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Dynamic Modeling of an Immobilized Cell Reactor

Application to Aerobic Reactions

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ABSTRACT

A mathematical model has been developed to describe the dynamic aerobic reaction occurring in a semibatch type of mixed flow reactor, containing cells immobilized in gel beads. This modeling is an extension of that developed in our previous study, for an immobilized cell reactor involving ethanol fermentation. In contrast to anaerobic reactions such as ethanol fermentation, (wherein the influent substrate concentration can be set at any desired level), aeration becomes necessary to provide additional substrate (oxygen) for most aerobic reactions occurring in immobilized cell reactors. Tobacco cell cultivation was chosen as a representative aerobic reaction, and the effect of aeration was assessed in terms of the volumetric coefficient of oxygen from gas to liquid phases.

Index Entries: Immobilized cell reactor; mathematical modeling, immobilized cell reactor; dynamic aerobic reaction; immobilized cell reactor; effect of aeration, immobilized cell reactor; containing cells in gel beads, immobilization of growing cell; tobacco cell, immobilization of growing cell; cell concentration change.

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INTRODUCTION

Immobilization of growing cells means that it has been confined or localized within a permeable matrix like gels. Various kinds of cells, such as microorganisms, plant, and animal cells have been immobilized and used for a variety of chemical processes (*1–5*).

Numerous mathematical modelings of the immobilized cell reactor have been developed, some of them assuming aerobic reactions (*6–8*). Steady-state systems were studied by Adlercreutz (*6*) and Shieh et al. (*7*). Unsteady mass balances were used for both biofilm and continuous phase, but the biofilm growth was neglected in the study by Worden and Donaldson (*8*). A model capable of predicting time courses of substrate and product concentrations, as well as the distribution of cell concentration in gel beads has been developed in our previous paper (*9*). This model, however, has been applied only to anaerobic reaction (ethanol fermentation) wherein the substrate concentration in the inlet flow can be set at almost any desired level. By contrast, the level of oxygen concentration in the inlet nutrient stream is limited by oxygen solubility in the medium. Oxygen is rate limiting in most of the aerobic reactions. Aeration, therefore, is commonly employed to provide additional oxygen supply for aerobic reactions in immobilized cell reactors.

The objective of this study is to extend the previous study (on an anaerobic reaction in immobilized cell reactors) to an aerobic reaction by taking into account the effect of aeration. The tobacco cell was chosen as a case study, and numerical calculations were carried out to assess the effect of aeration.

