EFFECTIVENESS FACTORS FOR TWO-STEP CONSECUTIVE BIOCHEMICAL REACTIONS WITH IMMOBILIZED BIOCATALYSTS

Ho-Nam Chang

Department of Chemical Engineering, Korea Advanced Institute of Science and Technology
P.O. Box 131 Dongdaemun, Seoul 131, Korea

(Received 29 August 1983 • accepted 10 December 1983)

Abstract—Effectiveness factor for irreversible two step consecutive reaction with the Michaelis-Menten kinetics was calculated numerically. The effect of the first reaction on the second reaction was studied by varying the Thiele modulus, M-M constants and the relative rate of the film mass transfer as compared with that of the pore. In all cases the first reaction enhanced the second reaction of which effectiveness factor sometimes exceeded one.

INTRODUCTION

Many of the reactions occurring in biological systems are complex in nature and carried out sequentially by several enzymes immobilized in cellular membranes or microorganisms growing on surfaces. Substrates are supplied by diffusion and convection from the bulk fluid to the solid surfaces where these are converted by the biocatalysts. The utilization of substrate in the solid-supported catalysts can be siginificantly different from that in liquids. For this reason it has been of great importance for biochemical engineers to understand the effectiveness of biochemical reactions occurring in the solid supports.

Most of biochemical reactions can be represented by simple Michaelis-Menten or Monod type reaction kinetics. Using this type of kinetics various investigators have studied mass transfer in immobilized enzymes, microbial films or flocs, which are limited to single step reactions[1-5]. However, in order to understand the complex biotransformations occurring in the nature, it becomes necessary to look into the sequential nature of these reactions. Unlike the studies on the single step reactions, those on sequential reactions are not abundant and are found mainly in immobilized enzyme systems. Two enzymes carrying out consecutive reactions can be coimmobilized in one bead or separately immobilized in two beads. In connection with the work initiated by Gunn and Wood for seeking optimal catalyst profile in the catalyst packed bed reactor[6], similar studies have been performed for the two separately immobilized enzyme systems[7-9]. Other investigators have performed theoretical analysis on the two step sequential enzymic reactions in one solid support[10-15]. The two-immobilized enzyme reaction systems such as a amylase-pullulanase, glucose oxidase-catalase and invertast—glucose oxidase have also been studied experimentally[16-20]. Owing to the nonlinear nature of Michaelis-Menten kinetics these works deal mostly with first or zero order kinetics and a few of them have dealt with M-M kinetics[13,15,19].

As in the immobilized enzyme systems, anaerobic digestion of organic waste proceeds with the two distinct reaction steps, namely acid formations catalyzed by microbes called, "acid formers" and methane formation by microbes called, "methane formers" even though the real systems are represented by more complicated reaction kinetics[21]. The anaerobic digestion of organic waste in mixed tank required longer treatment time, however the development of anaerobic filter made it possible to shorten the time considerably[22]. In this study with the application to anaerobic filter process in mind, we intend to investigate the effectiveness factor of the two step reactions with the Monod type kinetics numerically and compare the results of the numerical solution with those of the analytical solution in the limiting cases.

Governing Equations

Consider irreversible two-step sequential reactions catalyzed by biocatalyst X_a, X_b and where each step is specified by the Michaelis-Menten type rate equation. That is,

$$A \underset{X_a}{\rightarrow} B \underset{X_b}{\rightarrow} C \quad (1)$$

Assuming that the solid support is in the form of a slab geometry as shown in Fig. 1,

$$D_a\frac{d^2A}{dz^2}=\frac{k_a X_a A}{K_a+A} \quad (2)$$

$$D_b\frac{d^2B}{dz^2}=\frac{k_b X_b B}{K_b+\beta}-\frac{k_a X_a A}{K_a+A} \quad (3)$$

