



Free boundary problem for an initial cell layer in multispecies biofilm formation

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ABSTRACT

The initial attached cell layer in multispecies biofilm growth is considered. The corresponding mathematical model leads to discuss a free boundary problem for a system of nonlinear hyperbolic partial differential equations, where the initial biofilm thickness is equal to zero. No assumptions on initial conditions for biomass concentrations and biofilm thickness are required. The data that the problem needs are the concentration of biomass in the bulk liquid and biomass flux from the bulk liquid. The method of characteristics is used to convert the differential system to Volterra integral equations for which an existence and uniqueness theorem is proved. Subsequently, we show that the free boundary is an increasing function of time and biomass concentrations are positive in agreement with the biological process.

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1. Introduction

Jones [1], by using electron microscope, discovered in 1969 that a biofilm is characterized by several kinds of cells. Since then, the combination of high resolution three-dimensional imaging techniques, specific molecular fluorescent stains, molecular reporter technology, and biofilm-culturing apparatus have shown that biofilms are not simply a passive assemblage of cells that are stuck to surfaces, but are structurally and dynamically complex biological systems [2].

The start of the biofilm formation is characterized by the interaction between microbial cell and support. The first step is the adhesion to support or solid–liquid interface by planktonic bacteria, Fig. 1.1. The adhesion to support is a process depending on both probability that the bacteria get in contact with the support surface and the origin of an attractive force that allows the adhesion of bacteria. After the initial step of adhesion, the subsequent microcolonies develop in mature biofilms. This step is coupled with the production of extracellular polymeric substances.

Mathematical modelling of biofilm growth was extensively performed during the past decades. Essentially, two different classes of models have been developed: continuum models, e.g. among others [3–6], and differential-discrete models, e.g. [7,8]. In principles, methods of statistical mechanics can be used to derive macroscopic equations from the underlying description at the cellular scale [9].

Usually, an initial nonzero thickness in biofilm growth is assumed, and the formation of attached cell layer is neglected, Fig. 1.1(A) and (B). Nevertheless, this biological process can last several days or months, since it depends on many factors such as physical and chemical characteristics of substratum, nutrient concentration, hydrodynamic conditions and concentration of planktonic bacteria in the bulk. Therefore, the formation of attached cell layer is very important in environmental industrial application for wastewater treatment, in particular in the start-up of fixed-growth treatment reactors.

In this paper, we present a qualitative analysis of a mathematical model for the attached cell layer in multispecies biofilm formation. This biological process is described by a free boundary problem for nonlinear hyperbolic equations where the

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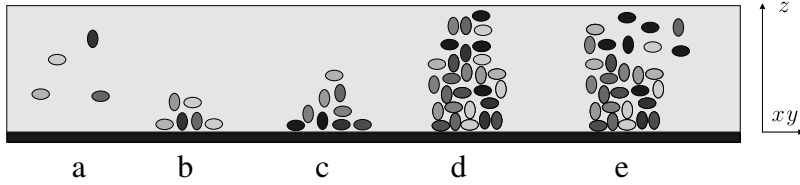


Fig. 1.1. Schematic biofilm formation. (A) Planktonic cells; (B) Attached cell layer; (C) Cell proliferation; (D) Mature biofilm; (E) Detachment.

initial biofilm thickness is zero. This problem is different from similar free boundary problems for biofilm growth, since the free boundary is a space-like line. Furthermore, no fictitious initial conditions for biomass concentrations and biofilm thickness are required. We only need to know the concentrations of biomass in the bulk liquid and the biomass flux from bulk liquid.

The objective of this paper is the qualitative analysis of solutions and of properties of the free boundary. The mathematical model is introduced in Section 2, where the complete free boundary problem is described. The differential equations are converted, in Section 3, into an equivalent system of Volterra integral equations. Subsequently, an existence and uniqueness theorem is proved by the classical fixed point theorem and suitable weighted norms. Section 4 is devoted to the analysis of the properties of solutions. It will be shown that the solutions are positive and the sum of fraction volumes is equal to 1. In addition, it is proved that the free boundary is an increasing function of time.

