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Mathematical modelling of biodegradation of monochloroacetic acid by *Xanthobacter autotrophicus* GJ10 immobilized in polyacrilamide gel

E. K. Vasileva*, K. K. Petrov, V. N. Beschkov

Institute of Chemical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev St., Block 103, 1113 Sofia, Bulgaria

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The biological method of waste water remediation includes the participation of peculiar bacterial strains, capable of utilizing the halogenated aliphates as a carbon source.

In the present paper a kinetic model of the process of monochloroacetic acid biodegradation via glycolic acid production was developed. This model allowed the evaluation of the effects of microbial growth and diffusion limitations inside the gel particles on the process rate and the separate contributions of the free and immobilized cells for the overall fermentation process upon multiple uses.

The model results were tested on the process carried out by the strain *Xanthobacter autotrophicus* GJ10 used in the process of degradation of the highly toxic 1,2-dichloroethane and suitable for remediation of monochloroacetate contaminated media.

Key words: biodegradation, monochloroacetic acid, immobilization, *Xanthobacter autotrophicus* GJ10, polyacrylamide, mathematical modeling.

INTRODUCTION

The short-chain halogenated aliphatic compounds, such as 1,2-dichloroethane, are frequent constituents of industrial waste waters [1]. They are produced in millions of tons annually and because of the toxic effects of these compounds on humans as well as on the natural environment, there is growing interest in technologies for their removal. The monochloroacetic acid (MCA) is an intermediate product of degradation of the strongly toxic 1,2-dichloroethane. Biotechnologies, involving the use of microorganisms, able to degrade both pollutants to nontoxic final products, were developed in the last years. For this purpose, specialized bacterial strains that are able to use halogenated aliphatics as sole carbon and energy sources were used [2, 3]. As one of the most successful MCA degrading strains was evaluated the strain *Xanthobacter autotrophicus* GJ10.

The multiple use of bacteria requires their immobilization, either by entrapment in gels or fixed on solid supports. It both cases problems associated with mass transfer resistance may arise as well as with the cell detachment from the support. The correct performance of the process requires the knowledge of the contribution of the immobilized cells and of the free ones growing independently after detachment from the matrix. Another important feature is the estimation of the inhibition effects enhanced due to the mass transfer resistance in the gel particles and in the biofilms.

In the present paper a mathematical model is proposed to evaluate all these effects accompanying the microbial biodegradation of monochloroacetic acid.

This model enables to evaluate quantitatively these effects and to estimate their importance on the net process.

MATHEMATICAL MODELLING

The purpose of the mathematical modelling in the present paper was to estimate quantitatively the contribution of the cells entrapped in the gel particles, and in the free culture, after leaking from the gel. Leakage of cells from external layers of the particles may lead to cell growth in the bulk phase, which would compete and could even suppress the contribution of immobilized cells to the studied biodegradation [4–6]. Another effect of the cell detachment is the particle exhaustion and the failure of further use. Therefore, the mathematical modelling helps to estimate quantitatively the sustainability of the immobilized biocatalyst.

Our mathematical model is based on the following assumptions:

i) The monochloroacetic acid (MCA, *S*) is considered as a carbon source for microbial growth

* To whom all correspondence should be sent:
E-mail: vasileva@ice.bas.bg

but as an inhibitor at higher concentrations as well. It is converted into glycolic acid (GA, *P*) according to the net scheme:

$$\mathrm{CH_2Cl–COOH} \rightarrow \mathrm{CH_2(OH)–COOH} \rightarrow \text{mineralization}$$

$$\frac{dc_S}{dt} = -k_1 \frac{dX}{dt}$$
$$\frac{dc_P}{dt} = k_1 \frac{dX}{dt} - k_2 X c_P \qquad (1)$$

As a simplification, we considered the second step of further glycolic acid mineralization as a single consecutive first-order reaction.

ii) The process for immobilized cells follows kinetic equations with a structure identical to that for a free culture (1) but possibly with different rate constants because of different conditions the cells are living under. The yield coefficients are considered the same as those for a free culture.

iii) There is no migration of bacterial cells within the particles although they are not uniformly distributed because of different supply of substrate [4, 7, 8]. Leakage of cells growing at the periphery of the particles into the broth may take place.

iv) No partitioning effects for the solutes exist, i.e. the solubility of the species is considered to be the same in the bulk and in the gel particles.

