Bioflocculation as a Microbial Response to Substrate Limitations

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Previous theories of nutrient supply to microbial flocs assumed that transport within the floc was by molecular diffusion, and they predict that overall nutrient uptake is reduced in flocs compared to dispersed cells. Calculations, supported by recent advances in understanding fluid flow through suspended aggregates, however, have shown that substantial fluid flow may occur through highly permeable bacterial flocs. Since bioflocculation of microorganisms in bioreactors is known to occur under conditions of low substrate availability, the rate of substrate uptake is assumed to be mass transfer limited. The hydrodynamic environment of a cell then determines cellular uptake rates. Through development of a relative uptake factor, the overall uptake by cells in flocs in sheared fluids and flocs attached to bubbles are compared with the uptake by an identical quantity of dispersed cells. Bioflocculation is found to increase the rate of substrate transport to cells in permeable flocs compared to dispersed cells, particularly for large-molecular-weight substrates and when bubbles are present.

INTRODUCTION

The attachment of microorganisms to surfaces and other microorganisms is ubiquitous in the natural environment and in engineered biological reactors. Preferential microbial growth on surfaces has been known for some time, and attached organisms are frequently dominant compared to freely suspended cells in many environments ranging from the human digestive system to natural streams. Freely suspended aggregates are observed for bacteria, yeasts, cellular slime molds, filamentous fungi, and algae. In suspended growth biological reactors for cell or product production, or for waste treatment, cell separation from the suspending fluid is critical. Gravitational settling, centrifugation, and filtration are enhanced if cells form larger, faster settling aggregates. While biological and inorganic particles can be aggregated by the addition of chemical coagulants, the ability of organisms to self-associate or bioflocculate is of considerable importance in process design. Understanding why microorganisms bioflocculate is then of importance in operating suspended growth biological reactors.

For inorganic particles, the chemical control of particle aggregation is well established from developments in colloidal science. A wide variety of mechanisms for microbial aggregation are reported, including polymeric materials and various appendages such as pili, fimbriae, cilia, filaments, fuzz, and hairs. The microscale phenomena of attachment involves some combination of hydrogen bonding and ionic, dipolar, and hydrophobic interactions. Subsequently, covalent bonds may be formed in some systems at cell–cell and cell–substratum interfaces.

Calleja has defined cell aggregation as the “gathering together of cells to form fairly stable continuous, multicellular associations under physiological conditions.” Microbial aggregation is genetically inducible, but the environmental conditions causing the physiological change from a dispersed to an aggregated state are subject to considerable uncertainty. A review of the literature on conditions promoting microbial aggregation indicates that the only common condition inducing aggregation is microbial starvation, since in batch cultures, bioflocculation is frequently observed at the end of logarithmic growth. Literature data also imply that macromolecules of either proteins or polysaccharides are implicated in the inducement of aggregation.

While the importance of bioflocculation is recognized in the natural and applied sciences, a mechanistic explanation of why cells associate into aggregates has not been proposed. Since bioflocculation is a microbial characteristic, there should be some advantage of growth within an aggregate. There are a number of possibilities. First, there may be
interactions between adjacent organisms such as commensalism, mutualism, parasitism and genetic exchange. Second, growth within an aggregate may protect cells from predation. Third, microbial growth within aggregates or films may be selected by the hydraulics of the system. For example, cells that settle in an activated sludge clarifier are recycled to the aeration basin while nonflocculent cells are not selected. Finally, growth within an aggregate may increase nutrient uptake compared to freely dispersed cells. For a pure culture of organisms to bioflocculate when substrate is nearly depleted implies that the cell associations confer some advantage over freely dispersed cells relative to increasing substrate uptake by aggregated cells. This final advantage is counter to the prevailing view that cells within flocs are at a disadvantage compared to dispersed cells due to diffusion limitations of substrate. The proposed theory, based on advective flow through microbial aggregates, predicts that greater substrate uptake by cells is possible within an aggregate compared to freely dispersed cells. This suggests a reevaluation of models for nutrient supply to microbial aggregates, and offers a logical explanation for a commonly observed phenomenon.

**NUTRIENT TRANSPORT TO MICROBIAL FLOCS**

Models for nutrient supply to suspended aggregates of microorganisms have been based on an approach adopted for heterogeneous catalyst pellets that account for reactant diffusion to a solid-liquid interface and diffusion of products away from the interface. In general, the steady-state transport of substrate into a porous aggregate can be described by the equation

\[ u \nabla C - D \nabla^2 C = -kC \]  

(1)

where the first term represents advective flow of substrate at an intrafloc velocity \( u \), and a substrate concentration \( C \). The second term represents the diffusive transport of substrate with an effective molecular diffusion coefficient, \( D \), and the term on the right-hand side represents substrate uptake, taken as first order for convenience, where \( k \) is the rate parameter. The importance of substrate transport by diffusion compared to advection is determined by the magnitude of the Peclet number for the floc, \( Pec(a_f) = a_f u / D \), where \( a_f \) is the floc radius.

