STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LIV, Sp.Iss. 2, 2009

BIOHYDROGEN PRODUCTION WITH PHOTOSYNTHETIC BACTERIA

ÉVA HARAI MOLNOS[a], SZABOLCS SZILVESZTER[a], BEÁTA ÁBRAHÁM[b], IOSIF NAGY[a], SZABOLCS LÁNYI[b], OVIDIU MUNTEAN[a]

ABSTRACT. The hydrogen obtained through biological processes, using anaerobic or photosynthetic bacteria, is called biohydrogen and is considered to be the energy of future. In the presence of light, the photosynthetic bacteria are able to use organic compounds as carbon source while liberating hydrogen. The aim of our research is to study the photoheterotroph fermentation process and to optimize the parameters to obtain higher conversion rate.

Keywords: biohydrogen, photoheterotroph fermentation, organic acid, photo-reactor, kinetic model

INTRODUCTION

Since hydrogen, respectively biohydrogen is a clean source of energy, it is considered to be the „energy of future". It has the highest energy content per mass (122 MJ/kg) [1], its combustion produces water, which is not detrimental to the environment. It is an energy carrier (a secondary source of energy), which is used to move, store and deliver energy in an easily usable form. Therefore, future energy technology will use hydrogen with an increasing trend in steady as well as unsteady combustion processes [2]. As part of this increasing trend, new processes need to be developed for cost efficient hydrogen production.

Biological hydrogen production technologies are still under development, they provide a wide range of approaches, including direct biophotolysis, indirect biophotolysis, photofermentation, dark fermentation or a combination of these processes [2, 3, 4]. Among these bioprocesses, the photofermentation has attracted our attention, because it has relatively higher substrate-to-hydrogen yields, it is possible to use renewable sources, like biomass and/or some food industry wastewaters which contain high amount of carbohydrates and organic acids, in addition, the process can potentially be driven by solar energy with minimal non-renewable energy inputs [5].

[a] *Universitatea „Politehnica" din Bucureşti, Facultatea de Chimie Aplicată şi Ştiința Materialelor, Str. Polizu, Nr. 1-7, RO-011061 Bucureşti, Romania, www.chim.pub.ro*

[b] *Universitatea „Sapientia", Facultatea de Ştiințe Tehnice şi Sociale, Str. Libertății, Nr. 1, RO-530104 Miercurea Ciuc, Romania, www.csik.sapientia.ro*

Photofermentation is the fermentative conversion of organic substrate to biohydrogen manifested by a diverse group of photosynthetic bacteria. They produce hydrogen under anaerobic conditions with illumination either from reduced substrates such as organic acids (purple non-sulphur bacteria) or from reduced sulphur compounds (purple sulphur bacteria) [6].

Hydrogen-intensive research work has already been carried out on the advancement of biological processes, such as the development of genetically modified microorganisms, metabolic engineering, improvement of the reactor designs, use of different solid matrices for the immobilization of whole cells, biochemical assisted bioreactor, development of two-stage processes etc., for higher H_2 production rates [2, 7]. The combination of dark fermentation and photofermentation seems to be an ideal biohydrogen producing model leading to the highest theoretical and practical H_2 yield [1, 8]. However, in such systems, the photofermentation constitutes limiting step for the overall hydrogen producing process, because it has poor hydrogen production rate due primarily to slow growth of photosynthetic bacteria and low light conversion efficiency of photobioreactors [1].

Accordingly, the aim of the research is to unveil the photofermentative processes, and once understood, to find possibilities to enhance the biohydrogen production. In order to achieve this goal, cheap raw materials were chosen, for instance, wastewater with high organic content (in addition, the treatment of this wastewater proved to be difficult and expensive). As a first step in this quest, literature data and kinetic equations were used to predict the result of the hypothetic process. The central question to be answered is whether it is worth to carry on with this pathway or not? In this paper an empiric model is developed which describes the undergoing processes, with the aim of selecting the optimal operation parameters, necessary to design an improved photoreactor. This model makes possible to predict the kinetic behavior of bacterial growth, product formation and substrate consumption rate in given conditions.