$$D_c\frac{d^2C}{dz^2}=-\frac{k_b X_b B}{K_b+B} \quad (4)$$

Boundary conditions for Eqs. (2)-(4) are

at $z = L_t$

$$D_a\frac{dA}{dz}=k_{fa}(A_o-A) \quad (5)$$

$$D_b\frac{dB}{dz}=k_{fb}(B_o-B) \quad (6)$$

$$D_c\frac{dC}{dz}=k_{fc}(C_o-C) \quad (7)$$

and at $z = 0$

$$\frac{dA}{dz}=\frac{dB}{dz}=\frac{dC}{dz}=0 \quad (8)$$

Summing Eqs. (2)-(4), we have

$$D_a\frac{d^2A}{dz^2}+D_b\frac{d^2B}{dz^2}+D_c\frac{d^2C}{dz^2}=0 \quad (9)$$

Solving Eq. (9) with the boundary condition given in Eq. (8), we have

$$D_aA+D_bB+D_cC=E \quad (10)$$

Adding Eqs. (5)-(7) leads to

$$k_{fa}A_o+k_{fb}B_o+k_{fc}C_o=k_{fa}A_i+k_{fb}B_i+k_{fc}C_i=F \quad (11)$$

where the subscript "i" denotes the concentrations at the liquid-solid interface. The interfacial concentrations A_i, B_i and C_i satisfy Eq. (11) and Eq. (9) as well.

Calculation of Effectiveness Factors

The reaction rate of A in the biocatalyst will be equal to the flux of A at the interface, $z = L_t$. For B the reaction rate in the catalyst will be equal to the consumption rate of A plus the amount of B transported through the interface. Thus η_a, η_b will be

$$\eta_a=\frac{\int\frac{k_a A X_a}{K_a+A}dV}{\int\frac{k_a A_o X_a}{Ka+A_o}dV}=\frac{D_a\frac{dA}{dz}\big|_{z=L_t}}{\frac{k_a A_o X_a}{K_a+A_o}L_t} \quad (12)$$

$$\eta_b=\frac{\int\frac{k_b B X_b}{K+B}dV}{\int\frac{k_b B_o X_b}{K_b+B_o}dV}=\frac{D_a\frac{dA}{dz}|_{z=L_t}+D_b\frac{dB}{dz}|_{z=L_t}}{\frac{k_b B_o X_b}{K_b+B_o}L_t} \quad (13)$$

Rewriting Eqs. (12) and (13) in terms of dimensionless variables

$$\eta_a=\frac{(K_A^*/A_C^*+1)}{\phi_a^2}\frac{dA^*}{dz^*} \quad (14)$$

$$\eta_b=\frac{(K_b^*/B_o^*+1)\left(\frac{dB^*}{dz^*}+\phi_m^2\frac{dA^*}{dz^*}\right)}{\phi_b^2} \quad (15)$$

where the dimensionless variables are as follows.

$$A^*=\frac{A}{C_t},\quad B^*=\frac{B}{C_t},\quad C^*=\frac{C}{C_t},\quad z^*=\frac{z}{L_t},$$

C_t = Reference Conc, $K_a^*=\frac{K_a}{C_t}$, $K_b^*=\frac{K_b}{C_t}$,

$$\phi_a=L_t\sqrt{\frac{k_A X_a}{D_a C_t}},\quad \phi_b=L_t\sqrt{\frac{k_b X_b}{D_b C_t}},\quad \beta_a=\frac{D_a}{L_t k_{fa}},$$

$$\beta_b=\frac{D_b}{L_t k_{fb}},\quad \phi_m=\sqrt{\frac{D_a}{D_b}}$$

Method of Numerical Solution

Rewriting Eqs. (2), (3) and boundary conditions of (5), (6) and (8) in terms of the dimensionless variables defined above

$$\frac{d^2A^*}{dz^{*2}}=\phi_a^2\frac{A^*}{K_a^*+A^*} \quad (16)$$

$$\frac{d^2B^*}{dz^{*2}}=\phi_b^2\frac{B^*}{K_a^*+B^*}-\phi_m^2\phi_a^2\frac{A^*}{K_a^*+A^*} \quad (17)$$

