Two Parameters Account for the Flocculated Growth of Microbes in Biodegradation Assays

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Abstract: Microbes in activated sludge tanks mostly occur in flocs rather than in cell suspensions. Flocculation results in a limited supply of substrate to the bacteria inside the flocs, which reduces the biodegradation rate of organic compounds by several orders of magnitude. This article presents a simple two-parameter extension of growth models for cell suspensions to account for the ensuing reduction of the degradation rate. The additional parameters represent floc size at division and diffusion length. The biomass of small flocs initially increases exponentially at a rate equal to that of cell suspensions. After this first phase, the growth rate gradually decreases and finally the radius becomes a linear function of time. At this time flocs are large and have a kernel of dead biomass. This kernel arises when the substrate concentration decreases below the threshold level at which cells are just able to pay their maintenance costs. We deduce an explicit approximative expression for the interdivision time of flocs, and thereby for the growth of flocculated microbial biomass at constant substrate concentrations. The model reveals that the effect of stirring on degradation rates occurs through a reduction of the floc size at division. The results can be applied in realistic biodegradation quantifications in activated sludge tanks as long as substrate concentrations change slowly. © 2000 John Wiley & Sons, Inc. Biotechnol Bioeng 70: 677–684, 2000.

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INTRODUCTION

Biodegradation of household chemicals primarily takes place by activated sludge in wastewater treatment plants (WWTPs). The quantification of this biodegradation process is important in judging and developing household chemicals.

A lot of data on biodegradation rates relate to free cell suspensions. However, it would not be realistic to use these rates for WWTPs, because most biomass present in these systems is coagulated and only a thin outer layer of the aggregates is metabolically active. This substantially reduces degradation rates.

Microorganisms in activated sludge mainly exist in flocs (flocs). Flocculation depends on the ability of cells themselves to form flocs, on cell density, and on favorable hydrodynamic shear forces. Mixing regime and cell density determine the collision frequency of cells and fragments, which, in combination with a sticking probability, determines the rate of floc formation.

Floc sizes have a wide range in continuous activated sludge processes. For instance, Knudson et al. (1982) observed flocs with sizes from 0.5–1,000 μm, with most of the flocs being smaller than 100 μm. In contrast, Zhang et al. (1997) found that 77% of the flocs were larger than 100 μm. Sizes ranging from 400 μm to 4 mm were also reported (Logan and Wilkinson, 1991). Flocs of brewers’ yeast during fermentation were found ranging in size from 60–400 μm (van Hamersveld et al., 1997).

The average mass-transfer rate from the bulk fluid to individual bacteria reduces with floc size. This results in a decrease in the biodegradation rate of organic compounds of several orders of magnitude compared with that by cell suspensions. Growth rates of cell suspensions cannot easily be used to predict growth rates of flocs. However, such a prediction may help to link degradation rates by flocs to those by cell suspensions.

In this article, we present a mathematical model for the growth of floc suspensions in terms of that of free cell suspensions. In order to describe mass-transfer and microbial activity, we assume a simple spherical floc geometry. We use this floc growth model to analyze the biodegradation of compounds in activated sludge, and show how the maximum floc size affects the degradation rates.

The combination of diffusion-limited degradation and microbial floc growth is new, to our knowledge, although rather complex models exist for some of the underlying processes. Winkler (1981) and Du et al. (1996), for instance, described the degradation by flocs, but did not account for growth. Logan and Wilkinson (1991) used fractal geometry to describe the floc structure accurately; the fractal dimen-
sion of flocs has been found to be around 2.5 (Logan and Wilkinson, 1991; Snidaro et al., 1997; van Hamersveld et al., 1997; Zartarian et al., 1997). Zartarian et al. (1997) modeled the three-dimensional activated sludge floc structure using ‘discrete smooth interpolation’ of digitized microtome sections. Their method makes it possible to quantify size, surface area, and volume of the floc. These rather detailed models of floc geometry are too complex to be useful for implementation in a dynamic context, where substrate concentration and biomass densities change simultaneously. The few useful and scattered data on degradation do not contain information enough to extract the large amount of required parameter values.