v) It is established that the first step of the monochloroacetic acid biodegradation is associated with considerable substrate inhibition, particularly for the microbial growth [9, 10]. We shall consider that the substrate inhibition follows the equation proposed by Andrews [11]. Hence, the equation of microbial growth takes the form:

$$\mu = \mu_{\max} \frac{c_S}{K_s + c_S + K_i c_S^2} . \qquad (2)$$

No product inhibition was considered, neither for the first step, nor for the final mineralization. Based on these assumptions, the mathematical model for spherical particles represents the following set of partial differential equations written in dimensionless form, cf. the List of symbols:

$$\frac{\partial \bar{c}_S}{\partial T} = \frac{\partial^2 \bar{c}_S}{\partial \rho^2} + \frac{2}{\rho}\frac{\partial c_S}{\partial \rho} - \Phi_S^2 . \bar{X} c_S$$
$$\frac{\partial \bar{c}_P}{\partial T} = R_D \left(\frac{\partial^2 \bar{c}_P}{\partial \rho^2} + \frac{2}{\rho}\frac{\partial \bar{c}_P}{\partial \rho} \right) + \Phi_S^2 . \bar{X} - \Phi_P^2 X \bar{c}_P \qquad (3)$$

with the following initial and boundary conditions:

$$T = 0, \ \bar{c}_i = 1 \qquad (4a)$$

$$\rho = 0, \ \partial \bar{c}_i / \partial \rho = 0, \qquad (4b)$$

$$\rho = 1, \ \partial \bar{c}_i / \partial \rho = Bi_i (\bar{c}_{is} - \bar{c}_{i\infty}), \qquad (4c)$$

The notations in Eqn. (6) are as follows:

$$T = D_S t / R^2, \ \rho = r/R, \ \bar{c}_i = c_i / c_S^0,$$
$$\Phi_S^2 = k_1 \mu_{\max,im} R^2 X_{im}^0 / (D_S c_S^0),$$
$$\Phi_P^2 = k_2 R^2 X^0 / (D_S c_S^0), \qquad (5)$$
$$\bar{\mu}_{im} = \mu_{im} R^2 / D_S, \ \bar{\mu}_{\max,im} = \mu_{\max,im} R^2 / D_S$$

For a batch culture, the variation of product concentration in the broth with time is given by the following equations, with the associated initial conditions:

$$V \frac{dc_{P\infty}}{dt} = -AD_P \left(\frac{\partial c_P}{\partial r} \right)_{r=R} + V(Y_{P/X} \frac{dX_\infty}{dt} - k_2 X c_{P,\infty}) \qquad (6)$$

$$V \frac{dX}{dt} = V\mu_\infty X_\infty + \beta A \left[\mu_{im} X_{im} \right]_{r=R}$$
$$t = 0, X_\infty = X_\infty^0, c_P = c_{P,\infty}^0 \qquad (7)$$

The cells leakage is taken into account by the multiplier β, being the apparent thickness of the peripheral layer of the gel particles where cells leakage takes place, cf. the List of symbols.

Eqns (6, 7) can be rewritten in the following dimensionless form:

$$\bar{c}_P = -R_{DA} L \int_0^t \left(\frac{\partial \bar{c}_P}{\partial \rho} \right)_{\rho=1} dT + Y_{P/X} \bar{\mu} . \bar{X}_\infty - (k_2 R^2 / D_S c_S^0) \bar{X}_\infty \bar{c}_P \qquad (8)$$

$$\frac{d\bar{X}}{dT} = \bar{\mu}_\infty \bar{X}_\infty + \bar{\beta} L \left[\bar{\mu}_{im} \bar{X}_{im} \right]_{\rho=1},$$
$$T = 0, \bar{X}_\infty = 0, \eta = 0, \bar{\mu} = \mu R^2 / D_S \qquad (9)$$

The term $\bar{\mu}_\infty \overline{X_\infty}$ in Eqn. (9) accounts for the growth of the cells leaked to the broth. The second term on the right hand side of Eqns. (5, 9) takes into account the share of the cells leaked to the broth from the periphery of the gel particles.

The meaning of the dimensionless parameters in Eqns. (6–9) is as follows:

Φ_S^2 is the Thiele modulus being proportional to the ratio of the characteristic reaction time (the growth-associated biotransformation) and the time for diffusion $R2/D$.

Φ_P^2 is the Thiele modulus for the second step of the consecutive reactions, i.e. the mineralization of glycolic acid to water and carbon dioxide.

$R_D = D_S/D_P$ *is* the ratio of diffusivities of MCA to GA. This ratio was taken as $R_D = 1$, accounting for the similar structures and masses of the MCA and GA molecules.

The Biot numbers *Bi*i for the different species (*i*th) are considered high enough to neglect the external mass transfer resistance.

