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Evaluation of substrate uptake by microbial film in a gel-like medium

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A B S T R A C T

Mathematical model was developed to demonstrates the predominantly reaction controlled region and diffusion limited region for $\varnothing p > 3$ with high or large diffusion in this case, but when $\varnothing p = 0.3$ the chemical reaction and diffusion onset occurred. The model developed illustrates the relationship between K_3C^* and N/N_{max} for various incremental steps of $K_2 L$. The result obtained reveals that increase in K_3C^* resulted to an increase in N/ N_{max} until an optimum value of N/N_{max} was achieved after then the values remain constant with incremental value on K_3C^*. The Kinetics of substrate is dependent of K_3C^* as well as K_2L values of the system, which in overall influence the substrate uptake by microbial film in gel- like medium.

Capsule Summary: The developed mathematical model to evaluate the substrate uptake by microbial film in a gel-like medium was investigated and the results obtained illustrates the predominantly reaction controlled region and diffusion limited region.

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INTRODUCTION

Bacteria are relatively small organisms usually enclosed by rigid walls. In many species the outer surface of the cell wall is covered with a jelly gummy coating called a capsule or microbial slim layer. Bacteria are typically unicellular. From the physiological analysis of microorganism the rate of substrate uptake is shown to depend on both diffusion of substrate into the microbial floc and the rate of the biochemical reaction in the microbial floc and film (Atkinson, 1974; Luedeking and Piret 1959; Pearl and Reed 1920; Williams, 1967).

In chemical reactor theory the empirical nth order rate equation for the reaction kinetics has resulted in the development of equations which describe the operating characteristics of ideal reactors. These design equations have proved extremely useful as an aid devising reactor configurations for particular duties. In laboratory studies the concept of ideal reactor has assisted in the design of experiments in the development of experimental data. Thus it is reasonable to anticipate that in biochemical reactor theory the availability of generalized rate equation for a gel-like medium is a necessary prerequisite for the development of generalized design procedures and for the interpretation of experimental data. More so the systematic design of a fermented demands knowledge of the overall rate of

substrate uptake by microbial floc and film (Cookson, 1990; Irwin, 1970; Andrews, 1988; Haldame, 1965; Powell, 1942; & Lindgren 1983).

Careful review of literature has indicated that a lot of research work has been done in this area by researchers in the need for more precise and specific knowledge in this field. Most of the works done by researchers lack ease in applicability as per literature review findings (Alkinson and Mavituna, 1991; Topiwala and Sinelair 1971; Ryu and Mateeles 1968; Tessier, 1958; Tomlinson and Snaddon, 1960 & Altinson and How, 1974). Oosterhuis and Kossen (1983) predicted a two compartment model to calculate the oxygen transfer capacity of a production scale bioreactor.

The Michaelis-Menten and Monod equation did not consider the diffusion parameters of the substrate through the medium of the microbial floc. From the knowledge of the cells biochemistry, it is apparent that the Monod equation is probably a great over simplification. It is evident that the growth of most bacteria is limited by the rate of active transport of glucose, oxygen and other nutrients therefore any equation that should predict the substrate uptake or biodegradation in a microbial floc must involve the diffusion parameters (Green et al., 1965; Powell etal, 1967; Norick, 1955; Monod, 1989; Moser, 1958; Richardson and Peacock, 1994 & Tomlinson and Snaddon, 1960).

The purpose to this work is to develop mathematic expressions that will predict the physical and chemical phenomena taking place in the uptake of substrate and oxygen in a gel-like and non-gel-like media. Monod equation for the rate of substrate uptake did not consider the contribution of the diffusion parameters of the substrate transport from the outer region of the micro-organism. In this work effort will be made to model an overall rate equation. The overall rate equation will provide a Pseudo-analytical solution for the problem of diffusion with biochemical reaction within microbial mass. The equation will be substantiated by the use of glucose as a sole limiting substrate and in conjunction with the theory for biological film reactor. The equation will be able to predict the influence of both the substrate concentration and the film thickness. Much work has been done on predictive models of substrate uptake in a microbial medium. However less is known about substrate uptake in a gel-like medium, yet gel-like medium is an important medium because most micro-organisms are gel- like (Powell, 1967; Finn and Wilson, 1954; Moo Young, 1975; Lindgren, 1983 & Bungay et al., 1969; Richardson and Peacock, 1994; Met and Eddy, 1991; & Bungay et al, 1969).

The most familiar and commercially important products of microbial action are beer, wine and vinegar and other alcoholic beverage. The trickling filter uses microbial action for the treatment of wastewater and the rotating disk contactor uses microbial film in the treatment of waste. Microbial films also appear in animal tissue culture and the leaching of ores, gel-like microbial film will arise in almost any system where solid surface contacts a microbial suspension. For these reasons and others gel-like microbial films are of considerable significance in biochemical engineering (Cookson, 1990; Pearl and Reed, 1920; Luedeking and Piret 1959 & Alkinson and Mavifuna, 1991).

The availability of an overall rate equation will facilitate development of design equations for both ideal and real fermenter and the interpretation of experimental data obtained in the laboratory and on full-scale equipment. This work is design to develop rate equations for the overall rate of substrate uptake in a gel-like media, substrate uptake in a non-gel-like media, oxygen uptake in a gel-like media and oxygen uptake in a non-gel-like media.