RESULTS AND DISCUSSION

Kinetic model development for photofermentation

Kinetic models of the processes are based on material balances, which describe the behavior of the process components. In our case, these are substrates (*S*), biomass (*X*) and products (*P*).

Our goal is to study the photofermentative conversion of cheap raw materials with high amounts of different organic acids, like dairy wastewater, the effluent of fermentative processes realized by thermophil microorganisms, etc. Theoretically, the stoichiometric conversion of organic acids to hydrogen by photofermentative processes takes place according to the following hypothetical reactions: [9]

acetate: $C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2$

lactate: $C_3H_6O_3 + 3H_2O \rightarrow 6H_2 + 3CO_2$

malate: $C_4H_6O_5 + 3H_2O \rightarrow 6H_2 + 4CO_2$

The model described below is based on the model proposed by Gadhamshetty et al. [5]. They developed a model which describes the dynamics of cell growth and the hydrogen evolution taking into account the dependence of growth and hydrogen formation on substrate concentration and light intensity, as well as the inhibitory effects of substrate, biomass and light intensity. In our model, the inhibitory effect of substrate to bacterial cell growth and product formation is neglected, despite the fact that inhibitory levels of substrate concentration can result in decreased hydrogen production. However, we assume that the concentration of the substrate intended to be used will not reach inhibitory levels.

Starting from that the bacteria use the substrate present in the media and converts it to products while the biomass concentration increases, the biomass growth can be described as follows. Theoretically, the **cell growth rate** is expressed as

$$\frac{dX}{dt} = \mu \cdot X \tag{1}$$

where X is the cell dry weight concentration (g/l) and μ is the specific growth rate (h^{-1}), which might depend on substrate concentration and other factors. Several models provide an expression for μ [5, 10], the Monod expression being the most common:

$$\mu = \frac{\mu_{\max} \cdot S}{K_S + S} \tag{2}$$

where μ_{max} is the maximum specific growth rate (h^{-1}), S is the substrate concentration (g/l) and K_S is the half saturation constant (the concentration of the rate-limiting substrate at which the specific growth rate is equal to half the maximum growth rate).

But the growth rate of photosynthetic bacteria is not only a function of substrate concentration, but it depends on the light intensity, too. At the same time, the cell growth rate may be inhibited at higher biomass concentration, due to reduction of light intensity inside the reactor, self-shading, and limiting substrate diffusion [5, 10]. So, kinetic models relating the three aspects (equation 3.) can be of value in designing the process:

$$\mu = \frac{\mu_{\max} \cdot S}{K_S + S} \cdot \left(1 - \frac{X}{X_{\max}}\right) \cdot \left(\frac{I}{K_{XI} + I + K_I \cdot I^2}\right) \tag{3}$$

where: X_{max} is the maximum value of the cell concentration (that level at which the cell growth will cease) (g/l), K_{XI} is the light saturation constant of cell formation (W/m^2), I is the light intensity (W/m^2) and K_I is the light inhibition constant of cell formation (m^2/W).

The kinetic expression for **product formation** is based on that product formation depends on both growth rate and instantaneous biomass concentration in a linear manner (equation 4):

$$\frac{dP}{dt} = Y_{PX} \cdot \frac{dX}{dt} + \mu_{PX} \cdot X \tag{4}$$

where P is the product concentration (g/l), Y_{PX} is the yield coefficient of product formation due to cell growth (g/g) and μ_{PX} is the specific formation rate of the product (h^{-1}).

The substrate type and its concentration are important factors in hydrogen evolution rates, additionally, higher light intensities can reduce the hydrogen production, but these inhibitory effects are already included in the cell growth rate expression. Therefore, in equation 5. is described only the inhibitory effect of the product to product formation (unlike the basic model):

$$\frac{dP}{dt} = \left(Y_{PX} \cdot \frac{dX}{dt} + \mu_{PX} \cdot X\right) \cdot \left(1 - \frac{P}{P_{\max}}\right) \tag{5}$$

where P_{max} is maximum product concentration (g/l).