at $z^* = 1$

$$\beta_a\frac{dA^*}{dz^*}=A_o^*-A^* \quad (18)$$

$$\beta_b\frac{dB^*}{dz^*}=B_o^*-B^* \quad (19)$$

at $z^* = 0$

$$\frac{dA^*}{dz^*}=\frac{dB^*}{dz^*}=0 \quad (20)$$

Owing to the nonlinearity of Eqs. (16) and (17), the solution for these equations is obtained by using the shooting technique which assumes the value of A*, B* at $z^* = 0$ and checks whether A*, B*, dA*/dz* and dB*/dz* at $z^* = 1$ satisfy Eqs. (18) and (19). When Φ_a and Φ_b are very large, the shooting method may not work. In this case the Chang's method for high Thiele moduli may be applied[23]. Once the values of A* and B* at $Z^* = 1$ are known, C* at $z^* = 1$ can be obtained from Eq. (11). Then "E" in Eq. (10) can be evaluated,

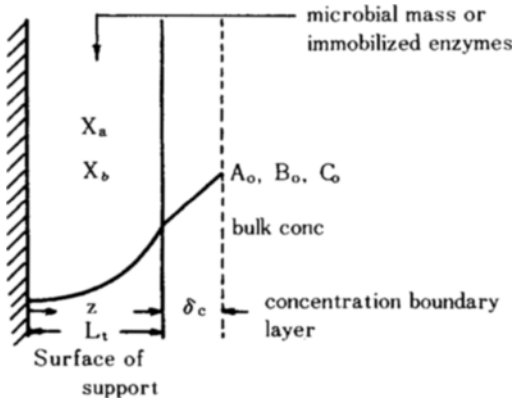

Fig. 1. Schematic Diagram of the Biocatalyst System in Consideration

which can subsequently be used to evaluted "C*" at the position other than $z^* = 1$.

Analytical Solution for the Limiting Cases

Eqs. (16), (17) are nonlinear differential equations whose analytical solutions are not immediately available. Many people have obtained analytical solutions for the first order or zero order kinetics which are limiting cases of Eq. (16) [1,4,24]. The same type of analytical solutions for B are possible in the case of the four combinations of the first order and zero order kinetics. However, the solutions are rather lengthy and complicated. For this reason these will be presented in the appendix.

The Values for the Typical Parameters

The parameters given in Table 1 are typical values in the reaction system occurring in the microbial films especially for methane production[22]. Depending on the microbial film thickness, activities of microbial cells, and substrate concentration, various ranges of dimensionless parameters can be generated, which has become the basis of the system simulation.

Table 1. Typical Ranges of Paramenters used for Effectiveness Factor Calculation (from Ref. 22)

Parameters	Ranges	units
D_a, D_b, D_c	0.5-2.0	cm^2/day
K_a, K_b	10-900	mg COD/l
k_a, k_b	4.0-20.0	gm COD/gm VSS/day
X_a, X_b	$1.0\text{-}10^{-5}$	gm VSS/cm^2-μ
L_f	50-500	μ
k_{fa}, k_{fb}, k_{fc}	2.2×10^{-3}–4.4×10^{2}	cm^2/day
A_0, B_0, C_0	300-12000	mg/l
	0.1-10	
	1.0-2.0	
	10^{-5}-50	

RESULTS AND DISCUSSION

Numerical Method

For the solution of Eqs. (15) and (16) Newton-Raphson's shooting method was successful and provided rapid conversion when Φ_a and Φ_b were low. However at high values of Φ_a and Φ_b this method failed to converge the solution, therefore we had to rely on the secant method in finding the values of dA^*/dz^* and dB^*/dz^* at $z^* = 1$. This method worked well at the values of Φ_a ad Φ_b as high as 30.

Fig. 2 shows a typical concentration profile of A*, B* and C* with respect to z^* when the bulk concentrations of A*, B* and C* are 1, 1 and 0 respectively. A* drops rapidly from 1 at the bulk fluid to a certain value A_i^* at the interface and decreases further inside the catalyst. The steepness of decrease will depend on the consumption rate of A in the catalyst. The higher the activity of the catalyst, the steeper it becomes. Thus the film diffusion limitation will be relative rather than absolute depending upon the activity of the catalyst in the support. The bulk concentration of B* is 1 which is the same as that of A*, however B* remains higher than A* inside the catalyst particle. Apparently this accumulation comes from the conversion of A* to B*. The concentration of the product C* is highest at $z^* = 0$ and decreases as z^* approaches 1 and will finally approach to the value in the bulk.