MATERIAL AND METHODS

Less familiar but of considerable importance is the propensity of some micro-organism to flocculate or to accumulate at solid surfaces in the form of a film. Many bacteria, for example are coated on their exterior surface by a layer of slimy material, which is predominantly polysaccharides (Bailey and Ollis, 1986). Upon contact, such organism tends to stick together, so that particles of agglomerated bacteria develop. The bacteria in the interior of such a flock particle arc enmeshed in a matrix of slime gel-like material, and they consequently do not experience direct contact with the medium. Instead the nutrient pass through a series of transport resistances, the relative magnitudes of which depend on nutrient hydro-dynamics, temperature, microbial activity and density, solution composition, interfacial phenomena and other factors. Consequently, the following may be regarded as resistances to material diffusion.

(1) Diffusion from bulk gas to the gas-liquid interface.
(2) Movement through the gas-liquid interface.
(3) Diffusion of the solute through the film (relatively unmixed liquid region) adjacent to the bubble into the mixed bulk liquid.
(4) Diffusion of the solute through the film (relatively unmixed liquid region) adjacent to the bubble into the mixed bulk liquid.
(4) Transport of the solute through the bulk liquid to a second film (relatively unmixed liquid region) surrounding the microbial species.
(5) Transport through the second film associated with the microbes.
(6) Diffusive transport into the microbial flock.
(7) Consumption of the solute by biochemical reaction within the organism.

A similar limitation applies to microbial films. Although the physical situation here differs somewhat from that in mold, the mathematical models usually used for the two cases are essentially identical. The situation obtained in the model for cell membrane extracellular transport and cell growth shows that all concentration gradients are confined to a boundary region in which there is no reaction. Similarly, reaction processes are restricted to the interior, which is uniform in
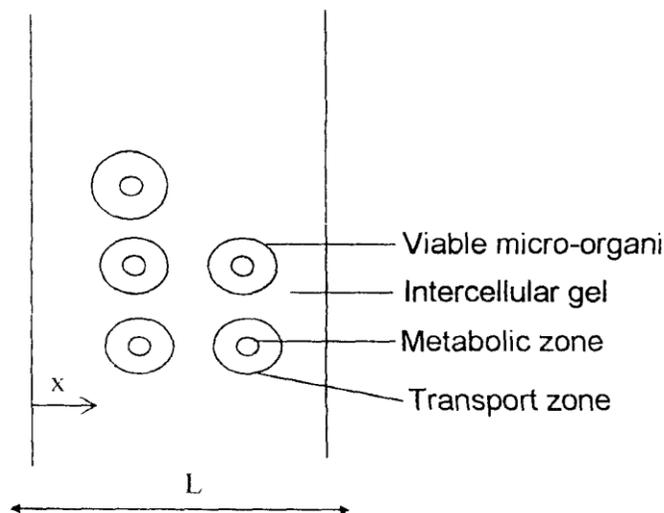
Fig. 1: Model for microbial film

all concentrations. In this kind of model, the mass transfer and reaction steps are viewed separately, they occur in series rather than in parallel. This is a lumped model (Tomlinson and Snaddon, 1960; Norick, 1955; Bungay et al., 1969; Met and Eddy, 1991 & Altinson and How, 1974).

Predictive model for substrate uptake by microbial film in a gel-like medium

The model consists of discrete viable micro-organism, homogenously dispersed in a continuous gel-like medium; it is assumed that the microbial monolayer is a uniform planar film consisting of two zones, an outer transport zone and an inner metabolic zone.

In steady state the rate of substrate utilization within the cell must be equal to the rate of substrate transport into the cell. The reactions involved in microbial and biochemical system is assumed to follow Michaelis-Menten or Monod Kinetics (Perret, 1969; Alkinson and Mavifuna, 1991; Ryu and Mateeles 1968; Green et al., 1965 & Powell, 1967).

$$r = \frac{\alpha c}{Ks + C} \qquad (1)$$

The overall rate of substrate utilization can be expressed as (1)

$$N = \int_o^L ar\,dx \qquad (2)$$

where a is the area of viable organisms per unit volume and r is the local rate of substrate uptake based upon the area a. Substrate diffusion through the gel-like region presumed to obey Ficks law of diffusion.

$$J = -De\,\frac{dc}{dx} \qquad (3)$$

J   =   diffusion flux

A mass balance on the stagnant gel-like region surrounding the cell, assuming constant diffusivity gives

$$\frac{d}{dx}\left(De\,\frac{dc}{dx}\right)$$

Since no reaction occurs in the film

$$(4)$$

In this model the mass transfer and reaction step are assumed to occur in series rather than in parallel.

Taking material balance between diffusion (substrate transport) and reaction (substrate utilization) at any point within the film gives

$$aD_e\,\frac{dC}{dx}\Big|_x - \left(-aD_e\,\frac{dC}{dx}\right)\Big|_{x+\Delta x} = a\,r\,\Delta x \qquad (5)$$

Rearranging gives

$$a\,D_e\,\frac{dC}{dx}\Big|_{x+\Delta x} - a\,D_e\,\frac{dC}{dx}\Big|_x = a\,r\,\Delta x \qquad (6)$$

Dividing by a Δ x

$$D_e\,\frac{\dfrac{dC}{dx}\Big|_{x+\Delta x} - D_e\,\dfrac{dC}{dx}\Big|_x}{\Delta x} = r \qquad (7)$$

As $\Delta x \rightarrow 0$

$$D_e\,\frac{d^2C}{dx} = r \qquad (8)$$

The material balance equation therefore becomes

$$D_e\,\frac{d^2C}{dx} = r = 0 \qquad (9)$$

Where

D_e = effective diffusion coefficient

r = rate of reaction given by Michaelis- Menten or Monod Equation

Hence equation (9) becomes

$$D_e\,\frac{d^2C}{dx^2} = \frac{aC}{K_S + C} = 0 \qquad (10)$$

In this material balance it is assumed that the Monod specific growth rate applies and that the yield factor is constant.

