anaerobic conditions were achieved by flushing argon through the culture medium [9–11]. However, in the present study, the photobioreactor was completely filled with culture medium and no argon was flushed. As a result the efficiency of the photobioreactor was increased since the working volume was equal to the photobioreactor volume and the need of using an inert gas (such as argon) to create an anaerobic atmosphere was eliminated. Since the photobioreactor did not have the top space containing the inert gas, the hydrogen collected had a high purity. Moreover, since argon gas was not used, the photobioreactor was simplified and the operation cost was greatly decreased. The dissolved oxygen in the medium and the air present in the capillary connection tube was neglected and the photobioreactor was assumed to be anaerobic. The small amount of oxygen which might be present in the medium was consumed by the bacteria during the early stages of growth. Evolved gas was collected in a water filled graduated cylinder attached to the photobioreactor by a capillary tube. A digital camera was placed in front of the gas collector. It was connected to a PC for on-line monitoring and recording of continuous hydrogen production. This system provided us very precise and accurate data for gas production rate and gas amount. The experimental setup was appropriate to carry out up to 6 runs in parallel. The photobioreactors used in this study were either a rubber-tapered glass tube of 4.1 ml or a glass bottle of 55 ml; however, we also applied this design successfully to larger scales up to 5.5 l (not shown).

2.3. Operating conditions

The photobioreactors were maintained at 30–33 °C in an incubator. The illumination was provided by a 150 W tungsten lamp. A uniform light intensity of 4000 lx was attained at the surface of photobioreactors unless otherwise indicated. Four thousand lux was found to be equivalent to 270 W/m^2 and to 1370 μmol photons/m^2/s (photosynthetically active radiation). The initial pH in photobioreactors was 6.6–6.8.

2.4. Analytical methods

Light intensity and spectrum measurements were made by a luxmeter (Lutron) and a spectroradiometer (StellarNet EPP2000-VIS-50). Evolved gas was analyzed by gas chromatography [8]. The bacterial cell concentration was determined spectrophotometrically. It was found that an optical density of 1.0 at 660 nm corresponded to a cell density of 0.6 g dry weight per liter of culture. Bacteriochlorophyll *a* was extracted from the cell with acetone–methanol mixture (7:2). The concentration of bacteriochlorophyll *a* in the extract was determined from the absorbance at 770 nm (extinction coefficient = 76 $mM^{-1}cm^{-1}$) [12]. Rhodamin B solution (6.5 mM) and $CuSO_4$ solution (135 mM) were used as optical filters in order to block specific ranges of light wavelength.

3. Results and discussion

3.1. Effect of light intensity on hydrogen production

This study was carried out to show the effect of incident light intensity on hydrogen production. Previously, Sasikala et al. [1] carried out a similar study and showed saturation of hydrogen production at 5000 lx. In this study, we performed a more precise experiment around this optimum to obtain a better insight: photobioreactors with very short light path (0.5 cm light path, 4.1 ml total volume) were used for high precision and photobioreactors were exposed to seven different light intensities between 88 and 405 W/m^2 (measured at the surface of photobioreactors).

The results were listed in Table 1. From the total hydrogen gas produced versus time data, the maximum hydrogen production rate (calculated from the linear hydrogen production phase during exponential bacterial growth), light conversion efficiency and the substrate conversion efficiency were estimated.

Substrate conversion efficiency for malate is determined as the ratio of moles of hydrogen that have actually been produced per moles of hydrogen that would have been produced if all

Table 1
The effect of light intensity on hydrogen production by *R. sphaeroides* in 4.1 ml photobioreactors

Light Intensity (W/m^2)	Maximum H_2 production rate ($ml/l_{culture}\,h$)	Total H_2 produced ($l/l_{culture}$)	Light conversion efficiency (%)	Substrate conversion efficiency (%)
88	18	0.68	1.11	34
118	22	0.58	0.82	27
169	28	0.64	0.76	31
209	31	0.65	0.65	30
277	33	0.80	0.50	36
338	34	0.75	0.45	37
405	34	0.60	0.25	30