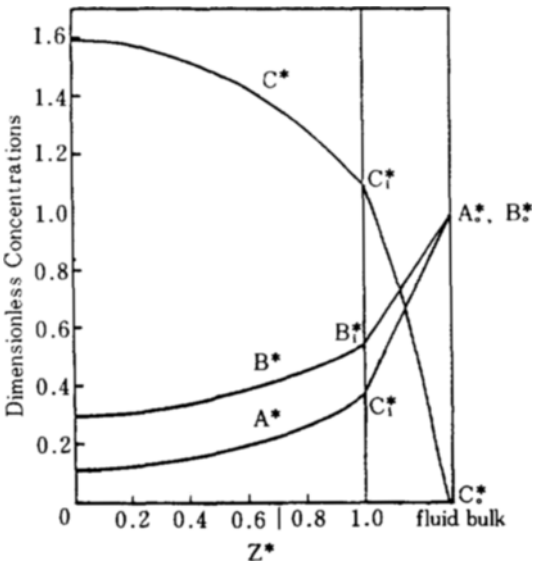

Fig. 2. Typical Concentration Profiles inside the Biocatalyst A_b^*, B_b^*, $C_b^* = 1.0, 1.0, 0.0$, and $C_t = A_o$.

Effect of Φ_a and Φ_b on η_a and η_b

The effectiveness factor η_b for the reaction $B^* \rightarrow C^*$ with the zero concentration of A* was shown in Fig. 3. η_b is essentially 1 when Φ_b is below 3. However as Φ_b increase, η_b decreases very rapidly. As the bulk concentration A* increases, η_b becomes larger than 1 and reaches a peak, and then decreases rapidly. Nevertheless η_b stays much higher than η_b with zero A*. From this figure it is clear that coimmobilization of biocatalyst X_a and X_b in one support provides effective reaction from $B^* \rightarrow C^*$ as has been found by many previous investigators[10,12,13,14,15].

Effect of K_a^*, K_b^* on η_a, η_b

With the fixed values of Φ_a, Φ_b the effect of K_a^* and K_b^* on was shown in Fig. 4. As K_b^* increases, the Michaelis-Menten kinetics approaches first order of

