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Modelling of immobilized cell systems

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A new model for the dynamic evolution of a membrane system containing immobilized cells is designed and theoretically studied. The analysis is based on the diffusion–reaction theory in which both the diffusion and reaction components are space and time dependent. The numerical treatment gives the time evolution of the system which tends toward a U-form cell distribution in the membrane, depending on its transport characteristics.

1. Introduction

There are many ways to consider immobilized cell systems [1]. The simplest theoretical approach is to study the system under steady-state conditions without taking into account its dynamic behaviour. The growth of the microorganisms in the gel is not considered, indeed the biocatalytic phase, i.e. the biomass, remains constant. The first studies developed simple diffusion–reaction systems [2], either by micro–macro models [3, 4] or by intrinsic models [5]. The new trend is to develop dynamic models by combining diffusion, reaction and also cellular growth [6–10], in order to describe how the rate of substrate consumption, the diffusion and the cell growth vary in the gel as a function of space and time. Both theoretical and experimental results show that the dynamical evolution of the systems leads to a heterogeneous distribution of the biomass, only the layer near the surface of the biocatalytic particle being active at the steady-state.

For the last few years, our laboratory has been engaged in the design and improvement of bioreactors [11] which, nowdays, require modelling. The aim of this paper is to present a new model which describes the dynamic evolution of membranes containing immobilized cells in order to reach a better understanding of the phenomena which control cell reactors.

2. Theory

The model is composed of two compartments separated by a gel slab containing the immobilized cells. A schematic representation of the system is shown in Fig. 1. The reaction occurring in the reactor corresponds to both the metabolic reaction which transforms substrate S into product P and the cell growth which increases the cell concentration inside the gel. The reaction can be represented by the equation:

$$n \text{ cells} + \text{substrate S} \rightarrow m \text{ cells} + \text{product P} \quad (1)$$

While cells are immobilized in the gel slab and not diffusing, substrate and product are involved in two coupled phenomena: the reaction and the diffusion.

The main assumptions on which the model is formulated are: (1) cells are homogeneously distributed inside the gel slab at the outset of the reaction; (2) no cell leakage will occur; (3) because of the geometry of the membrane, the diffusion phenomenon will be considered only in the direction perpendicular to the membrane; (4) complete mixing occurs in the reactor, hence the substrate concentration at the surface of the membrane is equal to the bulk substrate concentration; (5) the reaction catalysed by cells is assumed to be of the Michaelis–Menten type and the cell growth is assumed to follow a law of the Monod type without inhibition and death. The reaction parameters of the immobilized cells are equal to those of the free cells; and (6) the diffusion coefficients in the gel are dependent on the local cell concentration.

On the basis of these assumptions, the unsteady-state balance equation inside the membrane for a species Z is given, as for immobilized enzyme systems [12], by the classical diffusion–reaction law

$$\left(\frac{\partial Z(x,t)}{\partial t}\right)_{\text{total}} = \left[\frac{\partial Z(x,t)}{\partial t}\right]_{\text{diffusion}} + \left[\frac{\partial Z(x,t)}{\delta t}\right]_{\text{reaction}} \quad (2)$$

in which

$$\left[\frac{\partial Z(x,t)}{\partial t}\right]_{\text{diffusion}} = D_Z(B)\frac{\partial^2 Z(x,t)}{\partial x^2} \quad (3)$$

$$\left[\frac{\partial Z(x,t)}{\partial t}\right]_{\text{reaction}} = \varepsilon V_m \frac{S(x,t)}{K_m + S(x,t)} B(x,t) \quad (4)$$

$$\varepsilon = \begin{cases} +1 & \text{when } Z \equiv \text{P} \\ -1 & \text{when } Z \equiv \text{S} \end{cases}$$

V_m and K_m are the equivalent Michaelis–Menten constants corresponding to the immobilized cell, x is the abscissa coordinate along the thickness of the

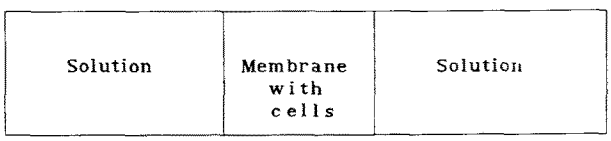

Figure 1 Schematic diagram of the model.

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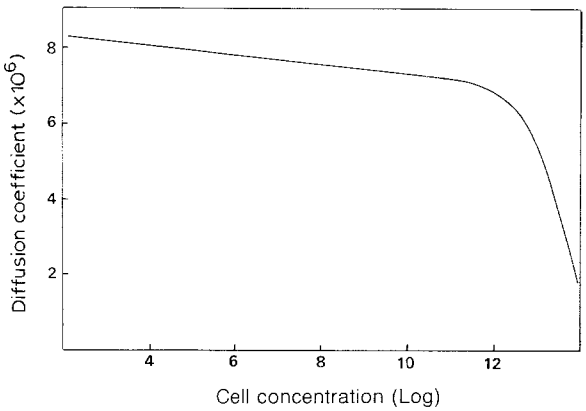


Figure 2 Evolution of the diffusion coefficient, D_Z, as a function of the local cell concentration (logarithmic scale).

membrane, t the time and D_Z the diffusion coefficient of species Z inside the membrane. In our model, D_Z is assumed to be a hyperbolic function of the local cell concentration (Fig. 2).