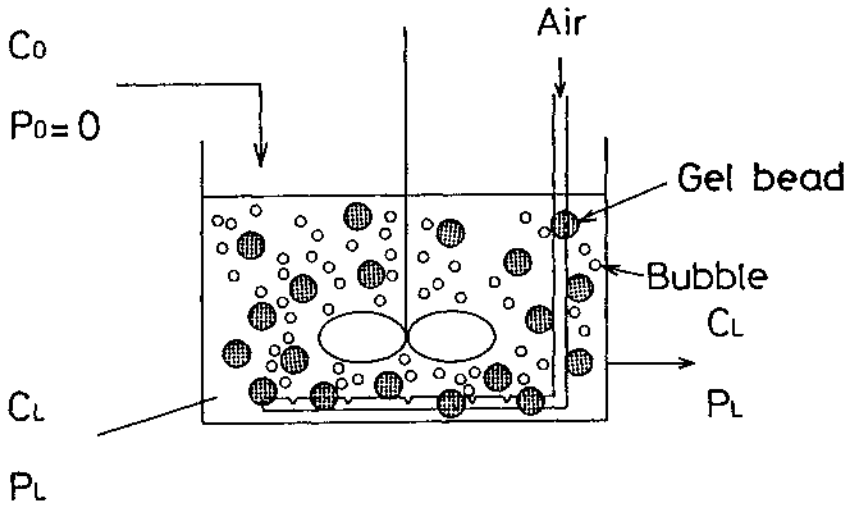
THEORY

Immobilized Cell Reactor

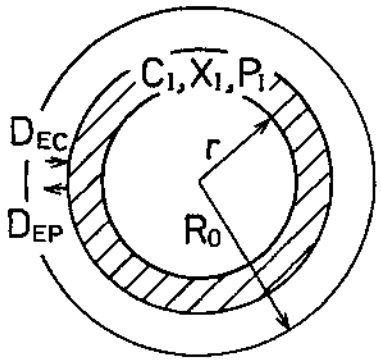
A model, as portrayed in Fig. 1a, has been proposed for a semibatch type of immobilized cell reactor. Substrate solution is continuously supplied into a mixed flow reactor containing cells immobilized in gel beads. This scheme is the same as that described in the previous study (*9*), except that aeration is added in the present one. Oxygen, the substrate, was supplied both in the incoming liquid medium and by means of aeration. The oxygen feed hereafter refers to the sum of these two forms of oxygen feeds.

A schematic representation of the distribution of cells immobilized within a gel bead is shown in Fig. 1b. The external mass transfer, internal diffusion, and reactions (within the gel beads) involving the time changes of the distribution of cell concentration must be treated simultaneously.

The present mathematical model is based on the same assumptions made in the previous study. The cells are entrapped within a permeable



(a) Over-all cross section of reactor



(b) Close-up view of gel bead

Fig. 1. Schematic representation of an immobilized cell reactor.

spherical gel beads and grow continuously and progressively throughout the gel beads. The reactant diffuses into and reacts throughout the gel beads at all times, most likely at different rates at different locations within the beads. The external mass transfer resistance is negligible. In addition, it is assumed that the oxygen supply is rate limiting.

The present mathematical model, based on the above assumptions, is represented by the following equations, and initial and boundary conditions:

$$\frac{\partial C_I}{\partial t} = D_{EC}\left(\frac{\partial^2 C_I}{\partial r^2} + \frac{2}{r}\frac{\partial C_I}{\partial r}\right) - \left(m + \frac{\mu}{Y_{X/C}}\right)X_I \tag{1}$$

$$\frac{\partial P_I}{\partial t} = D_{EP}\left(\frac{\partial^2 P_I}{\partial r^2} + \frac{2}{r}\frac{\partial P_I}{\partial r}\right) + Y_{P/C}\left(m + \frac{\mu}{Y_{X/C}}\right)X_I \tag{2}$$

$$\frac{\partial X_I}{\partial t} = \mu X_I \tag{3}$$

$$\mu = \frac{\mu_{max} C_I}{K_C + C_I} \tag{4}$$

$$\frac{dC_L}{dt} = \frac{F}{V_L}(C_0 - C_L) + k_L a(C_{st} - C_L) - \frac{3V_I D_{EC}}{R_0 V_L}\left(\frac{\partial C_I}{\partial r}\right)_{r=R_0} \tag{5}$$

$$\frac{dP_L}{dt} = \frac{F}{V_L}(P_0 - P_L) + \frac{3V_I D_{EP}}{R_0 V_L}\left(\frac{\partial P_I}{\partial r}\right)_{r=R_0} \tag{6}$$

I.C. $C_I(t=0, 0 \le r \le R_0) = 0, \quad C_L(t=0) = 0$ (7)

$P_I(t=0, 0 \le r \le R_0) = 0, \quad P_L(t=0) = 0$ (8)

$X_I(t=0, 0 \le r \le R_0) = X_{I0}$ (9)

B.C. $C_I(t>0, r=R_0) = C_L(t), \; \partial C_I(t>0, r=0)/\partial t = 0$ (10)