For \( Pec(a_f) \ll 1 \), diffusive transport of substrate within the floc dominates and the advective flow term can be dropped. Assuming spherical coordinates, the solution is given in terms of a diffusive effectiveness factor, \( \eta_d \), defined as the observed rate divided by the rate if there was no diffusion limitation within the floc. The solution becomes

\[ \eta_d = \frac{3 \Phi_d \coth 3 \Phi_d - 1}{3 \Phi_d^2} \]  

(2)

where \( \Phi_d = (a_f/3) \sqrt{k/D} \) is a dimensionless diffusive Thiele modulus. Aris' reviews the historical development of this theory and provides an extensive summary of experimental results in agreement with theoretical predictions in the catalysis literature. Mass transfer is recognized to be important for cells immobilized in biofilms, entrapped in a porous matrix, encapsulated behind some barrier, and for suspended, self-aggregating cells. For suspended growth bioreactors, experimental verification of the theory of combined reaction and diffusion has not been possible to date due to uncertainties in cell activity, in the diffusivity of substrates within an aggregate, and in the cell distribution inside the aggregate. For very porous aggregates, fluid streamlines can pass through the aggregates and large Peclet numbers within the floc are possible. Returning to eq. (1) and neglecting the diffusive transport term gives

\[ \frac{dC}{dx} = -kC \]  

(3)

for a linear coordinate system where \( u \) is the average intrafloc velocity in direction \( x \). Assuming for simplicity that the floc has a cube of length \( 2a_f \), then the cross-sectional area for advective flow is \( 4a_f^2 \) and the volume-to-cross-sectional-area ratio is \( 2a_f \). The advective effectiveness factor, \( \eta_a \), is the ratio of the observed uptake rate by a floc (the cross-sectional area times the flux removed during flow through the floc) to the uptake rate if all cells in a floc experienced the bulk substrate concentration, \( C_b \),

\[ \eta_a = \frac{4a_f^2 u C_b [1 - e^{-2a_f k/u}]}{Ba_f^2 k C_b} = \frac{1 - e^{-\Phi_a}}{\Phi_a} \]  

(4)

where \( \Phi_a = 2a_f k/u \) is an advective Thiele modulus for first order uptake.

The consequences of advective flow through porous catalyst particles were recognized by Nir and Pisma. They calculated effectiveness factors for catalyst particles of various configurations when there was reactant transport either by diffusion or by diffusion plus advection. By including advective flow through the catalyst particle they predicted a shift in the region of \( \eta = 1 \) out to greater dimensionless Thiele moduli. Their application was a packed bed of porous catalyst particles where intraparticle flow was induced by flow through the packed bed.

The diffusive and advective effectiveness factors in eqs. (2) and (4) are plotted in Figure 1 as functions of the respective dimensionless Thiele moduli. The two effectiveness factors are not substantially different. Experiments reported in the literature supporting the use of a diffusive model for mass transfer to microbial flocs via an effectiveness factor analysis therefore do not disprove an advective transport model. Since experimental data are usually fitted to the diffusive transport model by adjustment of the effective molecular diffusivity, a corresponding adjustment of intrafloc velocity would also allow fitting an advective model to the same data. However, the presentation of both diffusive and advective model results in terms of effectiveness factors always implies some hindrance to substrate uptake because of microbial association in a floc. An alternative analysis is developed below based on mass transfer limited growth such
that cellular uptake is a function of the fluid mechanical regime of the cells.

**ADVECTIVE FLOW THROUGH MICROBIAL FLOCS**

The prediction of advective flow through individual flocs requires information on floc size, floc porosity, and floc permeability under conditions of turbulent flow both in the presence and absence of bubbles in the bioreactor. At this time only limited empirical data are available on microbial floc size and porosity as a function of reactor conditions.

