Modeling Bacteria Flocculation as Density-Dependent Growth

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Introduction

Biological reactors are commonly used to remove pollutants from wastewater. One standard technology is the two-step activated sludge (AS) process. Both in the reaction and the settling tank, bacteria naturally aggregate and form flocs. It is well known—but poorly understood—that both floc formation and settling capacity strongly depend on the loading rate. To optimize this bioprocess it is, therefore, necessary to better understand the flocculation phenomenon.

Mathematical modeling has proven to be a valuable tool in the study of wastewater treatment plants. The activated sludge models describe the different biological processes (for example, chemical oxygen demand removal, (de) nitrification and phosphorus removal) involved in the AS process. Its core consists of the mass-balance equations, including the reaction kinetics as a function of the limiting substrates, which read in their simplest form

$$\begin{aligned}\frac{dx}{dt} &= h(s)x - Dx \\ \frac{ds}{dt} &= -h(s)x + D(s_{in} - s).\end{aligned} \tag{1}$$

where x is the biomass concentration, s the substrate concentration, $h(s)$ the specific growth rate, D the dilution rate, and s_{in} the substrate concentration in the inflow.

The description of floc formation and settling remains the weakest part of AS models. The problem has been studied by a variety of approaches (see[1,2] for reviews). Population balance models (PBM) describe floc aggregation and breakage and allow to compute the floc-size distribution as a function of time.[3,4] Computational-fluid dynamics (CFD) simulators describe the hydrodynamics in the clarification tank and try to predict the settling properties of the flocs.[5] Individual-based models (IBM) take both physicochemical and biological processes into account at the level of a single floc.[6]

These modeling approaches have in common a high-dimensional parameter space. Although these parameters can be identified from experiments, the resulting model is often too complex to provide insight in the governing mechanisms. Moreover, to compute the settling properties of the ensemble of interacting flocs in the clarifier, one has to combine a CFD with a PBM approach, which leads to even more intricate models.

Instead of using advanced simulators, we propose to take the simple model (Eq. 1) as a starting point. In particular, we investigate how these equations are modified when the biomass is organized in flocs. We propose a PBM-like model where both the floc interactions (as in standard PBM), and the bacteria growth are included. This qualitative model is

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sufficiently transparent to be manipulated analytically. Our approach is primarily intended to model the floc dynamics in the reaction tank, where both physicochemical and biological processes have to be taken into account. Nevertheless, our model can also be useful to check the common assumption of PBM that biological growth can be neglected in the settling tank.

Our analysis naturally leads to an effective model of the form

$$\frac{dx}{dt} = h(s,x)x - Dx$$
$$\frac{ds}{dt} = -h(s,x)x + D(s_{in} - s). \quad (2)$$

Note that the specific growth rate $h(s,x)$ depends both on the substrate concentration s, and the biomass concentration x, in contrast with the substrate-dependent growth rate $h(s)$ of model (Eq. 1). The specific growth rate $h(s,x)$ is called density-dependent. In fact, density-dependent growth rates have been proposed to describe bioreactor kinetics more accurately.[7,8] From an ecological point of view, this change has important consequences, as it allows microorganisms to coexist in a medium where classical, that is, substrate-dependent, models predict extinction by wash-out.

This work is not the first to study the influence of a heterogeneous biomass structure on the growth rate (see, for example,[9,10]). However, we present here, to the best of our knowledge, an original derivation of an effective model with density-dependent growth dynamics, starting from a PBM description including bacterial growth.

The article is organized as follows. First, we introduce the bioreactor model, including bacterial growth, floc aggregation and breakage, and hydrodynamics. Next, we present an analytical study, under the hypothesis that the timescale associated with the floc interactions is much shorter than the other processes. We show analytically how this hypothesis leads to a density-dependent growth rate. Finally, we discuss some numerical computations, that go beyond the hypothesis of separate timescales.

Flocculation Model for Growing Bacteria

Consider a bioreactor in which a biomass grows on a substrate. The density of the biomass is denoted by x, the density of the substrate by s. The biomass consists of bacteria which naturally aggregate in flocs. A floc containing n bacteria will be denoted by F_n. Define u_n as the density of flocs of size n. Expressing the densities x resp. u_n as the number of particles (bacteria resp. flocs) per unit of volume, we have

$$x = \sum_{n=1}^{\infty} n u_n. \quad (3)$$

The dynamics of the floc densities u_n is given by

$$\frac{du_n}{dt} = \left(\frac{du_n}{dt}\right)_{\text{bacterial growth}} - D\,u_n + \left(\frac{du_n}{dt}\right)_{\text{floc interaction}}. \quad (4)$$

The second term in the righthand side represents the bacteria disappearing in the effluent of the reactor with dilution rate D. The two other terms are now described in more detail.

