Diffusion-Controlled Autocatalytic Reaction: A Possible Driving Force for Microbial Flocculation

HENG-KWONG TSAO AND JYH-PING HSU[1]

Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan, Republic of China

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Assuming that intercellular messages are a requisite to microbial flocculation, we show that, on the basis of the effectiveness factor, the diffusional resistance in a biofloc can be advantageous to an autocatalytic reaction. In other words, the metabolic efficiency of flocculated microbes can be higher than that of the dispersed microbes. Therefore microbes tend to flocculate in certain circumstances.

I. INTRODUCTION

Flocculated microbes several orders of magnitude larger than individual microbes are found in various physicochemical processes. These microbes include bacteria, yeasts, cellular slime molds, filamentous fungi, algae, and protozoa (3). Separation of microbes from the medium in a bioreactor is essential for downstream treatments. Microbes in a flocculated form are more easily separated from the bulk phase than those in dispersed form through conventional operations, for instance, sedimentation, filtration, and centrifugation. Although chemical flocculant can be introduced to initiate flocculation, the self-association phenomenon of microbes is no doubt a significant factor one should consider. A detailed understanding of why and how it occurs is necessary for both design and control of a bioreactor.

The experimental results concerning bioflocculation are ample in the literature; its mechanism, however, is still not well understood. Although it is generally believed to be genetically inducible, the sufficient conditions for the initiation of the phenomenon are uncertain in the present state. It seems that microbes in a substrate-insufficient condition tend to flocculate. This is justified by the fact that flocculation is often observed at the end of the log phase in a batch reactor (11). The mechanistic explanation for the phenomenon is also not clear. Possible causes for the phenomenon include (1) interactions between microbes, for example, amensalism, mutualism, commensalism, and parasitism; (2) better protection for the microbes inside a floc than for those dispersed in the bulk phase; and (3) easier nutrient uptake. Logan and Hunt (9) indicate that in certain circumstance the metabolic efficiency of flocculated cells is higher than that of the cells in the dispersed phase. They define the following measure for the metabolic efficiency ϵ:

$$\epsilon = \frac{\text{metabolic rate of flocculated cells}}{\text{metabolic rate of dispersed cells}}.$$

If ϵ is greater than unity, cells tend to flocculate. It is assumed that the high porosity of floc will induce a uniform flow field inside it. This is in contrast to the shear flow field of the bulk liquid phase. If the mass transfer of substrate is the rate-controlling step, the transfer of substrate inside the floc is more efficient than the transfer of substrate outside the floc. In other words, under a certain shear rate of the bulk phase, substrate is more readily utilized by flocculated cells, and ϵ will be greater than unity (9). It should be pointed out, however, that for the operating conditions normally en-

[1] To whom correspondence should be addressed.

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countered, the porosity of floc is not high enough for a uniform flow field to be established inside it. It seems that a more realistic mechanistic description is needed.

II. THEORY

The experimental evidence reveals that in a nutrient-insufficient environment cAMP (adenosine 3′:5′-cyclic monophosphate) will be released by an amoeba cell to attract other cells (8). Also, it is proposed that the extracellular cAMP will initiate the production of cAMP, and thus, the production of cAMP is autocatalytic in nature (6, 13). Note that since cAMP is derived from ATP, its production is related to microbial metabolism. Therefore we assume that due to the interaction with the environment microbes will secrete a certain chemical substance X. This substance plays the role of a message conveyer. The concentrations of X inside microbes, in the floc, and in the bulk phase are denoted by C_1, C, and C_b, respectively.

1. Substrate Uptake

We assume that the Monod equation is applicable in the present case, i.e.,

$$\mu = \mu_{max} \frac{S}{K_m + S}, \qquad [1]$$

where μ, μ_{max}, S, and K_m denote the specific substrate uptake rate, the maximum value of μ, the concentration of substrate, and a constant, respectively. For small S, [1] can be rewritten as

$$\mu = K'_s S, \qquad [2]$$

where $K'_s = \mu_{max}/K_m$. Note that μ can be functionally dependent on C_1. For convenience, it is assumed that μ and C_1 are linearly correlated. In other words, $K'_s = K''_s C_1$, where K''_s is a constant. Suppose that the amount of biomass, B, remains constant. A steady-state material balance on the substrate for the floc yields the equation (2)

$$D_s \nabla^2 S = k_s C_1 S, \qquad [3]$$

where D_s denotes the effective diffusivity of the floc for substrate, ∇^2 represents the Laplacian operator (12), and $k_s = K''_s B$.