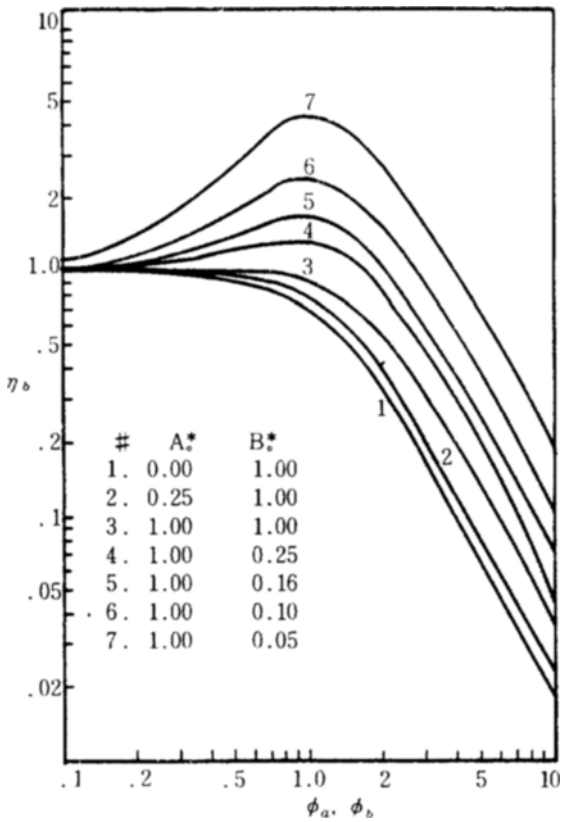

Fig. 3. Effect of ϕ_a and ϕ_b on η_a and η_b, $\phi_a = \phi_b$

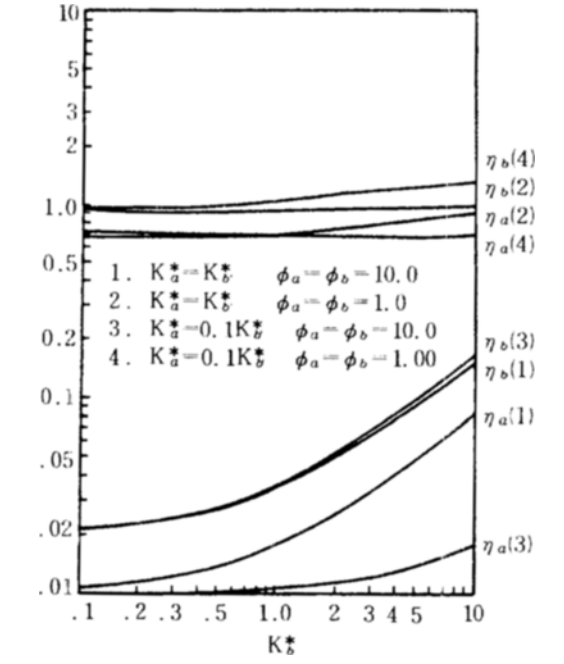

Fig. 4. Effect of K_a K_b on η_a and η_b

which effectiveness factor is lower than that of zero order. However Fig. 4 shows the increase of η_a and η_b with the increase of K_b^*. This is due to the decrease of apparent thiele moduli which is determined by dividing Φ_a and Φ_b by $\sqrt{K_a^*+1}$ and $\sqrt{K_b^*+1}$, respectively. Thus increasing K_a^* and K_b^* produces two separate contradictory effects on η_a and η_b. However, the overall effectiveness factor increases since the decrease in Φ_a and Φ_b increases η_a and η_b more rapidly than the change of reaction kinetics from the zero order to the first order lowers η_a and η_b. η_b is always higher than η_a. The effect is more pronounced when K_a^* is much smaller than K_b^* at Φ_a and Φ_b values of 10. When Φ_a and Φ_b are both 1, η_b are close to or slightly larger than 1 of which trend is the same as in high Φ_a and Φ_b.

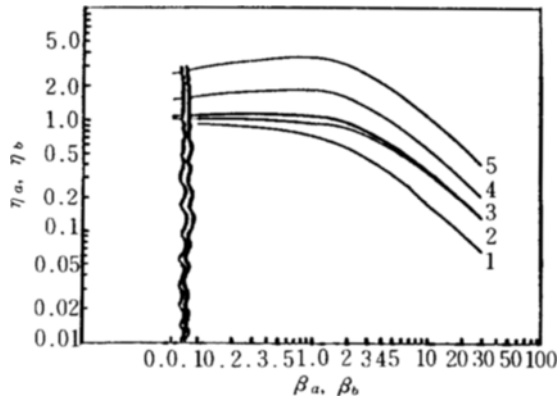

Fig. 5. Effect of β_a β_b on η_a and η_b

#	,	$\phi_a = \phi_b$	$\alpha_a = \alpha_b$	A_o^*, B_o^*
1.	η_a,	1.0,	1.0, 1.0	1.0, 1.0
2.	η_b,	1.0,	1.0, 1.0	1.0, 1.0
3.	η_b,	1.0,	0.1, 1.0	1.0, 1.0
4.	η_b,	1.0,	0.1, 1.0	1.0, 0.25
5.	η_b,	1.0,	0.1, 1.0	1.0, 0.10

Effect of β_a and β_b on η_a and η_b

Fig. 5 shows the effect of β_a and β_b on η_a and η_b. Like the effect of Φ_a and Φ_b on η_a and η_b, higher β decreases the effectiveness factors. This is clear since higher β means lower mass transfer coefficient which results in lower interfacial concentration. When the reaction of A → B approaches zero order ($\alpha_a = 0.1$), η_a increases relatively to $\eta_a = 1$. As the ratio of bulk concentration (A*/B*) increases, η_b increases as happened in the case of Thiele modulus.