2. Mathematical modelling of an initial cell layer

Consider the initial phase in one-dimensional multispecies biofilm growth. Let $f_i(z, t)$ be the volume fraction of the microbial species i , $\sum_{i=1}^n f_i = 1$, ρ_i the constant density, $X_i = \rho_i f_i(z, t)$ the concentration of the microorganism i , $\mathbf{X} = (X_1, \dots, X_n)$, $r_{M,i}(z, t, \mathbf{X}_i)$ the specific growth rate, $u(z, t)$ the velocity of the microbial mass, $L(t)$ biofilm thickness. In addition, denote by $\sigma(t)$ the biomass flux from bulk liquid to biofilm. This is the most used convention. On the other hand, if an opposite definition is adopted, as in [6], $\sigma(t)$ must be replaced by $-\sigma(t)$ and represents the biomass flux from biofilm to bulk liquid.

The initial growth process for multispecies biofilms in one space dimension may be described by the following free boundary problem

$$\frac{\partial}{\partial t} X_i(z, t) + u(z, t) \frac{\partial}{\partial z} X_i(z, t) = \rho_i r_{M,i}(z, t, \mathbf{X}) - X_i(z, t) \frac{\partial}{\partial z} u(z, t), \quad (2.1)$$

$$\frac{\partial}{\partial z} u(z, t) = \sum_{i=1}^n r_{M,i}(z, t, \mathbf{X}), \quad 0 < z \leq L(t), \quad t > 0, \quad (2.2)$$

$$\dot{L}(t) = u(L(t), t) + \sigma(t), \quad t > 0, \quad (2.3)$$

where $i = 1, \dots, n$. Eqs. (2.1)–(2.3) are derived from the mass balance for the microbial species as in [3,4,6]. The following boundary conditions will be associated to system (2.1)–(2.3)

$$X_i(L(t), t) = \psi_i(t); \quad i = 1, 2, \dots, n; \quad u(0, t) = 0; \quad \sigma(t) \geq \sigma_L > 0, \quad L(0) = 0. \quad (2.4)$$

Conditions (2.4)₁ state that the biomass concentrations at the biofilm boundary are the same as the bulk liquid. Therefore, they are the boundary conditions in this specific problem, although they are often named initial conditions in a general mathematical context. Eq. (2.4)₂ is a no flux condition between substratum and biofilm.

The characteristics $z = z(t)$ of system (2.1) are defined by $\partial z / \partial t = u$. Since they also depend on the initiation point $(L(t_0), t_0)$, Fig. 2.1, we will use the notation $z = c(t_0, t)$. Thus, the characteristics are defined by the following initial value problem

$$\frac{\partial}{\partial t} c(t_0, t) = u(c(t_0, t), t), \quad c(t_0, t_0) = L(t_0). \quad (2.5)$$

Condition (2.4)₃ essentially means that the initial curve is not a characteristic. By using the notations

$$G(z, t, \mathbf{X}) = \sum_{i=1}^n r_{M,i}, \quad F_i(z, t, \mathbf{X}) = \rho_i r_{M,i} - X_i G, \quad (2.6)$$

system (2.1) is rewritten as follows:

$$\frac{d}{dt} X_i(c(t_0, t), t) = F_i(c(t_0, t), t, \mathbf{X}(c(t_0, t), t)), \quad 0 \leq t_0 \leq t \leq T, \quad (2.7)$$

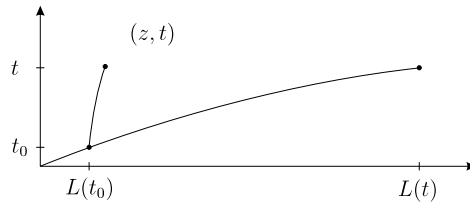


Fig. 2.1. Free boundary problem.

and after integrating over (t_0, t)

$$X_i(c(t_0, t), t) = \psi_i(t_0) + \int_{t_0}^t F_i(c(t_0, \tau), \tau, \mathbf{X}(c(t_0, \tau), \tau)) d\tau. \quad (2.8)$$