2. Chemical Substance

A steady-state material balance on X for the floc yields

$$D_c \nabla^2 C = k_d C - k_t (C_1 - C), \qquad [4]$$

where D_c denotes the effective diffusivity of the floc for X and k_d and k_t are constant. On the right-hand side of this equation, the first term represents the dissociating rate of X; the second term denotes the rate of release of X by microbes.

The production rate of X, R_x, can be a function of various factors. Since microbial flocculation occurs under a substrate-insufficient condition, this rate should be inversely proportional to substrate concentration. Let us consider the expression

$$R_x = dC_1/dt = f_1(C)/K_1 + S, \qquad [5]$$

where $f_1(C)$ is a function of C, and K_1 is constant. This equation implies that the production of X is autocatalytic in nature. For low substrate concentrations, we have

$$R_x \simeq f(C), \qquad [6]$$

where $f(C) = f_1(C)/K_1$. Expanding this expression in a Taylor series about $C = 0$ and neglecting terms of second order and higher for small C, we obtain

$$R_x = k_p(C_0 + C), \qquad [7]$$

where k_p and C_0 are constant. At steady state, the rate of increase of X through bioreaction equals its rate of decrease due to molecular diffusion. Hence,

$$R_x = k_p(C_0 + C) = k_t(C_1 - C). \qquad [8]$$

Substituting this expression into [4], we obtain

$$D_c \nabla^2 C = -(k_p C_0 + k_c C), \qquad [9]$$

where

$$k_c = k_p - k_d. \quad [10]$$

3. *Effectiveness Factor*

For convenience, we assume that the floc is spherical in shape with a radius a. In this case, [3] and [9] reduce, respectively, to

$$\frac{1}{r^2}\frac{d}{dr}\left(D_s r^2 \frac{dS}{dr}\right) = [k_s C_0 + (k_s + k_s')C]S \quad [11]$$

and

$$\frac{1}{r^2}\frac{d}{dr}\left(D_c r^2 \frac{dC}{dr}\right) = -(k_p C_0 + k_c C), \quad [12]$$

where $k_s = k_s' k_p / k_t$. The solutions of these two equations are subject to the conditions

$$\text{(i) } C = C_b \text{ and } S = S_b \text{ at } r = a \quad [13]$$

$$\text{(ii) } dC/dr = 0 \text{ and } dS/dr = 0 \text{ at } r = 0, \quad [14]$$

where S_b is the concentration of substrate in the bulk phase. Equation [14] states that the profiles of both C and S are symmetric about the center of the floc. To make the mathematical manipulations easier, the following dimensionless variables are defined:

$$x = r/a \quad [14a]$$

$$S' = S/S_b \quad [14b]$$

$$C' = C/C_b. \quad [14c]$$

Equations [11] through [14] become, respectively,

$$\frac{1}{x^2}\frac{d}{dx}\left(x^2 \frac{dS'}{dx}\right) = \alpha^2(\lambda_1 + C')S', \quad [15]$$

$$\frac{1}{x^2}\frac{d}{dx}\left(x^2 \frac{dC'}{dx}\right) = -\delta^2(\lambda_2 + C'), \quad [16]$$

$$C' = 1 \text{ and } S' = 1 \text{ at } x = 1, \quad [17]$$

and

$$dC'/dx = 0 \text{ and } dS'/dx = 0 \text{ at } x = 0, \quad [18]$$

where

$$\alpha = a[(k_s + k_s')C_b/D_s]^{1/2},$$

$$\delta = a(k_c/D_c)^{1/2},$$

$$\lambda_1 = k_s C_0/(k_s + k_s')C_b,$$

$$\lambda_2 = k_p C_0/k_c C_b.$$

According to the definition of ϵ, we have

$$\epsilon = \int_0^a k_s C_1 S(4\pi r^2)dr/[(4\pi/3)a^3(k_s S)_b C_1]$$

$$= \frac{3}{(\lambda_1 + 1)}\int_0^1 (\lambda_1 + C')S'x^2 dx, \quad [19]$$

where $(k_s S)_b$ denotes the value of $(k_s S)$ in the bulk phase. The solution of [16] subject to the conditions expressed in [17] and [18] takes the form

$$C'(x) = \frac{(1 + \lambda_2)\sin(\delta x)}{x \sin(\delta)} - \lambda_2. \quad [20]$$