The combination of diffusion-limited uptake and growth also occurs in tumor biology. Detailed quantitative and potentially useful descriptions have been formulated in this field of related interest (Groebe and Mueller-Klieser, 1991; McElwain and Ponzo, 1977; Ward and King, 1997). These models, however, are too complex to be applied in degradation studies.

Substrate availability, advective, and diffusive transport are important factors influencing the induction of biodegradative pathways as well as the biodegradation of chemicals themselves (Harms and Bosma, 1997). An incorporation of these factors in degradation studies would improve their realism.

Our aim is to study the reduction of mass-transfer rates due to the coagulation of bacteria and its implications for both biodegradation rates and growth rates. We clearly distinguish the different levels of organization: the individual cells in a floc, the floc as a ‘super individual,’ and the population of flocs in a reactor. The population model includes ‘birth and death’ of flocs. After disintegration of a floc, the fragments will start to form new aggregates or serve as substrate for other microbes. Ciliates, for example, could attack individual bacteria.

In the following sections we present a simplified model for diffusion-limited growth of individual flocs. Thereafter, we evaluate the growth of flocculated microbial biomass in a reactor and the degradation of substrate according to this model.

**APPROXIMATIVE GROWTH OF FLOCS**

The irregular floc structure is approximated by a sphere. This geometry defines the relationship between the growth of active microbial biomass in the outer shell of the floc and the generation of dead mass in the kernel. Although the detailed growth of this idealized floc must be evaluated numerically (partial differential equations), approximations can be given when a steady-state substrate concentration profile builds up in the floc. Such a steady state can be expected if growth is slow enough with respect to diffusive transport. We take the volume-specific amount of biomass (mass/volume) within the floc to be constant, which entails that the floc expands if biomass increases.

Cells are assumed to die when they cannot pay their maintenance costs. Hence, our approach to the growth of flocs involves a growth model for individual cells that accounts for maintenance costs. The well-known Marr-Pitt model (Marr et al., 1962; Pirt, 1965) provides the simplest way to take these costs into account. A more realistic but also a somewhat more complex one is given by the Dynamic Energy Budget theory, which is based on mechanistic rules for the uptake and use of substrates by organisms (Hanegraaf, 1997; Kooijman, 2000).

A spherical floc of radius $L_T(t)$ has a volume of $V_T(t) = \frac{4}{3}\pi L_T(t)^3$. Let $V_M(t)$ denote the maximum thickness of the living layer (Fig. 1). Index $M$ relates to the fact that living mass requires maintenance. For a floc without a dead kernel $L_T \approx L_M$ holds. In this case we assume that a floc grows exponentially at rate $r$, thus:

$$\frac{dV_M}{dt} = rV_M$$

However, when the floc consists of a dead core and an outer living layer, the following equation gives the change in total volume:

$$\frac{dV_T}{dt} = \frac{d}{dt}[V_M(t) + V_i(t)] = rV_M(t)$$

where $V_M$ and $V_i$ denote the volume of living and dead biomass, respectively. Living volume relates to total floc radius as:

$$V_M(t) = \frac{4}{3}\pi [L_T(t)^3 - (L_T(t) - L_M)^3] = 4\pi [L_T(t)^2 L_M - L_T(t)L_M^2 + \frac{L_M^3}{3}]$$

Furthermore, from the fact that the dead kernel has a radius $L_T - L_M$, it is easy to deduce

$$\frac{dV_i}{dt} = 4\pi(L_T - L_M)^2 \frac{dL_T}{dt}.$$

Substitution of the expressions for $V_T(t)$ and $V_M(t)$ in the differential Eqs. (1) and (2) leads to the equation describing the change in floc radius:

![Figure 1](image-url)
For a floc without a dead kernel, we have \( L_T \leq L_M \). The thickness of the living layer increases up to its maximum value, \( L_M \), which is associated with a given substrate concentration in the environment. The floc continues to grow; thus, \( L_T \) still increases and a dead kernel appears. For \( L_T \gg L_M \), the growth rate of floc radius becomes constant, that is, the cube root of floc volume increases linearly with time. This has been known for a long time (Emerson, 1950), and also applies to tumors (Mayneord, 1932; Steel, 1977), and mammalian fetuses (Huggett and Widdas, 1951; Kooijman, 2000), for the same reason: mass exchange between a (living) structure and its environment occurs across its surface area.

