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Simulation Study of the Effect of Temperature and of Light Intensity on Biohydrogen Production by *Rhodobacter Capsulatus*

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ABSTRACT

In this work, a theoretical analysis of the photo-fermentation for bio-hydrogen production is established. For this objective, a mathematical model including bacterial growth, kinetic of substrate and of hydrogen formation was used to highlighting the ability of Rhodobacter capsulatus for biohydrogen production in batch mode reactor. The simulation study of the photo-fermentation bioprocess shows that the bio-hydrogen productivity is strongly affected by temperature and by light intensity. It was found that the bacterial growth is optimal at high temperature 38 °C, whereas an inhibitor effect on hydrogen generation was detected in the same condition. In the other hand, it is noteworthy that high light intensity increases the performance of biohydrogen production. In addition, it was found that under the investigated conditions, the bacteria convert preferentially lactic acid compared to acetic acid.

Key words: Biohydrogen, Temperature, Light intensity, Rhodobacter capsulatus, Photo-Fermentation

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1. INTRODUCTION

Our energy requirements are almost totally provided from carbon-containing fossil sources, such as oil, coal and natural gas. Unfortunately, these ones cause serious environmental problems during combustion, such as acid rain, carbon dioxide emissions and climate changes. Moreover, oil, coal and natural gas are finite resources, and their consumption is much faster than their formation. In addition to these destructive environmental constraints, we add the organic solid wastes discharged from various sources which cause serious environmental pollution. It should be noted that their treatment presents an economic challenge. Thus the conversion of these wastes to energy could be considered a sustainable waste management strategy by various waste-to-energy technologies such as biological hydrogen production processes.

Currently, hydrogen is one of the future energy vectors and can be an alternative energy to fossil energies. It can be considered as an energy carrier that has been proved to be one of the best fuels for transportation. In addition, the combustion of hydrogen produces only water vapour without carbon oxide, and since it can be produced without causing any environmental problems, hydrogen [1, 2] as a future fuel has been drawing more and more attention. It can be produced by several methods, namely the chemical and biological processes. Among the biological routes for biohydrogen production, the photo or dark-fermentation of organic substrates is considered of great importance [1, 3-5] in the energy industry. The non-photosynthetic bacteria are usually, the dominants for hydrogen producing by dark-fermentation, while in photo-fermentation, the photosynthetic or some photo-heterotrophic bacteria are capable to convert organic acids such as acetic, lactic and butyric to hydrogen and other by-product (carbon dioxide) under anaerobic conditions in the presence of light. Furthermore, photo-fermentative hydrogen production is one of the feasible options for the effective management of organic solid wastes into clean energy. This method is less polluting and gainful for hydrogen production. In addition the biohydrogen which can be produced by fermentation bioprocesses using an appropriate bacterium can be used for a hydrogen bio-fuel cell for generating electricity. *Rhodobacter* species are photosynthetic that can produce hydrogen from small-chain organic acids derived from biomass. Nevertheless,

biohydrogen production by fermentation process can be affected by several operating parameters [6,7] which make it complex. This work deals with a numerical study of the performances of the bioprocess of photo-fermentation carried out in a batch reactor for biohydrogen production. Indeed, an analysis of the effect of the key parameters such as temperature and light intensity is established for *Rhodobacter capsulatus* by use of acetic and lactic acids as substrates in a batch mode bioreactor.

2. MATERIALS AND GROWTH CONDITIONS

Different types of agricultural residues can be used for bio-hydrogen production [8] by using photo-fermentation process. Photo-fermentative hydrogen production is generally carried out by prokaryotic microorganisms called purple non-sulfur photosynthetic bacteria (PNSB), in which are effective for hydrogen production from different kinds of substrate [9]. The strains used for photo-fermentation hydrogen production in this study and their characteristics are taken from Sevinç et *al* [10]. It is characterized by a rapid growth in the exponential phase. Acetate, lactate and glutamate constitute the carbon and nitrogen source respectively; they were utilized [11] for biosynthesis, growth and hydrogen production. The medium contained a mixture of 40mM acetic acid, 7.5mM lactic acid and 2mM glutamate. The photo-fermentation bioreactoris in the form of a transparent circular glass bottle of V=55 ml of volume containing 50 ml of culture. The culture media was inoculated with 10% bacteria. The photo-fermentation bioreactor is maintained at a constant incubation temperature (Nuve, ES250) and it was illuminated by 100W tungsten lamps. The light intensity was measured with a luxmeter (Lutron LX-105 Light Meter) [10].

3. MATHEMATICAL MODEL AND CALCULATIONS

In this study, a mathematical approach was used to analyze the impact of temperature and light intensity on biohydrogen production and bacterial growth using a photo-fermentative bacterium performed in a batch mode reactor. The substrates consumption was also analyzed under the investigated conditions. The quantitative description of photo-fermentative hydrogen production seems to be quite complex, due to the large number of parameters that have to be taken into account. Simple models such as Monod kinetics and the Gompertz equation have been used in this work. The modified Gompertz model was employed to describe the cumulative hydrogen production. For describing bacterial growth and substrates consumption, the following generalized expression was used to express the mass balance in the reactor of volume (V):

$$[Input] + [Production] = [Output] + [Accumulation] \quad (1)$$

a) Microbial growth

Based on equation 1, microbial growth can be written as:

$$F_{in} + \mu.V = F_{out} + \frac{d(X.V)}{dt} \quad (2)$$

Here, X is the bacterial concentration and t is the time and μ is the specific growth rate.