For cell growth, the Monod equation is used

$$\frac{\partial B(x,t)}{\partial t} = \mu B(x,t) \tag{5}$$

in which

$$\mu = \frac{S(x,t)}{K_s + S(x,t)}\mu_m \tag{6}$$

The initial and boundary conditions are:

$$\begin{aligned} t = 0 : S(x,0) &= 0, \quad \text{for } 0 < x < e \\ P(x,0) &= 0, \quad \text{for } 0 \leqslant x \leqslant e \\ B(x,0) &= B_0, \text{ for } 0 \leqslant x \leqslant e \end{aligned} \tag{7}$$

where e is membrane thickness

$$t > 0 : S(0,t) = S(e,t) = S_0$$

where S_0 is initial concentration

Owing to the complexity of the partial derivative equations, no analytical solutions can be calculated. A numerical simulation is required and the explicit scheme will be used leading to

$$B_x^{t+\Delta t} = B_x^t + \Delta t\,\mu_m\left[\frac{S_x^t}{K_s + S_x^t}\right]B_x^t \tag{8}$$

and

$$S_x^{t+\Delta t} = S_x^t + \frac{\Delta t}{\Delta x^2}[D_Z(B_x^t)(S_{x+\Delta x}^t + S_{x-\Delta x}^t - 2S_x^t)] + \Delta t\, V_m\frac{S_x^t}{K_m + S_x^t}B_x^t \tag{9}$$

3. Results

Equations 8 and 9 give the time evolution of the system which shows the simultaneous substrate consumption and cell growth. These two phenomena are coupled: the cell growth is dependent on the local substrate concentration inside the membrane and the diffusion (Equation 3) and reaction (Equation 4) parts of the global substrate equation are functions of the local cell concentration. This explains why the system tends toward a stabilization of the concentration profiles, which we call steady-state distribution. Three types of result will be shown: (i) the shape of the steady-state cell distribution inside the membrane, (ii) the different steps which lead the system to its steady-state, and (iii) the influence of diffusion and reaction parameters on the steady-state cell distribution.

3.1. Intramembrane cell concentration profiles

Because of the cell growth, the cell concentration increases with time inside the membrane. Owing to the diffusion constraints which affect the uptake of substrate, the best conditions for cell growth will be obtained near the membrane–solution interfaces and the increase in cell concentration will be maximum in these regions. The heterogeneity in the cell distribution inside the membrane will be reinforced by the increase of the diffusion constraints in these regions due to the fact that the cells progressively occupy the free space in the membrane. The result is strong heterogeneity of the cell concentration inside the membrane as shown in Fig. 3 where the cell concentration is plotted as a function of membrane thickness. The greatest part of the biomass is located near the membrane–solution interfaces.

3.2. Time evolution of the system

Because of the cell growth, the catalyst concentration and thus the reaction rate inside the membrane is not constant as a function of time. Three periods can thus be distinguished.

(i) The first period is characterized by a low cell concentration leading to a diffusion–reaction balance in favour of the diffusion. The substrate uptake by the membrane can be seen both in the substrate concentration profile (Fig. 4) and in the reaction rate profile (Fig. 5). The substrate is consumed as soon as it diffuses into the gel, leading to a cell growth limited to the borders of the membrane. Conversely, the cell growth is very slow in the centre of the membrane because of the low concentrations of substrate and cells.

(ii) When the membrane is filled by the substrate, the reaction rate increases and the net rate becomes

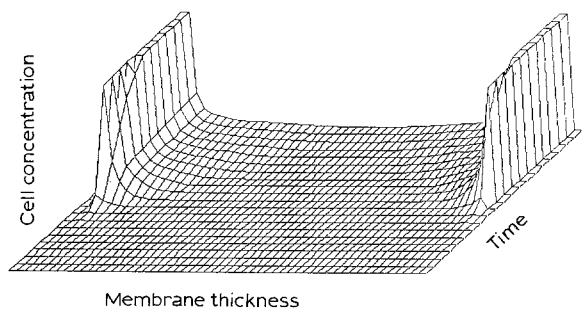


Figure 3 Time evolution of the cell distribution inside the membrane. At the steady-state, the greatest part of the biomass is located near the membrane–solution interface.

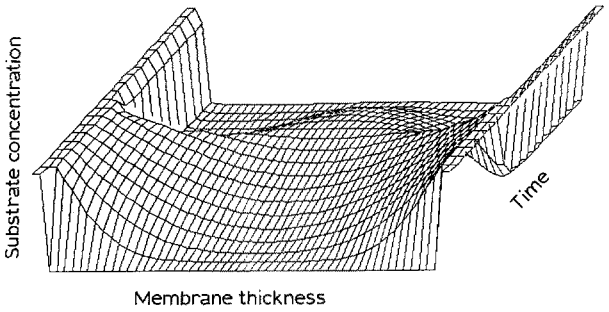


Figure 4 Time evolution of the substrate concentration profile inside the membrane.

negative, driven by the reaction (Fig. 5). Owing to the curvilinear cell concentration profile, the reaction rate is higher near the membrane–solution interfaces. Cell growth is thus maximum and substrate is entirely consumed in these regions. Because of the very high cell concentration, the diffusion coefficients strongly decrease and no substrate remains in the membrane–solution regions; the reaction front is thus progressively displaced towards the centre of the membrane because of the previously accumulated substrate (Fig. 4).