When a floc develops an increasing dead kernel, it eventually becomes mechanically unstable and falls apart. This event depends on turbulent shear forces and on the size and porosity of the floc (Ruiz and Izquierdo, 1997; van Hamersveld et al., 1997). The porosity generally changes with size and thus with age of the floc. However, we refrain from modeling these details and assume that the floc falls apart at a given volume \( V_p \), which depends on environmental conditions. We denote the radius at division as \( L_d \).

At fragmentation of the floc, the dead material becomes suspended and the living shell falls apart into \( n \) small flocs without a dead kernel (Fig. 2). We suppose that the living shell partitions into daughter flocs without changing thickness, which implies that these newly formed flocs have a radius of \( L_b = \frac{3}{2} L_M \) for \( L_d \geq L_M \) (if \( L_d < L_M \), \( L_b = \frac{1}{2} L_d \)). The number of daughter flocs per mother floc is thus

\[
\frac{d}{dt} L_T = \begin{cases} \frac{r F}{3} \frac{L_T}{3} & \text{for } L_T \leq L_M \\ r L_M \left(1 - \frac{L_M}{L_T} + \frac{L_M^2}{3L_T^2}\right) & \text{for } L_T > L_M \end{cases} \tag{3}
\]

\( n = \begin{cases} \frac{L^3_d}{L^3_b} & \text{for } L_t = 0 \\ \frac{L^3_M - L^3_t}{8} - \frac{3L^2_M - 3L_t L_M + L^2_M}{L^2_M} & \text{for } L_t > 0. \end{cases} \]

The growth equation (3) can be solved explicitly for the interdivision time \( t_d \) of flocs.

For \( t^*_d = \frac{L^*_d}{L^*_M} \), we obtain:

\[
r_{t_d} = \begin{cases} 3 \ln 2 & \text{for } t^*_d \leq 1 \\ 3 \ln 2 - 1 + \frac{\pi}{3\sqrt{3}} + \frac{1}{\sqrt{3}} \arctan \left(\sqrt{3}(2l^*_d - 1)\right) \\ + \frac{1}{2} \ln \left(1 - 3l^*_d + 3l^*_d^2\right) & \text{for } t^*_d > 1 \end{cases} \tag{4}
\]

The values of \( r \), \( L_M \) (thus also of \( n \), \( l^*_d \) and \( t_d \)) depend on the substrate concentration \( X \). The specific growth rate of the floc population, \( r_F \), is given by:

\[
r_F(X) = \frac{\ln n(X)}{t_d(X)} \tag{5}
\]

The equations above quantify the relationship between the specific growth rate of flocs and that of free cells via the interdivision time:

\[
r_F(X) = \frac{\ln n(X)}{rt_d(X)} \tag{6}
\]

Figure 3 shows the ratio of \( r_F \) to \( r \) as a function of the size at division \( l^*_d \). Figure 4 shows the specific population growth rate \( r_F \) as a function of the scaled substrate concentration. The specific growth rate of suspended cells, \( r \), required to calculate \( r_F \), is given by the assumed cell growth model. In the Appendix an expression for the growth rate of a floc is derived, using the Dynamic Energy Budget theory to describe cell dynamics.

The maximum thickness of the living layer, which is needed to calculate \( n \), follows from a closer analysis of the substrate concentration profile in the floc. This subject is addressed in the next section.

### STEADY-STATE SUBSTRATE PROFILES

In this section we analyze the steady-state substrate profiles in a floc. We start with introducing a number of parameters and equations. The analysis results in an equation to calculate the maximum thickness of the living layer, \( L_M \).

A large floc of radius \( L_T \) consists of a living layer of thickness \( L_M \) around a dead kernel of radius \( L_d \), that is,
The scaled functional response at the edge of the dead kernel is zero; that is, \( f_0 = 0 \). This means that the substrate assimilation rate equals the maintenance rate. The scaled functional response multiplied by the maximum assimilation rate yields the amount of energy which is taken up. Thus, \( f_0[p_{Am} - p_M] = 0 \), where \([p_{Am}]\) is the volume-specific maximum assimilation rate and \([p_M]\) the volume-specific maintenance rate (Kooijman, 2000). Hence, the value of \( f_0 \) follows from solving \( r = 0 \), where \( r \) is the specific growth rate of cells. The substrate concentration at the edge of the dead kernel is \( X_t = X(L_M) = x_t X_K \), where \( x_t = f_0/(1 - f_0) \).