Note that since both ϵ and C' are positive, δ and λ_1 satisfy the conditions $0 < \delta < \pi$, and $0 < (1 + \lambda_1)$. Substituting [20] into [15] yields

$$\frac{1}{x^2}\frac{d}{dx}\left(x^2 \frac{dS'}{dx}\right) = \gamma^2 \frac{[\lambda + \sin(\delta x)]}{x \sin(\delta)} S', \quad [21]$$

where

$$\gamma^2 = \alpha^2(1 + \lambda_2) \quad [21a]$$

and

$$\lambda = (\lambda_1 - \lambda_2)/(1 + \lambda_2). \quad [21b]$$

Substituting [20] into [19] gives

$$\epsilon = \frac{3}{(\lambda + 1)}\int_0^1 \left(\lambda + \frac{\sin(\delta x)}{x \sin(\delta)}\right)S'x^2 dx. \quad [22]$$

Solving [21] analytically is not straightforward, if not impossible, and a numerical approach seems inevitable. Here, the method of orthogonal collocation (4) is adopted. Note that ϵ is dependent upon three parameters: γ, δ, and λ. The effect of these parameters on ϵ is examined in the following discussion.

3.1. Parameter γ. Figures 1 and 2 show the variation of the effectiveness factor as a func-

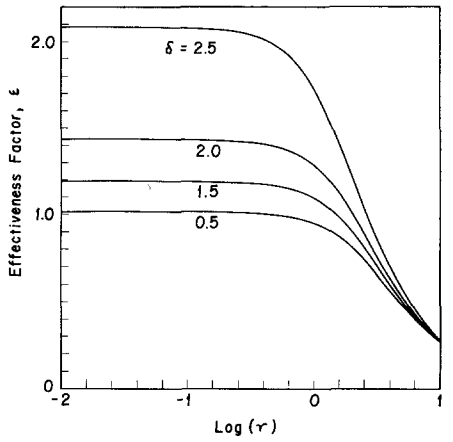

FIG. 1. The variation of the effectiveness factor as a function of γ with various values of δ for the case $\lambda = 0$.

tion of γ with various values of δ for the cases $\lambda = 0$ and $\lambda = \pi/2$, respectively. As γ increases, the diffusion of substrate becomes the rate-determining step and the effectiveness factor will decrease. For large enough γ, ϵ will be below unity, and there is no advantage for microbes to stay in the flocculated state.

The definitions of α^2 and γ^2 indicate that they are a measure of the ratio

$$\frac{\text{uptake rate of substrate on floc surface}}{\text{diffusion rate of substrate in floc}}.$$

As γ^2 approaches zero, the diffusion rate of the substrate is much greater than its uptake rate. In this case, the concentration of the substrate is uniform throughout the floc and the metabolic reaction becomes the rate-determining step. In other words, $S' = 1$ and ϵ has its maximum value ϵ_{max}, which is a function of δ and λ. Equation [22] can be integrated analytically to obtain

$$\epsilon_{max} = \frac{3}{\lambda + 1}\left[\frac{3}{\lambda} + \frac{1}{\delta}\left(\frac{1}{\delta} - \frac{1}{\tan(\delta)}\right)\right]. \quad [23]$$

3.2. Parameter δ. The effect of δ on ϵ_{max} is shown in Fig. 3. Note that δ^2 is a measure of the ratio

$$\frac{\text{net production rate of extracellular X}}{\text{diffusion rate of extracellular X}}.$$

If the net production rate of extracellular X is much greater than its diffusion rate, the autocatalytic nature of the production of X will prohibit the existence of a steady state. This occurs if δ approaches π. As δ^2 approaches zero, $\sin(\delta x)/\delta$ approaches x, and [20] reduces to $C'(x) = 1$. This means that the production of X is the rate-determining step and the formation of floc has no advantage. Rather, it provides a resistance for the diffusion of substrate.

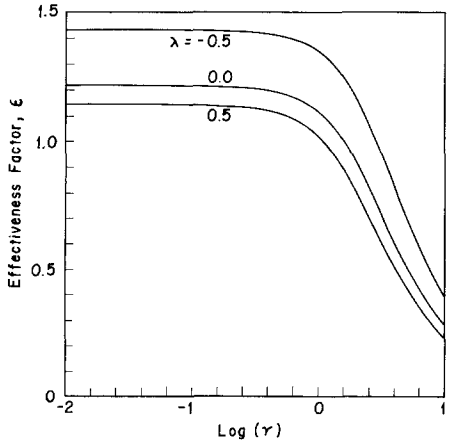

FIG. 2. The variation of the effectiveness factor as a function of γ with various values of δ for the case $\lambda = \pi/2$.