(iii) The third period starts when all substrate molecules present in the central area of the membrane are consumed. At this time, the only active regions of the membrane are the layers near the interfaces.

3.3. Influence of the parameters on the cell distribution

At the onset of the reaction, the cells are homogeneously distributed in the membrane. The final distribution is dependent on the local conditions and, in particular, on the local substrate concentration. In diffusion–reaction systems, the balance between diffusion and reaction drives the steady-state substrate profile. In these diffusion-reaction-growth systems, the balance between diffusion and reaction will induce the distribution of the cell growth rate which, in turn, influences the cell profile.

When diffusion is very fast, i.e. when either the membrane is thin or the diffusion coefficient is high or the substrate concentration is low, the substrate distribution will be homogeneous and cells will grow at the same rate throughout the membrane. As long as the cell concentration is lower than a critical value, the phenomena remain uniform because diffusion is still

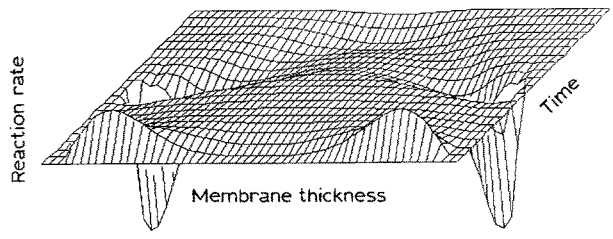
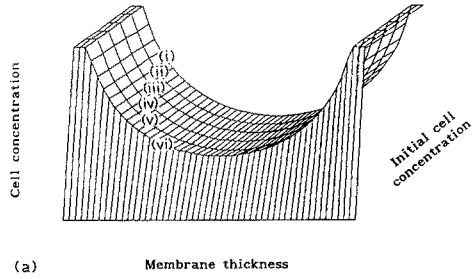


Figure 5 Time evolution of the reaction rate profile inside the membrane.



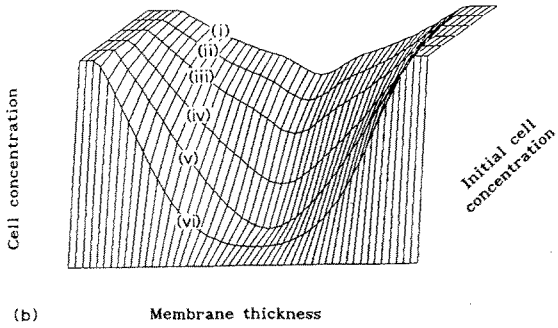


Figure 6 Steady-state cell distribution inside the membrane as a function of the initial cell concentration: (i) 10^4, (ii) 10^6, (iii) 10^8, (iv) 10^{10}, (v) 10^{11} and (vi) 10^{12} cells cm^{-3}, (a) for a reaction-controlled system ($e = 2$ mm, $S_0 = 10^{-3}$ mmol cm^{-3}), and (b) for a diffusion-controlled system ($e = 10$ mm, $S_0 = 10^{-1}$ mmol cm^{-3}).

faster than reaction. Above the critical value, reactions prevail over diffusion and the curvilinear substrate and cell profiles appear. This means, under these conditions, that whatever the initial cell concentration, as long as it is lower than the critical value, the final cell distribution remains roughly unchanged (Fig. 6a).

In contrast, when diffusion is slower than reaction, i.e. when either the membrane is thick or the diffusion coefficient is low or the substrate concentration is high, the substrate distribution will be heterogeneous and the growth rate distribution will not be uniform, even shortly after the onset of the reaction. In this case, the critical cell concentration, for which reaction prevails over diffusion, is very low. For an initial cell concentration below the critical value, the previously described phenomena is still obtained. It is different for an initial cell concentration above the critical value: the curvilinear diffusion–reaction profile appears very quickly and is amplified by the cell growth. This results in a heterogeneous distribution of the cell concentration at the steady-state which depends on the initial cell concentration (Fig. 6b).

4. Discussion and conclusion

A new model for the dynamic evolution of a membrane system containing homogeneously distributed immobilized cells has been designed and theoretically studied. The paper shows that the diffusion–reaction–growth system leads to a final heterogeneous U-form cell distribution which depends on the transport characteristics of the membrane and on the substrate concentration of the surrounding solution. The

numerical simulation clearly describes the dynamics of the system. The conclusions are in agreement with the theoretical and experimental works already published.

Nevertheless, this remains a single model and other constraints, such as cell death, inhibition phenomena, external mass transfer, etc, have to be taken into account in a more sophisticated approach, in order to be able to interact with experiments and improve our understanding of such systems.

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