Now we are able to calculate the minimum substrate concentration that is needed to support life. The position \( L_M \), where this concentration \( X_t \) is reached, is calculated from the substrate concentration profile. This is the solution of Eq. (7), which describes the diffusion in a sphere. Since biomass takes up substrate, a consumption term \( f(L,t)X_{fm} X_F \) is present in this equation. The factor \((L_T - L)^2\) appears because the coordinate origin is located on the surface of the sphere.

\[
\frac{\partial}{\partial t} X(L,t) = \frac{D}{(L_T - L)^2} \frac{\partial}{\partial L} \left( (L_T - L)^2 \frac{\partial}{\partial L} X(L,t) \right) - f(L,t) X_{fm} X_F
\]

\( X_F \) denotes the biomass density in the floc (C-mol/ volume), \( j_{Xfm} \) the maximum mass-specific uptake rate for biomass, and \( D \) the substrate diffusion coefficient. The initial condition \( X(L,0) \) and the boundary condition \( X(0,t) \) are assumed to be given. The derivative of the substrate concentration with respect to \( L \) in the center of the floc or at the edge of the dead kernel is zero; that is,

\[
\frac{\partial}{\partial L} X(L_T, t) = 0 \quad \text{or} \quad \frac{\partial}{\partial L} X(L_M, t) = 0.
\]

Let the substrate concentration in the environment be constant and let growth be slow with respect to the change in the substrate profile. Let \( L_D \) denote the ‘diffusion length’

\[
L_D = \sqrt{\frac{DX_K}{j_{Xfm} X_F}}.
\]

At steady state

\[
\frac{\partial}{\partial t} X = 0 \text{ and } X(L, t)
\]

is constant in time. In this case, substitution of the dimensionless scaled length

\[
l = \frac{L}{L_D}
\]

and the scaled functional response

\[
f(l) = \frac{x(l)}{x(l) + 1}
\]

in Eq. (7) yields:

\[
0 = \frac{1}{(l_T - l)^2} \frac{d}{dl} \left[ (l_T - l)^2 \frac{dx}{dl} \right] - f(l)
\]

with boundary conditions \( x(0) = x_0 \) and \( dx(l_M)/dl = 0 \). The latter condition results in \( x(l_M) = x_t \) if \( l_F > l_M \).

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**Figure 3.** The scaled specific growth rate of flocs \( r_T/L_t \) as a function of its scaled radius at division \( (l_F = L/L_M) \). The specific growth rate of small flocs equals the specific growth rate of suspended cells.

**Figure 4.** The specific growth rate of flocs as a function of the scaled bulk substrate concentration. The numbers next to the curves indicate the length at division as fraction of diffusion length. The specific growth rate of cells according to the DEB theory is \( r = (k_g f - k_g g)(f + g) \) (Appendix). The minimum scaled substrate concentration \( x_t \), found by solving \( r = 0 \), equals \( k_g g/(k_E - k_g g) \) (with \( k_E = 0.8 \) h\(^{-1} \), \( k_M = 0.05 \) h\(^{-1} \), \( g = 1 \).
The steady-state substrate profile can be further simplified if \((l_f - l) \gg 2dx/dl\). This occurs, for instance, if the curvature of the floc surface is negligibly small. Equation (8) then reduces to:

\[
0 = \frac{d^2x}{dl^2} - f(l)
\]

The implicit solutions for the profiles of the scaled substrate concentration \((x)\) and the scaled functional response \((f)\) are:

\[
l(x) = \frac{1}{\sqrt{2\pi}} \int_{x_f}^{x_l} dy \left( y - x + \ln \frac{1 + x}{1 + y} \right)^{-1/2}
\]

\[
l(f) = \frac{1}{\sqrt{2\pi}} \int_{f_l}^{f_f} dz \left( \frac{z}{1 - z} \right)^{1/2} \left( \frac{1 - f}{1 - f_t} + \ln \frac{1 - z}{1 - f_t} \right)
\]

This directly leads to the scaled maximum thickness of the living layer:

\[
l_M(x_i) = 2^{-1/2} \int_{x_f}^{x_l} \left( y - x + \ln \frac{1 + x}{1 + y} \right)^{-1/2} dy
\]  

Figure 5 illustrates the \(x\)- and \(f\)-profiles. Figure 6 shows the scaled thickness of the living layer \(l_M\) as a function of the scaled substrate concentration.