Boundary conditions

The outer surface of the film is exposed to medium where the substrate concentration is c, and the solid surface supporting the film is not permeated by the substrate. Consequently, the boundary condition s for c are

$$x = L \quad \left. \begin{array}{l} C = C* \\[2mm] \dfrac{dC}{dx} = 0 \end{array} \right\}$$

$$x = 0 \qquad\qquad\qquad\qquad (11)$$

These boundary conditions specify the substrate concentration at the solid. Liquid interface and the fact that the support surface is impermeable to the surface.

Model solution

Equation (10) can be solved analytically if C is very small.

When C is very small then

$$\frac{d^2C}{dx^2} - \frac{1}{D}\frac{aC}{K_S} = 0 \qquad\qquad (12)$$

Let $\dfrac{a}{DK_S} = K_2^2$

$$\therefore K_2 = \left(\frac{a}{DK_S}\right)^{\frac{1}{2}} \qquad\qquad (13)$$

The characteristic equation will be

$$m^2 - K_2^2 = 0$$

$$m^2 = K_2^2$$

$$m = \pm K_2$$

The solution then will be:

$$C = A_1\, e^{K_2 x} + A_2\, e^{-K_2 x}$$

or $\quad C = B_{\cosh} K_2 x + D_{\sinh} K_2\, x \qquad (14)$

Where, A_1, A_2, B and arbitrary constants depend on the initial conditions. Equation (14) is the solution to the problem when C is high the problem become complex and ordinary analytical method of solution cannot be used readily to provide equations of closed algebraic form which describe

the operating characteristics of ideal fermenter as well as for the development of equation for assessment of laboratory data, thus a functional relationship is to be preferred.

Functional relationship

By solving equations (1), (9) and (11) we can express the mathematical problem in dimensionless terms because this will reduce the effective number of parameters from six to three.

$$\left. \begin{array}{l} \dfrac{d^2 f}{dx^2} - \dfrac{m^2 f}{1 + \beta f} = 0 \\[3mm] x = 1 \qquad f = 1 \\[3mm] x = 0 \qquad \dfrac{df}{dx} = 0 \end{array} \right\}$$

$$(15)$$

Where f $\quad = \dfrac{C}{C*}$

$$X = \frac{X}{L}$$

$$M = L\left(\frac{a}{K_S D_e}\right)^{\frac{1}{2}}$$

$$\beta = \frac{C*}{K_S}$$

In equation (15) the parameter M represents a dimensionless microbial film thickness and the parameter β represents a dimensionless substrate concentration. Inspection of the definitions of M and β suggest that it is convenient to define two biological rate equation coefficients such that

$$\left. \begin{array}{l} K_2 = \left(\dfrac{a}{K_S D_e}\right)^{\frac{1}{2}} \\[4mm] K_3 = \dfrac{1}{k_s} \end{array} \right\}$$

$$(16)$$

So that M = K₂L

and $\quad \beta = K_3 C*$

Where K₂ has the dimensionless of L^{-1} and represents a solid phase diffusional limitation while K₃ has the dimension of $M^{-1} L^3$.

The mathematical solution to equation (15) takes the form

$$F = g(x, m, \beta) \qquad (17)$$

This solution is in terms of concentration profile, and has limited application. It is more convenient to express the solution as a flux at the interface of the microbial mass with the nutrient solution. This is because at steady state the total rate of consumption of substrate is equal to the total flux.

$$N = \left| D_e \frac{dc}{dx} \right|_{x=L} \qquad (18)$$

Assuming there is no solid phase diffusional limitation the substrate concentration throughout the film is everywhere equal to c* therefore the rate of substrate uptake will be given by

$$N^* = (L) \frac{aC^*}{k_s + C^*} \qquad (19)$$

Equation (19) can be written as

$$N^* = \frac{k^1 LC^*}{1 + k_3 C^*} = N_{max} \frac{k_3 C^*}{1 + K_3 C^*} \qquad (20)$$

Where

$$K_1 = \frac{a}{k_s}$$

$$N_{max} = \frac{k_1 L}{k_3}$$

$$\left(K_1 = S^{-1}, K_3 = cm^3 / g, L = cm \right)$$

K_1 is a biological rate equation coefficient with dimensions of T^{-1} the term N^* is the limiting value of the flux N for a given value of C* and N_{max} represents the maximum possible rate of the reaction for a film of thickness L.

In the terms of deviation from the flux given in equation (17) and (18).

$$N = \left| De \frac{dc}{dx} \right|_{x-L} = \lambda N^* \qquad (21)$$

Where λ is an effectiveness factor

$$= \frac{flux\,with\,diffusion}{flux\,without\,diffusion}$$

We can deduce from the definition given a solution to equations (15), (20) and (21) that:

$$\lambda = \frac{1 + \beta}{M^2} \frac{df}{dx} \Big|_{x=1} \qquad (22)$$

I.e. at x = if =1

Atkinson and Daoud (1968) have given a solution to equation (13) for small values of β as:

$$\lambda = \frac{\tanh k_2 L}{k_2 L} = \frac{\tanh M}{M} \qquad (23)$$

This may be rewritten as:

$$\lambda = \frac{\tanh \phi_\rho}{\phi_\rho}$$

Since ϕ_ρ tends to K2L as K3C* approaches zero.