Let us now consider Eq. (2.2)

$$\frac{\partial}{\partial z} u(c(t_0, t), t) = G(c(t_0, t), t, \mathbf{X}(c(t_0, t), t)). \quad (2.9)$$

Hence,

$$\frac{\partial u}{\partial t_0}(c(t_0, t), t) = G(c(t_0, t), t, \mathbf{X}(c(t_0, t), t)) \frac{\partial c}{\partial t_0}(t_0, t),$$

and

$$\frac{\partial u}{\partial \tau}(c(\tau, t), t) = G(c(\tau, t), t, \mathbf{X}(c(\tau, t), t)) \frac{\partial c}{\partial \tau}(\tau, t), \quad 0 < \tau \leq t_0.$$

Integrating over $(0, t_0)$ yields

$$u(c(t_0, t), t) = \int_0^{t_0} G(c(\tau, t), t, \mathbf{X}(c(\tau, t), t)) \frac{\partial c}{\partial \tau}(\tau, t) d\tau, \quad (2.10)$$

since $u(c(0, t), t) = u(0, t) = 0$.

Consider Eq. (2.3)

$$\dot{L}(t_0) = u(L(t_0), t_0) + \sigma(t_0) = u(c(t_0, t_0), t_0) + \sigma(t_0), \quad (2.11)$$

and use (2.10)

$$\dot{L}(t_0) = \sigma(t_0) + \int_0^{t_0} G(c(\tau, t_0), t_0, \mathbf{X}(c(\tau, t_0), t_0)) \frac{\partial c}{\partial \tau}(\tau, t_0) d\tau. \quad (2.12)$$

Hence,

$$\dot{L}(\theta) = \sigma(\theta) + \int_0^\theta G(c(\tau, \theta), \theta, \mathbf{X}(c(\tau, \theta), \theta)) \frac{\partial c}{\partial \tau}(\tau, \theta) d\tau, \quad 0 < \theta \leq t_0,$$

and, after integration over $(0, t_0)$,

$$L(t_0) = \int_0^{t_0} \sigma(\theta) d\theta + \int_0^{t_0} d\theta \int_0^\theta G(c(\tau, \theta), \theta, \mathbf{X}(c(\tau, \theta), \theta)) \frac{\partial c}{\partial \tau}(\tau, \theta) d\tau. \quad (2.13)$$

Consider Eq. (2.5)

$$\frac{\partial}{\partial \theta} c(t_0, \theta) = u(c(t_0, \theta), \theta), \quad c(t_0, t_0) = L(t_0), \quad t_0 < \theta \leq t.$$

Integrate over (t_0, t)

$$c(t_0, t) = L(t_0) + \int_{t_0}^t u(c(t_0, \theta), \theta) d\theta,$$

and use (2.10)

$$c(t_0, t) = L(t_0) + \int_{t_0}^t d\theta \int_0^{t_0} G(c(\tau, \theta), \theta, \mathbf{X}(c(\tau, \theta), \theta)) \frac{\partial c}{\partial \tau}(\tau, \theta) d\tau. \quad (2.14)$$

Differentiation of (2.14) with respect to t_0 and some simple manipulations yield

$$\frac{\partial}{\partial t_0} c(t_0, t) = \sigma(t_0) + \int_{t_0}^t G(c(t_0, \theta), \theta, \mathbf{X}(c(t_0, \theta), \theta)) \frac{\partial c}{\partial t_0}(t_0, \theta) d\theta. \tag{2.15}$$

The differential system (2.5), (2.7) and (2.11) is equivalent to the integral system (2.8) and (2.13)–(2.15). Indeed, if a solution exists for the integral system, this can be differentiated and the differential system is recovered.

In the most general biological process the function $r_{M,i}$ can also depend on substrates. This dependence has been omitted here, since inessential in the mathematical problem discussed in this work.