K_S is the MCA saturation constant for free culture and μ is the specific cell growth rate taking into account the consumption of MCA as well as the substrate inhibition effects according to the Eqs. (2–4).

The multiplier β takes into account the rate of cell leakage from a layer at the periphery of the spherical gel particles. If no gel destruction takes place, it is reasonable to assume that the maximum possible value of the dimensionless factor $\overline{\beta}$ may reach a unity if the cells from the whole particle volume leak into the surrounding medium. It means that in the latter case $\overline{\beta} = 1$, $\mu_{im}X_{im} \approx \mu_\infty X_\infty$. In case of no cell leakage, $\beta = 0$.

$L = A.R/V$ is a dimensionless parameter taking into account the particles interfacial area and their concentration. In case when the particles concentration is low and provided the spherical solid particles are of a uniform radius, this parameter is related to the bed porosity, ε, by the equation:

$$L = \frac{N_p \pi d_p^2}{V}\frac{d_P}{2} = \frac{3N_pV_p}{V} = 3(1-\varepsilon), \qquad (10)$$

Where N_p and V_p are the number of particles and the volume of a single particle, respectively. Hence, the parameter L can be calculated knowing the particles volume and the volume of the fluid.

There are eleven parameters in the model (2–9). Some of them can be evaluated from independent sources or experiments and thus facilitating the parameter estimation from experimental data for lactic acid fermentation by entrapped cells. These parameters are given in Table 1 with explanation of the way they are estimated or used further.

Many of the parameters could be taken from experimental data for free culture of the same strain, namely K_s, K_P, $Y_{P/X}$ and μ_{max} [9, 10]. The diffusivity D_S is taken from the literature for aqueous media [12].

The effect of immobilized cells on the solute's diffusion coefficients is considered quantitatively by Lefebvre and Vincent [7] and Gutenwik *et al*. [8]. One may expect that the solutes diffusivities may vary during one run within the gel pores because of microbial growth. In our case we shall consider constant diffusivities during one run, taking into account only their change from run to run.

The Biot number is considered to be high enough to provide well-stirred state conditions in the broth. The specific particle area parameter L was determined from the void volume of the mixture particles/broth.

The left four parameters (Φ_L^2, $\mu_{max,\,im}$, Φ_P^2, and β) have to be estimated from the model, cf. Eqns. (6–9) and based on the experimental data.

The set of differential equations (6) with the initial and boundary conditions (Eqns. (6a–c), (7), (8)) could be solved numerically. We used an implicit finite difference scheme coupled with the Thomas algorithm for solving the resulting linear algebraic equations.

For the purpose of parameter estimation the experimental results on the studied microbial biodegradation, published recently [13] were used. The parameter estimation was accomplished by minimization of sum of the squares of the differences between the experimental and the calculated values of monochloroacetic (S) and glycolic acid (P) concentrations at each moment of sampling.

$$Sum = \sum_j \left[(S_j^{calc} - S_j^{exp})^2 + (P_j^{calc} - P_j^{exp})^2 \right] \qquad (11)$$

The sums of the square differences of the normalized concentrations were very low, i.e. ~10^{-4}–10^{-6} orders of magnitude and always less than 0.001 for the estimated parameters.

RESULTS AND DISCUSSION

The values of the estimated model coefficients are given in Tables 1, 2, 3. It was interesting to note, that the maximum growth rate for the immobilized cells was determined with very high accuracy, namely:

$$\mu_{max,im} = 0.023\ h^{-1} \qquad (13)$$

Slight differences in the initial approximations during the estimation procedure gave very large discrepancies in the sum of least squares. This strong sensitivity is an indication of the model reliability. This value for $\mu_{max,im}$ is much lower, i.e. one order of magnitude than the specific growth rate for free cells. Probably it is due to the spatial limitations for the cell growth inside the particles.

