МАТЕМАТИКА И МАТЕМАТИЧЕСКО ОБРАЗОВАНИЕ, 2007
MATHEMATICS AND EDUCATION IN MATHEMATICS, 2007
Proceedings of the Thirty Sixth Spring Conference of
the Union of Bulgarian Mathematicians
St. Konstantin & Elena resort, Varna, April 2–6, 2007

SYSTEM ANALYSIS OF HOMO–HETEROGENOUS MECHANISMS IN BIOFILM REACTOR*

Kliment I. Mladenov, Elitca M. Petrova, Ludmil N. Nikolov, Vjara S. Mamatarkova, Stoyan S. Stoytchev

At recent time the biofilm systems are studied using mathematical modeling which is the subject of the present work. This research presents the development of authors previous experience in the problems of formulation and improvement of the mathematical model of a biofilm system based on the information about the subsystems and on verbal model of oxidation of ferrous ions in biofilm reactors. Our investigations are realized by evolution strategy which consists in gradual complicating of the mathematical model by including additional terms. The model sensitivity is tested with respect to its parameters.

1. Introduction. Biofilm is a complex [1] and self organized system [2, 3] consisting of spontaneously fixed cells of microorganisms, plants or animals on interphase surfaces with specific dynamics of forming and functioning. Due to the important role of biofilms in nature as well as in the artificial systems, they are object of high interest of scientists and engineers [4]. The biofilms, formed on solid inert carriers, are entirely used in bioprocess systems in ecological engineering being the key part of tail gas and wastewater treatment technologies as well as in processes of hydrometallurgy [5]. They are also an object of intensive research in corrosion prevention and development of ecologically friendly industrial technologies, in medicine, transport, navy and building construction. [5, 6].

At recent time the biofilm systems are object of mathematical modeling due to the potentialities of this approach for studying the bioprocess mechanisms from system analysis point of view. There are scarce literature date about mathematical models of biofilm systems based on the knowledge about the main phenomena such as attachment, microorganism growth and death, change or lost of microbial activity during their residence in the biofilm, excretion of cells, formation of swimming aggregates etc. [1]. An attempt is made to formulate a verbal, and later – a mathematical model, of biofilm system reflecting these phenomena. The preliminary numerical experiment showed that mathematical model could exactly describe the features of a fed-batch processes of bacterial oxidation of ferrous ions in a biofilm formed by Acidithiobacillus ferrooxidans. The aim of the present study is to report a mathematical model which summarizes the interaction among

*This work is supported in part by NSF (Bulgaria) Grasnt TN-1502/2005.
2000 Mathematics Subject Classification: 92-99, 92B05
Key words: biotechnology, bioreactor, biofilm, bioprocess systems, mathematical modeling

different subsystems of the bioprocess system like Xsc, Xbf, S, P and the bioreactor parameters.

2. Bioprocess system. The main points in treating the bioprocess system are : stoichiiometry, bioreactor, biofilm systems and bioprocess essentials considered further .

2.1. Bioprocess stoichiometry. *Acidithiobacillus ferrooxidans* oxidizes the ferrous ions according to the following stoichiometric equation (1):

$$4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{Acidithiobac\ ferrooxidants} 2Fe_2(SO_4)_3 + 2H_2O \quad (1)$$

During the oxidation, bacteria form biofilm system which consists of microbial cells and mainly of jarosite - nonstoichiometric compound of the type $Me_xFe_y(SO_4)_{...}$

2.2. Bioreactor and biofilm system. The bioreactor (Fig. 1) plays key role for bioprocess performing and naturally, for data acquisition, especially when it concerns the investigations of main phenomena having place in the biofilms (Fig. 2). This device ensures appropriate conditions for biofilm formation, its development and functioning due to low shear stress and rather high values of external mass transfer coefficients. Its main shortcomings are lack of biofilm thickness control and limited specific surface of the carrier.

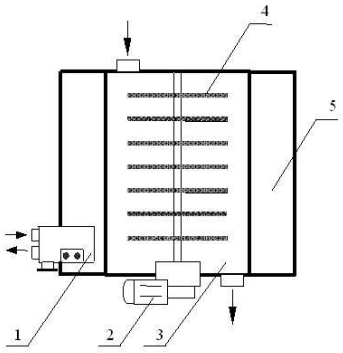

Fig. 1. Scheme of biodisk reactor 1 – thermostat system, 2 – electric motor with reductor, 3 – reaction volume, 4 – biodisks, 5 – thermostatic volume.