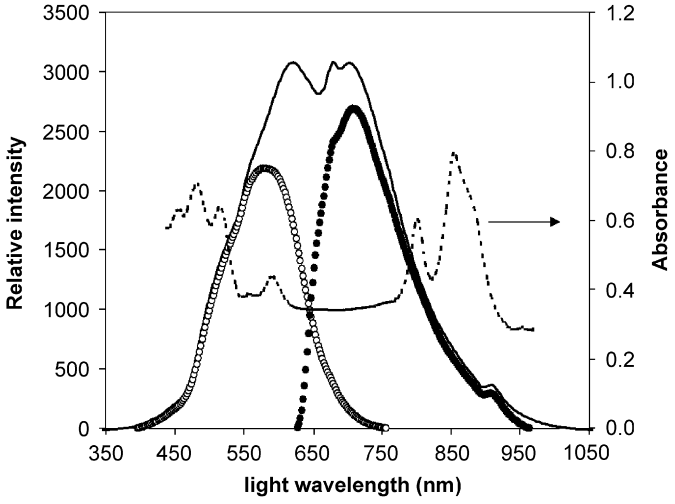

Fig. 1. The light spectrum at the surface of the photobioreactors and the absorption spectrum of *R. sphaeroides* O.U. 001. (—): control photobioreactor, no filters used, 370–1035 nm range, (○): Rhodamin B solution filter (> 760 nm blocked), (●) $CuSO_4$ solution filter (< 630 nm blocked), (- - -) absorbance spectrum of *R. sphaeroides* O.U. 001 (maxima at 375 (not shown in figure), 590, 805 and 860 nm by bacteriocholoropyll *a* and 450, 482 and 514 nm by spheroidene).

of the substrate had been converted to hydrogen through the stoichiometric equation:

$$C_4H_6O_5 + 3H_2O \rightarrow 6H_2 + 4CO_2.$$

Light conversion efficiency is determined as the ratio of the total energy value of the hydrogen that has been obtained to the total energy input to the photobioreactor by light radiation. It is calculated by

$$\eta = [(33.61 \cdot \rho_{H_2} \cdot V_{H_2})/(I \cdot A \cdot t)] \times 100, \quad (1)$$

where V_{H_2} is the volume of produced H_2 in l, ρ_{H_2} is the density of the produced hydrogen gas in g/l, I is the light intensity in W/m^2, A is the irradiated area in m^2 and t is the duration of hydrogen production in h. Incident light intensity was used in the calculations instead of the absorbed light intensity since the runs were carried out in batch mode, where the cell concentrations and thus the absorbed light intensities vary throughout the process. More information about calculation and discussion