In turbulent reactors steady-state floc size for either microbial or mineral flocs is controlled by a balance between floc aggregation and floc breakup. Experimentally, flocs are approximately the size of the Kolmogorov microscale

\[ a_t \approx \left( \frac{\nu^3}{\varepsilon_t} \right)^{1/4} \]  

where \( \varepsilon_t \) is turbulent kinetic energy dissipation rate per unit mass and \( \nu \) is kinematic viscosity.\(^{12,13}\) For convenience the turbulence is characterized by a mean fluid shear rate, \( G = (\varepsilon_t/\nu)^{1/2} \), such that for water at 20°C an approximate relationship between floc radius and mean fluid shear rate is

\[ a_t = 1000G^{-1/2} \]  

where the floc radius is in μm and the fluid shear rate is in s\(^{-1}\). Since floc sizes are comparable to the turbulence microscale, the floc Reynolds number, \( a_t^2 G/\nu \), is one. The size-shear rate relationship is not applicable to the stronger, filamentous activated sludge flocs described by Parker et al.\(^{14}\) and Sezgin et al.\(^{15}\)

Microbial and inorganic flocs are highly porous, with reported porosities for 100-μm radius flocs summarized in Table I.\(^{8,16-21}\) The data show considerable variability and uncertainty because floc characterization is hindered by the fragile nature of suspended flocs. An empirical relationship relating floc porosity to floc size is obtained from the data of Tambo and Watanabe\(^{20}\) (their Fig. 14) where density differences were calculated from measured microbial floc settling velocities

\[ 1 - \varepsilon = 8a_f^{-1.6} \]
where $a_r$ is in $\mu$m. The coefficient of 8 in eq. (7) was chosen such that a floc of 5 $\mu$m radius has a porosity of 0.4. Similar increases in porosity with floc size have been observed with microbial and inorganic flocs.\textsuperscript{22,23} Equation (7) predicts that a 100-$\mu$m radius floc would have a porosity of 0.995 which is within the range of values reported in Table I.

Fluid flow through porous, permeable aggregates in sheared and settling environments is predictable from theoretical calculations by Adler.\textsuperscript{25} For flocs in laminar shear, the average intrafloc velocity is

$$
\mu = \frac{2}{3\pi} \sqrt{E(\xi)} Ga_f \tag{8}
$$

where $E$ is a tabulated function of $\xi = a_r/\sqrt{\kappa}$, given by Adler,\textsuperscript{25} and $\kappa$ is the floc permeability. For $\xi < 10.9$, advective flow within aggregates is predicted to occur while for $\xi \geq 10.9$ the intrafloc velocity is zero and only diffusive flow is possible. Adler also analyzed the case of a permeable particle moving through fluid and predicted the intrafloc velocity, $\mu$, is

$$
u = U \frac{a_f^2(\xi)}{a_r} \tag{9}
$$

where $U$ is the external fluid velocity relative to the floc, and $a_f^2(\xi)$ is a drainage radius function given by Adler. For floc motion relative to fluid motion such as settling, intrafloc fluid flow always occurs if floc permeability is nonzero.

Utilization of Adler's predictions in estimating intrafloc fluid flow requires an expression for floc permeability as a function of floc characteristics that is approximately valid over the high porosities expected for microbial flocs. As discussed by Dullien,\textsuperscript{26} the usual Kozeny–Carman equation for the permeability of packed spheres is not valid since flow at high porosities is around objects rather than through an equivalent conduit as assumed in the Kozeny–Carman analysis. Empirically, Davies (see ref. 27) observed that the permeability of fibrous mats could be fitted to the equation

$$
\kappa = \frac{a_r^2}{16(1 - \varepsilon)^4[1 + 56(1 - \varepsilon)^2]} \tag{10}
$$

where $a_r$ is the radius of the cylindrical filaments. Experimental data used in the correlation were over a porosity range of 0.7–0.994. Matsumoto and Suganuma\textsuperscript{28} report a similar dependence of permeability on porosity over a porosity range of 0.9–0.998 for model flocs composed of steel wool. An alternative expression for the permeability of a swarm of spheres was derived by Brinkman (see ref. 29) as

$$
\kappa = \frac{a_r^2}{18} \left( \frac{3}{1 - \varepsilon} + \frac{4}{1 - \varepsilon} - 3 \sqrt{\frac{8}{1 - \varepsilon} - 3} \right) \tag{11}
$$

where $a_r$ is the radius of the spherical particles. Calculations by Sutherland and Tan\textsuperscript{30} using Brinkman's permeability expression concluded that very little advective flow was possible through settling, porous aggregates.

The permeability relationships in eqs. (10) and (11) depend only on either cylinder or sphere radius and floc porosity. The porosity in turn is a function only of aggregate size through the empirical relationship in eq. (7), and floc size is determined by reactor turbulence as characterized by a fluid shear rate through eq. (6). Thus, advective flow velocities within flocs can be calculated from Adler's relationship in eq. (8) with only the fluid shear rate as an independent variable. Intrafloc velocities predicted by the Davies correlation are shown in Figure 2 for three cylinder radii representing bacterial cells in a dendritic pattern. For 1-$\mu$m cells, the intrafloc velocity reaches a maximum of ca. 1500 $\mu$m/s at a fluid shear rate of 1000 $s^{-1}$. At higher fluid shear rates, the decreasing floc size and decreasing porosity and permeability result in lower intrafloc velocities which rapidly approach zero as $a_r/\sqrt{\kappa}$ nears 10.9. When the fluid shear rate is less than 1000 $s^{-1}$, the intrafloc velocity decreases with decreasing fluid shear rate because the increasing floc size dominates over the increase in porosity and permeability. Intrafloc velocity is very sensitive to cylinder or cell size.