We carried out a study of the concentration profile inside the floc and obtained an expression for the maximum thickness of the living layer, Eq. (9). The value of \(L_M = l_M L_D\) is required in the previous section to calculate the interdivision time of flocs, Eq. (4), given that \(r\) is the specific growth rate of cells. Nevertheless, a direct substitution of \(L_M\) into Eq. (4) gives an overestimation of the floc population growth rate. This overestimation is due to the gradual decrease in the specific growth rate of individual cells from the surface of the floc to the edge of the dead kernel. The derivation of \(l_M\) does not account for the decreasing curvature of the growing floc, which gives rise to an error in the opposite direction.

**REACTOR DYNAMICS**

The specific growth rate of flocculated microbial mass at substrate concentration \(X\), as given by Eq. (5), depends on the following parameters: length at division \(L_D\), diffusion length \(L_D\), and the parameters included in the specific growth rate of cell suspensions (for the Marr-Pirt model: the substrate to biomass conversion factor, \(y_{XM}\), the maintenance rate coefficient, \(k_M\), the mass-specific maximum uptake rate, \(j_{XM}\), and the saturation coefficient, \(X_K\)). This means that steady-state growth of flocs has two extra parameters compared to that of cell suspensions. If we use the Marr-Pirt model for cellular growth, the floc dynamics of a batch reactor amounts to:

\[
\frac{d}{dt}X = -y_{XM} \left( \frac{d}{dt}X_V + \frac{d}{dt}X_{V_f} \right) - j_{XM}X_V
\]

\[
\frac{d}{dt}X_V = X_{f,f}(X)
\]

\[
\frac{d}{dt}X_{V_f} = \frac{V_f(t_f) - V_f(t_b)}{V_M(t_f) - V_M(t_b)} \frac{d}{dt}X_V
\]

\[
= \left( \frac{L_D}{L_M} \right)^3 - 2 - 3 - 1 \right)^{-1} X_{f,f}(X)
\]

with \(X_V\) and \(X_{V_f}\) the density of living and dead biomass, \(y_{XM}\) the ratio of substrate uptake to biomass production, and \(j_{XM}\) the mass-specific maintenance rate of biomass. The first equation in (10) represents the use of substrate. Substrate is going to the growth of biomass (first term) and to the maintenance of living biomass (second term). The second equation represents the growth of living biomass. The third equa-
tion describes the increase of dead biomass. The production rate of dead biomass is proportional to the growth rate of living biomass. The proportionality constant is the change in dead volume $\Delta V_d$ relative to the change in living volume $\Delta V_M$ during the floc life cycle. Remember that the value of $V_l(t_b)$ is zero in the present model.

The derivation of the relationship between the specific growth rate $r_F$ and the substrate concentration only applies at steady state. We expect, however, that the approximations are satisfactory as long as the medium-concentration changes slowly. If the substrate concentration rises fast, and the thickness of the living layer increases faster than the growth of the radius, a formal problem of resurrection occurs. We expect this to be of minor quantitative significance and the problem disappears if the kernel is dormant, rather than dead. The situation of a rapidly rising substrate concentration can only occur at the start of the experiment.

The present formulation includes neither the further degradation of dead biomass nor the process of cometabolism. The extension to a CSTR is straightforward.

**DISCUSSION**

Mass transfer into an organism takes place across its surface area. Organisms can be classified on the basis of how surface areas that are involved in the uptake of substrate grow. Organisms can be classified on the basis of how surface area. Mass transfer into an organism takes place across its surface area. Organisms can be classified on the basis of how surface area grows proportional to volume $V_0$ in V0-morphs (such as biofilms), and proportional to volume $V_1$ in V1-morphs (such as filaments). For V0-morphs this means that surface area remains constant. Spherical flocs with a dead kernel represent a dynamic mixture between a V1-morph and a V0-morph, with an increasing weight on the latter during the division interval (Kooijman, 2000).