Equation (22) can be represented as:

$$\lambda = g(m, \beta) \qquad (24)$$

The corresponding substrate uptake is then obtained by combination of equation (20), (21), and (24) to give:

$$N = \lambda N_{max} \frac{k_3 C^*}{1 + k_3 C^*} \qquad (25)$$

This equation describes the substrate uptake as a flux N, which depends upon the gel or slime thickness L, the interfacial concentration C* and the coefficients k1, k2 and k3 that are characteristics of the microbe substrate system.

When the rate of substrate uptake by a single microorganism is given by the Monod, the parameters k1, k2 and k3 are related to the Monod coefficients as follow;

$$\left. \begin{array}{l} K_1 = \dfrac{\mu_{max} \, \rho o}{Y_0 + K_S} \\[2mm] K_2 = \left(\dfrac{k_1}{De} \right)^{\frac{1}{2}} \\[2mm] K_3 = \dfrac{1}{k_s} \end{array} \right\}$$

Where

Y0 = is the yield of microbial mass per unit mass of substrate consumed.

$\rho_o =$ is the density of the microbial mass

Equation (25) can be written as:

$$\frac{N}{N_{max}} = \frac{\lambda \beta^*}{1 + \beta^*} \qquad (26)$$

Where

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$$\beta^* = \frac{C^*}{K_S}$$

$$K_3 = \frac{1}{K_S}$$

Hence

$$\beta^* = K_3 C^*$$

So that

$$\frac{N}{N_{max}} = \lambda \left(\frac{K_3 C^*}{1 + K_3 C^*} \right) \tag{27}$$

The effectiveness factor $0 \langle \lambda \leq$ is defined separately as follow: (Mueller et al., 1966).

(1)   For the predominantly reaction controlled region

$$\lambda = 1 - \frac{\tanh M}{M} \left(\frac{\phi_\rho}{\tanh \phi_\rho} - 1 \right)_{For \phi_\rho \leq 1} \tag{28}$$

Where

M = k_2 L

$$\leq 1 \, then \, \lambda = \frac{\tanh \phi_\rho}{\phi_\rho} \, and \, if \, \phi_\rho \leq 1 \, then \, \lambda = 1$$

If M

(2)   For the predominantly diffusion limited region i.e. in the gel-like region.

$$\lambda = \frac{1}{\phi_\rho} - \left[\frac{\tanh M}{M} \left(\frac{1}{\tanh \phi_\rho} - 1 \right) \right]_{For \phi_\rho \geq 1} \tag{29}$$

$$\phi_\rho \geq 1 \, then \, \lambda = \frac{1}{\phi_\rho}$$

If

The Thiele modulus $\left(\phi_\rho \right)$ is defined by Atkinson and Mavituna (Atkinson and Mavituna, 1991) as

$$\phi_\rho = \left[\frac{(K_2 L)(K_3 C^*)}{1 + K_3 C^*} \right] [2(k_3 c^* - In(1 + K_3 C^*))]^{\frac{1}{2}} \tag{30}$$

$$N_{max} = \frac{K_1 L}{K_3}$$

$$M = K_2 L$$

$$\beta = K_3 C^*$$

From the above simplification in equation (30)

$$N = \frac{\tanh \phi_\rho}{\phi_\rho} N_{max} \frac{k_3 C^*}{1 + K_3 C^*} \tag{31}$$

If K2L <1 for all K3C* or K3 C*< 0.1 for all K2L

$$N = N_{max} \frac{K_3 C^*}{1 + K_3 C^*} \, for \phi_\rho \leq 1 \tag{32}$$

Criteria for assessing the magnitude of mass transfer effects on overall kinetics (Table 1)

Predictive model for oxygen uptake by microbial film in a gel-like medium: Many micro-organisms are capable of respiring in the absence of molecular oxygen, these micro-organisms are called anaerobes and, in some cases, oxygen is a necessary condition for either their well-being or for the production of some desired product. On the other hand some micro-organisms cease to function when deprived of oxygen, these are called obligate anaerobes. The oxygen requirements of a microbial fermentation present series of problem in the design of a fermenter.

The respiration rate of aerobic microbes is dependent on dissolved oxygen concentration in the growth medium. Since oxygen is an essential but sparingly soluble nutrient of many molds used industrially, transport of oxygen into mold film is an important problem to explore.

The case in consideration is that of a uniform film which has infinite area but finite depth. As was previously discussed the local rate of reaction is assumed to be described by Michaelis- Menten or Monod Kinetic, so that at steady state the material balance at any point in the film gives:

$$De \frac{d^2 C_A}{dx^2} = \frac{\mu_{max} C}{K_s + C} \tag{33}$$

Where

De is the effective diffusion of oxygen in the film, and

X is the distance measured from the surface of the film

Expressing the equation in dimensionless form by putting

$$\beta = \frac{C}{K_s}$$

$$x = \frac{x}{L}$$

Where L is the total thickness

Equation (33) then becomes:

Table 1: Criteria for mass transfer limitation

Criterion	Limiting Rate Process	Extent of Mass Transfer Limitation
$\phi_\rho \langle 0.3$	Chemical Reaction	Negligible
$\phi_\rho \rangle 3$	Diffusion	Large
$\phi_\rho = 0.3$	Chemical reaction/ diffusion	Onset