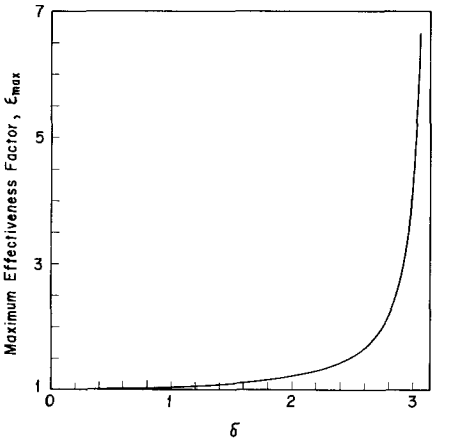

FIG. 3. The variation of ϵ_{max} as a function of δ for the case $\lambda = 1$.

3.3. Parameter λ. Equation [21b] indicates that λ is a monotone decreasing function of λ_2. Note that λ_2 is a measure of the ratio

$$\frac{\text{rate of production of X of microbes}}{\text{adjusted dissociation rate of X}}.$$

The larger the value of λ_2, the greater the concentration of extracellular X; the autocatalytic characteristic of the production of X makes cell flocculation advantageous. Thus λ is a measure of the inverse concentration of extracellular X. If λ approaches infinity (λ_2 approaches -1), the concentration of extracellular X is negligible and ϵ_{max} approaches unity. On the other hand, if λ approaches -1 (λ_2 approaches infinity), the rate of production of X increases and ϵ_{max} increases rapidly. Microbes tend to flocculate. Figure 4 illustrates the effect of λ on ϵ_{max}.

III. DISCUSSION

A parameter sensitivity analysis based on [15], [16], and [19] suggests that ϵ will have a large value if δ is large and γ and λ are small. In other words, the flocculated state is favorable for rapid X production, high X diffusion resistance, and low substrate diffusivity. Note that δ/γ is a measure of the ratio

$$\frac{\text{diffusion rate of X}}{\text{diffusion rate of substrate}}.$$

For a fixed value of λ, ϵ is always greater than unity if the ratio δ/γ is greater than a certain value; otherwise, it is less than unity. As an example, if $\lambda = 0$, then $\epsilon > 1$ for $\delta/\gamma > 1$, and $\epsilon < 1$ for $\delta/\gamma < 1$.

It has been observed experimentally that a diffusional resistance exists in the floc formed by aggregated microbes (7, 1, 10). This resistance hinders the transport of substrate from the bulk phase outside the floc to the surface of microbes inside the floc. Hence, the uptake of substrate by flocculated microbes is less efficient than that of the dispersed microbes. The effectiveness factor is less than unity in this case, and staying in the flocculated state is disadvantageous to microbial metabolism. This, however, overlooks the potential positive effect of the diffusional resistance on the microbial metabolism. The experimental evidence reveals that cAMP performs the role of a message conveyer for *Dictyostelium discoideum* in a substrate-insufficient condition. Furthermore, the production of cAMP is essentially an autocatalytic reaction (5, 6). Due to this characteristic and the existence of the diffusional resistance, the concentration of the message conveyer inside the floc will be higher than its concentration outside the floc. This will result in a higher metabolic efficiency. On the basis of this consideration, the choice between the dispersed state and the flocculated state depends upon whether the diffusional resistance favors the metabolic reaction. That is, the result of comparison of the relative significance between the resistance for substrate diffusion and that for message diffusion determines the state of microbes. Therefore the present analysis provides a way of interpreting why microbes tend to flocculate under certain conditions.

It should be pointed out that the assumption made in deriving [3], i.e., the rate of substrate

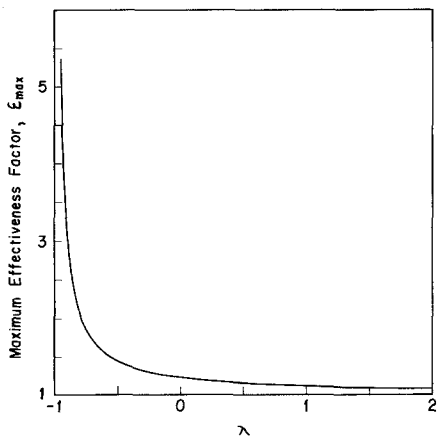

FIG. 4. The variation of ϵ_{max} as a function of λ for the case $\delta = \pi/2$.

uptake is proportional to X, is an oversimplification of a real process. The present analysis, however, can be extended to the case of a general rate expression without much difficulty.

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