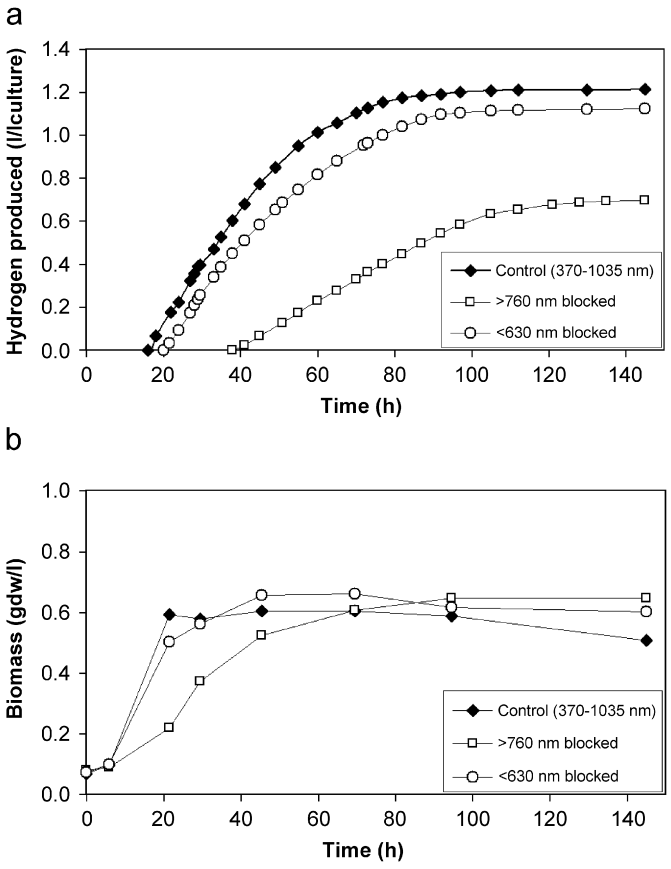

Fig. 2. Effect of incident light wavelength: (a) on hydrogen production, (b) on growth. (⧫): no filters used, 370–1035 nm range (control), (□): Rhodamin B solution used as optical filter (> 760 nm blocked), (○): $CuSO_4$ solution used as optical filter (< 630 nm blocked).

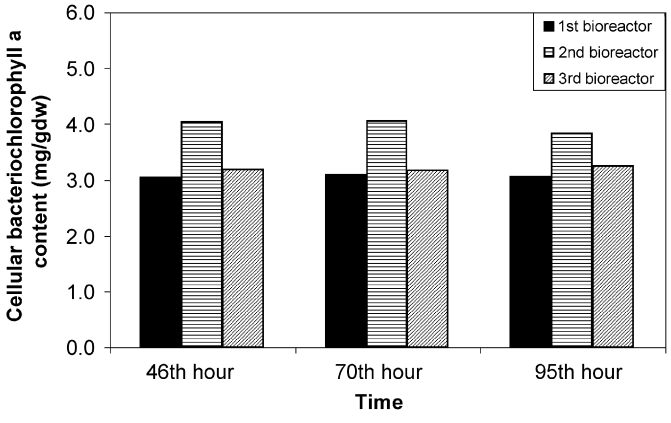

Fig. 3. Effect of incident light wavelength on cellular bacteriochlorophyll *a* levels. Bioreactor 1: no filters 370–1035 nm (control), bioreactor 2: > 760 nm blocked, bioreactor 3: < 630 nm blocked.

of the substrate and light conversion efficiencies are given in previous works [2,8].

According to the results an increase in light intensity up to $270\,W/m^2$ increased the maximum hydrogen production rate up to $33\,ml/l_{culture}\,h$. Further increase in light intensity did not change the rate and no photoinhibition was observed. On the