Discussions on Analytical Solutions

In Fig. 6 the analytical solutions are compared with the numerical solution of the Michaelis-Menten kinetics. B_{10} remains highest among the concentrations of B. This is due to the accumulation of B converted from A by the zero order reaction shown in the bottom of the figure.

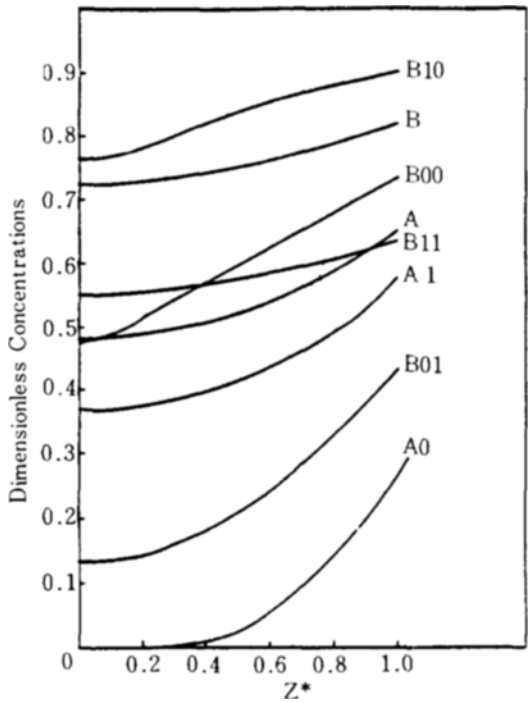

Fig. 6. Comparison of exact solutions with analytical solutions of the limiting cases (see the appendix) $\phi_a = \phi_b = 1.00$, $\beta_a = \beta_b = 1.00$, $\alpha_a = \alpha_b = 1.00$, $\phi_m = 1.00$

Then B_{00} starts high, but it decreases rapidly due to the depletion of B by the zero order kinetics. B_{01} is lowest among all since slow conversion of A to B by catalyst X_a by first order reaction and rapid decrease of B by the zero order reaction. Obtaining an approximate solution for B remains to be done as in the case of single enzyme reaction[25].

NOMENCLATURE

A, B, C: Concentrations of substrates and products, M
C_t: Reference concentration, M
D: Diffusivities of species, A, B, and C, cm^2/sec
E: Parameter defined in Eq. (10)
F: Parameter defined in Eq. (11)
K_a, K_b: Michaelis-Menten or Monod constant, M
k_a, k_b: Kinetic constants, M/sec
k_{fa}, k_{fb}, k_{fc}: Mass transfer coefficients, cm/sec
L_t: Thickness of the support
X_a, X_b: concentration of biocatalysts
z : Distance from the surface of the support
β_a β_b: Inverse of Biot number
η_a η_b: Effectiveness factor for reaction 1 and 2
Φ_a Φ_b: Thiele modulus,
ϕ_m: Square root of the two diffusivities,

Subscripts and superscripts

c: concentration at z = 0
o: Bulk concentrations, zero order concentrations
i: Interfacial concentrations
*: Dimensionless parameters
a,b: Parameters for reaction "1" and "2" respectively.
l: first order concentrations
': derivative