We illustrated floc dynamics with the simplest possible model for cellular growth that takes maintenance into account. The Appendix shows how this dynamics combines with more realistic models based on cellular physiology.

The gist of our contribution is that we use simple diffusion arguments to reveal the relationship between substrate concentration and the thickness of the living layer, and a simple geometry of flocs to quantify the deactivation of microbial metabolism. Only two parameters appear to dominate the growth process: the floc volume at fragmentation and the diffusion length. The first parameter is affected by turbulence, thus by stirring. The latter parameter combines a number of properties of the microbes and the compound: the maximum specific uptake rate, the saturation coefficient, the density of biomass in the floc, and the diffusion rate of the compound through the floc, which depends on the porosity of the living biomass. The set of equations (10) show how one can account for growth of microbes in flocs and the occurrence of inactive biomass in a very simple and yet mechanistically inspired way.

Although we are fully aware of the limitations of our idealizations, we believe that this relatively simple model does capture the main features of the effect of floculation on microbial degradation of compounds. Our formulation can be used to link degradation rates by cell suspensions to that by flocs, and to quantify the effects of increased stirring (via a reduction of the floc size at division).

**NOMENCLATURE**

$$D$$ diffusion coefficient
$$[E]$$ energy density
$$[E_c]$$ volume-specific costs of growth
$$[E_m]$$ maximum energy density

$f(L)$ scaled functional response: $X(L) / X_k + X(L)$
$f_0$ scaled functional response at $L = L_m$
$f_T$ average scaled functional response
$g$ energy investment ratio: $[E_c]/[E_m]$
$k_e$ specific energy conductance: $[p_{Am}]/[E_m]$
$k_m$ maintenance rate coefficient: $[p_{Am}]/[E_c]$

$\dot{M}_{km}$ mass-specific maximum uptake rate

$\dot{M}_{km}$ mass-specific maintenance rate

$L_d$ diffusion length: $\sqrt{D X_k X_F}$

$L_b$ radius at birth
$L_T$ total radius of floc
$L_d$ diffusion length at division: $L_d/L_T$
$L_M$ maximum thickness of living layer: $L_d/L_T$
$L_m$ scaled total radius of floc: $L_d/L_T$

$L_{M}$ total volume of biomass
$L_{M}$ volume at division
$L_{d}$ volume of living biomass
$L_{T}$ volume of dead biomass

$x$ scaled substrate concentration: $X/X_k$

$X(L)$ substrate concentration at $L$

$X_k$ saturation coefficient of scaled functional response

$X_F$ amount of biomass / volume floc
$X_V$ structural biomass / volume
$X_{Vd}$ dead biomass / volume

$\beta$ minimum substrate concentration for support
$\gamma_{VX}$ substrate (X) needed per biomass (V) formed

$\gamma_{X}$ substrate (X) needed per reserve (E) formed

Quantities which are expressed per unit of biovolume have square brackets, [ ]. The following symbols are used for the dimensions: −, no dimension; $e$, energy; $t$, time; $l$, length; #, amount.
The Marr-Pirt model for growth is attractively simple, but not very realistic. A substantial increase in realism can be obtained on the basis of the Dynamic Energy Budget (DEB) theory (Kooijman, 2000). Here we derive the steady-state growth of the floc radius, given that the growth of bacterial cells follows the DEB theory.

This theory delineates structural mass and reserves (mixtures of carbohydrates, lipids, and proteins) as state variables; maintenance and growth of structural mass are at the expense of reserves. The volume-specific maintenance rate \([p_M]\) and volume-specific energy costs of growth \([E_G]\) are assumed to be constant. The reserve energy density (i.e., the ratio of reserve energy to structural volume) follows first-order dynamics, thus the scaled functional response, \(X_k\) is the saturation coefficient, and \([p_{Am}]\) is the volume-specific maximum assimilation rate of substrate into reserves.