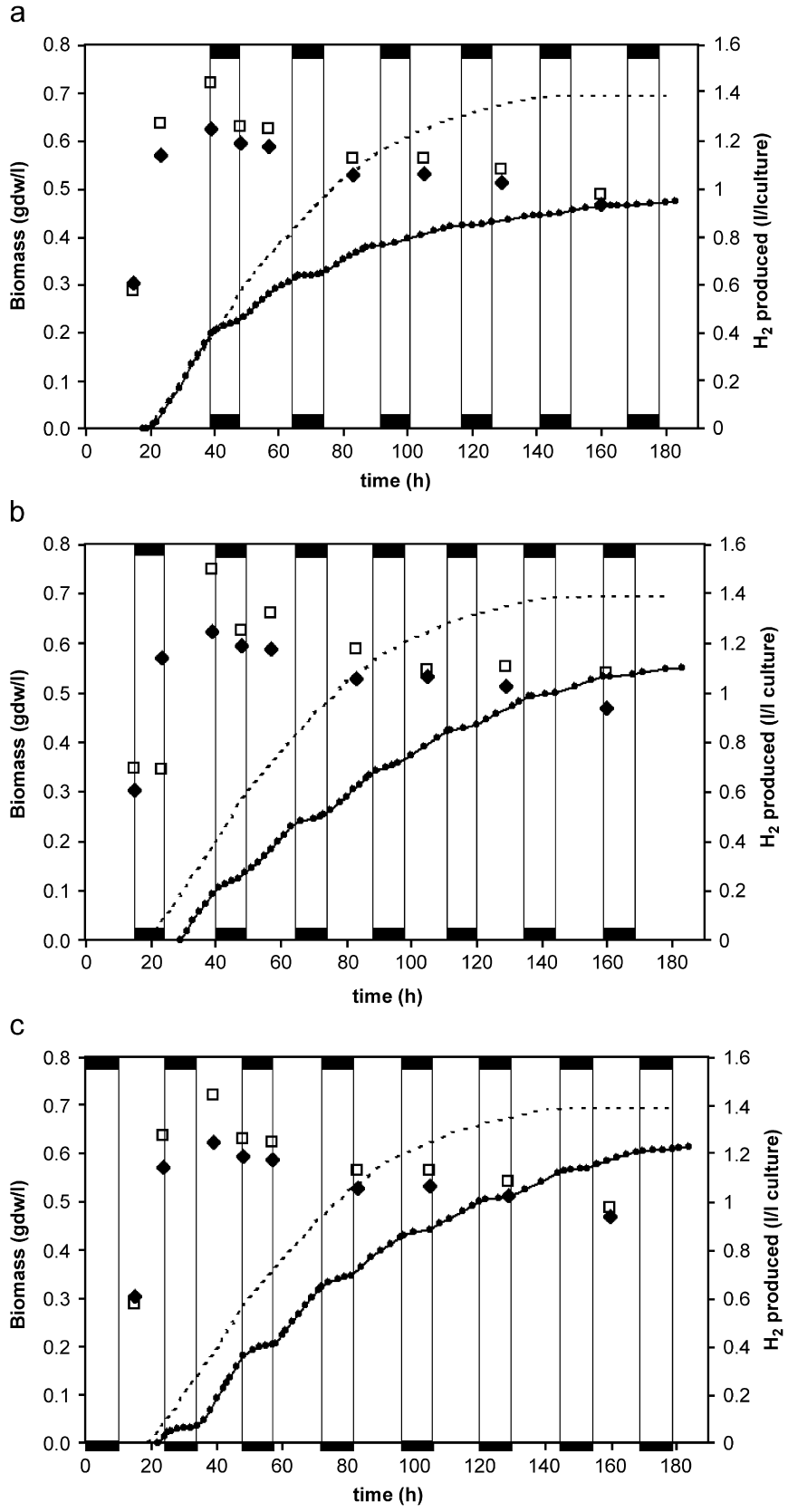

Fig. 4. Effect of light–dark cycles on growth and hydrogen production, black bands indicate dark periods (a) illuminated until hydrogen production starts, then 14 h light–10 h dark cycles were applied, (b) illuminated with 14 h light–10 h dark cycles from the start on, (c) illuminated with 10 h dark–14 h light cycles from the start. (□): growth under cyclic illumination, (♦): growth under continuous illumination (control); (—-): hydrogen production under cyclic illumination; (- - -): hydrogen production under continuous illumination (control).

Table 2
The effect of different illumination protocols on hydrogen production by *R. sphaeroides* in 55 ml photobioreactors

Illumination protocol	Average H_2 production rate ($ml/l_{culture}$ h)	Total hydrogen ($l/l_{culture}$)	Light conversion efficiency (%)	Substrate conversion efficiency (%)
Illuminated continuously	15	1.36	0.54	68
Illuminated until hydrogen production, then 14 h light–10 h dark cycles	8	0.95	0.53	47
14 h light–10 h dark cycles after inoculation	8	1.11	0.81	55
10 h dark–14 h dark cycles after inoculation	8	1.25	0.66	62
10 h at dark after inoculation, then continuous illumination	11	1.00	0.42	50
24 h at dark after inoculation, then continuous illumination	6	0.56	0.27	28

other hand, the substrate conversion efficiency was not significantly influenced by the light intensity within the given range.

Light conversion efficiency decreased from 1.11% to 0.25% as light intensity increased from 88 to 405 W/m^2. The decrease in light conversion efficiency was not actually a drawback since produced hydrogen (V_{H_2}) remained the same. The decrease resulted from the high value of (I) that is the incident light intensity in Eq. (1). In solar bioreactors the light intensity is not the limiting factor since in a sunny summer day, light intensities up to 850–950 W/m^2 are common for most of the Europe (40°–55°N).