Table 2: Experimental result

K_3C^*	N/N_{max}						
	$K_2L=0.5$	$K_2L=1$	$K_2L=3$	$K_2L=5$	$K_2L=10$	$K_2L=20$	$K_2L=30$
0.2	0.11	0.07	0.03	0.02	0.01	0	0
0.4	0.175	0.145	0.06	0.04	0.02	0.01	0.01
0.6	0.25	0.225	0.1	0.07	0.04	0.02	0.01
0.8	0.35	0.325	0.17	0.1	0.05	0.03	0.02
1	0.475	0.45	0.25	0.16	0.07	0.03	0.02
2	0.55	0.52	0.3	0.19	0.08	0.04	0.03
4	0.68	0.67	0.450	0.28	0.14	0.7	0.04
6	0.775	0.77	0.625	0.4	0.20	0.1	0.07
8	0.85	0.83	0.775	0.56	0.28	0.14	0.09
10	0.90	0.9	0.870	0.75	0.38	0.19	0.125
20	0.25	0.925	0.910	0.85	0.45	0.225	0.150
40	0.960	0.960	0.950	0.950	0.60	0.3	0.20
60	0.975	0.975	0.970	0.960	0.80	0.4	0.26
80	1	1	0.980	0.98	0.90	0.525	0.35
100	1	1	0.990	0.99	0.97	0.70	0.46
200	1	1	1	1	0.98	0.80	0.525
400	1	1	1	1	0.99	0.98	0.68
600	1	1	1	1	1	0.99	0.98
800	1	1	1	1	1	1	0.99
100	1	1	1	1	1	1	1
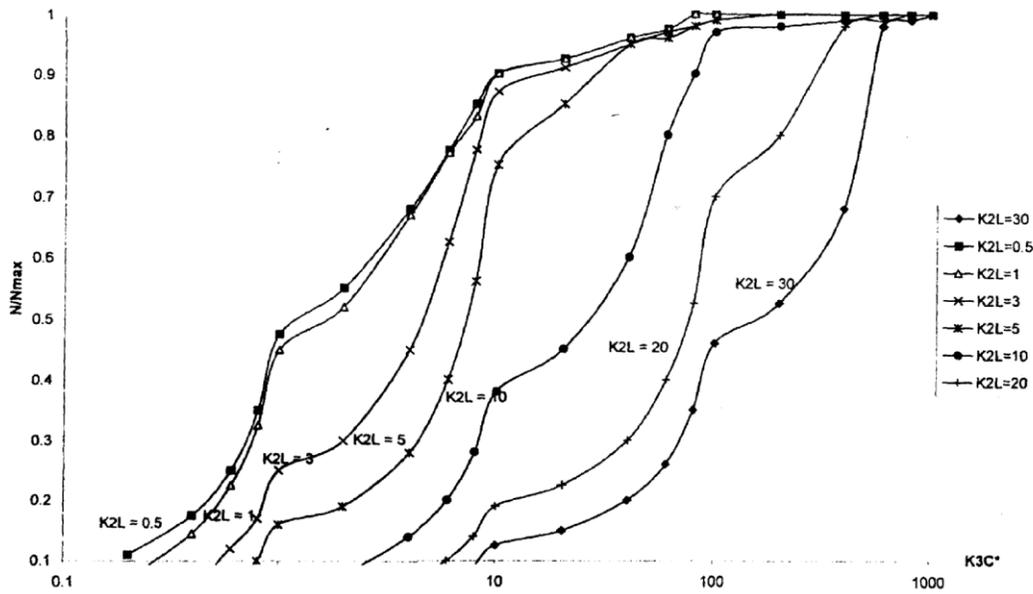
Fig. 2: Graph of N/N$_{max}$ against K$_3$C* at K$_2$L = 0.5, 1, 5, 10, 20, 30
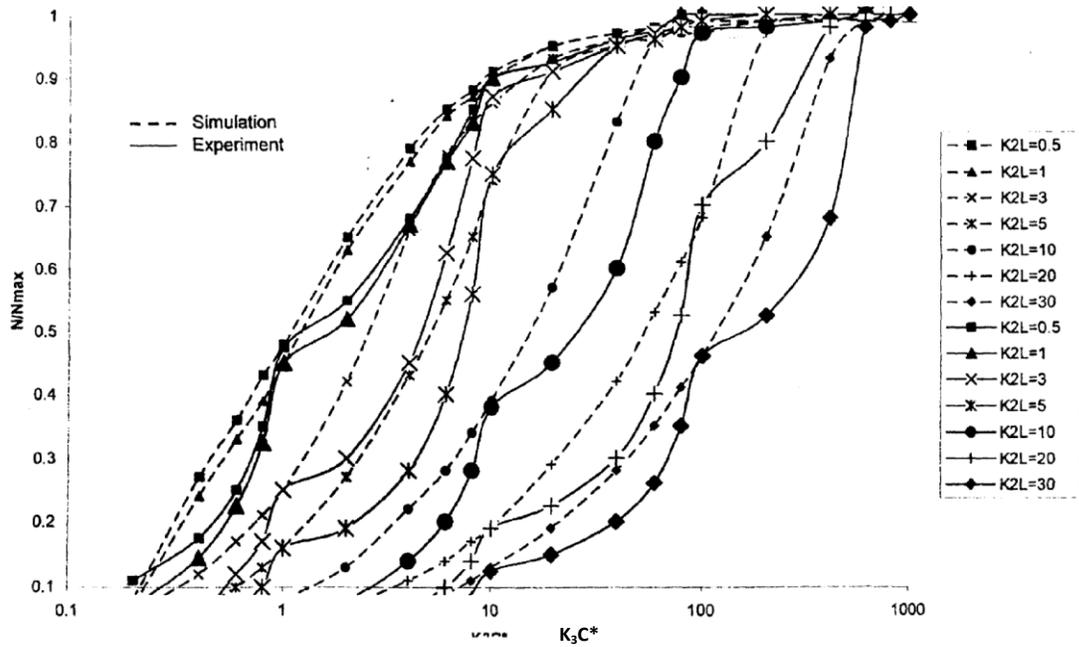
Fig. 3: Graph of N/N$_{max}$ against K$_3$C* at k$_2$L = 0.5, 1, 3, 5, 10, 20, 30