Table 1. List of the model parameters and their values

Parameter	Values	Reference
Bi, Biot number, dimensionless	> 1000	Own assumption
D_S, diffusivity of monochloroacetic acid, (m^2/s)	1.37×10^{-9}	[15]
D_P, diffusivity of glycolic acid, (m^2/s)	1.37×10^{-9}	Own assumption
k_1, rate constant for monochloroacetic to glycolic acid fermentation, included in Φ_L^2, dimensionless		Included in the Thiele modulus, Φ_S^2,
k_2, rate constant of the second step of microbial degradation, included in Φ_P^2, $((kg/m^3)^{-1}\cdot h^{-1})$	-	Included in the Thiele modulus Φ_P^2
K_i, substrate inhibition constant, $(kg/m^3)^{-1}$	0.0406	From the free cells experiments [14]
K_s, saturation constant for monochloroacetic acid in Monod's equation, (kg/m^3)	0.0123	Own data from the free cells experiments
L, particle specific surface area parameter, dimensionless, Eqn. (10)	Different values	Own data
$Y_{P/X}$, product yield factor for free cells, dimensionless	1.0	Own data from the free cells experiments
$\overline{\beta}$ cells leakage factor, ($0 < \overline{\beta} < 1$), dimensionless	Different values	To be determined in the present paper
μ_{max}, specific maximum microbial growth rate for free cells, (h^{-1})	0.155	Own data from the free cells experiments
$\mu_{max,\,im}$, specific maximum microbial growth rates for immobilized cells, (h^{-1})	Different values	To be determined in the present paper
Φ_S^2, Thiele modulus on monochloroacetic acid conversion, dimensionless, Eqn. (5)	Different values	To be determined in the present paper
Φ_P^2, Thiele modulus on glycolic acid conversion, dimensionless, Eqn. (5)	Different values	To be determined in the present paper

Table 2. Parameter values estimated at MCA initial concentration of 5 mM.

Run No.	Φ_S^2, [-]	Φ_P^2, [-]	$\mu_{max,\,im}$ [h^{-1}]	$\overline{\beta}$, [-]
R1	11.15	1×10^{-4}	0.023	2.4×10^{-2}
R2	3.5	1×10^{-4}	0.023	1.7×10^{-2}
R3	6.32	1×10^{-4}	0.023	1.86×10^{-2}
R4	8.97	1×10^{-4}	0.023	1.87×10^{-2}
R5	8.6	1×10^{-4}	0.023	1.93×10^{-2}

Table 3. Parameter values estimated at MCA initial concentration of 10 mM.

Run No.	Φ_S^2, [-]	Φ_P^2, [-]	$\mu_{max,\,im}$, [h^{-1}]	$\overline{\beta}$, [-]
R1	2.14	0.1×10^{-4}	0.023	4.2×10^{-4}
R2	10.9	1×10^{-4}	0.023	1.52×10^{-2}
R3	17.36	0.51×10^{-4}	0.023	1.53×10^{-2}
R4	8.84	0.51×10^{-4}	0.023	1.54×10^{-2}
R5	8.647	0.1×10^{-4}	0.023	1.55×10^{-2}
R6	1×10^{-6}	0.1×10^{-4}	$\sim10^{-7}$	3.2×10^{-5}

The comparison of the values for the Thiele modulus Φ_S^2 for the first process shows that it stays stable at the lower initial concentration, i.e. there is slight cell leakage into the broth, which is compensated by the microbial growth inside the particles. This statement corresponds to the stable and very low values of the leakage factor $\overline{\beta}$. In the case of higher MCA initial concentration (i.e. 10 mM) the Thiele modulus passes through a maximum tending to zero for the last run. Obviously, in this case, the particles are already exhausted due to the leakage and the growth cannot compensate these losses, probably due to substrate inhibition. The initial values at the immobilized cell concentration X_{im}^0 prior to each run estimated from Φ_S^2 vary between 90 and 880 kg/m^3. For a reference, the free cell concentrations in the stationary phase are about 5 kg/m^3.

It is important to mention, that the leakage factor remains stable during the runs at both initial concentrations. Its values are very close to each other except for the first and last runs at 10 mM. The last fact could be explained by the extremely low initial cell concentrations in the first and the last experiments.

A review of the obtained parameter values shows that the Thiele modulus Φ_P^2 for the second process is practically constant at both initial MCA concentrations, no matter of the runs. This fact shows that the mineralization of glycolic acid is not inhibited by MCA or GA and that the immobilized cells are sufficiently active for all experiments.

A comparison of the modelling and experimental results from [13] is shown in Fig. 1. It illustrates the transient processes for a single run and the process stability for multiple repeated runs.

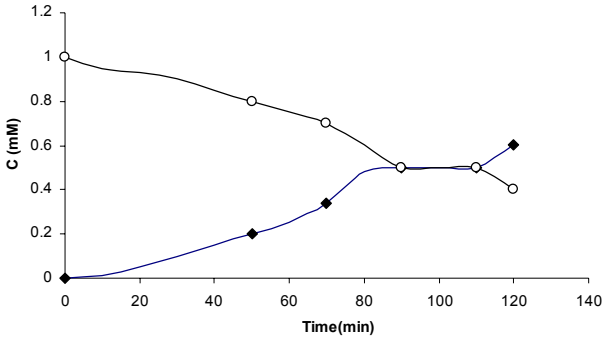

Fig. 1. Comparison of experimental data for monochloroacetic and glycolic acid with the model ones. (–o–) - monochloroacetic acid; (–♦–) - glycolic acid. The lines present results of mathematical modelling.