The light intensity that should be attained is at least 270 W/m^2 at the darkest point of the photobioreactor for obtaining high hydrogen production rate. That could be one of the limitations in the design of photobioreactors. The light intensity at low intensities is the rate determining parameter of hydrogen production thus it is one of the most important parameters that should be monitored and controlled closely; if the sunlight intensity in an outdoor photobioreactor is below this threshold value, additional artifical illumination may be provided to keep the hydrogen production rate high.

Kitajima et al. [5] estimated the effect of the hydrogen uptake on the hydrogen production rate from lactate by *R. sphaeroides* RV in reactors with agitation and various depths. They showed that the hydrogen production rate decreased as the bioreactor depth increased (due to the insufficient light penetration into the bioreactors), to a compensation point where the rates of the hydrogen production and uptake were balanced in the reactor, so that no apparent production of hydrogen would be observed. They estimated that point to occur if the photobioreactor was 27 cm deep, under the highest outdoor sunlight illumination. They anticipated that the values of light compensation points or rates of hydrogen production and uptake will differ depending on the strain of bacteria, substrate composition, and the coloration of the medium. Our results confirm with their findings.

Nakada et al. [4] have reported that alteration of light intensity and light spectrum upon passage of the light through the reactor affected light energy conversion efficiencies to hydrogen. They also found that light energy decreased exponentialy with depth of the photobioreactor. According to their results, light energy conversion was low in the first compartment, that was the closest one to the light source, but light energy conversion was high in the last photobioreactor compartment which received the lowest light energy. In the present study, all photobioreactors received the same light spectrum at different intensities.

3.2. Effect of the wavelength of the light on hydrogen production

In this study, the absorbance spectrum of *R. sphaeroides* O.U. 001 was determined first (Fig. 1). The absorption maxima at 375, 590, 805 and 860 nm are due to the bacteriochloropyll *a* and 450, 482 and 514 nm are due to the spheroidene (carotenoid) content. Then bacteria were exposed to light at specific wavelengths in order to observe the effect of the light wavelength on growth and hydrogen production. In this set of experiments photobioreactors with 55 ml working volume were used. The control photobioreactor received the normal bell-shaped light spectrum from the lamp (370–1030 nm) whereas optical filters were placed in front of the other two photobioreactors to obtain the light spectra given in Fig. 1 at the surface of the photobioreactors. The other sides of photobioreactors were wrapped by aluminum folio to prevent light scattering. As illustrated in Fig. 1, it can be seen that Rhodamin B solution acted as an optical filter that transmitted all of the light with wavelength less than 560 nm, while blocking the light with wavelength greater than 760 nm completely. On the other hand, $CuSO_4$ solution acted as an optical filter that transmitted almost all of the light with wavelength greater than 720 nm while blocking the light with wavelength less than 630 nm completely.

The results are presented in Figs. 2 and 3. It is clearly seen that in case of infrared light blocked photobioreactor, growth and hydrogen production are affected negatively: Hydrogen production lag time was 40 h compared to 17 h for the control photobioreactor, produced hydrogen gas was 57% of the