The only bacterial growth present in our model is through cell division. As a bacterium present in a floc of size n divides, we assume the daughter bacteria to stick to the floc, which will then consists of $n+1$ bacteria. This growth can be written as

$$F_n \rightarrow F_{n+l} \quad \text{with reaction rate } h_n(s). \quad (5)$$

As we assume the reactor to be perfectly mixed, all flocs have the same substrate density s available. However, the dependency of the growth rate $h_n(s)$ on the floc size n takes into account that bacteria at the surface of the flocs have easier access to the substrate than the bacteria inside the flocs. While realistic functions $n \rightarrow h_n(s)$ could be derived from detailed models,[6,11] our analysis does not require such an explicit expression.

The corresponding part of the dynamics is

$$\left(\frac{du_1}{dt}\right)_{\text{bacterial growth}} = -h_1(s)u_1$$
$$\left(\frac{du_n}{dt}\right)_{\text{bacterial growth}} = h_{n-1}(s)u_{n-1} - h_n(s)u_n, \quad n \geq 2. \quad (6)$$

Indeed, a growth event $F_n \rightarrow F_{n+1}$ corresponds to the consumption of a floc of size n and the production of a floc of size $n+1$. Mass action kinetics are assumed for this reaction.

The floc interactions we consider are the aggregation of two flocs to form one bigger floc and the breakage of one floc into two smaller ones. As Eq. 4 is continuous in time, processes involving three or more flocs are implicitly included. The floc interactions can be written as

$$F_m + F_n \rightarrow F_{m+n} \quad \text{with reaction rate } a_{m,n}$$
$$F_{m+n} \rightarrow F_m + F_n \quad \text{with reaction rate } b_{m,n}. \quad (7)$$

Many studies have been carried out to obtain these coefficients both theoretically[2] and experimentally.[3,4] Again, our analysis does not need explicit expressions for the reaction rates $a_{m,n}$ and $b_{m,n}$.

The corresponding part of the dynamics is

$$\left(\frac{du_n}{dt}\right)_{\text{floc interaction}} = \sum_{m=1}^{\lfloor \frac{n}{2} \rfloor} a_{m,n-m}u_m u_{n-m} - \sum_{m=1}^{\infty}(1+\delta_{m,n})a_{m,n}u_m u_n + \sum_{m=1}^{\infty}(1+\delta_{m,n})b_{m,n}u_{m+n} - \sum_{m=1}^{\lfloor \frac{n}{2} \rfloor} b_{m,n-m}u_n, \quad (8)$$

where $\lfloor x \rfloor$ is the largest integer smaller than x, and $\delta_{m,n}$ equals 1 when $m = n$, and 0 otherwise. These are the standard PBM equations,[12] in which, for example, the first term corresponds to the aggregation of two flocs to form a floc F_n.

Fast Flocculation Dynamics

The application of PBMs to the AS process assumes that the flocculation can be uncoupled from other processes. It is argued that in the settling tank the substrate concentration s is sufficiently low to justify this assumption. We now derive an effective model for this situation.

To make the separation in timescales explicit, we introduce a small parameter $\varepsilon > 0$

$$\frac{du_n}{dt} = \left(\frac{du_n}{dt}\right)_{\text{bacterial growth}} - D\,u_n + \frac{1}{\varepsilon}\left(\frac{du_n}{dt}\right)_{\text{floc interaction}}. \quad (9)$$

Taking $\varepsilon \to 0$, we introduce a sharp distinction between

- the fast dynamics, consisting of the floc interaction, for times $t \sim \varepsilon$, and
- the slow dynamics, consisting of the bacterial growth and the dilution, for times $t \sim 1$.

The idea now is as follows. On the short timescale, the system evolves to fast dynamics equilibria ($u_n^{\text{fast}}(x)$), parameterized by the total bacteria density x. On the large timescale, the system evolves on the manifold of these equilibrium distributions. As this manifold is 1-D and parameterized by x, we obtain autonomous dynamics for the biomass density x.