$P_I(t>0, r=R_0) = P_L(t), \; \partial P_I(t>0, r=0)/\partial t = 0$ (11)

where C, P, X are the concentrations of oxygen, product, and cell, respectively. The subscripts I, L, 0 refer to the immobilized gel beads, the external liquid medium, and the initial value, respectively. C_{st} is the saturated concentration of oxygen which would be in equilibrium with the gas phase oxygen concentration. D_{EC} and D_{EP} are the effective diffusivities of oxygen and product, respectively. V_I and V_L stand for the volume of the immobilized gel beads and the liquid medium, respectively. R_0 is the bead radius, F the volumetric flowrate of the inlet nutrient solution as well as the effluent, m the specific maintenance rate, μ_{max} the maximum specific growth rate, K_S is the value of oxygen concentration at which the specific growth rate is half its maximum value, r the radius, and t the time. We make use of yield factors $Y_{X/C}$, $Y_{P/C}$ in the substrate balance. k_La is the volumetric coefficient for the oxygen transfer from the gas to the liquid phase. Note that Eq. (5) differs from the previous one (*9*), since it contains the term associated with aeration. To obtain C(t), P(t), and X(t), Eqs. (1) through (6), subject to Eqs. (7) through (11), were solved simultaneously by using a finite difference method.

Table 1
The Constants Used for Calculation of *Nicotiana tabacum*

C_0	$= 6.56\times10^{-3}$	(kg/m^3)
F	$= 1.13\times10^{-6}$	(m^3/h)
V_I	$= 5\times10^{-5}$	(m^3)
V_L	$= 5\times10^{-5}$	(m^3)
k_La	$= 36$	(1/h)
R_0	$= 2\times10^{-3}$	(m)
D_{EC}	$= 6.8\times10^{-6}$	(m^2/h)
D_{EP}	$= 2.3\times10^{-6}$	(m^2/h)
μ_{max}	$= 0.045$	(1/h)
K_C	$= 1.32\times10^{-4}$	(kg/m^3)
m	$= 6.4\times10^{-3}$	(kg/kg-cell/h)
$Y_{P/C}$	$= 1.0$	(kg/kg-oxygen)
$Y_{X/C}$	$= 0.45$	(kg-cell/kg-oxygen)

RESULTS AND DISCUSSION

The tobacco cell, *Nicotiana tabacum* was chosen for a case study of the aerobic reaction in the immobilized cell reactor, because there exists substantial data (in reference to tobacco cells) available for the model calculation. The tobacco cell yields products such as phenolic-type compounds (*10*) and Quinones (*11*) while it grows in a medium containing minerals, growth factors, carbon sources, and plant growth regulators under aerobic conditions. Many substrates may affect tobacco cell activities, but oxygen is the most important one, because the equilibrium concentration of dissolved oxygen is extremely low when compared to the dissolved concentrations of other major nutrients, i.e., oxygen is the rate limiting substrate. Therefore, oxygen is treated as the sole substrate affecting the tobacco cell culture in the model calculation. Table 1 summarizes the numerical values used for the model calculation of this study. $Y_{X/C}$ and μ_{max} of the cells are taken from Azechi (*12*), and m from Sasaki (*13*). D_{EC} and D_{EP} were calculated according to Furusaki and Seki (*14*), K_C according to Taguchi et al. (*15*). There is no data available in the above literature with regard to $Y_{P/C}$, so a few values (in the range 10^{-3}–1 kg/kg-oxygen) were arbitrarily chosen. No significant differences were observed in the general configurations of the substrate and product concentration profiles with varying $Y_{P/C}$ values, thus only the case of $Y_{P/C} = 1.0$ kg/kg-oxygen will be discussed in this study. The maximum cell concentration in gel beads was also arbitrarily chosen.