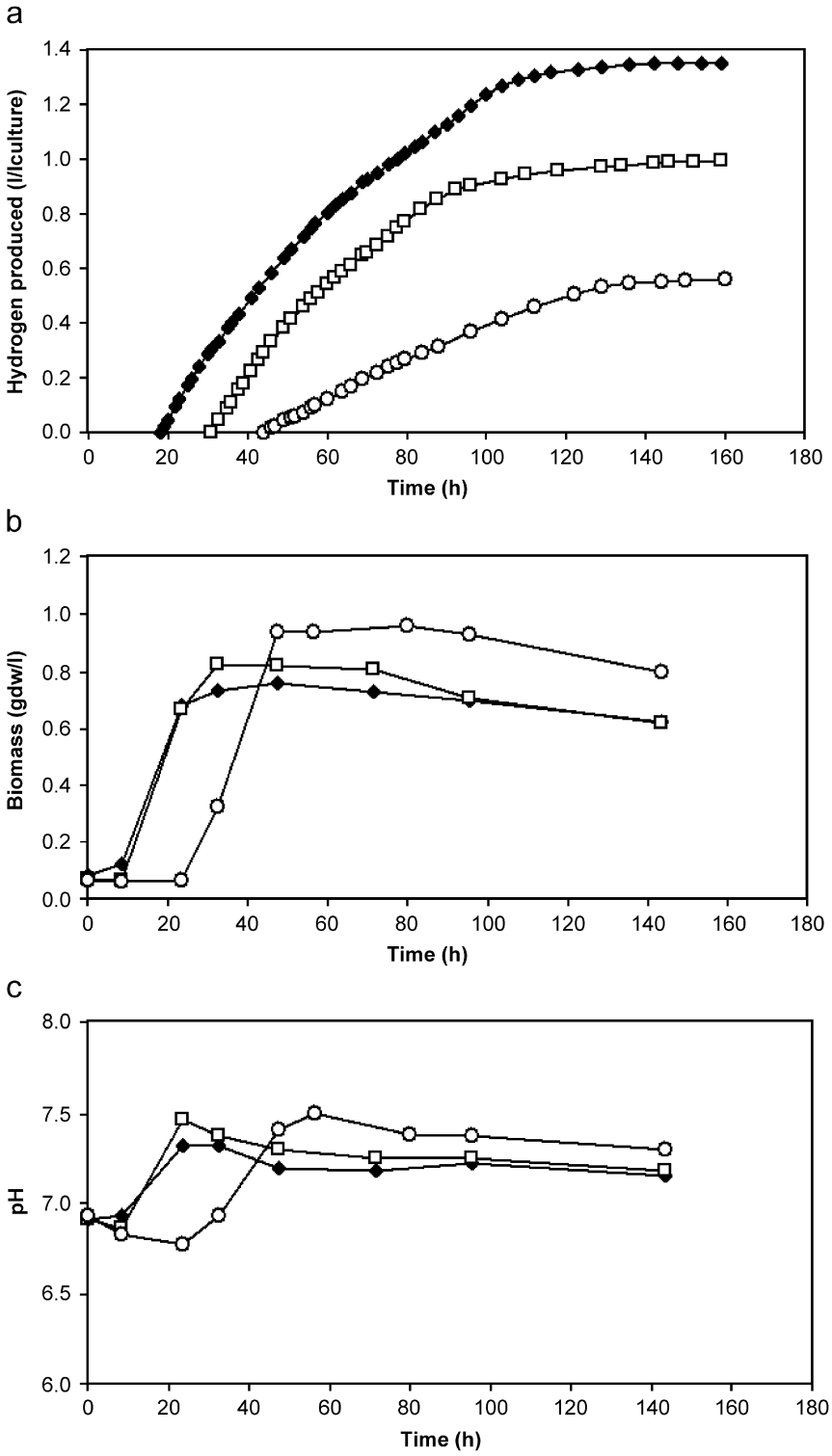

Fig. 5. Effect of initial illumination (a) on hydrogen production, (b) on growth, (c) on pH change. (♦): continuous illumination from the start (control); (□): 10 h of dark period at the start, then continuous illumination was applied; (○): 24 h of dark period at the start, then continuous illumination was applied.

control and growth was slower. Moreover, cellular bacteriochlorophyll *a* content in this photobioreactor was 30% higher than in the control photobioreactor (Fig. 3), which means that there is a great energy stress on bacteria and they produce more bacteriochlorophyll *a* to overcome that shortage. In case of the blue light blocked photobioreactor, hydrogen production was slightly affected compared to the control: Hydrogen production lag time was 4 h longer, 7% less hydrogen gas was obtained. Cellular bacteriochlorophyll *a* content in this photobioreactor was quite close to that in the control photobioreactor.