For batch reactors, the volume is constant so,

$\frac{dV}{dt} = 0$ and $F_{in} = F_{out}$.

So, the rate of growth is expressed by the following equation:

$$\frac{dX}{dt} = \mu \quad (3)$$

The following logistic model [12] was used for evaluating μand to model the growth.

$$\mu = X.k_c\left(1 - \frac{X}{X_{max}}\right) \quad (4)$$

The growth rate is expressed as:

$$\frac{dX}{dt} = k_c X\left(1 - \frac{X}{X_{max}}\right) \quad (5)$$

k_c is the apparent rate of specific growth and X_{max} is the maximal concentration.

Initial condition: at$t = t_0 = 0, X = X_0$,

WhereX_0 is the initial bacterial concentration (g/l).

The following data (Tables 1, 2 and 3) taken from Sevinç et *al*. [10] are used in simulation run to examine the effect of temperature and light intensities on microbial growth.

Table -1 Constants of *Rb. capsulatus* at 20 °C and at different light intensities

Parameter	Light intensity (lux)				
	1500	2000	3000	4000	5000
$X_0\ (g\ l^{-1})$	0.206	0.182	0.113	0.113	0.180
$X_{max}\ (g\ l^{-1})$	0.859	0.795	0.585	0.591	0.513
$k_c(h^{-1})$	0.022	0.023	0.053	0.050	0.053

Table -2 Constants of *Rb. capsulatus* at 30 °C and at different light intensities

Parameter	Light intensity (lux)				
	1500	2000	3000	4000	5000
$X_0\ (g\ l^{-1})$	0.158	0.158	0.153	0.127	0.193
$X_{max}\ (g\ l^{-1})$	0.858	0.729	0.721	0.655	0.868
$k_c(h^{-1})$	0.059	0.057	0.059	0.074	0.066

Table -3 Constants of *Rb. capsulatus* at 38 °C and at different light intensities

Parameter	Light intensity (lux)				
	1500	2000	3000	4000	5000
$X_0\ (g\ l^{-1})$	0.118	0.169	0.166	0.162	0.197
$X_{max}\ (g\ l^{-1})$	0.938	1.044	1.071	1.070	1.071
$k_c(h^{-1})$	0.066	0.045	0.057	0.054	0.040

b) Biohydrogen generation

To evaluate cumulative hydrogen production, a model of modified Gompertz equation is used. Then, the productivity can be expressed as follows [13]:

$$H = H_{max}\, exp\left\{-exp\left[\frac{R_{max}\, e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (6)$$

λ is the lag phase (hr) of biomass growth and the constant $e = \exp(1) = 2.71828$.

H_{max} is the maximal cumulative hydrogen production, R_{max} is the maximal hydrogen productivity and t is the incubation time. The effect of temperature, of light intensities and of time incubation was examined in this study. The different parameters relative to the Modified Gompertz Model [14] are summarized in the following tables 4, 5 and 6.

Table -4 Modified Gompertz Model parameters at 20 °C and at different light intensities

Parameter	Light intensity (lux)				
	1500	2000	3000	4000	5000
$H_{max}\ (mmol\ l^{-1})$	30	37.4	39.9	43.3	57.8
$R_{max}\ (mmol\ l^{-1})$	0.22	0.39	0.34	0.30	0.43
$\lambda(h)$	118	54	56	47	17

Table -5 Modified Gompertz Model parameters at 30 °C and at different light intensities

Parameter	Light intensity (lux)				
	1500	2000	3000	4000	5000
$H_{max}\ (mmol\ l^{-1})$	37.0	59.1	63.2	59.1	58.7
$R_{max}\ (mmol\ l^{-1})$	0.44	0.56	0.51	0.48	0.49
$\lambda(h)$	42	40	36	38	23

Table -6 Modified Gompertz Model parameters at 38 °C and at different light intensities

Parameter	Light intensity (lux)				
	1500	2000	3000	4000	5000
$H_{max}\ (mmol\ l^{-1})$	21.8	31.4	36.1	32.5	29.0
$R_{max}\ (mmol\ l^{-1})$	0.16	0.38	0.29	0.43	0.22
$\lambda(h)$	36	44	27	33	34

d) Substrate consumption

The mass balance for both acids can be expressed by the following expression:

$$F_{in} - v.X.V = F_{out} + \frac{d(S.V)}{dt} \quad (7)$$

In batch mode and at a constant volume, we obtain:

$$\frac{dS}{dt} = -v.X \quad (8)$$

Assume Monod kinetic:

$$v = \frac{v_m S}{k_m + S} \quad (9)$$

So, it was obtained:

$$\frac{dS}{dt} = -\frac{v_m . S}{k_m + S} X \quad (10)$$

Initially, acetic and lactic acid were used as substrates in this study where the reactions for hydrogen formation are given as follows [15]:

$$C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (11)$$

$$C_3H_6O_3 + 3H_2O \rightarrow 6H_2 + 2CO_2 \quad (12)$$

Theoretically four and six moles of hydrogen can be generated from one mole of acetic and lactic acid, respectively. In this work concentrations of the following by-products (formic acid, butyric acid and propionic acid) are not taken into account. Because it is known that for the irreversible processes, the rate equation becomes simpler. The consumption was supposed follow a first order kinetics [14]. Then the concentration of both acids is given by the following equation:

$$\frac{dS}{dt} = -k.S \quad (13)$$

k is the rate constant given as fuction of temperature according to Arrhenius equation.