3. Volterra system

Consider the following positions

$$x_i(t_0, t) = X_i(c(t_0, t), t), \quad \mathbf{X}(x_1, \dots, x_n), \tag{3.1}$$

$$\Phi_i(\mathbf{X}(t_0, t), c(t_0, t), t) = F_i(c(t_0, t), t, \mathbf{X}(t_0, t)), \quad i = 1, \dots, n. \tag{3.2}$$

$$\Phi_{n+1}(\mathbf{X}(t_0, t), c(t_0, t), c_{t_0}(t_0, t), t) = G(c(t_0, t), t, \mathbf{X}(t_0, t))c_{t_0}(t_0, t), \tag{3.3}$$

$$\Phi_{n+2} = \Phi_{n+1}. \tag{3.4}$$

By using definitions (3.1)–(3.4), Eqs. (2.8) and (2.13)–(2.15) are rewritten as

$$x_i(t_0, t) = \psi_i(t_0) + \int_{t_0}^t \Phi_i(\mathbf{X}(t_0, \tau), c(t_0, \tau), \tau) d\tau, \tag{3.5}$$

$$c(t_0, t) = \int_0^{t_0} \sigma(\theta) d\theta + \int_0^{t_0} d\theta \int_0^\theta \Phi_{n+1}(\mathbf{X}(\tau, \theta), c(\tau, \theta), c_\tau(\tau, \theta), \theta) d\tau + \int_{t_0}^t d\theta \int_0^{t_0} \Phi_{n+1}(\mathbf{X}(\tau, \theta), c(\tau, \theta), c_\tau(\tau, \theta), \theta) d\tau, \tag{3.6}$$

$$c_{t_0}(t_0, t) = \sigma(t_0) + \int_{t_0}^t \Phi_{n+2}(\mathbf{X}(t_0, \theta), c(t_0, \theta), c_{t_0}(t_0, \theta), \theta) d\theta, \tag{3.7}$$

$$L(t_0) = \int_0^{t_0} d\theta \int_0^\theta G(c(\tau, \theta), \theta, \mathbf{X}(\tau, \theta))c_\tau(\tau, \theta) d\tau + \int_0^{t_0} \sigma(\theta) d\theta, \tag{3.8}$$

where $i = 1, \dots, n$ and $0 \leq t_0 \leq t \leq T$.

Note that Eq. (3.8) is separated from system (3.5)–(3.7). Thus, this system is solved firstly. Then, the solution is used in Eq. (3.8) to find L . The following theorem holds for system (3.5)–(3.7).

Theorem 1. Assume $\sigma, \psi_i, i = 1, \dots, n$, continuous and Φ_j Lipschitz continuous

$$\sigma, \psi_i \in C([0, T]), \quad i = 1, \dots, n, \tag{3.9}$$

$$|\Phi_i(\mathbf{X}, c, t) - \Phi_i(\tilde{\mathbf{X}}, \tilde{c}, t)| \leq L_i \left[\sum_{h=1}^n |x_h - \tilde{x}_h| + |c - \tilde{c}| \right], \quad i = 1, \dots, n, \tag{3.10}$$

$$|\Phi_i(\mathbf{X}, c, c_{t_0}, t) - \Phi_i(\tilde{\mathbf{X}}, \tilde{c}, \tilde{c}_{t_0}, t)| \leq L_i \left[\sum_{h=1}^n |x_h - \tilde{x}_h| + |c - \tilde{c}| + |c_{t_0} - \tilde{c}_{t_0}| \right], \quad i = n + 1, n + 2. \tag{3.11}$$

Then, there exists a unique continuous solution $x_i, c, c_{t_0} \in C(I)$ to Volterra system (3.5)–(3.7), where $I = \{(t_0, t) : 0 \leq t_0 \leq t \leq T\}, T > 0$.

