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MATHEMATICAL MODELING FOR STUDYING MICROBIAL PROCESSES – SOME EXAMPLES

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Abstract. Mathematical modeling may have different purposes in chemical and biochemical engineering sciences. One of them is to confirm or to reject kinetic models for certain processes, or to evaluate the importance of some transport phenomena on the net chemical or biochemical reaction rate. In the present paper different microbial processes are considered and modeled for evaluation of kinetic constants for batch and continuous processes accomplished by free and immobilized microbial cells. The practical examples are from the field of wastewater treatment and biosynthesis of products, like enzymes, lactic acid, gluconic acid, etc.

By the aid of mathematical modeling the kinetics and the type of inhibition are specified for microbial wastewater denitrification and biodegradation of halogenated hydrocarbons. The importance of free and immobilized cells and their separate contribution to the overall microbial process is also evaluated for some fermentation processes: gluconic acid production, dichloroethane biodegradation, lactic acid fermentation and monochloroacetic acid biodegradation.

ACM Computing Classification System (1998): G.1.7, G.1.8.

Key words: microbial kinetics, free and immobilized cells, mathematical modeling, cell detachment, parameter evaluation.

1. Biochemical kinetics evaluation. The traditional kinetic experiments are carried out under batch conditions in well stirred tank reactors at fixed temperature, depending of the specific microbial cells. The mathematical models consist in sets of ordinary differential equations, involving inhibition, either by substrate, or products with different number of known and unknown parameters. The latter have to be evaluated.

There are different kinetic models taking into account the inhibition. Including different kinetic models we can estimate the most appropriate one using mathematical modeling. Here are some examples.

1.1. Microbial denitrification. Denitrification is used to mark the reduction of oxidized nitrogen compounds to gaseous nitrogen. As a result of the reduction, different intermediate and end products are produced: nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), or molecular nitrogen (N_2), by the following consecutive steps:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO\ (g) \rightarrow N_2O\ (g) \rightarrow N_2\ (g)$$

It was established that the process is retarded at higher nitrate concentrations, due to substrate S (i.e. NO_3^-) or product P (NO_2^-) inhibition.

The evaluation of the process kinetics requires a certain model of inhibition. In this case the following set of ordinary differential equations has been solved:

$$\begin{aligned} \frac{dS}{dt} &= -\alpha\mu X \\ \frac{dP}{dt} &= \alpha\mu X - \beta_x P \\ \frac{dX}{dt} &= \mu X - \beta_x X \end{aligned} \tag{1}$$

with the appropriate initial conditions:

$$t = 0, \quad S = S_0, \quad P = 0, \quad X = X_0. \tag{2}$$

Two types of substrate inhibition were checked: the Andrews equation [1], Eq. (3a), and the one of Aiba et al.[2], Eq. (3b).

$$\mu = \mu_{\max} \frac{S}{K_s + S + K_i S^2} \tag{3a}$$

(3b) $$\mu = \mu_{\max} \frac{S}{K_s + S} \exp(-K_2 S)$$

The product inhibition was described by:

(4) $$\mu = \mu_{\max} \frac{S}{K_s + S} \frac{K_3}{K_3 + P}$$

Unknown parameters to be determined are: α, β_x, K_2 (or K_i) and K_3. The remaining ones have previously been determined experimentally. The system (1–4) has been solved by the 20-sim simulator [3, 4], coupled with a Nelder-Mead optimization procedure for kinetic parameter evaluation. As a minimized function the sum of the squares of the differences between the experimental values and the calculated ones for nitrate concentration was adopted:

(5) $$F = \sum_i (S_{i,\exp} - S_{i,calc})^2,$$

for six different initial nitrate concentrations with 7 experimental points for each experiment. Model adequacy was tested by the scatter of the kinetic parameters evaluated for different substrate concentrations and other conditions and the F-test. It was shown that the substrate inhibition better follows Aiba's model, Eq. (3b), than the Andrews one. The comparison between the experimental data and the ones predicted by the model shows that there is a very good agreement for nitrate reduction and only qualitative one for nitrite reduction:

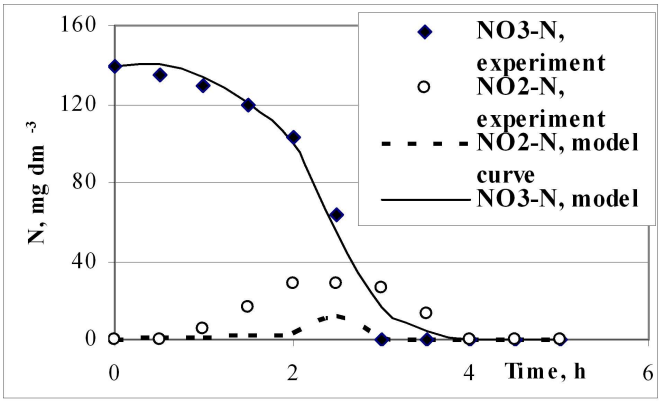

Fig. 1. Comparison of model and the experimental data. Initial NO_3^--N concentration: 139.5 mg/l

1.2. Bacterial production of cyclodextrin-glucano-transferase (CGT-ase). The enzyme cyclodextrin-glucano-transferase (CGTase) is produced by different bacteria. We have used experimental results for the strain *Bacillus circulans* at batch conditions [5] to evaluate the microbial kinetics. The similar system (1–3) of differential equations with the kinetic models has been tested by the same evaluation procedure differing by the rate of product (i.e. CGTase) formation, Eq.(6):

$$\frac{dP}{dt} = \alpha \cdot \mu \cdot X + \beta \cdot X \tag{6}$$

and the model parameters by sum of the least-squares of the product concentrations. In this case substrate inhibition followed the Andrews model better [6].

1.3. Dehalogenation by microbial cells attached to solid support. The mathematical modeling was used in this case to estimate the contribution of the immobilized cells and the free ones detached from the support to the overall biodegradation of 1,2-dichloroethane [7] in continuous process:

$$\text{DCE} \rightarrow \text{chloride ions} + \text{other products}$$

$$\frac{dX_1}{dt} = \mu X_1 + k_{im} X_{im} + D_1(X - X_1) \tag{7}$$

$$\frac{dX_{im}}{dt} = \mu_{im} X_{im} - k_{im} X_{im} \tag{7a}$$

$$\frac{dS_1}{dt} = -r(X_1) - r(X_{im}) - \beta X_1 - \beta_{im} X_{im} + D_1(S - S_1) \tag{7b}$$

with the initial conditions:

$$t = 0, X = X_1 = 0, X_{im} = X_{im}^0, S_1 = 0, P_1 = 0. \tag{7c}$$

The total biomass concentration in the liquid phase X_1 is due to cell leakage and the consequent microbial growth. Theoretically the substrate biodegradation could be accomplished either by the free or immobilized cells, as given by Eqs. (7b, c). The contribution of the free cells depends on the leakage factor k_{im}. The higherk_{im}, the bigger the contribution of the free cells. If $k_{im} = 0$, there is no cell leakage and the process is carried out by the immobilized cells only.

Table 1. The parameter values estimated for a continuous process for 0.5 mM initial DCE concentrations and different dilution rates for cells attached to solid support

Parameter/Dilution rate, h^{-1}	2.65	5.9	7.35
$k_{im,}$, $m.h^{-1}$	0.0008	0	0
$\mu_{\max,im}$, h^{-1}	0.26	0.17	0.29
Sum of squares (–)	0.007	0.006	0.008

Handling of experimental data for 3 different initial DCE-concentrations by the technique described above and minimizing the sum of squares for the product (i.e., chloride) concentrations we have shown that there is practically no leakage of cells and the biodegradation was due to the immobilized cells only, cf. Table 1. For reference, the estimated $k_{im,}$ was less than 0.001.