$$\frac{d^2\beta}{dx^2} = M^2 \frac{\beta}{1+\beta}$$

(34)

Where

$$M = L\frac{\mu_{max}\,\rho_o}{Y_o D_e K_s}$$

Y$_0$ = overall yield coefficient for growth

$\rho_O = $ Biomass concentration

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If the rate of reaction obtained with no diffusional restriction is denoted by μ_k^1 then an effectiveness factor may be defined as:

$$\lambda = \frac{\mu_1}{\mu_k^1}$$ (35)

We can deduce from equation (34) and (35) that (Chu and Hougen, 1962)

$$\lambda = \frac{1+\beta}{M^2} \frac{dC}{dx}\bigg|_{x=1}$$

Where x = 1, L=1

The flux of substrate is related to the rate of reaction by use of the effectiveness factor λ.

In general, the flux of substrate is given as:

$$N = \lambda N \max \frac{\beta}{1+\beta}$$ (36)

For $\beta = k_3 C^*$

Equation (36) can be written as:

$$N = \lambda N \max \frac{\beta}{1+\beta}$$

So that

$$\frac{N}{N\max} = \lambda \frac{\beta}{1+\beta}$$ (37)

Where the effectiveness factor λ is expressed in terms of the modified Thiele modulus ϕ_ρ which is defined by Atkinson (36) as:

$$\phi\rho = \frac{\beta}{1+\beta} \frac{K_2 L}{\left[2\left(\beta - In\left(1+\beta\right)\right)\right]^{1/2}}$$ (38)

So that

$$\lambda = 1 - \frac{\tanh K_2 L}{K_2 L}\left(\frac{\phi_\rho}{\tanh \phi_\rho} - 1\right) For \; \phi_\rho \geq 1$$ (39)

and

$$\lambda = \frac{1}{\phi_\rho} - \frac{\tanh K_2 L}{K_2 L}\left(\frac{\phi_\rho}{\tanh \phi_\rho} - 1\right) For \phi_\rho \leq 1$$ (40)

Assumptions

The following assumptions were made in the model development

(1) Steady state i.e. constant composition, concentration, temperature; constant liquid holp up and wetted area; no net accumulation of microbial mass constant net metabolic rate.
(2) No chemical reaction or biological oxidation by micro-organisms in suspension in the liquid phase.
(3) Thickness of gel-like region is uniform.
(4) Diffusion through the membrane is uniform everywhere. There is no longitudinal mixing.
(5) There is only soluble substrate removal. Contribution of suspended solids to slime thickness as a result of filtration and subsequent bio-oxidation is ignored.
(6) BODS taken as single substrate i.e. oxidation rates of all chemical species are identical.
(7) Excess oxygen available i.e. gas/liquid interface area and liquid phase mass transfer coefficients are sufficiently large so that the dissolved oxygen concentrations at the liquid/gelly interface result in bio-oxidation reactions which are zero order with respect to oxygen.
(8) There is uniform temperature distribution. The temperature is independent of position in the microbial membrane i.e. local equilibrium between heat generation by oxidation reactions and heat loss to the atmosphere.

RESULTS AND DISCUSSION

Experimental details are collected from Atkinson and Daoud (1968) and Atkinson and Mavituna (1991).

Figure 2 illustrates the behaviour of the ratio of N/Nmax against $K_3 C^*$ at various values of $K_2 L$ (0.5, 1, 5, 10, 20, 30). The result obtained illustrates an increase in N/Nmax ratio with increase in $K_3 C^*$ until a unity value of 1 was obtained upon the incremental values on $K_3 C^*$. These variations in the ratio of N/Nmax can be attributed to the variation in $K_3 C^*$ as well as other functional parameters that controls the system.

Figure 3 shows the relationships between the simulated results obtain from the model with the experimental results. Increase in the ratio of N/N_{max} was observed with increase in $K_3 C^*$. The variation in the ratio of N/N_{max} for both theoretical and experimental results can be attributed to the variation in $K_3 C^*$.

The rate of substrate uptake in a gel-like microbial medium depends not on the substrate concentration but rather on the "reduced substrate concentration", "relative substrate concentration" or "normalized substrate concentration" C^*/ K_s or $K_3 C^*$.

If we examine the N/N_{max} versus $K_3 C^*$ curve, we observe three distinct regions where the N/N_{max} responds in a characteristic way to change in $K_3 C^*$.