First, we look at the short timescale and the flocculation interactions. The reaction scheme (Eq. 7) suggests an analogy between chemical reactions and floc interactions. Indeed, a derivation like the one in equilibrium chemistry yields a set of conditions for the equilibrium between flocs of different size

$$K_{m,n} = \frac{u_{m+n}}{u_m u_n}, \quad \text{for all } m, n,$$

with $K_{m,n}$ the equilibrium constant, independent of any density u_k. As these conditions are not independent, we consider a basis of floc interactions, that is, a set of independent interactions from which the others can be obtained by taking linear combinations. One such basis is given by

$$nF_1 \rightleftharpoons F_n \quad \text{with equilibrium constant } K_n.$$

The equilibrium conditions then read

$$u_n = K_n u_1^n, \quad \text{for all } n \geq 2.$$

All floc densities u_n for $n \geq 2$ are expressed in terms of u_1, which for a given biomass density x can be obtained from Eq. 3. It is not difficult to prove that this equilibrium is unique, and thermodynamics guarantees that it is stable.

Next, we consider the other processes on the large timescale. We write the dynamics for the total bacteria density x by combining Eqs. 3, 4, 6 and 8

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \sum_{n=1}^{\infty} h_n(s) u_n - Dx.$$

We then replace the floc densities u_n by the fast dynamics equilibrium ($u_n^{\text{fast}}(x)$)

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \sum_{n=1}^{\infty} h_n(s) u_n^{\text{fast}}(x) - Dx = h(s,x)x - Dx,$$

where we have introduced the specific growth rate $h(s,x)$

$$h(s,x) = \frac{\sum_{n=1}^{\infty} h_n(s) u_n^{\text{fast}}(x)}{x}. \tag{10}$$

If the floc growth rate $h_n(s)$ is proportional to the floc size n, a factor x can be divided out in Eq. 10, and we obtain a substrate-dependent growth function $h(s)$, see Eq. 1. In all other cases, we find a genuine density-dependent growth rate $h(s,x)$, see Eq. 2.

Slow Flocculation Dynamics

For the reaction tank, assuming the parameter ε to be small is not obvious. Literature reports flocculation times of the order of 1 to 10 min,[4,13] to be compared with bacterial growth times of 1 h to 1 day, and with retention times of a few hours to a few days. We use numerical simulations to investigate how well density-dependent growth (Eq. 2) approximates the full flocculation model (Eq. 9).

The parameter values used in the simulations are given in Table 1. The floc growth rate behaves as $h_n(s) \sim n^{\alpha}$. The growth rate per bacterium decreases with increasing floc size, indicating a limited access to the substrate inside the flocs. The exponent $\alpha = 2/3$ can be interpreted as a surface-to-volume ratio for spherical flocs. Instead of considering all possible floc interactions (Eq. 7), we assume that only individual bacteria attach to and detach from the flocs. Therefore, $a_{m,n} = b_{m,n} = 0$ if both $m \neq 1$ and $n \neq 1$. Moreover, the aggregation and breakage coefficients behave as $a_{m,1} \sim m^{\alpha}$ and $b_{m,1} \sim m^{\alpha}$, which can again be considered as the surface of spherical flocs.

From a numerical point of view, the infinite sequence of dynamical (Eq. 9) were truncated at $n = 300$. By appropriately choosing the parameters and the initial conditions, we took care that this truncation did not influence the simulation results. Figure 1 compares the full dynamics (Eq. 9, together with Eqs. 6 and 8), for different values of the parameter ε with the reduced dynamics (Eq. 2, together with Eq. 10). For small ε, the solutions of Eq. 9 for different initial conditions converge rapidly (after a time of the order $t \sim \varepsilon$) to each other. The solution of Eq. 2 almost coincides with those of the full dynamics, indicating that the latter can be approximated as dynamics on the manifold of distributions ($u_n^{\text{fast}}(x)$). When ε increases, the solutions for different initial conditions differ more and more. This indicates that there are no longer autonomous dynamics in the variable x, and, thus, no well-defined specific growth rate.

We conclude that for larger values of ε, the system cannot be described by a dynamical equation like Eq. 2. Nevertheless, Figure 1 shows that for all values of ε, the different initial conditions lead to the same equilibrium. On the other hand, the nontrivial equilibrium of Eq. 2, satisfies $h(s,x) = D$. If we want the reduced dynamics to predict the correct equilibrium, the specific growth rate should satisfy this condition. In this way, we obtain a well-defined density-depend-