To describe the **substrate consumption** during photofermentation, we have to take into consideration that substrate is necessary for bacterial cell growth, for product formation and for bacterial maintenance. Thus, the substrate consumption kinetics can be expressed by the equation:

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{XS}} \cdot \frac{dX}{dt} + \frac{1}{Y_{PS}} \cdot \frac{dP}{dt} + \mu_{SX} \cdot X\right) \tag{6}$$

where Y_{XS} is the yield coefficient of cells on substrate (g/g), Y_{PS} is the yield coefficient of product formation due to cell growth (g/g) and μ_{SX} is substrate consumption rate constant (h^{-1}).

In contrast to the basic model [5], the model proposed by us neither take into account the auto-inhibition by substrate.

The majority of the studies on biohydrogen production from organic waste have focused on the use of photosynthetic bacteria *Rhodobacter* sp. Therefore, batch experimental resulting kinetic parameters were selected from the literature, which are necessary to simulate the photofermentative processes with the proposed model. The selected parameters are listed in table 1.

Table1. Typical values of kinetic parameters found in the literature for *Rhodobacter* sp. (batch cultivation using malate as substrate)

Kinetic parameter		Used for simulation	References
Maximum growth rate, μ_{max}	0.09-0.17 h^{-1}	0.12	[5, 11]
Yield coefficient of hydrogen formation due to bacterial growth, Y_{PX}	2.45-3.2 gH_2/g malate	0.024	[5, 11]
Specific formation of the product, μ_{PX}	0.009 h^{-1}	0.009	[5]
Monod saturation constant, K_S	0.0912 g/l	0.0912	[5]
Light saturation constant of cell formation, K_{XI}	15-22 W/m^2	22	[5]
Light inhibition constant of cell formation, K_I	0.0001 m^2/W	0.0001	[5]
Yield coefficient of cells on substrate, Y_{XS}	3.7 g/g	3.7	[11]
Yield coefficient of products on substrate, Y_{PS}	0.492 g/g	0.09	[11]
Substrate consumption rate constant, μ_{SX}	0.091 h^{-1}	0.091	[11]
Maximal biomass concentration, X_{max}	1-1.8 g/l	1.8	[5]
Maximal product concentration, P_{max}	0.25-0.4 g/l	0.4	[5, 11]

The efficiency of conversion of light energy into hydrogen in the presence of an appropriate substrate and optimum cell growth conditions is a key factor for economic photofermentative biohydrogen production [5, 12]. The efficiency (η) of the conversion of light energy to hydrogen is determined as a ratio of the total energy value of the hydrogen that has been obtained to the total energy input to the photobioreactor by light radiation (equation 7.), and can be calculated using the equation 8. [13, 14]:

$$\text{Efficiency (\%)} = \frac{\text{Combustion enthalpy of hydrogen}}{\text{Absorbed light energy}} \cdot 100 \tag{7}$$

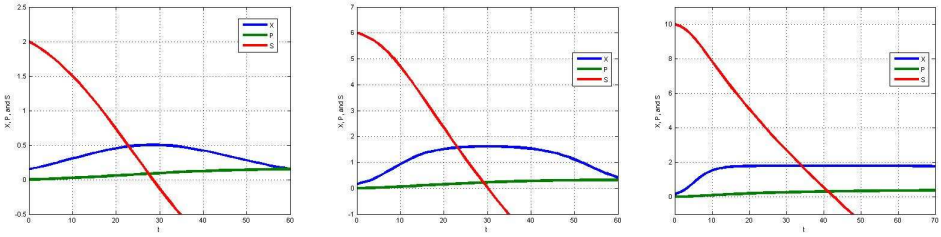
$$\eta = \frac{33.61 \cdot \rho_{H_2} \cdot V_{H_2}}{I \cdot A \cdot t} \cdot 100 \tag{8}$$

where 33.61 is the energy density of hydrogen gas (W·h/g), ρH2 is the density of the produced hydrogen gas (g/l), VH2 is the volume of produced H2 (l), I is the light intensity (W/m2), A is the irradiated area (m2) and t is the duration of hydrogen production (h).