Growth was also not significantly affected by that light-filter. These results showed that the infrared region of light spectrum where the bacteriochlorophyll *a* absorption maxima exist is very important for hydrogen production, whereas the left part of spectrum where the carotenoid absorption maxima exist is not significantly effective. Thus, it is recommended that the outdoor photobioreactors should be located and oriented such that they receive the sunlight during sunset and sunrise to ensure that they receive plenty of red and infrared light. Moreover, it can be concluded that the artificial light source which does not emit light in the red-infrared region (750–950 nm) are not suitable for illumination of the photobioreactor for hydrogen production.

3.3. Effect of light/dark cycles and illumination on hydrogen production

To get information about the tolerance of the growing cells to dark periods, four photobioreactors (55 ml in volume) were run in parallel; each one was subjected to different illumination protocols. The first photobioreactor was illuminated continuously (control); the second photobioreactor was illuminated after inoculation until hydrogen production started, then 14 h light–10 h dark cycles were applied; the third photobioreactor was illuminated by 14 h light–10 h dark cycles after inoculation; the fourth photobioreactor was illuminated by 10 h dark–14 h light cycles, that is, it started with a dark period after inoculation and received the first light after 10 h.

The data in Fig. 4 illustrate the comparison of the variation of total hydrogen gas produced and cell concentration with respect to time for each illumination protocol with the results of the continuously illuminated photobioreactor. Fig. 4 indicates that in the photobioreactors that are exposed to light–dark cycles, hydrogen production stopped during dark periods, and was restored when illumination started again. However, as summarized in Table 2, the average hydrogen production rate and the total hydrogen produced decreased compared to the continuously illuminated photobioreactor; from 15 to 8 $ml/l_{culture}$ h and from 1.36 to 0.95–1.25 $l/l_{culture}$, respectively. This decrease is possibly due to the consumption of available substrates during dark periods by the bacteria. These results confirmed the findings of Miyake et al. [13], who reported that hydrogen production stopped during dark periods, and was restored when illumination started again. Similarly, cells did not grow during dark periods but survived and growth was restored when illumination started again (Fig. 4b). It is also observed that the dark period extends the lag time of hydrogen production from 18 to 22–28 h. These results suggest that the overall hydrogen production rate and the total amount of hydrogen produced in an outdoor solar bioreactor which will be exposed to day–night cycle will be lower compared to the continuously illuminated photobioreactor. Artificial illumination during night might be considered based on the gain reported and the cost of such an installment.

Fig. 5 illustrates the triggering effect of illumination after inoculation to biological hydrogen production by giving the total hydrogen production, growth and pH change. The photobioreactors were kept either 10 or 24 h at dark after inoculation, and then they were illuminated continuously. In order to see stimulation of light on hydrogen production, the results were compared with the results from a continuously illuminated photobioreactor. The results are compared with effect of the light/dark cycle runs and summarized in Table 2.

If the reactor was kept at dark after inoculation, the lag time of both growth and hydrogen production increased, and the total amount of produced hydrogen and the rate of hydrogen production decreased. It is deduced from Fig. 5 that if the photobioreactor was kept at dark after inoculation, the cell did not grow until illumination started. *R. sphaeroides* could not grow under dark anaerobic conditions; however, it survived in fermentation mode by consuming malate. The decrease of pH during the dark period also confirmed that the bacteria survived in fermentation mode. Hydrogen production could not be achieved until a threshold cell concentration was obtained. These results confirm the literature: Gurgun et al. [14] reported slow growth under dark anaerobic conditions. Uffen et al. [15] reported growth to a limited extent only when heavy inoculations were made under dark anaerobic conditions.

Either inoculation should be made in the morning to allow the solar bioreactor to receive daylight during the first phase of the process or the artificial illumination should be provided after inoculation if there is not enough light.

4. Conclusion

It is concluded that the photobioreactor depth should be limited depending on the light intensity received by the photobioreactor. Hydrogen production rate drops significantly below the light intensity of 270 W/m^2 thus that intensity should be attained at the darkest point of the photobioreactor for obtaining high hydrogen production rate.

It is shown that the infrared light (750–950 nm) plays an essential role for photoproduction of hydrogen. The photobioreactors should receive infrared light. In case the light source does not provide such light, optical systems that allow shifting the light to the 600–800 nm wavelength range need to be employed to enhance the hydrogen production by these bacteria.