Table 1. Parameter Values used in the Simulations

floc-growth rate $h_n(s)$	$h_n(s) = h(s)n^{\alpha}$ with $\alpha = \frac{2}{3}$
bacterium growth rate $h(s)$	$h(s) = \frac{0.2s}{s+6}$
aggregation rates $a_{m,n}$	$a_{m,n} = \begin{cases} m^{\alpha}\delta_{n,1} & \text{if } m \geq n, \\ n^{\alpha}\delta_{m,1} & \text{otherwise.} \end{cases}$ with $\alpha = \frac{2}{3}$
breakage rates $b_{m,n}$	$b_{m,n} = \begin{cases} 0.1m^{\alpha}\delta_{n,1} & \text{if } m \geq n, \\ 0.1n^{\alpha}\delta_{m,1} & \text{otherwise.} \end{cases}$ with $\alpha = \frac{2}{3}$
dilution rate D	$D = 0.04$
inflow-substrate concentration s_{in}	$s_{\text{in}} = 20$

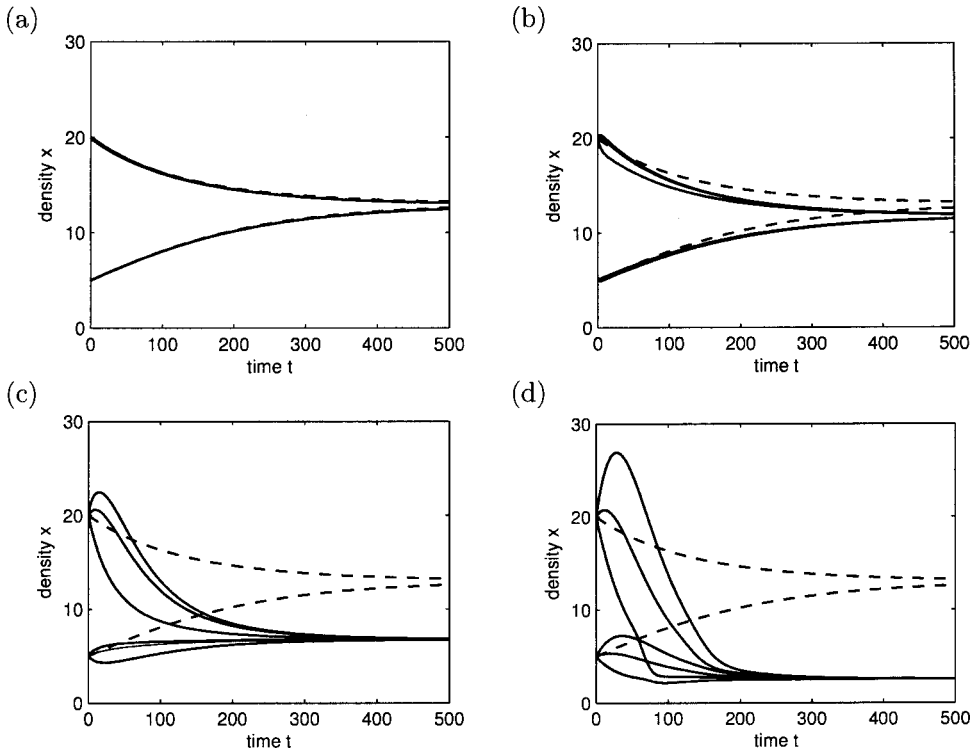

Figure 1. Comparing full and reduced dynamics for relaxation to equilibrium.

The full dynamics (Eq. 9) for different ε and the reduced dynamics (Eq. 2) are compared. Parameter values of Table 1 were used. The full dynamics, shown in full line, were integrated for six initial conditions: three with $x(0) = 20$ ($u_n(0) = 20\ \delta_{n,1}$, $u_n(0) = 2\ \delta_{n,10}$, and $u_n\ (0) = 0.2\ \delta_{n,100}$), and three with $x(0) = 5$ ($u_n(0) = 5\ \delta_{n,1}$, $u_n(0) = 0.5\ \delta_{n,10}$, and $u_n\ (0) = 0.05\ \delta_{n,100}$). The reduced dynamics, shown in dashed line, were integrated with initial conditions $x(0) = 20$, and $x(0) = 5$. (a) $\varepsilon = 0.001$; (b) $\varepsilon = 0.01$; (c) $\varepsilon = 0.1$, and (d) $\varepsilon = 1$.

ent growth rate, which we call the specific growth rate at equilibrium $h^{\text{equi}}(s,x)$.

Figure 2 plots the specific growth rate at equilibrium for different values of the parameter ε. For small ε, the specific growth rate at equilibrium coincides almost with the explicit formula (Eq. 10). As ε increases, the difference with Eq. 10 becomes substantial.

The reconstructed growth rates $h^{\text{equi}}(s,x)$ can now be used to integrate Eq. 2. By construction, this model will tend to the same equilibrium as the full model (Eq. 9). To test how well it approximates the dynamics, we perturb the system out of equilibrium and look at the resulting dynamics. As shown in Figure 1, perturbations which disturb too heavily the floc-size distribution cannot be correctly modeled by an equation like Eq. 2. We therefore apply a perturbation in the dilution rate D, which acts similarly on the different floc densities u_n. Figure 3 shows that the reduced model predicts with rather good precision the reaction of the full system to this perturbation.