Model simulation results

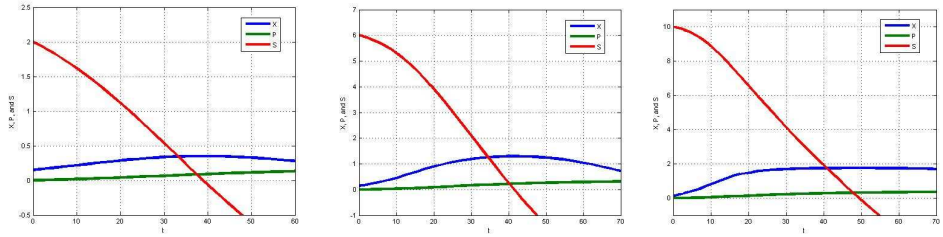
The three differential equations of the mathematical model were solved together using MATLAB software package (version 7.7.0.) to evaluate the biomass growth, product formation and substrate consumption rates. The initial conditions were the following: product concentration P = 0 g/l, biomass concentration X = 0.15 g/l, the value of substrate concentration varied between S = 2, 6 and 10 g/l and the value of light intensity varied too between 50 – 200 W/m^2. For modeling and simulation the parameters listed in table 1. were used. The values of Y_{PS} and Y_{PX} used for simulation differ significantly from the ones reported in literature, since theoretically the yield coefficient of hydrogen on malate has to be Y_{PS} = 12/134 = 0.09 g/g malate. Hence, the yield coefficient of hydrogen formation due to bacterial growth: $Y_{PX} = Y_{PS}/Y_{XS}$ = 0.09/3.7 = 0.024 g H_2/g bacterium.

The representative simulation results are presented in the figures below (Figure 1 – 4.).



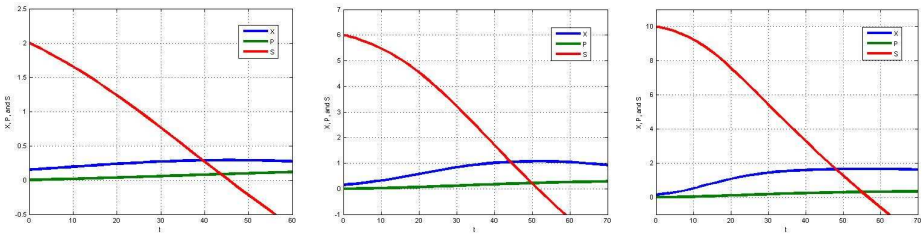
(A) (B) (C)

Figure 1. The variation of biomass (X), substrate (S) and product (P) concentrations at 50 W/m^2 light intensity when the initial substrate concentration was set to (A) 2 g/l, (B) 6 g/l, (C) 10 g/l



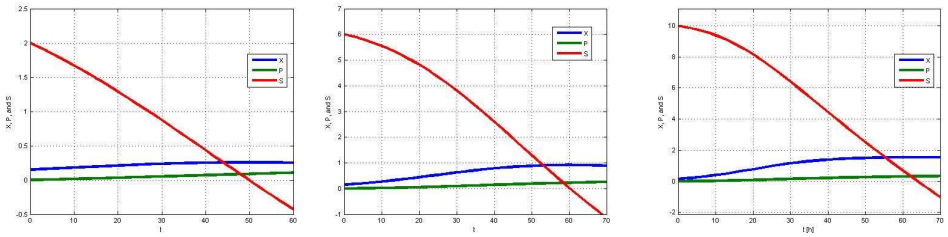
(A) (B) (C)

Figure 2. The variation of biomass (X), substrate (S) and product (P) concentrations at 100 W/m^2 light intensity when the initial substrate concentration was set to (A) 2 g/l, (B) 6 g/l, (C) 10 g/l



(A) (B) (C)

Figure 3. The variation of biomass (*X*), substrate (*S*) and product (*P*) concentrations at 150 W/m^2 light intensity when the initial substrate concentration was set to (A) 2 g/l, (B) 6 g/l, (C) 10 g/l



(A) (B) (C)

Figure 4. The variation of biomass (*X*), substrate (*S*) and product (*P*) concentrations at 200 W/m^2 light intensity when the initial substrate concentration was set to (A) 2 g/l, (B) 6 g/l, (C) 10 g/l

Taking into consideration the applied light intensity values, it can be observed that the obtained product concentration at 50 W/m^2 intensity was the lowest in all cases. Additionally, at 100, 150 and 200 W/m^2 light intensities, the obtained product concentration is nearly equal, so the most economical solution is to illuminate the bacterial strains with 100 W/m^2 light intensity. The same conclusion can be drawn from the comparison of substrate consumption rates: for instance, an initial concentration of 10 g/l is consumed in 60 h at a light intensity of 200 W/m^2, in 55 h at 150 W/m^2 and in 50 h at 100 W/m^2.