REFERENCES

1. Atkinson, B.. Pion Limited, 1974.
2. Kobayashi, T. and Laidler, K.J.: Biotech. and Bioeng., **16** (1974) 77.
3. Toda, T.: Biotech. and Bioeng., **17** (1975) 1729.
4. Jennings, P.A., Snoeyink, V.L. and Chian, E.S.K.: Biotech. and Bioeng., **18** (1976) 1249.
5. Williamosn, K. and McCarty, P.L.: J. Water Poll. Cont. Fed., **48** (1976) 9.
6. Gunn, D.J. and Thomas, W.J.: Chem. Eng. Sci., **20** (1965) 89.
7. Chang, H.N. and Reilly, P.J.: Chem. Eng. Sci., **31** (1976) 413.
8. Choi, C.Y. and Perlmutter, D.D.: AIChE J., **23** (1977) 319.
9. Chang, H.N. and Reilly, P.J.: Biotech. and Bioeng. **20** (1978) 243.
10. Goldman, R. and Katchalski, E.: J. Theoretical Biol., **32** (1971) 243.
11. Broun, G., Thomas, D. and Selegeny, E.: J. Membrane Biol., **8** (1972) 313.
12. Krishna, R. and Ramachandran, P.A.: J. Appl. Chem. Biotechnol., **25** (1975) 623.
13. Ramachandran, P.A., Krishna, R. and Panchal, C.B.: J. Appl. Chem. Biotechnol., **26** (1976) 214.
14. Gondo, S.: The Chemical Engineering J., **13** (1977) 229.
15. Lin, S.H.: Biophys. Chem., **7** (1977) 229.
16. Martensson, K.: Biotech. and Bioeng., **16** (1974) 1567.
17. Buchholz, K. and Godelmann, B.: Biotech. and Bioeng., **20** (1978) 1201.
18. D'souza, S.F. and Nadkarni, g.B.: Biotech. and Bioeng., **22** (1980) 2179.
19. Mercer, D.G. and L'dnscoll, K.F.: Biotech. and Bioeng., **23** (1981) 2447.
20. Lawrence, R.L. and Okay, V.: Biotech. and Bioeng., **15** (1973) 213.
21. Pohland, F.G.: Advances in Chemistry Series 105, ACS., Washington, D.C. (1971).
22. Young, J.C. and McCarty, P.L.: Stanford Univ., Dept.

$$\frac{d^2B^*_{01}}{dz^2} = \phi_b^2 - \frac{\phi_a^2\phi_m^2}{\alpha_a}A^* \qquad (A-5)$$

where $A^*_1 = a_{11}\cosh(\phi'_a(z-z_a))$

(1) $z_a < z < 1$

$B^*_{01} = 1/2\,\phi_b^2 z^2 - \phi_m^2 a_{11}\cosh(\phi'_a(z-z_a)) + b_{31}z + b_{32}$

$B^{*'}_{01} = \phi_b^2 z - \phi_m^2\phi'_a a_{11}\sinh(\phi'_a(z-z_a)) + b_{31}$

where

$b_{31} = -\phi_b^2 z_a$

$b_{32} = 1 + \phi_b^2\{z_a(1+\beta) - \frac{1}{2} - \beta\} + \phi_m^2 a_{11}\{\cosh(\phi'_a(1-z_a)) + \beta_b\phi'_a\sinh(\phi'_a(1-z_a))\}$

(2) $z_b < z < z_a$

$B_{01} = -1/2\,\phi_b^2(z_a)^2 - \phi_m^2 a_{11} + b_{31}$

6. The zero order kinetics for B with the zero order kinetics of A*.

$$\frac{d^2B^*_{00}}{dz^2} = \phi_b^2 - \phi_m^2\phi_a^2 \qquad (A-6)$$

(1) The solutions are $z_a < z < 1$. $(z_a > z_b)$

$B^*_{00} = 1/2(\phi_b^2 - \phi_m^2\phi_a^2)z^2 + b_{41}z + b_{42}$

$B^{*'}_{00} = (\phi_b^2 - \phi_m^2\phi_a^2)z + b_{41}$

where $b_{41} = (\phi_m^2\phi_a^2 z_a - \phi_b^2 z_b)$

$b_{42} = 1 - \{(\frac{1}{2}+\beta)(\phi_b^2 - \phi_m^2\phi_a^2) + (\phi_m^2\phi_a^2 z_a - \phi_b^2 z_b)(1+\beta_b)\}$

(2) The solutions are $z_b < z < 1$.

$B^*_{00} = 0.5(\phi_b^2)z^2 - \phi_b^2 z_b + b_{43}$

$B^{*'}_{00} = \phi_b^2 z - \phi_b^2 z_b$

where

$b_{43} = \phi_b^2 z_a z_b - \phi_m^2\phi_a^2 z_a^2 + b_{41}z_a + b_{42}$