The region of advective transport dominance over diffusive transport can be determined from the criterion $u a_r/D \gg 1$. For a simple, soluble substrate having a molecular diffusivity of $10^{-8} \text{ cm}^2/\text{s}$ or $10^3 \mu\text{m}^2/\text{s}$, and using eq. (6), this Peclet number criterion becomes $u \gg \sqrt{G}$ where $u$ is in $\mu$m/s and $G$ is in $s^{-1}$ units. For 1-$\mu$m radius cells, the intrafloc velocities in Figure 2 dominate transport at fluid shear rates over the range $1 \leq G \leq 3000 \text{ s}^{-1}$.

### MASS TRANSFER

The benefits of bioflocculation are examined based on a comparison of mass transfer rates between cells inside flocs

<table>
<thead>
<tr>
<th>System</th>
<th>Porosity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>0.999</td>
<td>16</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>0.91 ± 0.15</td>
<td>17</td>
</tr>
<tr>
<td>Zoogloea ramigera</td>
<td>0.66 ± 0.10</td>
<td>18</td>
</tr>
<tr>
<td>Mold pellets</td>
<td>0.83 ± 0.03</td>
<td>8</td>
</tr>
<tr>
<td>Computer-generated aggregate</td>
<td>0.984</td>
<td>19</td>
</tr>
<tr>
<td>Computer-generated aggregate</td>
<td>0.975</td>
<td>20</td>
</tr>
<tr>
<td>Necessary for advective flow</td>
<td>0.984</td>
<td>21</td>
</tr>
<tr>
<td>in sheared fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assuming $(1 - \varepsilon) = 8a_f^{-1.6}$</td>
<td>0.995</td>
<td>this study</td>
</tr>
</tbody>
</table>

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**Table I. Porosities of 100 $\mu$m aggregates.**
and freely dispersed cells. One of the major assumptions in this analysis is that substrate uptake by microorganisms can be modelled using a mass transfer analysis. Since biofloculation is observed at the end of exponential phase growth, or when the substrate concentration is low, mass-transfer-limited growth is reasonable. Empirical evidence supporting mass-transfer-limited microbial growth, is indicated by increased microbial substrate uptake when fluid flow or mixing is increased. Nutrient supply to suspended phytoplankton is of considerable interest since substrate levels are typically very low in natural environments. Munk and Riley \(^{31}\) used a mass transfer analysis to show the importance of particle settling on nutrient uptake. Subsequent work by Pasciak and Gavis \(^{32,33}\) and Gavis \(^{34}\) found by calculation and laboratory measurements that phytoplankton nutrient uptake increased when the fluid shear rate increased. Similarly, phosphate uptake by diatoms supported on a filter increased with increasing fluid flow velocity. \(^{35}\) These observations of substrate uptake limited by mass transfer are significant in that the mass transfer rates calculated to spheres of the same size as the organism are at least 50-fold greater than observed uptake rates. If a cell surface is not completely covered by receptors, as for facilitated transport across cell membranes, then only limited surface coverage by specific enzymes is expected, and mass transfer rates to the receptors can limit growth rather than internal enzymatic processes.

There are several reasons to utilize a mass transfer limited uptake model to estimate cellular uptake rates. The alternative use of Michaelis–Menten kinetics, for example, requires that total enzyme concentration be much less than the substrate concentration which may not be reasonable during endogenous growth of microorganisms. Steady state Michaelis–Menten kinetic models also underestimate the maximum uptake rates of starved cells, that is luxury uptake, as observed in bacteria \(^{36}\) and phytoplankton. \(^{37}\) Microorganisms also contain multiple enzymatic pathways that shift in dominance, causing different uptake kinetics depending on substrate concentration. \(^{38,39}\)

Three distinct fluid mechanical environments are considered and illustrated in Figure 3. In Figure 3(a), a sphere is shown suspended in a uniform shear flow that causes the
sphere to rotate, representing dispersed cells or low permeability flocs in turbulent flow fields. Figure 3(b) shows a sphere fixed in a uniform fluid flow field as would be the case for a cell inside a floc that has intrafloc fluid flow. A sphere attached to a rising bubble is illustrated in Figure 3(c) where the sphere may project outside the bubble’s laminar boundary layer.