The changes in distributions of oxygen, product, and cell concentration with time, within a gel bead, are illustrated in Fig. 2. The initial oxygen and product concentrations were assumed to be zero, and the initial cell

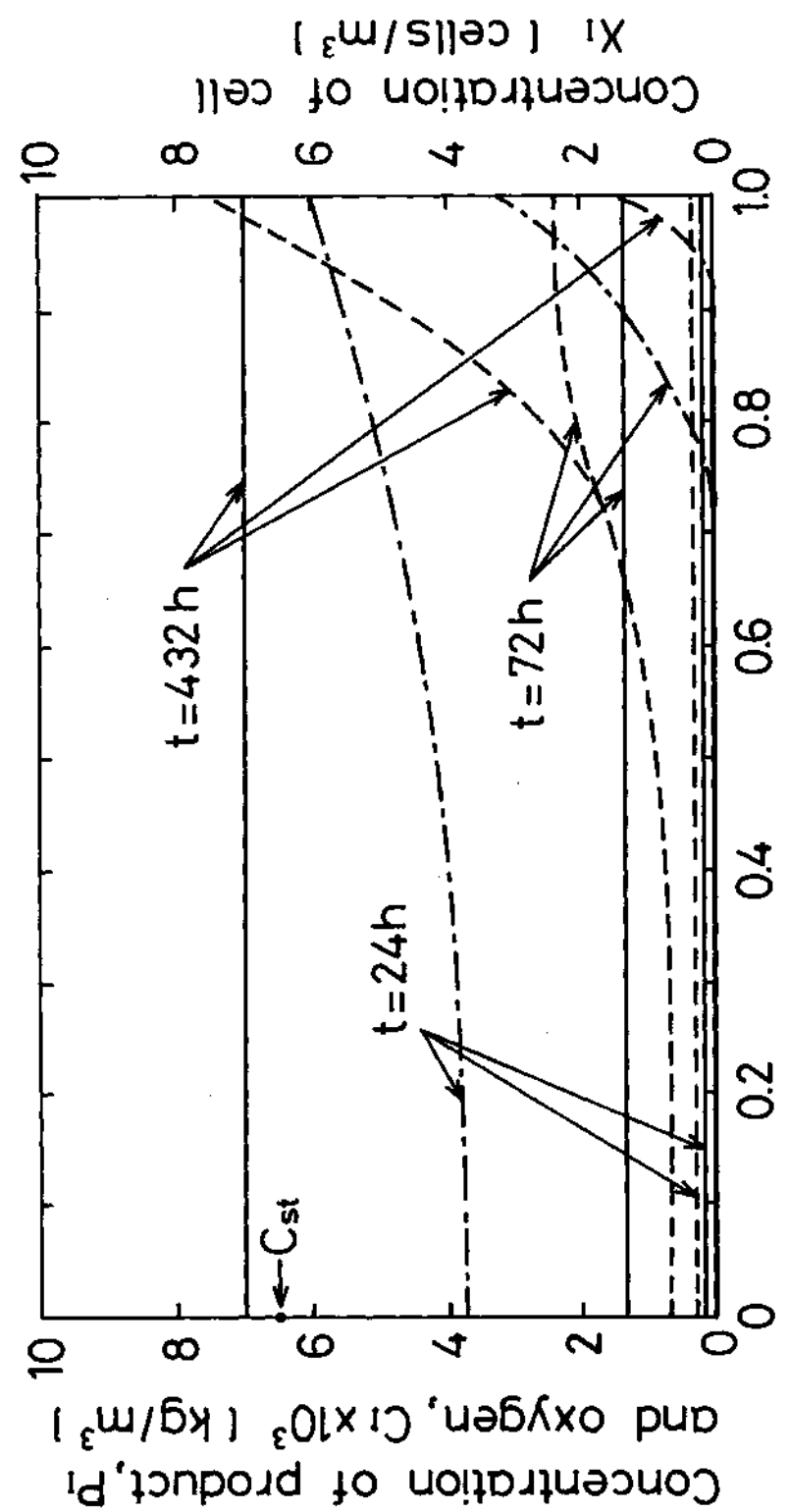


Fig. 2. Change in distributions of oxygen, product and cell concentrations with time. — · — · — :oxygen, ——— :product, and – – – – : cell.

concentration was set at 10^{11} cells/m^3 throughout the gel bead in the model calculation. Figure 2 shows that during the early stages of the process when the cell concentration is still at low levels ($t < 24$ h), the oxygen concentration increases (with moderate concentration gradient) throughout the gel bead. The oxygen uptake by cells increases as the cell population increases, and the depth of oxygen penetration shortens ($t = 72$ h), resulting in steeper cell and oxygen concentration gradients until an equilibrium state is reached ($t = 432$ h).