$$k = k_0 \exp(-E_a/RT) \quad (14)$$

Where, $k_0$is a constant,E_a is the activation energy, R is the universal gas constant and T is the temperature in Kelvin.

By comparison of equation 10and equation 13, we obtain that the rate constants of lactic and acetic acids:

$$k_{lactic} = \frac{v_m . X}{k_m + S_{lactic}} \quad (15)$$

And

$$k_{acetic} = \frac{v_m . X}{k_m + S_{acetic}} \quad (16)$$

So, the equations gouverning the consumption of lactic and acetic acids are respectivelly, as follows:

$$\frac{dS_{lactic}}{dt} = -k_{lactic}\, S_{lactic} \quad (17)$$

$$\frac{dS_{acetic}}{dt} = -k_{acetic}\, S_{acetic} \quad (18)$$

The integration of these equations from initial time (t_0) leads to predict the cocentration of acetic and lactic acid as function of initial concentration and time.

The effect of temperature, of light intensities and of time incubation was examined in this study for lactic and acetic acids. The initial concentrations at initial time (t_0) are $S^0_{lactic} = 7.5mM$ and$S^0_{acetic} = 40mM$.

The rate constants for lactic acid consumption at 20 °C, 30 °C, 38 °C and at different light intensity [10] are given in the following tables 7 and 8.

Table -7 Rate constants (k_{lactic}) for lactic acid consumption

Temperature(°C)	**Light intensity (lux)**				
	1500	2000	3000	4000	5000
20	0.0134	0.0273	0.0223	0.0264	0.0219
30	0.0306	0.0209	0.0320	0.0272	0.0273
38	0.0381	0.0337	0.0248	0.0294	0.0228

Table -8 Rate constants (k_{acetic}) for acetate acid consumption

Temperature (°C)	**Light intensity (lux)**				
	1500	2000	3000	4000	5000
20	0.0106	0.0139	0.0131	0.009	0.012
30	0.0167	0.0105	0.0428	0.0125	0.0147
38	0.0138	0.0192	0.0154	0.0107	0.0142

4. SIMULATION RESULTS AND DISCUSSIONS

In this work, a mathematical model was developed for simulation of a batch mode bioreactor. The obtained set of equations is solved numerically by the Runge-Kutta method [16] performed on Matlab Software. Since the photo-fermentation process is an enzymatic process using PNS bacteria that are strongly affected by several parameters. Indeed, the effect of temperature and light intensity on bacterial growth, biohydrogen production and substrate consumption were numerically studied.

4.1. Effect of temperature on bacterial growth and on biohydrogen production

The main purpose of this work is to determine the influence of temperature and of the intensity of light on bacterial growth and on biohydrogen production. Several studies [17] have shown that temperature has even a pronounced effect on microbial activity. The obtained results from the logistic model are shown in the Figure1. It should be noted that the temperature has a significant effect on the growth of *Rhodobacter capsulatus* and it exhibits a better growth at 38 °C and 30 °C. However, it presents a poor growth at 20 °C. This environment mesophilic bacterium needs or requires a temperature of a range of 30 and 35°C for its growth. The results presented in Figure 2 correspond to the modified Gompertz model that shows the evolution of biohydrogen levels at different temperatures. The biohydrogen being produced by this bacterium follows an exponential way. Regarding the effect of temperature on the concentration of the bio-hydrogen, it was found that the production of bio-hydrogen is important at 20 and 30 °C. By increasing the temperature up 38 °C, there was a decrease of biohydrogen. Temperatures below 21°C and above 33°C had a negative effect on productivity; yields decreased highly in many bacteria species [17, 18]. It should be noted that the production rate of hydrogen changes differently with the used temperatures. Generally, low or high temperatures can affect the response of a microorganism by direct or indirect manner. Direct effects consist of decreased of growth rate, enzyme activities, alteration of cell composition and differential nutritional supplies. The indirect effects concern the solubility of solute molecules, diffusion of nutrients, osmotic effects on membranes and cell density [18]. Cell growth and hydrogen formation both occur during photo-fermentative hydrogen production. Since hydrogen is produced by cells, increasing the number of cells should increase the hydrogen produced.