Conclusion

In this article, we investigated how flocculation influences the bacterial growth dynamics in a bioreactor. In the context of the activated sludge process, this coupling of physico-chemical and biological phenomena is mostly relevant for the reaction tank. In particular, we studied the possibility of an effective model on the level of the biomass density, without explicitly taking flocculation into account.

Such an effective description is only possible when the flocculation dynamics are sufficiently fast compared to the other processes. In this case, the specific growth rate, which for isolated bacteria depends only on the substrate density, gains an additional dependence on the biomass density. It is interesting to note that such a density-dependent growth rate has recently

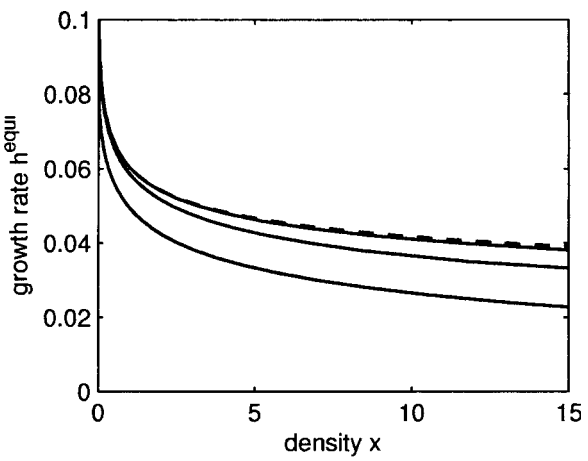

Figure 2. Specific growth rate at equilibrium.

The specific growth rate at equilibrium $h^{\text{equi}}(s,x)$ as a function of the biomass density x for a fixed substrate density $s = 6$. Parameter values of Table 1 were used. The curves in full line correspond to, from bottom to top, $\varepsilon = 1$, $\varepsilon = 0.1$ and $\varepsilon = 0.01$. The specific growth rate (Eq. 10) is shown in dashed line.

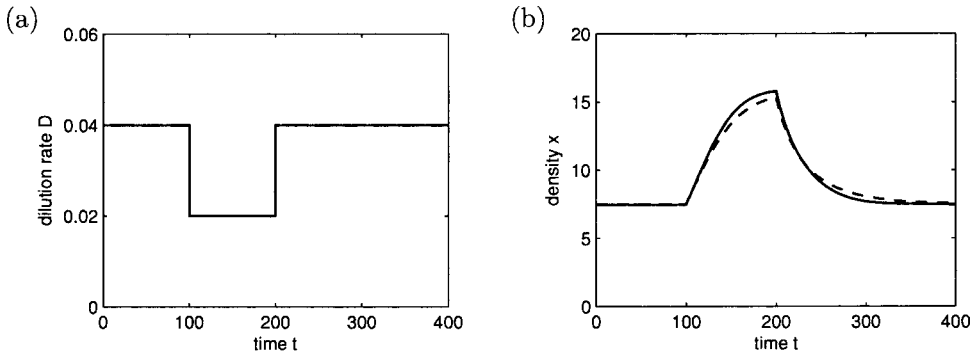

Figure 3. Comparing full and reduced dynamics for response to perturbation.

The full dynamics (Eq. 9) and the reduced dynamics (Eq. 2) are compared for a step in the dilution rate D. (a) Excitation in the dilution rate D, and (b) reaction of the two models. Parameter values of Tab. 1 were used. The full line corresponds to the system (Eq. 9) with $\varepsilon = 1$. The dashed line corresponds to the system (Eq. 2) with specific growth rate $h^{\text{equi}}(s,x)$. For both simulations, the initial condition was taken as the equilibrium for dilution rate $D = 0.04$.

been proposed as a mechanism to explain the coexistence of many bacterial species growing on a limited number of substrates. We will investigate the link between flocculation and species coexistence in a forthcoming contribution.

When the flocculation dynamics have timescales comparable to the bacterial growth, the details of the floc-size distribution do affect the global system dynamics. In that case, dynamics autonomous in the biomass density do not exist, and the notion of specific growth rate is ill-defined. However, if the reactor evolves such that the floc-size distribution remains equilibrated, it makes sense to define a specific growth rate at equilibrium. We showed in a simple example, that such a growth rate, which is again density-dependent, can yield an accurate description of the system dynamics.

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