Expressions for mass transfer rates to spheres within flocs and spheres freely suspended in turbulent fluids are available from the literature. In general, the rate of mass transfer to a sphere of radius \( a \) is given by

\[
Q = k' 4\pi a^2 (C_b - C_s)
\]  
(12)

where \( k' \) is a mass transfer coefficient, and \( C_b \) and \( C_s \) are the bulk phase and surface concentrations of substrate, respectively. Since mass transfer is assumed to limit growth, the surface substrate concentration is much less than the bulk phase, so setting \( C_s = 0 \) is reasonable. The mass transfer coefficient is expressed in terms of a dimensionless Nusselt number, \( Nu = k'a/D \), which is the ratio of mass transfer in a given fluid mechanical regime to mass transfer by molecular diffusion alone, following Batchelor\(^{40}\) and others. Equation (12) then becomes

\[
Q = 4\pi Nu Da C_b
\]  
(13)

For cells or impermeable flocs in turbulent shear flow, as characterized in Figure 3(a), the Nusselt number depends on the dominance of advective transport compared to diffusive through a Peclet number, which in shear flow is \( Pe_G = a^2G/D \), where

\[
Nu_G = 1 + 0.26Pe_G^{12}; \quad Pe_G \ll 1
\]  
(14)

and

\[
Nu_G = 1 + 0.55Pe_G^{10}; \quad Pe_G \gg 1
\]  
(15)

Equation (14) was derived by Frankel and Acrivos\(^{41}\) for laminar shear flow and Batchelor\(^{42}\) developed eq. (15) for turbulent flow when the particle Reynolds number, \( a^2G/\nu \), was small. The expressions in eqs. (14) and (15) differ little over the range \( 0.1 < Pe_G < 10 \) and an empirical formula for interpolating within this region is

\[
Nu_G = 1.54Pe_G^{1.51}; \quad 0.1 \leq Pe_G \leq 10
\]  
(16)

For a fixed sphere in an advective flow field as depicted in Figure 3(b), mass transfer depends on the advective flow Peclet number, \( Pe_a = ua/D \), as correlated to experimental observations by Brian and Hales,\(^{43}\)

\[
Nu_a = [1 + 0.48Pe_a^{23}]^{1/2}
\]  
(17)

which is valid up to very large values of the advective Peclet number.

**RELATIVE UPTAKE**

As previously discussed, an effectiveness factor cannot be used to examine the possible advantages of bioflocculated growth. In our analysis, cells are assumed to be mass transfer limited so that uptake rates are controlled by the fluid mechanical regime around the cells whether dispersed or aggregated. Unlike an effectiveness factor analysis based on a fixed reaction rate constant, an alternative presentation based on relative uptake is required. The relative uptake, \( \gamma \), is defined as

\[
\gamma = \frac{\text{observed rate}}{\text{rate if all cells are dispersed in the fluid}}
\]  
(18)

where the floc and the dispersed cell are in the same fluid mechanical environment. It is only under conditions of relative uptake greater than unity that bioflocculated growth is advantageous; otherwise, dispersed cells could remove substrate faster than aggregated cells and in continuous cultures would out-compete cells within flocs.

To calculate relative uptake between cells inside flocs and dispersed cells, first-order uptake rate parameters are needed for the different fluid mechanical regimes. Volume-averaged rate parameters are calculated by multiplying the cellular uptake rate by the number concentration of viable cells within a floc. The uptake rate for a mass-transfer-limited cell of radius \( a_c \) is given by a slight modification of eq. (13) to include a correction factor, \( f_a \), which incorporates the efficiency of substrate uptake and accounts for surface area coverage by cell membrane bound transport enzymes as:

\[
Q = 4\pi Nu Da_c C_b f_a
\]  
(19)

The number concentration, \( N_c \), of viable cells of size \( a_c \) within a floc is
\[ N_c = \frac{1 - \varepsilon}{4} f_s \]
\[ \frac{1}{3} n a_c \]

where \( f_s \) is the fraction of the cells that are viable. The volume-averaged rate parameter becomes

\[ k(Nu) = \frac{3NuD(1 - \varepsilon)E_b}{a_c^2} \]

(21)

where the rate parameter dependence on the fluid mechanical regime is specifically indicated by the inclusion of the Nusselt number. For simplicity, the product of the surface area availability and the cell viability was combined into an overall collector efficiency, \( E_b \), where \( E_b = f_s f_c \approx 1 \).