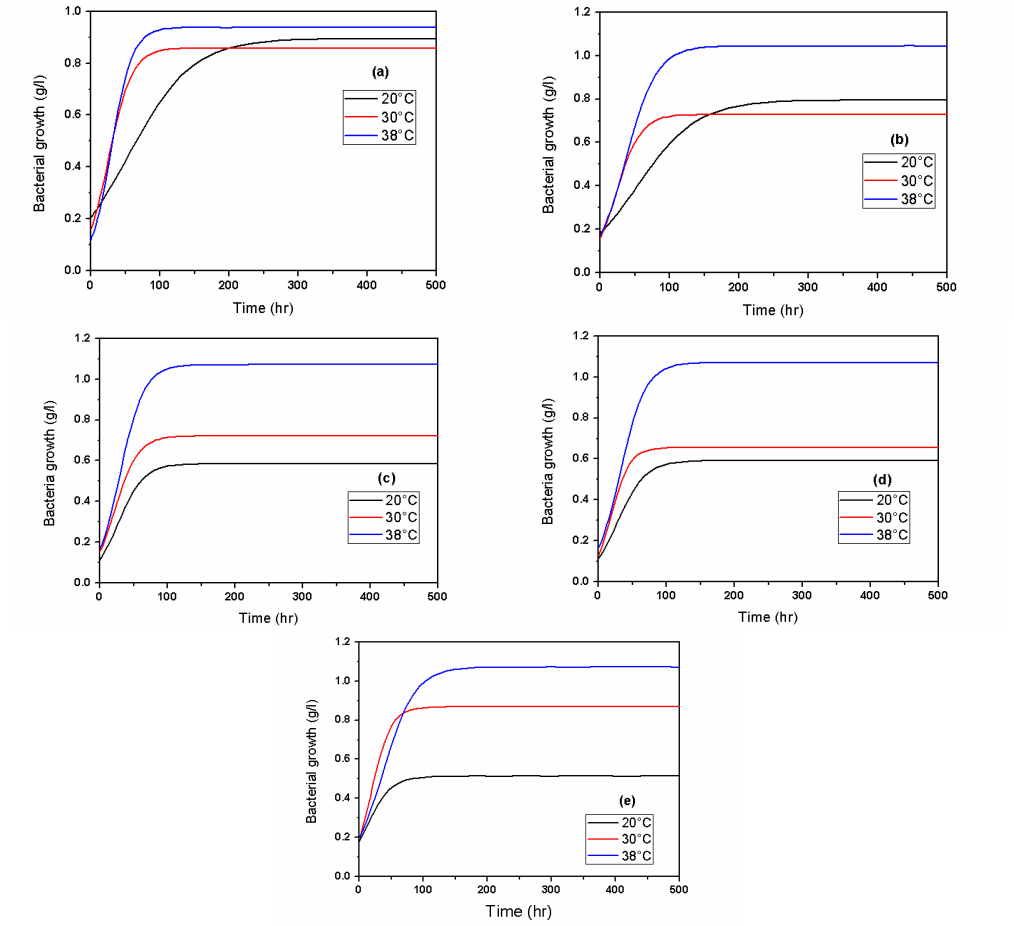

Fig. 1 Temperature effect on *Rhodobacter capsulatus* growth at different light intensities: (a): 1500lux, (b): 2000lux, (c): 3000lux, (d): 4000lux and (e): 5000lux.