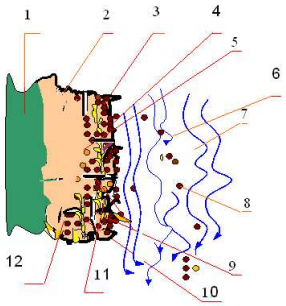

Fig. 2. Scheme of thin, young biofilm system formed on biodisc by Acidithipbacillus ferrooxidans during of first cycles (1–3 cycles). 1.Carryer; 2.Biofilm; 3. Strongly linked cells in the biofilm; 4. Weakly linked cells in the biofilm; 5. Dead cells; 6. Stream lines; 7. Substrate; 8. Swimming aggregates; 10. Suspended cells; 11. Pore;12. Closed area; 13. Exsopolysaccharides; 14. Jarosite.

2.3. Bioprocess essentials. The oxidation process is realized following the homo-heterogenous mechanism. It means that the cells live, grow, reproduce, transform ferrous ions to ferric ones, form the biofilm and die in the reaction zones of the biodisk reactor simultaneously both in the liquid and in the biofilm formed on the disks. During the oxidation, bacteria form biofilm system which consists of microbial cells and mainly of jarosite-nonstoichiometric compound of the type MexFey(SO4)z .

3. Mathematical model. Our mathematical model is built according to the following assumptions:

1) All the processes are realized without diffusion limitation, which allows to use for their mathematical description only equations of microbial kinetics. It means that the oxidation rate does not depend on hydrodynamics, heat- and mass-transfer.

2) The mass balance follows the stoichiometry (Eq. (1)).

3) The physiological constants of the swimming cells and those in the biofilm have the same numerical values.

4) In the first cycle, that is up to 24-th hour, swimming cells are in lag-phase. The attachment of the cells on the inert carrier begins in log-phase after 24-th hour.

5) There is an exchange of cells between the biofilm and the liquid phase in the bioreactor reaction zone, and there is an excretion of swimming aggregates from the biofilm.

6) The velocity of oxidation could be inhibited or not, by the product of the reaction, i.e. the ferric ions.

7) The microbial cells sink in biofilm constantly, loosing the possibility to be fed by the substrate. This is the reason for their dying.

8) Sedimentation rate of feric ions follows zero order kinetics.

The initial mathematical model of the process (Variant 1.) is a system of five ordinary differential equations, namely:

$$\frac{dX_{sc}}{d\tau} = \frac{\mu^S_{\max}}{K_M + S + K_I P} X_{sc} + K_{sc} F_{bf} X_{bf} - D_{fix} X_{sc},$$

$$\frac{dX_{bf}}{d\tau} = \frac{\mu^S_{\max}}{K_M + S + K_I P} X_{bf} - K_{sc} F_{bf} X_{bf} - K_d X_{bf} + D_{fix} X_{sc},$$

$$\frac{dX_{sa}}{d\tau} = \frac{\mu^S_{\max}}{K^+_M S + K_I P} X_{sa} + K_{sa} F_{bf} X_{bf},$$

$$\frac{dS}{d\tau} = -\frac{1}{Y_{x/s}} \frac{\mu^S_{\max}}{K_M + S + K_I P} \left(X_{sc} + X_{sa} + X_{bf} \right),$$

$$\frac{dP}{d\tau} = -\frac{dS}{d\tau} - K_{sed.}.$$

Further, the biofilm is investigated numerically during the initial phases of growth, i. e. using the data of the first three cycles. In this case it is supposed that there is no excretion of swimming aggregates and the system is reduced to four equations (the third equation is eliminated) – Variant 2.

The numerical and identification procedures based on experimental data, obtained from a biodisk reactor with a strain of Acidithiobacillus ferrooxidans in fed batch regime during 14 cycles, is described in details in [4]. Based on the obtained results, some

conclusions could be made, namely:

i) the physiological parameters (constants) of the thick biofilm model show very large variations and it is supposed that this is due to internal diffusion limitations, when the biofilm is rather thick;

ii) in the first three cycles of the process development the biofilm thickness is not more than 100μm, and obviously the processes occur without internal diffusion limitations.

Further on, the mathematical model is based on experimental data obtained in our previous studies having in mind the following considerations:

– the laboratory data from the biodisk reactor is used more effectively;

– only the first three cycles of experimental data, which express the bioprocess development without diffusion limitations, are observed.

On the base of these considerations an evolution strategy is realized.

The strategy consists of gradual complication of the mathematical model through adding additional terms. At first, we make difference in the phisiological constant $M_{\max}$ and K_M between swimming cells and biofilm , and later we assume differences in economical coefficients, as well.