In contrast to this, the product concentration always remains nearly uniform throughout the gel bead and increases with time, indicating that the rate of product diffusion is much greater than the rate of product formation. This occurs owing to the small rate of oxygen supply when compared to the rate of product diffusion as evidenced by the gradual decrease of the oxygen concentration on the gel bead surface: The concentration is smaller than the saturation concentration, C_{st}, in equilibrium with the gas phase, which is an important point to note.

Figure 3 demonstrates the significance of aeration for the aerobic reaction in the immobilized cell reactor. The calculated time courses with aeration ($k_La = 36$ 1/h) and without aeration ($k_La = 0$) are shown for the oxygen and product concentrations and for the mean concentration of cells (in gel beads). The mean concentration of cells, $\overline{X_I}$, was calculated from Eq. (12).

$$\overline{X_I} = \int_0^{R0} 4\pi r^2 X_I dr \,/\, \int_0^{R0} 4\pi r^2 dr \qquad (12)$$

Figure 3 indicates that when no aeration is employed, the oxygen concentration in the liquid medium instantaneously decreases to a very small value, (5.8×10^{-7} kg/m^3), and the product to a small yield, (6.6×10^{-3} kg/m^3), whereas the mean concentration of cells hardly changes with time (10^{11} cells/m^3). By contrast, when aeration is employed, the oxygen concentration does not reduce to such a small value. It levels off, after a gradual decrease in the early stages of the reaction, to an equilibrium value of 2.0×10^{-3} kg/m^3. The cell concentration (after a gradual increase) also reaches an equilibrium value at approximately the time (100 h) when the oxygen concentration levels off. There is an interesting feature in Fig. 3 that is different from that of ethanol fermentation. That is, the product concentration continues to increase (up to about 430 h) even after the oxygen and cell concentrations leveled off at earlier times. This appears to occur owing to the interaction between the rates of liquid feed and product formation. Compared with ethanol fermentation, the retention time for tobacco cell cultivation must be kept long. One should note that the rates of product formation of aerobic reactions (of animal and plant cells) is generally much smaller than that of anaerobic reactions. After reaching an equilibrium state, the cell population consumes oxygen at a constant rate. The concentration of the product in the reactor, however, continues to increase for some time because the effluent rate is smaller than the rate of product formation. When the effluent rate is set considerably high,