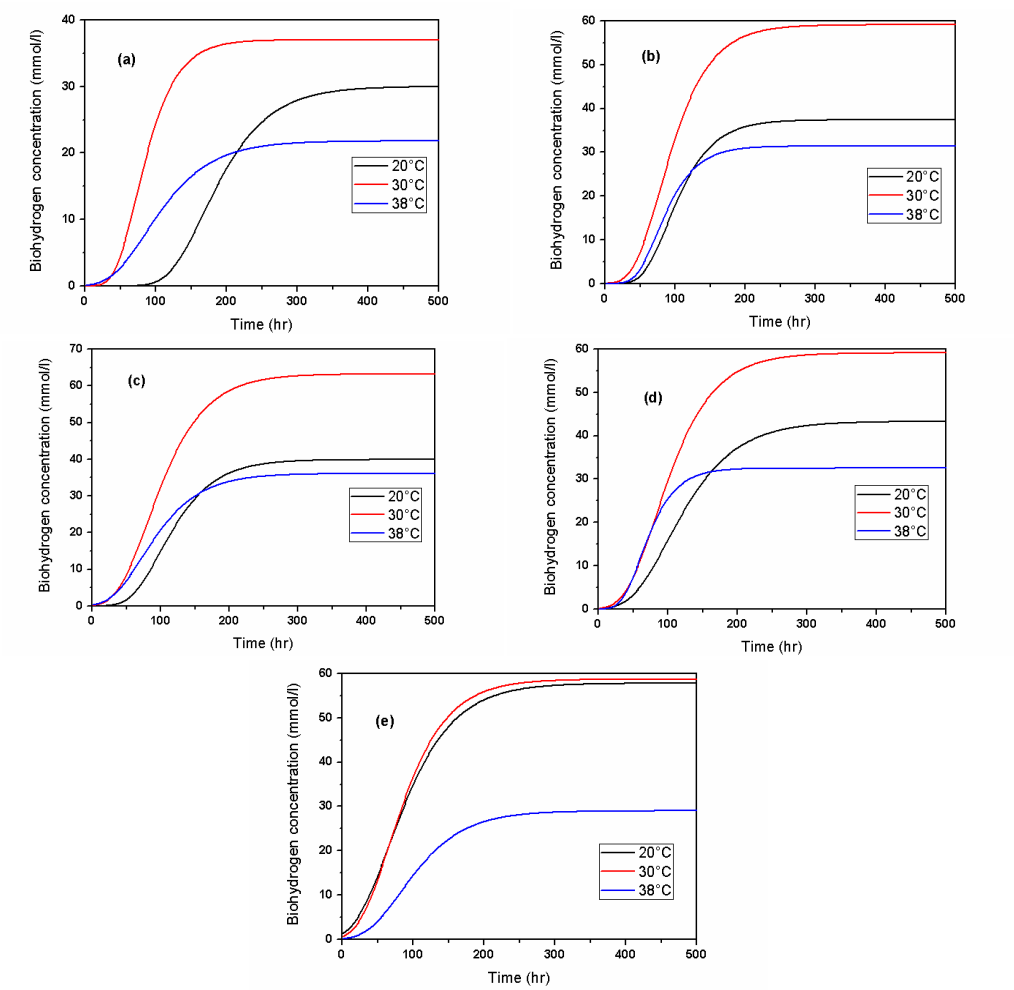

Fig. 2 Biohydrogen production at different temperatures and at different light intensities in *Rhodobacter capsulatus;* (a): 1500lux, (b): 2000lux, (c): 3000lux, (d): 4000lux and (e): 5000lux

4.2. Effect of light intensity on bacterial growth and biohydrogen production

Light intensity is one of the most important factors that affect hydrogen production by PNS bacteria. Hydrogen production by PNS bacteria is mediated by nitrogenase enzyme and the required energy for hydrogen production is provided by the conversion of light energy to ATP by photosynthetic membrane apparatus. It is known that the photo-fermentative hydrogen production is a microbial process in which electrons and protons generated through oxidation of organic compounds are used to produce molecular hydrogen under anaerobic, nitrogen-limited conditions, utilizing light as energy source.

Results of the simulation study of the effect of the light intensity on bacterial growth are shown in Figure 3. The kinetic growth varied also exponentially with the used values of the light intensities. It should be noted that the light intensity has a significant impact on biohydrogen bacterial growth and especially on productivity. Bacterial growth is important when the light intensity is up1500lux. It is obvious that bacterial growth (*Rhodobacter capsulatus*) is optimal at low light intensity. In the other hand, produced biohydrogen level achieved high values at high light intensity (5000lux) as shown in Figure 4. It is noteworthy that biohydrogen synthesis and bacterial growth didn't take the same behaviour. Hydrogen production and maximum biomass values appear to be in opposite variations. Increasing light intensity resulted in an obvious increase in hydrogen production in comparison to 1500lux exposure at 30°C. These results are in consistence with previous studies [19]. For *Rhodobacter sphaeroides* for example, the rate of hydrogen production increased with increasing light intensity up to 4000lux at 30°C [20]. Furthermore, the rate of hydrogen production increases highly when light intensity is above 1000lux than lower, and the activity of bacteria were improved drastically. As to 1200lux, 1600lux and 2000lux, the increase of hydrogen production rate is not significant. The rate of hydrogen production reached a maximum at approximately 1600lux. These may be indicating that the positive impact of the bacteria on

activity of hydrogen production will decline when light intensity increases to a certain level. The effect of light intensity on rate and on the amount of hydrogen production decreases gradually and any increase in light intensity does not have any effect while light intensity reaches a certain value [21].The growth and hydrogen production of photo-fermentation bacteria need to apply energy by light condition. So, light intensity also was an important limiting factor for photo-hydrogen production. For example, the optimum light intensity of *Rhodopseudomonas* RLD-53 strain for hydrogen production was at 3000-5000lux. At low light intensities, the hydrogen production decreases significantly and the biomass growth decreases moderately. Some studies show that the hydrogen production by *Rhodobacter sphaeroides* was better whereas biomass growth was slow. Furthermore, increasing light intensity in the infrared region can result in a significant increase in photo-biologically generated hydrogen [21]. So, an optimal light utilization and optimal penetration of light in the photo-bioreactor being essential for achieving high yield of hydrogen production by phototrophic bacteria [22].