Those changes are included in the third variant (Variant 3.) of the system of equations given below:

$$\frac{dX_{sc}}{d\tau} = \frac{\mu^{sc}_{\max} S}{K_M + S + K_I P} X_{sc} + K_{sc} F_{bf} X_{bf} - D_{fix} X_{sc} \tag{1}$$

$$\frac{dX_{bf}}{d\tau} = \frac{\mu^{bf}_{\max} S}{K^{bf}_M + S + K_I P} X_{bf} - K_{sc} F_{bf} X_{bf} - K_d X_{bf} + D X_{sc} \tag{2}$$

$$\frac{dS}{d\tau} = -\frac{1}{Y_{x/s}} \frac{\mu^{sc}_{\max} S}{K_M + S + K_I P} X_{sc} - \frac{1}{Y_{x/s_{bf}}} \frac{\mu^{bf}_{\max} S}{K^{bf}_M + S + K_I P} (X_{sa} + X_{bf}) \tag{3}$$

At this place we explain the equations in details. The first equation describes the rate of swimming cells growth which depends on: the physiological constants $\mu_{max}[\mathrm{h}^{-1}]$ – the maximum specific growth rate of suspended microorganisms Acidithiobacillus ferrooxidans, K_M [kg/m^3] – Michaelis-Menten constant which expresses the bacterial affinity to the substrate in suspended cells; concentration of swimming cells X_{sc} [kg/m^3] in solution; subsrat concentration – S [g/l], concentration of the cells in the biofilm X_{bf} [kg/m^3] and on that part of biofilm surface, which is going to excrete cells [m^2]. In the equation (1) a possibility to investigate the process velocity, inhibited by the product of reaction (I) – ferric ions, is specified by the term $K_I P$ [kg/m^3].

The second equation describes the rate of cell growth in the biofilm. Additionally, it depends on $\mu^{bf}_{\max}$ – the maximum specific growth rate of the cells from biofilm, K^{bf}_M – the Michaelis-Menten constant of the cells from biofilm and K_d [h^{-1}] – the constant of the velocity of cells dying due to the sink into the biofilm.

The third equation depicts the velocity of oxidation of the substrate. It includes the constants μ_{max}, K_M and K_I. It depends also on the economical coefficient $Y_{x/s}$, which describes the quantity of the suspended biomass produced by unity of substrate concentration and $Y_{x/s_{bf}}$ – the economical coefficient of cells in biofilm.

The last equation describes the change of product concentration in the liquid phase

measured during the experiments. It depends on the substrate oxidation (Eq. (3)) and the velocity of sedimentation on zero order, i.e. only on the constant of sedimentation – K_{sed} [kg/m^{3}h].

The sedimentation of ferric ions in the liqid phase is also studied. It is supposed that it depends on the number of the swimming cells. They transform ferros to ferric ions and jarosite which ensures a sedimentation of ferric ions in the solution. Thus, in the latest model (Variant 4.) of initial phases of forming and functioning of the biofilm system, where the second term of the RHS of the fourth equation reflects the dependence of the sedimentation on X_{sc}, the equation (4) is replaced by

$$\frac{dP}{d\tau} = -\frac{dS}{d\tau} - K_{sed.} X_{sc}.$$

The system of four first-order ordinary differential equations is solved by fourth order Runge-Kutta formulae. The accuracy of the evaluation is tested comparing the results of a procedure of a single and a double increment at the time variable. The developed FORTRAN computer program automatically adjusts the increarement during the whole computation by its doubling or halving. The identification procedures based on the strategy of scanning of constant space for finding the minimum of the residues sum is applied. For this reason, the problem consists in finding the minimum of the following objective function:

$$F = (S_T - S_E)^2 + (P_T - P_E)^2,$$

where the subscribe T means theoretical values and E means experimentally measured values at the last hour of the cycle.

4. Results and discussion. The presented model equations (1-4) include twelve constants, namely : $\mu^{sc}_{\max}$, $\mu^{bf}_{\max}$, K_M, K_I, K_{sc}, F_{bf}, D_{fix}, K^{bf}_M, K_d, $Y_{x/s}$, $Y_{x/s_{bf}}$ and K_{sed}. Some of them, like F_{bf}, characterize the design of the bioreactor, others describe the of biofilm formation processes. Numerical experience data are shown on Fig. 3.