The expression for the interdivision time (Eq. 4) still applies, but the specific growth rate of the cells differs from the Marr-Pirt model. For the Marr-Pirt model, the specific growth rate of cells is \(k_E f - k_M g\). When the maintenance rate coefficient \(k_M\) is zero, \(k_E g\) represents the maximum specific growth rate.

Equations for batch reactor dynamics should account for reserves in living and dead biomass \((M_E, M_{E_1})\), respectively. The terms \(dX_{V_1}/dt\) and \(dX_{E_1}/dt\) appear because living biomass is transformed into dead biomass.

\[
\frac{d}{dt} X = -y_{xy} \left( \frac{d}{dt} X_V + \frac{d}{dt} X_{V_1} \right) - y_{x_e} \left( \frac{d}{dt} X_{E} + \frac{d}{dt} X_{E_1} \right)
\]

with \(g = [E_G]/[E_m], k_M = [p_M]/[E_G], k_E = [p_{Am}]/[E_m]\), and \(f = L_M E_G f(L)dL\), and \(f_0\) is the functional response at the surface of the floc. The energy investment ratio, \(g\), is defined as the quotient of the volume-specific costs of growth, \([E_G]\), and the maximum energy density \([E_m]\). The maintenance rate coefficient, \(k_M\), stands for the costs of maintenance with volume-specific maintenance rate, \([p_M]\), and cost of biovolume synthesis, \([E_G]\); the specific-energy conductance \(k_E\) is the quotient of the volume-specific maximum assimilation rate, \([p_{Am}]\), and \([E_m]\). The specific growth rate of suspended cells at steady state, according to the DEB theory, is \(r = (k_E f - k_M g)/(f + g)\) (Kooijman, 2000, p. 108; p. 315).

The denominator of the integrand in Eq. (11) stands for the local costs of growth, which consist of a contribution to the reserves, \([E_m]/f(L)\), and to the structural biomass, \([E_G]\). The first term of the numerator represents the assimilative input. The last term is the maintenance flux. The middle term stands for extra energy available from the reserves. As explained above, the functional response and reserve density of a typical cell decreases with time. The change in \(f\) is caused by the outward movement of the profile relative to the cell and equals \((df/dL) (dL/df)\); multiplied by \([E_m]\) this gives the small amount of extra energy available for growth. The second factor, \((1 - L f_L)^2\), weighs the contribution of the different shells in the floc as a function of the radius. This is necessary because more biomass is present in the outer shells, if the shells have a constant thickness \(dL\). The reserve density of the dead biomass equals \(f_t = [E_t]/[E_m]\). The approximation (Eq. 12) is obtained by replacing the profile of the functional response \(f/L\) by its mean value \(\bar{f}\).

The floc sizes for which this derivation holds are \(V_T \geq V_M\). If the floc is small enough, the inner cells can pay their maintenance costs and keep living, and \(V_T = V_M\), while \(V_1 = 0\). We simply replace \(L_M\), the upper boundary of the integral, by \(L_T\) and \(f_t\) by \(f_T = f(L_T)\) in (11) to obtain the expression for the growth of flocs without a dead kernel.

The expression for the interdivision time (Eq. 4) still applies, but the specific growth rate of the cells differs from the Marr-Pirt model. For the Marr-Pirt model, the specific growth rate of cells is \(k_E f - k_M g\). When the maintenance rate coefficient \(k_M\) is zero, \(k_E g\) represents the maximum specific growth rate.

Equations for batch reactor dynamics should account for reserves in living and dead biomass \((M_E, M_{E_1})\), respectively. The terms \(dX_{V_1}/dt\) and \(dX_{E_1}/dt\) appear because living biomass is transformed into dead biomass.
\[
\frac{d}{dt} X_E = X_F r_F(X) \frac{M_E(t_{d}) - M_E(t_{b})}{M_X(t_{d}) - M_X(t_{b})}
\]

\[
\frac{d}{dt} X_{V_f} = X_{V_f} r_F(X) \frac{M_{V_f}(t_{d}) - M_{V_f}(t_{b})}{M_X(t_{d}) - M_X(t_{b})}
\]

\[
\frac{d}{dt} X_{V_f} = X_{V_f} r_F(X) \frac{M_{E1}}{M_{V_f}}
\]

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