At a very low concentration (e.g. $K_3 C^* < 0.01$) the N/N_{max} versus $K_3 C^*$ curve is essentially linear, that is the rate
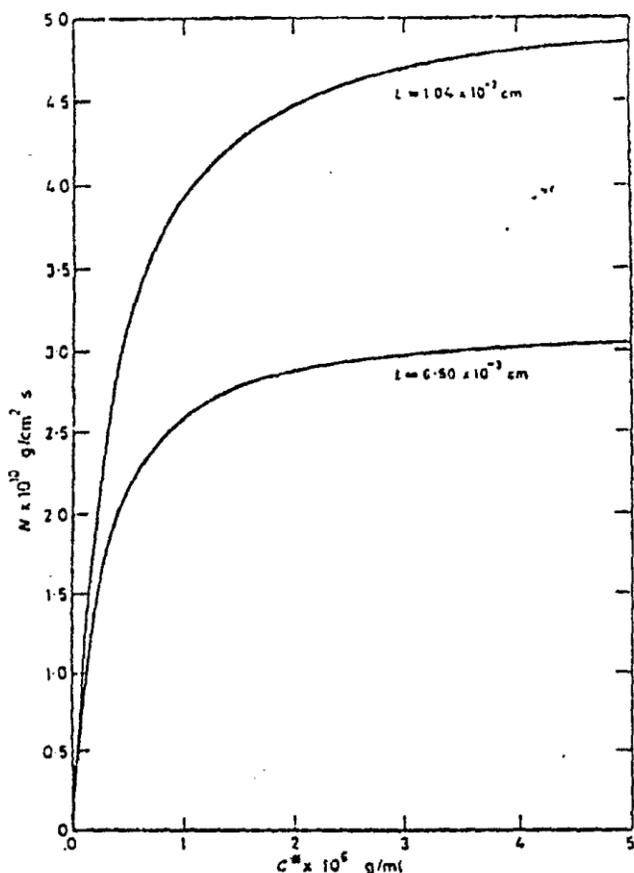
Fig. 4: Removal flux calculated using equations (28), (29), (30) and (41)

is directly proportional to the substrate concentration. This is the region of first-order kinetics. The linear relationship between

N/N_{max} and K_3C^* can be derived from the model equation so far developed.

$$\frac{N}{N\max} = \lambda \frac{K_3 C^*}{1 + K_3 C^*}$$

(41)

Where $K_3 C^* \leq K_S$ the K_3C^* in the denominator may be ignored and the equation reduces to

$$\frac{N}{N\max} = \lambda K_3 C^*$$

(42)

Where λ becomes a first order constant. At very high substrate concentration (e.g. $K_3C^* > 100$) N/N_{max} is essentially independent of K_3C^*. This is the region of zero-order kinetics.

The mathematical model for the overall rate of substrate uptake by a microbial film implies that there is a

diffusion resistance in a microbial mass. A quantitative comparison of the substrate concentration profiles predicted by the model with those obtained experimentally by Atkinson and Davies supports this view. The widely accepted Monod equation ignores any diffusion resistance in the microbial mass and is of the same algebraic form as one of the asymptotes of the complete equation.

The complete biological rate equations provide a useful analytical tool for all possible values of the biological rate coefficients K_1, K_2, K_3, and the physical variables (L, C*). This allows design equations to be developed on an analytical basis (Dean and Hinshellwood, 1986; Stanier et al., 1970) and allows for the quantitative interpretation of experimental data.

For a given set of rate coefficients (corresponding to a given microbial-substrate system) the removal flux can be expressed in terms of the substrate concentration and the film thickness (fig 4). The so-called "critical" substrate concentration is the substrate concentration beyond which the removal flux is essentially independent of concentration; the concentration is a function of film thickness.

The effective diffusion coefficient, De, of a substrate in microbial mass can be calculated from the biological rate equation coefficients, since

$$\left. \begin{array}{l} K_1 = \dfrac{a}{K_S} \\[12pt] K_2 = \left(\dfrac{a}{K_s\, De} \right)^{\frac{1}{2}} \end{array} \right\}$$

$K_1 =$

(43)

Therefore: De $= \dfrac{K_1}{K_2^2}$

The effective diffusion coefficient of oxygen in a gel-like bacteria film is given by Tom Iinson and Snaddon (Dean and Hinshellwood, 1986; Stanier et al., 1970) as about two-thirds the value of oxygen diffusion in a non gel-like medium. Bungay and Harold (1971) found that the theoretical oxygen concentration profile when the effective diffusion coefficient in non-gely medium.

This in the effective diffusion coefficient is compatible with the corresponding situation in inert gels where it is known that the diffusion coefficient of a substrate is reduced, compared with the diffusion coefficient in a non gel-medium (Chu and Hougen, 1962).

From equation (45) we have;

$$K_2 = \left(\frac{K_1}{De} \right)^{\frac{1}{2}}$$

(44)

It follows that K_2 can be estimated from a known value of K1 and a value of De assumed using the above data as a guide line.

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The value of De for glucose can be calculated from equation (43) and the data of Atkinson and Doud (1970). This suggest that the effective coefficient for glucose is 110% of that of non gel-like medium e.g. water.

$$\left.\begin{array}{ll} K_1 & = 2.13 \times 10^{-1}\, S^{-1} \\ K_2 & = 133.5\, cm^{-1} \\ K_3 & = 4.31 \times 10^{6}\, ml/g \end{array}\right\} \mathrm{Re}\, f1$$

(46)

When the rate of substrate uptake by a single micro-organism is given by the Monod equation the parameter K_1, K_2, and K_3 are related to the Monod coefficient as follows:

$$\left.\begin{array}{l} K_1 = \dfrac{G_{max}\, \rho_o}{Y_o\, K_m} \\[2mm] K_2 = \left(\dfrac{K_1}{De}\right)^{1/2} \\[2mm] K_3 = \dfrac{1}{K_m} \end{array}\right\}$$

Where, Y_o is the yield of microbial mass per unit mass of substrate consumed. In contrast, for those organisms which, as single cells exhibit a significant diffusion limitation no such simple relationship exist and the coefficient K_1, K_2, and K_3 have to be retained explicitly.