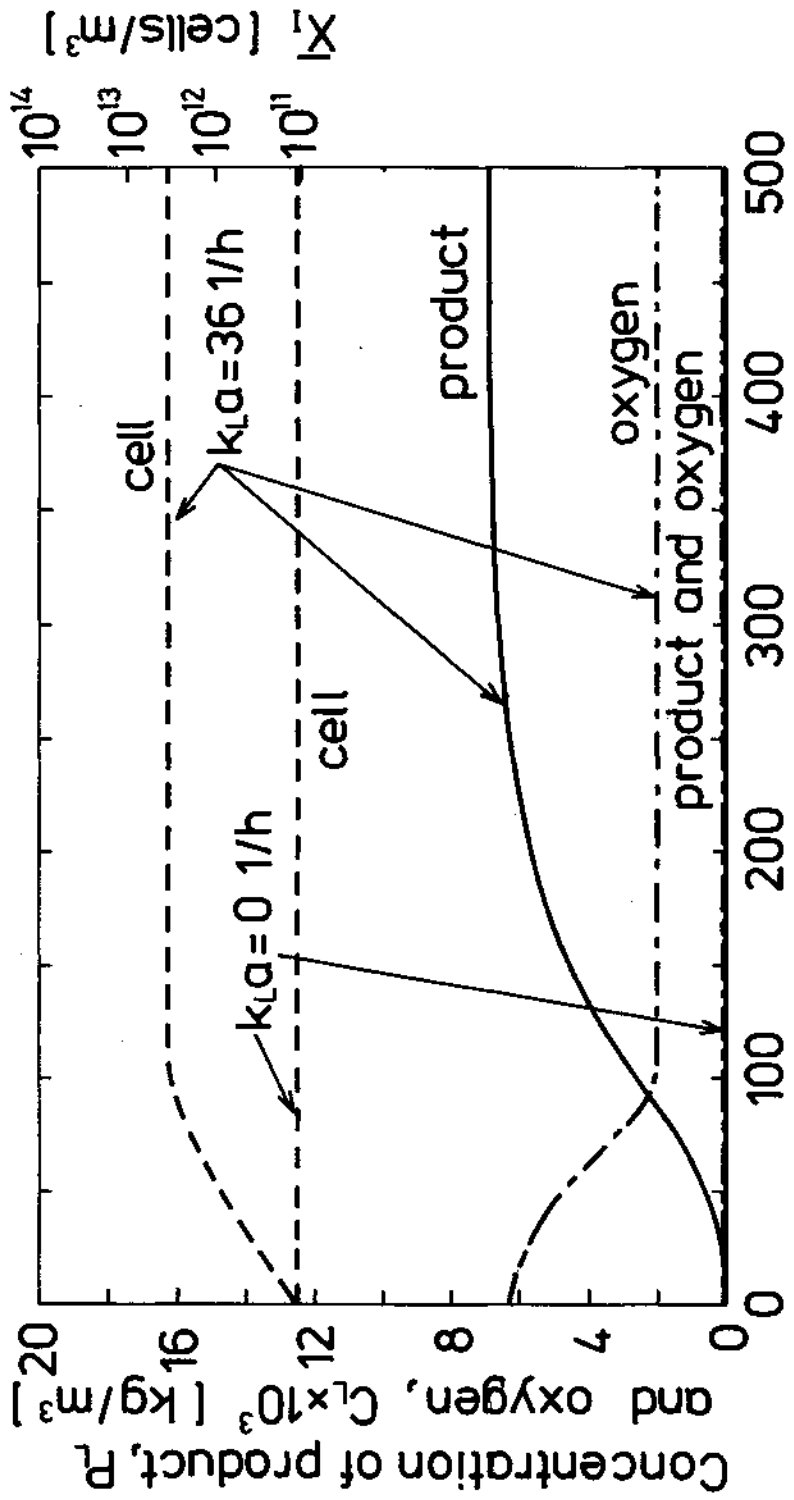


Fig. 3. Time courses of oxygen and product concentrations in the liquid medium and mean cell concentration in gel beads.