2. Microbial processes with entrapped cells (transport phenomena and cell detachment). In this case the cells are entrapped in gels, like calcium alginate, polyacrylamide, etc. There is a mass transfer resistance for substrate and product molecules in the gel pores and membranes and therefore the apparent bioconversion rates are lower than for free cells [8]. The retarded mass transfer due to molecular diffusion can worsen the situation in case the product is an inhibitor. On the other hand, cells may grow within the particles and form concentration profiles with different access to the substrate feed. The situation becomes more complicated when cells leak from the particle periphery into the broth, continuing to grow independently.

All these effects can be estimated and conclusions on the nature and contribution of immobilized cells and of the free ones can be drawn using adequate mathematical models and appropriate experimental data by minimizing the sum of the least squares. For the case of batch process with particles with defined shape (i.e. spherical one), one can write the following system of partial differential equations [9]:

$$\begin{aligned} \frac{\partial c_S}{\partial t} &= D_S \left(\frac{\partial^2 c_S}{\partial r^2} + \frac{2}{r} \frac{\partial c_S}{\partial r} \right) - k_1 \frac{dX}{dt} \\ \frac{\partial c_P}{\partial t} &= D_P \left(\frac{\partial^2 c_P}{\partial r^2} + \frac{2}{r} \frac{\partial c_P}{\partial r} \right) + k_1 \frac{dX}{dt} - k_2 X c_P \end{aligned} \tag{8}$$

With the following initial and boundary conditions:

$$t = 0,\ c_S = c_0;\ c_P = 0;\ X = X_0 \tag{8a}$$

$$r = 0,\ \partial c_i/\partial r = 0, \tag{8b}$$

$$r = R,\ D_i(\partial c_i/\partial r) = k(c_{is} - c_{i\infty}), \tag{8c}$$

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

$$V\frac{dc_{P,\infty}}{dt} = -AD_P\left(\frac{\partial c_P}{\partial r}\right)_{r=R} + V(Y_{P/X}\frac{dX_\infty}{dt} - k_2 X_\infty c_{P,\infty}) \tag{9}$$

$$V\frac{dX}{dt} = V\mu_\infty X_\infty + k_{im}A\left[\mu_{im}X_{im}\right]_{r=R}$$
$$t = 0, \quad X_\infty = X_\infty^0, \quad c_P = c_{P,\infty}^0 \tag{10}$$

The process rate depends on different factors, like the mass transfer coefficient in the bulk k, the interfacial area A, the specific microbial growth rates in the bulk μ and within the particles μ_{im}, the product yield coefficient $Y_{P/X}$, etc. The indices "∞" denote bulk concentrations and quantities, whereas "im" denotes quantities related to the gel particles. We shall consider two-step consecutive reactions involving product degradation in the second step:

$$S \rightarrow P \rightarrow \text{degradation products}$$

The microbial growth kinetics follows equations like Eqs. (3a, b) and (4), taking into account both substrate and product inhibition.

The system (9–10) was solved numerically for each separate practical case by an implicit difference scheme coupled with the Thomas algorithm for solving the resulting system of linear algebraic equations. There are some examples below.

2.1. Glucose to gluconic acid biotransformation [9]. The experimental difference between the performance of free and immobilized cells is shown in Fig. 2. It was shown that the product of the rate constant and the initial immobilized cell concentration.

Table 2

Glucose, kg m^{-3}	$\mu_{\max,\infty}$, h^{-1}	$\mu_{\max,im}$, h^{-1}	$k_1 X^0_{im}$, g dm^{-3}
40	0.31 ± 0.05	0.38 ± 0.04	21
90	0.30 ± 0.03	0.38 ± 0.03	22
180	0.34 ± 0.04	0.34 ± 0.05	24