CONCLUSIONS

On the basis of the analysis of experimental results applying mathematical modeling we have come to the following conclusions:

The mathematical modelling of the process showed that for the present experiments loss of activity due to cell leakage was compensated by microbial growth inside the particles for 5 consecutive runs. Moreover, microbial growth was strongly retarded inside the particles and the second step of microbial mineralization was slightly dependent on the initial substrate concentration or the glycolic acid.

LIST OF SYMBOLS

A	Particles total interfacial area [L^2]
Bi	Biot number; $Bi = kR/D_L$ [-]
c	Concentrations, [$M \cdot L^{-3}$]
D_i	Diffusivity of the ith species, [$L^2 \cdot T^{-1}$]
d_p	Particle diameter, [L]
k_1	Rate constant of the lactose fermentation, Eqn. (1), [-]
k_2	Rate constant of lactic acid degradation, Eqn. (1), [$(M \cdot L^{-3})^{-1} . T^{-1}$]
k	Mass transfer coefficient, Eqn. ($Bi = kR/D_L$), [$L \cdot T^{-1}$]
K_S	Saturation constant for lactose in Monod Eqn. (7), [$M \cdot L^{-3}$]
K_i	Constant of substrate inhibition, Eqn. (2), [$(M \cdot L^{-3})^{-1}$]
K_P	Constant of product inhibition, Eqn. (2), [$M \cdot L^{-3}$]
L	Parameter taking into account the effect of specific particles area; $L = 3(1-\varepsilon)$, [-]
N_p	Number of gel particles, [-]
R	Radius of the particles, [L]
r	Radial co-ordinate, [L]
R_D	Ratio of diffusivities related to that of lactose, [-]
t	Time, [T]
T	Dimensionless time, $T = D_L t/R^2$, [-]
V	Total volume of the system, [L^3]
X	Living cells concentration, [$M \cdot L^{-3}$]
$Y_{P/X}$	Product yield coefficient, [-]

Greek Symbols

β	Apparent radius of the peripheral layer of the particles emitting viable cells from interface into the bulk, [L]
$\bar{\beta}$	Share of viable cells leaking from the particles interface into the bulk, $\bar{\beta} = \beta/R$, between 0 and 1, [-]
ε	Void fraction, [-]
μ	Specific microbial growth rate, Eqn. (7), [T^{-1}]
$\rho = r/R$	Dimensionless radial coordinate, [-]
Φ_S^2	Thiele modulus for lactose conversion, Eqn. (7), [-]
Φ_P^2	Thiele modulus for lactic acid degradation, Eqn. (7), [-]

Subscripts

S	Denotes quantities related to lactose (substrate)
P	Denotes quantities related to lactic acid (product)
i	Denotes quantities related to certain species in the system
im	Denotes values, related to immobilized cells
max	Denotes maximum values
∞	Denotes values, related to the bulk phase

Superscript

–	Denotes dimensionless quantities
0	Denotes initial concentrations

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МАТЕМАТИЧНО МОДЕЛИРАНЕ НА БИОРАЗГРАЖДАНЕ НА МОНОХЛОРОЦЕТНА КИСЕЛИНА ОТ КЛЕТКИ НА ЩАМА *XANTHOBACTER AUTOTROPHICUS GJ10,* ИМОБИЛИЗИРАНИ В ПОЛИАКРИЛАМИДЕН ГЕЛ

Е. К. Василева*, К. К. Петров, В. Н. Бешков

Институт по инженерна химия, Българска академия на науките, ул. „Акад. Г. Бончев“, блок 103, 1113, София

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(Резюме)

Биологичните методи за пречистване на отпадни води се осъществяват чрез използване на специфични бактериални щамове, които са в състояние да използват халогенираните алифати като въглероден източник.

В настоящата статия е представен кинетичен модел на процеса на биоразграждане на монохлороцетна киселина с междинен продукт гликолова киселина. Този модел позволява оценката на ефекта от микробния растеж и дифузионните ограничения в гелните частици. Оценен е приносът на свободните и имобилизираните клетки в процеса на биоразграждане при многократно използване на гелните частици.

Резултатите от математичния модел са проверени експериментално с клетки на щама *Xanthobacter autotrophicus GJ10*, известен със способността си да разгражда токсичния 1,2-дихлоретан. Щамът е подходящ за обезвреждане на среди, замърсени с монохлорацетати.