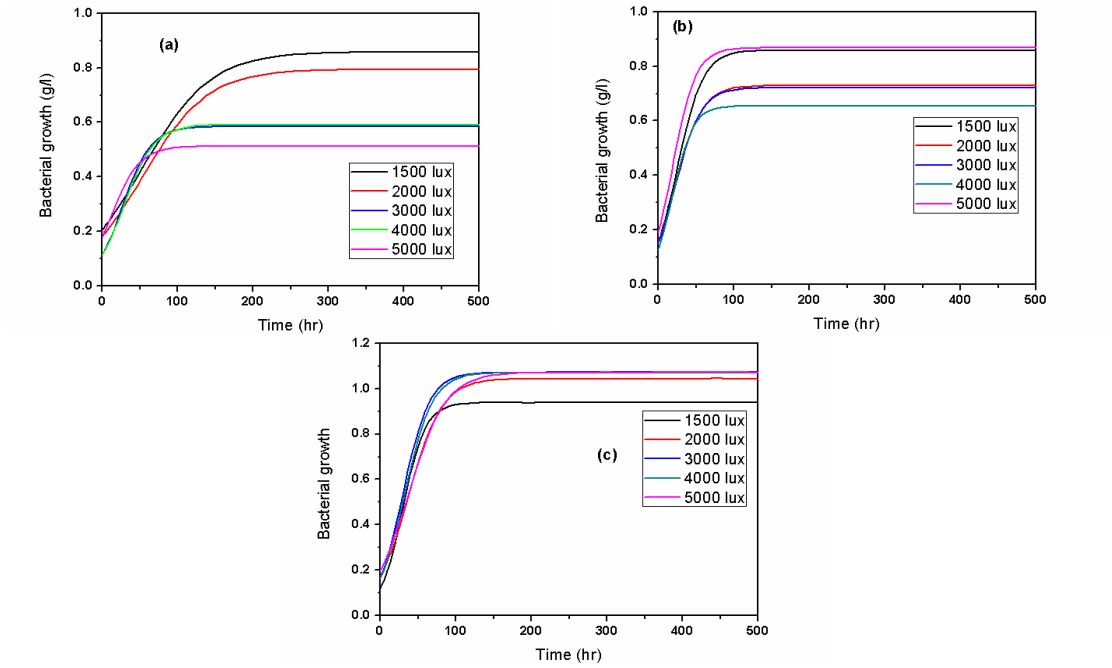

Fig. 3 Bacteria growth at different light intensities in *Rhodobacter capsulatus;* (a):20°C, (b):30°C and (c):38°C

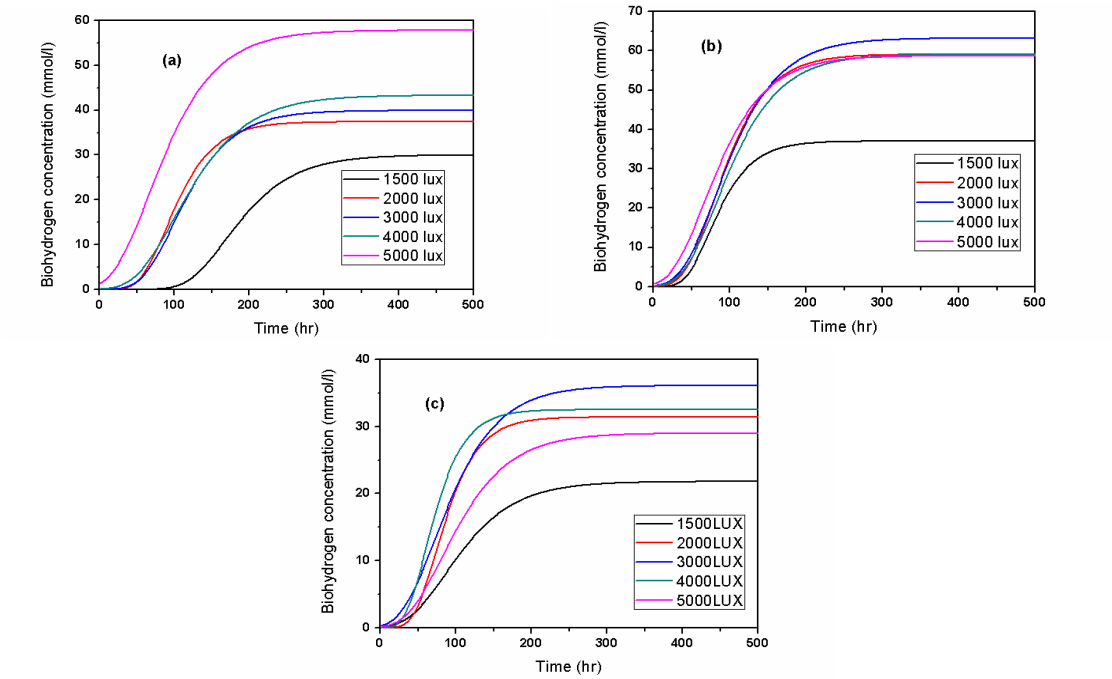

Fig. 4 Biohydrogen production at different light intensities in *Rhodobacter capsulatus;*(a):20°C, (b):30°C and (c):38°C

4.3. Temperature and light intensities effect on organic acids consumption

It is known that the light availability is one of the most important factors influencing the activity of photosynthetic bacteria and substrates consumption. Generally, promoting or inhibiting effect is related to the applied light spectrum and intensity. Photo-fermentative hydrogen production refers to the microbial process, during which organic substrates are oxidized under anaerobic conditions in the presence of light, generating hydrogen and carbon dioxide. The metabolic pathway of the hydrogen production is affected by three external factors: carbon source, light and oxygen availability. PNSB can utilize many carbon sources such as sugars, short chain organic acids, amino acids, alcohol and polyphenols. Lactate and acetate are the carbon sources. Generally, concentrations of these substrates decrease with time as they are consumed during photo-fermentation. In the following the concentrations profiles for lactic and acetic acids are discussed. For the variation of lactic acid concentration as shown in Figure 5, it was found that the effect of the temperature on acid consumption is very significant only for the values of 1500, 2000 and 3000lux of the applied light intensities. For higher values of intensities such as 4000 and 5000lux, the effect of temperature is not significant and may consider negligible. Whereas for the variation of acetic acid concentration as shown in Figure 6, it was found that the effect of temperature is more remarkable when varying the light intensity. The increase of light intensity enhances the substrates consumption and consequently the biomass growth. Lactic and acetic acids were consumed by *Rhodobacter capsulatus* both during their growth as well as in hydrogen generation process. However, too intense light can lead to the photo-inhibition and decrease of reaction rate.