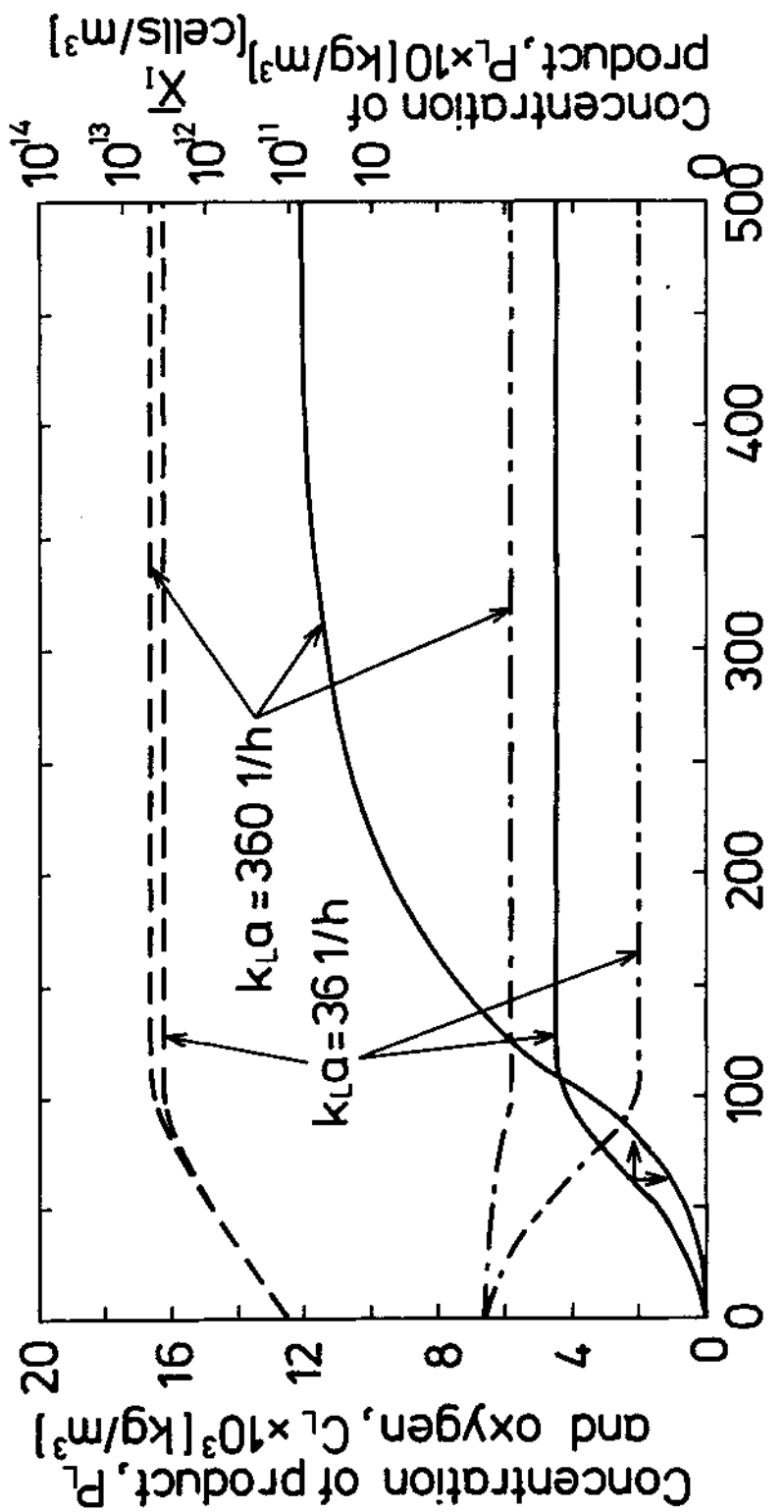


Fig. 4. Effects of volumetric flowrate and volumetric oxygen transfer coefficient on the courses of oxygen, product, and mean cell concentrations. — - — :oxygen, ——— :product, and ———— : cell.

$F = 1.82 \times 10^{-5}$ m³/h (which is impractical, however), the concentrations of oxygen, product, and cells level off at about the same time as is shown in Fig. 4. A parametric study with respect to k_La values (between 0 to 360 1/h) was made, but no significantly different features were observed, as is shown in Fig. 4. The same numbers listed in Table 1 (except for F value) were used for the calculation of the physical values in Fig. 4.

CONCLUSIONS

A mathematical model has been developed to describe a dynamic aerobic reaction occurring in a semibatch type of mixed flow reactor, containing cells immobilized in gel beads. The time courses of substrate and product concentrations, as well as the distribution of cell concentration were calculated. Oxygen, the limiting substrate, is supplied in incoming fluid and by aeration as well. In a practical range of operations it was found that the product would continue to increase long after the cell and oxygen concentrations had arrived at their equilibrium levels. This is caused, given sufficient supply of oxygen through aeration, by the combination of the slow rate of product formation and large retention time required for aerobic reactions in the immobilized cell reactor.

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