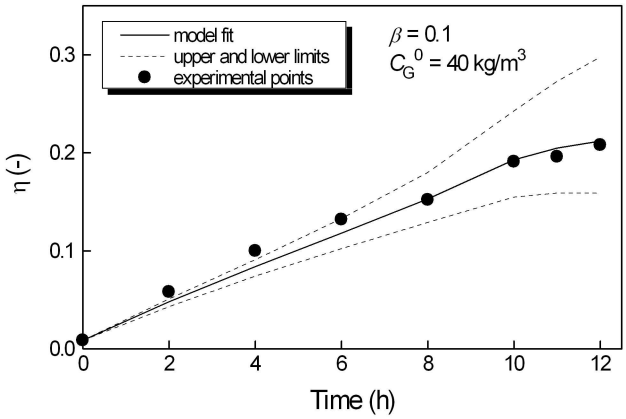

Fig. 2. Dynamics of glucose to gluconic acid conversion observed in a free culture and by immobilized cells of *Gluconobacter oxydans*. The leakage factor (here β) is 0.1

$k_1 X^0_{im}$ was very stable for a wide range of initial glucose concentrations (from 40 to 180 kgm^{-3}) arguing for the validity of the model, cf. Table 2. In this case the maximum microbial specific growth rate for the immobilized cells was comparable to (even somewhat higher than) that for free ones.

2.2. Lactic acid fermentation [10]. Lactic acid fermentation was treated as a case study for product inhibition and its impact on the bacteria entrapped in gel particles. The experimental data showed considerable cell leakage and therefore rapid exhaustion of the biocatalyst. These observations were quantified by a model similar to the one in Eqs. (8–10). Some parameters evaluated by the procedure described above are shown in Table 3.

The values of $\Phi_S^2 = k_1 \mu_{\max,im} X^0_{im} . R^2/(D_S c_0)$ are much lower at the first

Table 3. The values of the estimated kinetic parameters for lactic acid fermentation

Run No.	Φ_S^2, [–]	$\mu_{\max,im}$, [h^{-1}]	k_{im}, [–]
1	$\sim 10^{-5}$	0.022	2.10^{-10}
2	1.6	0.020	0.005
3	2.4	0.020	0.008
4	2.3	0.020	0.005
5	0.2	0.020	0.001

and the last run compared to the others because of the very low biomass concentration inside the particles, due to non-developed culture in the beginning and exhaustion because of the leakage at the end. The specific microbial growth rate is too low to compensate the cell leakage, although k_{im} is not too high.

2.3. Biodegradation of monochloroacetic acid [11]. The experiments on biodegradation of monochloroacetic acid (MCA) have been carried out with bacteria from the strain *Xanthobacter autotrophicus* GJ10, entrapped in polyacrylamide gel. The same set of equations (8a–c) was used taking into account the further mineralization of the intermediate product, e.g. glycolic acid. The rate constants (e.g. Φ_S^2 and Φ_P^2), the leakage factor and the maximum microbial specific growth rate in the particles were determined minimizing the following sum of squares including the data for the product too:

$$F = \sum_i (S_{i,\exp} - S_{i,calc})^2 + \sum_i (P_{i,\exp} - P_{i,calc})^2 \tag{11}$$

Some of the results for parameter values are shown in Table 4. The rate constant for the first reaction varies with the different runs because of bacterial growth within the cells and by the cell leakage, represented by k_{im}. The maximum microbial specific growth rate in the particles is constant but ten times lower than the one for free cells (0.155 h^{-1}). The leakage factor remains constant for the main runs, tending to zero when the particles are exhausted because of cell leakage.

Table 4. Estimated values of kinetic parameters for initial MCA concentration 10 mM.

Run No.	Φ_S^2, [-]	Φ_P^2,[-]	$\mu_{\max,im}$, [h^{-1}]	k_{im}, [-]
1	2.14	1.10^{-5}	0.023	$4.2*10^{-4}$
2	10.9	1.10^{-4}	0.023	0.015
3	17.36	$5.\ 10^{-5}$	0.023	0.015
4	8.84	$5.\ 10^{-5}$	0.023	0.015
5	8.647	$1.\ 10^{-5}$	0.023	0.015
6	$1*10^{-6}$	$1.\ 10^{-5}$	$\sim 10^{-7}$	$3.2*10^{-5}$

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