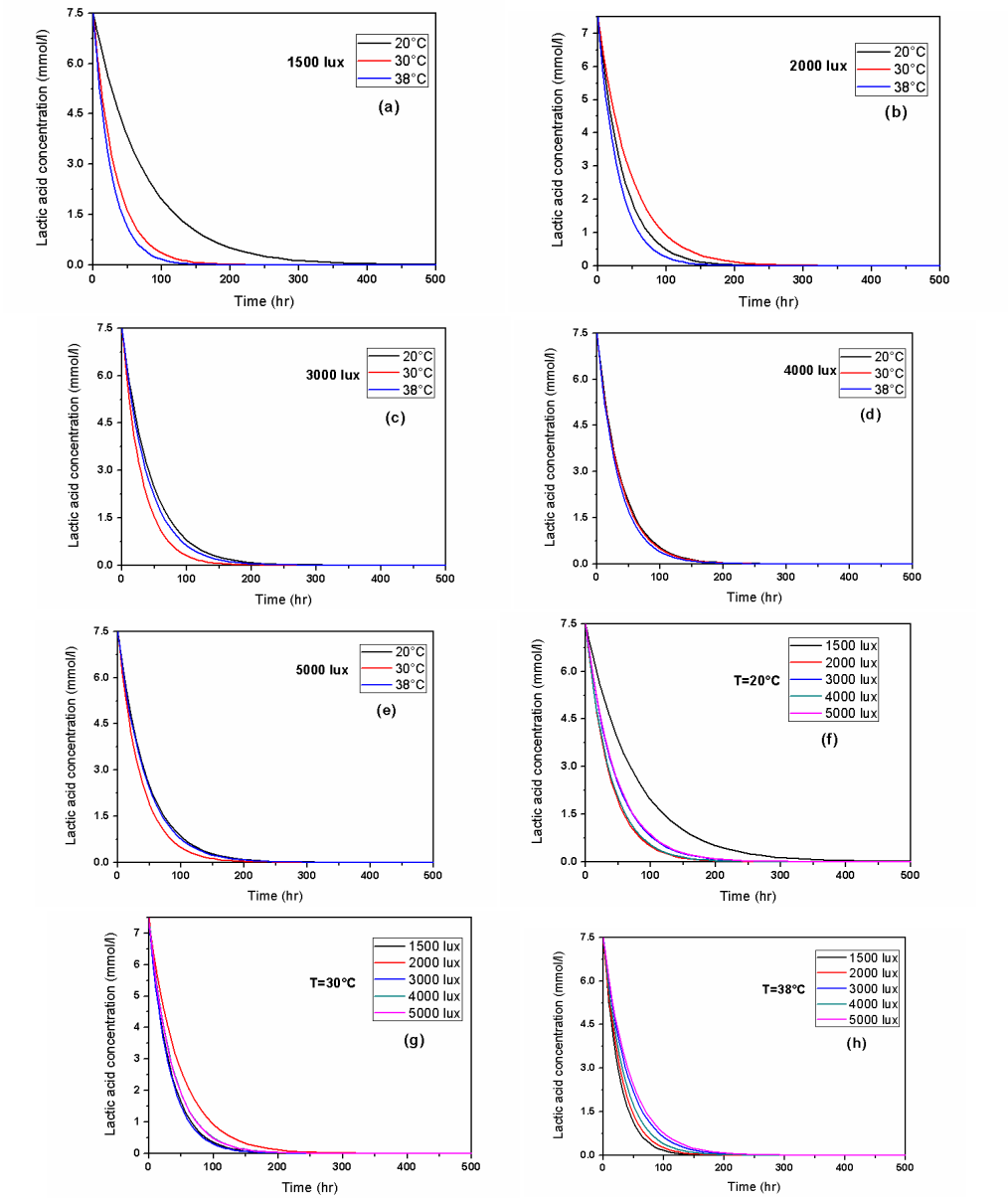

Fig. 5 Light intensities ((a), (b), (c), (d) and (e)) and temperature ((f), (g) and (h)) effect on lactic acid consumption