When calculating the relative uptake, the mass transfer rate to an equivalent number of dispersed cells is necessary. Since each cell is dispersed into a sheared fluid at the bulk substrate concentration, the mass transfer rate becomes \( k(Nu_c)C_b \), where

\[ k(Nu_c) = \frac{3Nu_c(a_c)D(1 - \varepsilon)E_b}{a_c^2} \]

(22)

and \( Nu_c(a_c) \) is the Nusselt number for a single cell in sheared fluid.

With the above mass transfer expressions, flocs under three different conditions are considered. The first analysis considers the traditional case of a porous but impermeable floc where substrate transport is dominated by diffusion. The next two cases allow for substrate transport into flocs by intrafloc fluid flow induced by floc shearing and floc attachment to bubbles.

The first case considers impermeable flocs with substrate uptake limited by external mass transfer resistance to the floc surface followed by diffusional resistance and substrate removal within the floc. By solving for the concentration profile within the floc using a flux matching boundary condition, Logan\textsuperscript{21} arrived at a relative uptake comparison of

\[ \gamma_d = \frac{Nu_c(a_c)}{3\Phi_d^2Nu_c(a_c)} \times \frac{3\Phi_d \cosh 3\Phi_d - \sinh 3\Phi_d}{3\Phi_d \cosh 3\Phi_d + [Nu_c(a_c) - 1] \sinh 3\Phi_d} \]

(23)

Two Nusselt numbers are indicated; one for the floc in shear flow, \( Nu_c(a_c) \), as is needed to represent the external mass transfer resistance, and the other, \( Nu_c(a_c) \), to account for the mass transfer to a dispersed cell in sheared fluid. The diffusive Thiele modulus appearing in eq. (23) is defined as \( \Phi_d = \frac{a_r}{3} \sqrt{k(1)/D} \) where \( k(1) \) is the first-order rate parameter defined in eq. (21) evaluated at \( Nu = 1 \), representing the stagnant fluid environment assumed within a floc for this case.

A form of eq. (23) that incorporates the traditional effectiveness factor analysis is available when external mass transfer resistance is negligible, or

\[ Nu_c \gg 3\Phi_d \coth 3\Phi_d - 1 \]

(24)

indicating the disadvantages of cell association in impermeable flocs. First, the effectiveness factor is less than or equal to one because of substrate consumption and diffusional limitations within flocs. Second, there is a mass transfer advantage for a single, dispersed cell in a sheared fluid, \( Nu_c(a_c) > 1 \) over a cell inside a floc containing stagnant fluid where \( Nu = 1 \).

The second case considers a permeable floc in sheared fluid. For this case the relative uptake derivation follows the analysis given in eq. (4) except that two different first-order rate parameters are included:

\[ \gamma_a = \frac{u}{2a_r k(Nu_c)} \left[ 1 - \exp \left( -\frac{2a_r k(Nu_c)}{u} \right) \right] \]

(26)

Inside the floc the rate parameter is \( k(Nu_a) \) when intrafloc fluid flow is induced by fluid shear outside the floc and for dispersed cells in the sheared fluid the rate parameter is \( k(Nu_c) \). Expressing eq. (26) in terms of an advective Thiele modulus, \( \Phi_a = 2a_r k(Nu_a)/u \), based on the first-order rate parameter within the floc, the advective relative uptake expression becomes

\[ \gamma_a = \frac{Nu_a}{Nu_c} \eta_a \]

(27)

where the advective effectiveness factor is given in eq. (4). This result again incorporates the substrate depletion component of the effectiveness factor analysis but also includes the effect of fluid mechanical regime on uptake through the ratio of Nusselt numbers in advective and shear flow. Since the advective Nusselt number can exceed the Nusselt number in shear flow, relative uptakes greater than unity are possible.

The third fluid mechanical regime of flocs in bioreactors considers interaction with bubbles, as was illustrated in Figure 3(c). Flocs attached to bubbles have advective flow induced by the bubble rise velocity. Assuming that the floc projects out of the bubble boundary layer, induced advective flow through the floc can be estimated from Adler’s analysis as summarized in eq. (9) using the bubble rise velocity, \( U_b \), for the fluid flow velocity past the floc. For a bubble Reynolds number greater than unity, a bubble of radius \( a_b \) has a rise velocity

\[ U_b = \sqrt{2ga_b} \]

(28)

which for a 0.1-cm bubble is 14 cm/s. The relative uptake analysis arrives at an expression similar to eq. (27), except that the fluid flow velocity inside the floc is calculated from floc motion relative to fluid rather than for a floc in sheared fluid. An alternative analysis based on a floc in the sheared bubble boundary layer gave similar results.\textsuperscript{21} For convenience, bubble size and rise velocity are fixed even though fluid shear will affect the bubble size as well as distort and rupture the bubble.
RESULTS

A complete sensitivity analysis of relative uptake predictions for various floc environments is not attempted; instead, a selective comparison of relative uptake over a broad range of fluid shear rates is presented. The parameters of most interest in the comparison of predictions are the overall collector efficiency, $E_B$, and the substrate diffusivity for the three floc cases considered: impermeable flocs, permeable flocs, and permeable flocs attached to bubbles.