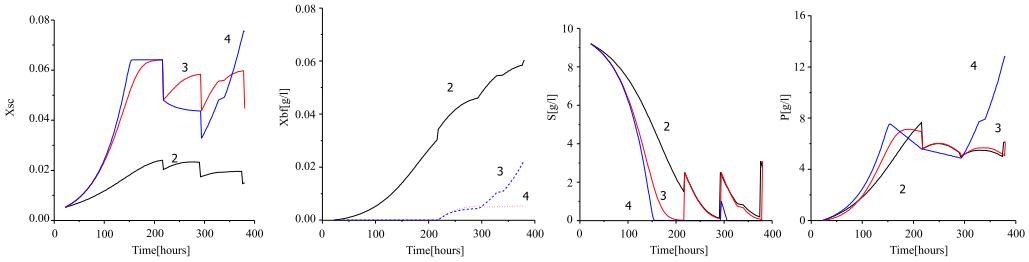

Fig. 3. Time course of X_{sc}, X_{bf}, S, P for the first three cycles of the process

The curves revel the time course of X_{sc}, X_{bf}, S, P for the first three cycles of the process. Those, numbered by 2, correspond to the Variant 2, those by 3 – to Variant 3, and by 4 – to Variant 4. The obtained results show that the used software is effective and helps to get a better understanding of the experimental results, which come from the starting phases of forming and functioning of biofilm systems at different bioreactors.

REFERENCES

[1] L. Nikolov, V. Mamatarkova, E. Petrova, St. Stoytchev. Basic Phenomena in the Bioprocess Systems with Fixed Film on Solid Supporting Beads. Part II. *New Data About Biofilm Growth and Functioning*, **2** (2005), 33–44.
[2] L. Nikolov, V. Mamatarkova, E. Petrova , St. Stoytchev. Modeling of the Base Phenomena in Bioprocess Systems with Spontaneously Fixed Biomass, Proc. 10-th Nat. Congress on Theoretical and Applied Mechanics, Varna 13-16 Sept. 2005, Prof. Marin Drinov Acad. Publ. House, Vol. **1**, 2005, 543-547.
[3] L. Nikolov, E. Petrova, V. Mamatarkova, St. Stoytchev. Study on Forming and Functioning of Biofilm by Means of Mathematical Model, *Ecological Engeneering and Enviroment Protection*, **2** (2006), 42–51.
[4] L. Nikolov, V. Mamatarkova. Dynamics of Sedimentation of Ferric Ions in Biodisk Reactor. *Ecological Engeneering and Enviroment Protection*, **1** (2005), 42–51.
[5] M. Valkova, L. Nikolov, T. Todorov. Microbiological Ferrous Ions Oxidation in a Rotating Biodisk Reactor. *Ann. of Sofia University "St. Kl. Ohridski"*, **75** (1982), 58–66 (in Bulgarian).
[6] L. Nikolov, V. Mamatarkova. Dynamics of Sedimentation of Ferric Ions in Biodisk Reactor. *Ecological Engeneering and Enviroment Protection*, **1** (2005), 42–51.

Kliment Ivailov Mladenov
Elitca Mihailova Petrova
Stoyan Stoyanov Stoytchev
Institute of Mechanics
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 4
1113 Sofia, Bulgaria
e-mail: kliment@imbm.bas.bg
e-mail: elitca@imbm.bas.bg
e-mail: stoyan@imbm.bas.bg

Ludmil Nikolov Nikolov
Vjara Savova Mamatarkova
Biological Faculty
Sofia University "St. Kliment Ohridski"
8, Dragan Zankov Str.
Sofia, Bulgaria
e-mail: lnikolov@biofac.uni-sofia.bg
e-mail: vsm@biofac.uni-sofia.bg

СИСТЕМЕН АНАЛИЗ НА ХОМОГЕННО-ХЕТЕРОГЕННИ МЕХАНИЗМИ В БИОФИЛМОВ РЕАКТОР

Климент И. Младенов, Елица М. Петрова, Людмил Н. Николов, Вера Маматаркова, Стоян Стоянов Стойчев

В последно време биофилмовите системи се изследват на базата на подходящо изградени математични модели, което е и темата на настоящата работа. Тя се явява и последователно развитите на изследванията на авторите по проблема за формулиране и подобряване на математичния модел описващ биофилмовата система на базата на информация за подсистемите и вербалния модел на окисляване на железни йони в биофилмов реактор. Стратегията на изследванията е еволюционна: от опита и експеримента – към модела, неговото третиране, усъвършенстване и развитие. Изпитана е и чувствителността на модела относно неговите параметри.