The highest biohydrogen concentration (0.4 g/l) was obtained at 10 g/l initial substrate concentration after 60 h fermentation. In this case the efficiency of conversion of light energy to hydrogen (calculated with equation 8.) is 0.23%. For comparison, the efficiency of conversion of light energy to hydrogen in case of 6 g/l initial substrate concentration was calculated, too. In this case, the highest biohydrogen concentration (0.2 g/l) was achieved after 40 h fermentation, so the calculated conversion efficiency is 0.17%. These conversion efficiency rates are too low for cost-efficient reactor operation. Thus, the model simulation results could provide the answer for the introductory dilemma regarding the future of this pathway.

CONCLUSIONS

The profitability of biohydrogen production processes with photosynthetic bacteria depends on the light to H_2 conversion efficiency. A possibility to increase this efficiency is, for example, to improve the photoreactor and to use cheap raw materials, such as organic compound-rich wastewater. For this, comprehensive knowledge is necessary about bacterial growth kinetics, light requirements of photosynthetic bacteria, light penetration in the photoreactor, about the processes which take place in the photoreactor.

The proposed model and the simulation results proved to be a reliable tool for process analyzing and are useful for making preliminary calculations of experimental parameters for the biohydrogen production. Also, the developed model may be used to determine optimal operation conditions and to design an improved photobioreactor.

REFERENCES

1. C.-Y. Chen, G.D. Saratale, C.-M. Lee, P.-C. Chen, J.-S. Chang, *International Journal of Hydrogen Energy*, **2008**, *33*, 6886.
2. A. Demirbas, "Biohydrogen–For Future Engine Fuel Demands", Springer Verlag, London, **2009**.
3. P.C. Hallenbeck, J.R. Benemann, *International Journal of Hydrogen Energy*, **2002**, *27*, 1185.
4. L. Gabrielyan, A. Trchounian, *International Journal of Hydrogen Energy*, **2009**, *34*, 2567.
5. V. Gadhamshetty, A. Sukumaran, N. Nirmalakhandan, M.T. Myint, *International Journal of Hydrogen Energy*, **2008**, *33*, 2138.
6. J. Rupprecht, B. Hankamer, J.H. Mussgnug, G.C. Dismukes, O. Kruse, *Applied Microbiology and Biotechnology*, **2006**, *72*, 442.
7. D. Das, N. Khanna, T.N. Veziroglu, *Chemical Industry & Chemical Engineering Quarterly*, **2008**, *14*, 57.
8. A.A. Tsygankov, *Russian Journal of General Chemistry*, **2007**, *77*, 685.
9. B. Uyar, M. Schumacher, J. Gebicki, M. Modigell, *Bioprocess and Biosystems Engineering,* **2009**, *32*, 603.
10. H. Koku, I. Eroglu, U. Gündüz, M. Yücel, L. Türker, *International Journal of Hydrogen Energy*, **2003**, *28*, 381.
11. I. Eroglu, K. Aslan, U. Gündüz, M. Yücel, L. Turker, *Journal of Biotechnology*, **1999**, *70*, 103.
12. M.J. Barbosa, J.M.S. Rocha, J. Tramper, R.H. Wijffels, *Journal of Biotechnology*, **2001**, *85*, 25.
13. E. Nakada, Y. Asada, T. Arai, J. Miyake, *Journal of Fermentation and Bioengineering*, **1995**, *80*, 53.
14. B. Uyar, I. Eroglu, M. Yücel, U. Gündüz, L. Türker, *International Journal of Hydrogen Energy*, **2007**, *32*, 4670.