Figure 4 plots predicted relative uptake factors for 1-μm cells and a simple, soluble substrate with molecular diffusivity of $10^{-4}$ cm$^2$/s and a wide range in the collector efficiency. For an impermeable aggregate, Figure 4(a) shows that relative uptake is always less than one because mass transfer to a dispersed cell in sheared fluid will exceed mass transfer to a cell by diffusion alone since the Nusselt number in sheared fluids exceeds one. Increasing the overall efficiency parameter toward one lowers the relative uptake as less substrate is available within the flocs. There is no advantage under any conditions considered here for cell association in an impermeable floc compared to a dispersed cell.

For permeable flocs, relative uptake factors can exceed unity. Figure 4(b) considers permeable flocs in sheared fluid without bubbles. For low values of the $E_B$ parameter, cell association in a floc is advantageous. The relative uptake factors are zero at fluid shear rates above 3300 s$^{-1}$ when the flocs have reduced permeability and no advective flow as shown in Figure 2. Figure 4(c) examines relative uptake factors for flocs attached to bubbles. For the lower fluid shear rates, the relative uptake exceeds unity. At higher fluid shear rates the smaller, less permeable flocs attached to bubbles have reduced intrafloc flow and the dispersed cells have increased mass transfer; both processes causing a decrease in relative uptake factor as the fluid shear rate increases.

The sensitivity of relative uptake predictions to the $E_B$ parameter is different for the three cases. At the lowest value, $E_B = 0.01$, relative uptake values approach a ratio of mass transfer to a cell in the aggregate to a dispersed cell, with both cells at nearly equivalent substrate concentrations since very little substrate depletion occurs, i.e. $\eta_d = \eta_a = 1$. For $E_B = 1$, relative uptake factors for impermeable and permeable flocs are greatly reduced while cells inside permeable flocs attached to bubbles are unaffected because of the high advective flow rates. The impermeable floc is most sensitive to $E_B$ since the diffusive flux is determined by the concentration gradient which is controlled by substrate uptake. Permeable flocs and flocs on bubbles have lesser dependences on this parameter because transport

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Figure 4. Relative uptake predictions for $a_c = 1$ μm and $D = 10^{-4}$ cm$^2$/s with $E_B = 1.0$; $E_B = 0.1$; and $E_B = 0.01$ in a sheared fluid for (a) impermeable flocs, (b) permeable flocs, and (c) permeable flocs attached to bubbles.
through the floc is controlled by advective flows that are independent of substrate uptake. Kinks in the curves represent a change in the mass transfer expressions over the Peclet numbers given in eqs. (14), (15), and (16).

Figure 5 presents the sensitivity of relative uptake to substrate diffusivity at low values of $E_b$. The lower diffusivities represent what would be expected for higher-molecular-weight compounds that are still far less than micrometer in size. Figure 5(a) considers the impermeable floc model and shows a general decrease in relative uptake with decreasing diffusivity, reflecting the inability of larger molecules to diffuse into flocs compared to diffusion to a dispersed cell. For permeable flocs in sheared fluid, Figure 5(b), and permeable flocs attached to bubbles, Figure 5(c), relative uptake factors generally exceed unity and increase with decreasing substrate diffusivity.

**DISCUSSION**

Suspended microbial aggregates are highly porous due to their formation by collisions of smaller, porous aggregates. One of the consequences of this high porosity is that fluid shear and bubbles can induce intrafloc fluid flow. While advective flow through microbial aggregates has not been previously considered in the traditional diffusive effectiveness factor analysis of substrate uptake, inclusion of advective transport and substrate removal arrived at very similar results as indicated in Figure 1. The effectiveness factor approach was modified here for an analysis of relative uptake to account for substrate uptake being determined by the fluid flow regime around a cell. Using reasonable empirical expressions relating floc size and porosity to fluid shear rate, relative uptake factors greater than unity identified conditions whereby cell association within flocs was more favorable to growth than dispersed cells. The two factors that were predicted to benefit cell growth within flocs are the presence of bubbles because of the large advective flow velocities induced, and substrates of higher molecular weight that have diffusivities less than $10^{-5} \text{ cm}^2/\text{s}$. Flocs must also be sufficiently permeable so that fluid shear induces substantial advective flow.