CONCLUSIONS

The biological rate equations provide an analytical solution for the problem of diffusion with biochemical reaction within gel-like microbial mass. The availability of these equations for both "ideal" and "real" fermenters and the interpretation of experimental data obtained in the laboratory and on full scale equipment.

The equation has been substantiated by the use of glucose as sole limiting substrates and in conjunction with the theory for the biological film reactor, appears to predict the influence of both the substrate concentration and the film thickness (Chu and Hougen, 1962; Oosterhuis and Kossen, 1983). The estimated values of the effective diffusion coefficients also provide a degree of substantiation. The applicability of the same functional expressions to both microbial flocks and films provides a useful basis for laboratory experiment to determine kinetic parameters since it may not be necessary to use the same geometry of microbial mass (i.e. flocks of film) in the laboratory as in full-scale fermenter.

The similarity between substrate diffusion coefficients in non-gel-like medium and within gel-like microbial medium allows an approximation to be made to the "solid" phase diffusion limitation (i.e. the effectiveness factor) on the basis of the coefficient K_1, K_2, and K_3. This is particularly helpful since the experimental determination of

these possibilities exists for their evaluation based upon batch experiments using shake flasks or similar apparatus containing microbial flocks of single organism proportion.

NOMENCLATURE

A, Cell external surface area (m^2)
Ap, External area of a wet microbial floe (m^2)
A, External surface area of viable micro-organisms per unit volume of microbial mass (m)
a_o, Concentration of nutrient at time zero (kg/m^3)
a', Average increase in cell critical substance per time per cell (kg/s)
a", Increase in critical substance due to internal cell production (kg)
B_n, Biomass concentration define by controls in equation (kg/m^3)
C, Substrate concentration (mass/volume) (kg/m^3)
C*, Substrate concentration at the interface between the micro-organism and the aqueous solution (kg/m^3)
Cc, External concentration (kg/m^3)
C_{10}, Initial interior substrate concentration (kg/m^3)
$C_{1(t)}$, Interior substrate concentration after time t (kg/m^3)
D, Rate of biomass decay (kg/s), (m^3/s)
Da, Damkohler number
Dc, Effective diffusion coefficient within microbial mass (m^2/s)
E, Enzyme concentration (kg/ms)
Ea, Activation energy (J/kmol)
ES, Enzyme substrate complex concentration (kg/m^3)
F, Dimensionless concentration defined by equation
F, Faradays constant (c/g euiv)
ΔG, free energy change accompanying the transport of materials (Kcal/ mol)
G, Monod specific growth rate (mol/cm.s)
G_{max}, Monod maximum specific growth rate ($mol/cm2/s$)
hD, Mass transfer coefficient (m/s)
H+, Hydrogen ion concentration (kg/m^3)
J, Diffusion flux (kg/m.s)
Ks, System coefficient (kg/m^3)
Km, System coefficient value of concentration when the specific growth rate is half its maximum value (kg/m^3)
K, Membrane permeability (s-1)
K_1, Biological rate equation coefficient (s1)
K, Maximum rate of substrate utilization per unit mass of micro-organism
Kd, Endogenous decay coefficient per time
K_2, Biological rate equation coefficient (m^3/kg)
K_3, Biological rate equation coefficient
L, "Wet" biological film thickness (m)
M, Dimensionless coefficient
N, Number of cells per unit volume (m/kg)
n_s, Size of microbial population when nutrient is exhausted and the population enters the stationary phase
N, Rate of substrate consumption per unit interfacial area
N_{max}, Maximum rate of substrate uptake ($m^3/cm^2 s$)
N, Rate of substrate uptake (m^3/cms)

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Q_{o2}, Average volumetric rate of oxygen utilization (kg/cm2s)

R, Rate of reaction per unit surface area of microbial film (mol/cm2s)

R, Local rate of substrate uptake per unit area of viable microorganism (mol/cm^{2}s)

t_{lag}, Lag time (time)

td, Time interval required to double the population (t)

Vmax, Maximum rate of degradation (mol/cm2s)

V, Cell volume (cm^3)

X, Dimensionless space

X, Space co-ordinate (m)

Yo, Yield of microbial mass per unit mass of substrate consumed

Z, $1 + \beta$

Z_1, Number of charges on transport molecules (c/mm')

α, Rate coefficient (cm-')

β, Bulk dimensionless coefficient

β^*, Surface dimensionless concentration

ε, Constant

η_e, Effectiveness factor

λ, Effectiveness factor

μ, Specific microbial growth rate (s-')

μ^1_k, Rate of reaction without diffusional restriction (mol/cm^2.s)

θ, Membrane permeability

μ_{max}, Maximum specific microbial growth rate (S)

$\mu(0)$, Specific microbial growth rate at temperature 0 (S 1)

μeff, Effective specific growth rate (S-I)

ϕp, Thiele modulus (dimensionless)

$\Delta\psi$, Potential difference across membrane (v)

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