The hydrogen production by photosynthetic bacteria stops at dark. Although it is not a must, the outdoor photobioreactor may be illuminated during night to decrease the batch duration, increase the overall hydrogen production rate and the total amount of hydrogen produced. If possible the batch should be started in the morning, to let bacteria grow to a threshold concentration for hydrogen production during daytime. Otherwise artificial illumination should be used for an efficient process.

Acknowledgment

This study was supported by DPT-YUUP, METU (BAP-08-11-DPT.2002K120510 OYP-FBE-BTEK1) and EU 6th framework project “HYVOLUTION”.

References

[1] Sasikala K, Ramana CV, Rao PR. Environmental regulation for optimal biomass yield and photoproduction of hydrogen by *Rhodobacter sphaeroides* O.U.001. Int J Hydrogen Energy 1991;16:597–601.

[2] Koku H, Eroglu I, Gündüz U, Yücel M, Türker L. Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides* O.U. 001. Int J Hydrogen Energy 2002;27:1315–29.

[3] Wakayama T, Miyake J. Light shade bands for the improvement of solar hydrogen production efficiency by *Rhodobacter sphaeroides* RV. Int J Hydrogen Energy 2002;27:1495–500.

[4] Nakada E, Nishikata S, Asada Y, Miyake J. Light penetration and wavelength effect on photosynthetic bacteria culture for hydrogen production. In: Zaborsky OR, editor. Biohydrogen. New York: Plenum Press; 1998.

[5] Kitajima Y, El-Shishtawy RMA, Ueno Y, Otsuka S, Miyake J, Morimoto M. Analysis of compensation point of light using plane-type photosynthetic bioreactor. In: Zaborsky OR, editor. Biohydrogen. New York, Plenum Press; 1998.

[6] Wakayama T, Miyake J, Toriyama A, Kawasugi T, Arai T, Asada Y. Photohydrogen production using photosynthetic bacterium *Rhodobacter sphaeroides* RV; simulation of the light cycle of natural sunlight using an artificial source. In: Zaborsky OR, editor. Biohydrogen. New York: Plenum Press; 1998.

[7] Biebl H, Pfennig N. Isolation of members of the family Rhodosprillaceae. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, editors. The prokaryotes, vol. 1. New York: Springer; 1981. p. 267–73.

[8] Koku H, Eroglu I, Gündüz U, Yücel M, Türker L. Kinetics of biological hydrogen production by the photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001. Int J Hydrogen Energy 2003;28:381–8.

[9] Sasikala C, Ramana VC, Raghuveer P. Regulation of simultaneous hydrogen photoproduction during growth by pH and glutamate in *Rhodobacter sphaeroides* O.U. 001. Int J Hydrogen Energy 1995;20(2):123–6.

[10] Hoekema S, Bijmans M, Janssen M, Tramper J, Wijffels R. A pneumatically agitated flat-panel photobioreactor with gas recirculation: anaerobic photoheterotrophic cultivation of a purple non-sulfur bacterium. Int J Hydrogen Energy 2002;27:1331–8.

[11] Eroglu E, Gündüz U, Yücel M, Türker L, Eroglu I. Photobiological hydrogen production by using olive mill wastewater as a sole substrate source. Int J Hydrogen Energy 2004;29(2):163–71.

[12] Clayton RK. Spectroscopic analysis of bacteriochlorophylls in vitro and in vivo. Photochem Photobiol 1966;5:669–77.

[13] Miyake J, Wakayama T, Schnackenberg J, Arai T, Asada Y. Simulation of the daily sunlight illumination pattern for bacterial photo-hydrogen production. J Biosci Bioeng 1999;88(6):659–63.

[14] Gurgun V, Kirschner G, Pfennig N. Fermentation of pyruvate by 7 species of phototrophic purple bacteria. Z Allg Mikrobiol 1976;16(8): 573–6.

[15] Uffen RL, Wolfe RS. Anaerobic growth of purple non-sulfur bacteria under anaerobic dark conditions. J Bacteriol 1970;104:462–72.