Some evidence exists in the literature indicating that fluid flows through microbial aggregates. When diffusive effectiveness factor models are fitted to experimental data, resulting substrate diffusivities occasionally exceed the molecular diffusivity of the substrate in water. Yoshida et al. evaluated oxygen transport into fungal pellets and found that diffusivities 4–12 times the molecular diffusivity were required to fit a diffusion model to the experimental data. Miura et al. showed that the transport of a high molecular weight tracer into a fungal pellet could not be explained by

![Figure 5](image)

**Figure 5.** Relative uptake predictions for $a_c = 1 \mu \text{m}$ and $E_b = 0.01$ with

- (---) $D = 10^{-7} \text{ cm}^2/\text{s}$;
- (-----) $D = 10^{-6} \text{ cm}^2/\text{s}$; and
- (----) $D = 10^{-7} \text{ cm}^2/\text{s}$ in sheared fluid for (a) impermeable flocs, (b) permeable flocs, and (c) permeable flocs attached to bubbles.
diffusion models. Ho and co-workers\textsuperscript{68} have summarized a number of these studies and they have attributed high apparent diffusivities to bulk penetration of fluid into the flocs.

The analysis as presented here has required a number of assumptions and the use of empirical data extrapolated from various sources. The fluid flow analysis as presented by Adler\textsuperscript{23} was for a single permeable aggregate in an infinite fluid subject to laminar fluid shear. Adler’s analysis was extrapolated to a turbulent suspension containing a high floc volume concentration. Turbulence as characterized by a mean fluid shear rate is a reasonable first approximation. Turbulence in stirred reactors has a distribution of turbulent energy dissipation rates and the highest dissipation rates near rotating paddles dominates aggregate breakup processes with reaggregation occurring in lower intensity zones.\textsuperscript{12,47,48} Such a process of breakup and reaggregation can increase the external surface area of a floc and thus alter transport processes unlike the steady state floc size used in this analysis. For concentrated suspensions, limited fluid volume outside the flocs would tend to force greater fluid flow through microbial flocs than for dilute suspensions. For example, a suspension containing 2000 mg dry cell mass/L in a bioreactor corresponds to 1% cell volume concentration, but if the cells are in flocs of 98% porosity, then 50% of the fluid volume is within flocs. Sludge volume measurements following quiescent settling give approximate porosities in this range and the high recycle rates required by activated sludge clarifiers reflects the poor compactability of microbial flocs. Considerable uncertainty exists in the literature on in situ floc characteristics such as size and porosity. Experimental difficulties hinder sampling such a dynamic system undergoing breakup and rapid reaggregation. The process of sample removal from the turbulent fluid will rapidly alter floc size in response to changing conditions — either higher shear rates in the sampling apparatus breaks up floc,\textsuperscript{49,50} or the lower shear within a sampling tube causes floc growth.

There are a number of implications of these results leading to understanding microbial growth in bioreactors. First, when mass transfer limits substrate uptake and cell growth, then kinetic expressions for substrate uptake require inclusion of the fluid mechanical regime as well as specific nutritional requirements of the microorganism. This complicates scaleup from batch and small volume reactors to conditions expected in large volume reactors having a different distribution of fluid shear rates. Second, for the case of oxygen uptake in aerobic cultures, considerable uncertainty exists on rates. Figure 4 shows that substrate uptake is strongly influenced by advective flow induced by fluid shear and the presence or absence of bubbles. Oxygen uptake experiments conducted without bubbles do not allow for dramatically accelerated uptake when flocs interact with bubbles. Third, the predicted relative uptake factors in Figures 4(a) and 4(b) for a simple, soluble substrate are not substantially different for impermeable and permeable flocs. Experiments with glucose-like substrates can usually be fitted to an impermeable floc model based on diffusive transport. Distinguishing between permeable and impermeable floc will require that experiments be conducted with larger molecular weight substrates since impermeable aggregates are predicted to have decreased uptake compared to dispersed cells while permeable aggregates have more favorable uptake of higher-molecular-weight substrates than dispersed cells.

CONCLUSIONS

Transport of substrate to cells within porous aggregates can be dominated by fluid flow through the aggregate rather than molecular diffusion. The traditional effectiveness factor comparison of uptake within flocs and dispersed cells can not distinguish between diffusive and advective dominated transport if model parameters can be arbitrarily adjusted to fit experimental data. When mass transfer limits substrate uptake, the presence of advective flow through flocs can offer an advantage to cells associated with highly porous and permeable flocs. Bioflocculation during substrate limited growth is thus seen as a microbial response to increase substrate uptake. Calculations indicate that either the presence of bubbles or growth on substances with diffusivities less than $10^{-6}$ cm$^2$/s can select microorganisms which form flocs over other dispersed organisms.

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References