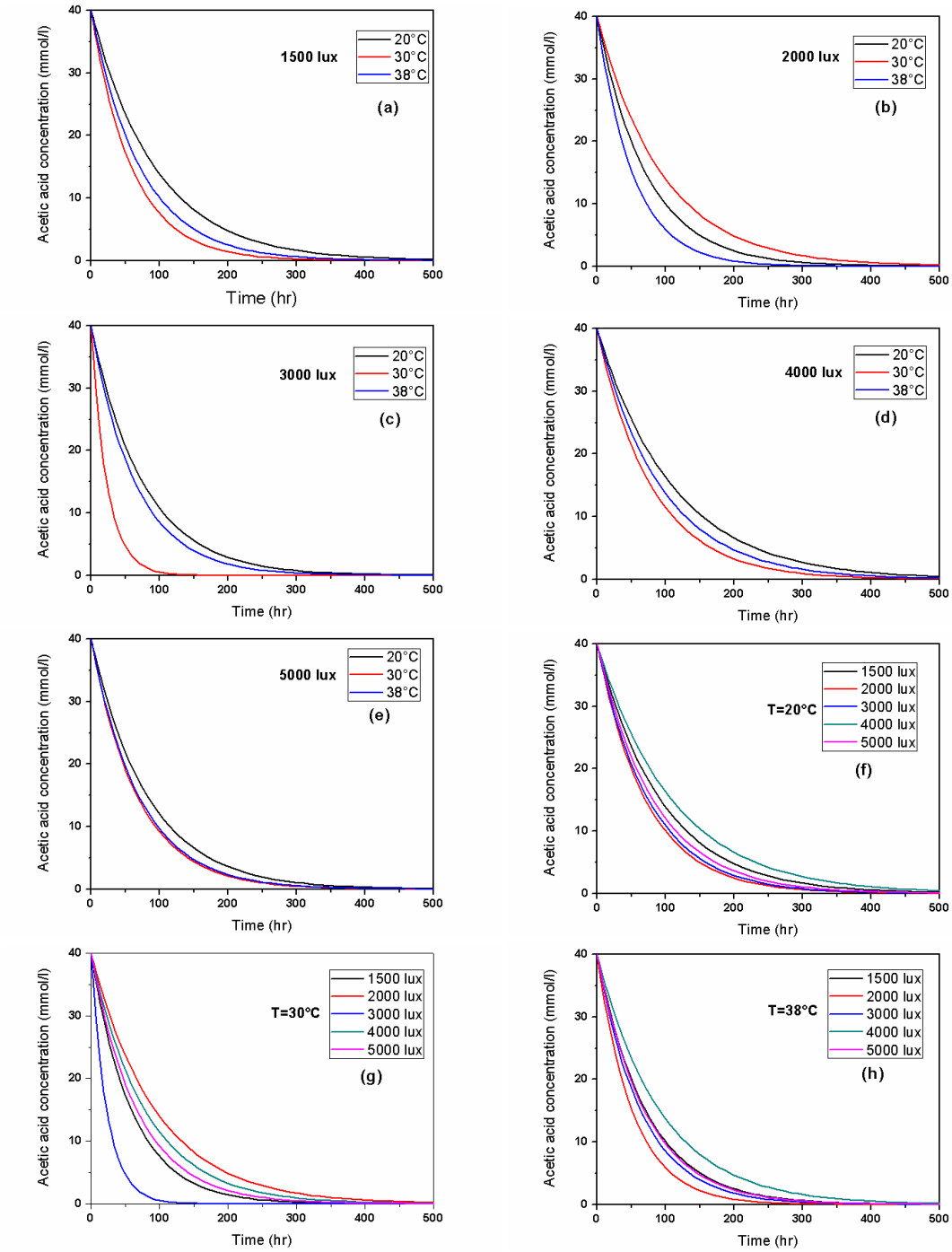

Fig. 6 Light intensities ((a), (b), (c), (d) and (e)) and temperature ((f), (g) and (h)) effect on acetic acid consumption

Finally, the obtained results at different temperatures and light intensities show that the consumption of lactic acid is very pronounced compared to the acetic acid. It was found that the bacteria use preferentially the lactic acid as substrate before acetic acid. This can be explained by the fact that the studied bacterium has suitable enzyme equipment for the degradation of lactic acid. Therefore, they do not need a long enough latency phase, and in addition the generation time is reduced.

5. CONCLUSION

The main obtained results, demonstrate the effect of temperature and light intensity on the performances of photo-fermentation process, in which concretized by bacterial growth and biohydrogen production by *Rhodobacter capsulatus* as principal metrics. It was found that the two measured metrics depend strongly on temperature and light intensity. A temperature of 30°C constitutes the ideal one for the both parameters namely for bacterial growth and for biohydrogen

generation. In the other hand, light intensity plays a critical role especially on biohydrogen yield. Our findings confirm that the high intensity influences positively on the productivity. Under the investigated conditions, the use of 5000lux illumination gives high levels of biohydrogen comparatively to the other ones. The study of cell growth and hydrogen production kinetics of *R. capsulatus* may provide an insight for further studies and guide for large scale hydrogen production processes. Therefore, the organic acids produced during the acidogenic phase of anaerobic digestion of organic wastes can be converted to hydrogen and carbon dioxide by using similar photosynthetic anaerobic bacteria. *Rhodobacter capsulatus*, which is a typical purple nonsulfur photosynthetic bacterium, is able to produce hydrogen under photosynthetic condition**.**

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