Proof. Consider the vector space S of the continuous functions $x_i, c, c_{t_0} \in C(I)$ with norm

$$\|(\mathbf{x}, c, c_{t_0})\| = \sum_{i=1}^n \max_t e^{-\gamma_1 t_0 - \gamma_2 t} |x_i(t_0, t)| + \max_t e^{-\gamma_1 t_0 - \gamma_2 t} |c(t_0, t)| + \max_t e^{-\gamma_1 t_0 - \gamma_2 t} |c_{t_0}(t_0, t)|,$$

where γ_1 and γ_2 are positive constants. Let $(\mathbf{y}, C, C_{t_0}) = A(\mathbf{x}, c, c_{t_0})$ be the map defined by the equations below

$$y_i(t_0, t) = \psi_i(t_0) + \int_{t_0}^t \Phi_i(\mathbf{X}(t_0, \tau), c(t_0, \tau), \tau) d\tau, \tag{3.12}$$

$$C(t_0, t) = \int_0^{t_0} \sigma(\theta) d\theta + \int_0^{t_0} d\theta \int_0^\theta \Phi_{n+1}(\mathbf{x}(\tau, \theta), c(\tau, \theta), c_\tau(\tau, \theta), \theta) d\tau + \int_{t_0}^t d\theta \int_0^{t_0} \Phi_{n+1}(\mathbf{x}(\tau, \theta), c(\tau, \theta), c_\tau(\tau, \theta), \theta) d\tau, \tag{3.13}$$

$$C_{t_0}(t_0, t) = \sigma(t_0) + \int_{t_0}^t \Phi_{n+2}(\mathbf{x}(t_0, \theta), c(t_0, \theta), c_{t_0}(t_0, \theta), \theta) d\theta, \tag{3.14}$$

where $i = 1, \dots, n$ and $0 \leq t_0 \leq t \leq T$. Denote by $(\tilde{\mathbf{y}}, \tilde{C}, \tilde{C}_{t_0}) = A(\tilde{\mathbf{x}}, \tilde{c}, \tilde{c}_{t_0})$ and consider Eq. (3.12)

$$|y_i(t_0, t) - \tilde{y}_i(t_0, t)| e^{-\gamma_1 t_0 - \gamma_2 t} \leq L_i \int_{t_0}^t \left[\sum_{h=1}^n |x_h(t_0, \tau) - \tilde{x}_h(t_0, \tau)| + |c(t_0, \tau) - \tilde{c}(t_0, \tau)| \right] e^{-\gamma_1 t_0 - \gamma_2 \tau} e^{-\gamma_2(t-\tau)} d\tau.$$

Hence,

$$|y_i(t_0, t) - \tilde{y}_i(t_0, t)| e^{-\gamma_1 t_0 - \gamma_2 t} \leq L_i \|(\mathbf{x}, c, c_{t_0}) - (\tilde{\mathbf{x}}, \tilde{c}, \tilde{c}_{t_0})\| / \gamma_2, \tag{3.15}$$

$i = 1, \dots, n$. Consider Eq. (3.13)

$$|C(t_0, t) - \tilde{C}(t_0, t)| e^{-\gamma_1 t_0 - \gamma_2 t} \leq L_{n+1} \int_0^{t_0} d\theta \int_0^\theta \left[\sum_{h=1}^n |x_h(\tau, \theta) - \tilde{x}_h(\tau, \theta)| + |c(\tau, \theta) - \tilde{c}(\tau, \theta)| + |c_\tau(\tau, \theta) - \tilde{c}_\tau(\tau, \theta)| \right] e^{-\gamma_1 \tau - \gamma_2 \theta} e^{-\gamma_1(t_0-\tau)} e^{-\gamma_2(t-\theta)} d\tau + L_{n+1} \int_{t_0}^t d\theta \int_0^{t_0} \left[\sum_{h=1}^n |x_h(\tau, \theta) - \tilde{x}_h(\tau, \theta)| + |c(\tau, \theta) - \tilde{c}(\tau, \theta)| + |c_\tau(\tau, \theta) - \tilde{c}_\tau(\tau, \theta)| \right] e^{-\gamma_1 \tau - \gamma_2 \theta} e^{-\gamma_1(t_0-\tau)} e^{-\gamma_2(t-\theta)} d\tau.$$

Hence,

$$|C(t_0, t) - \tilde{C}(t_0, t)| e^{-\gamma_1 t_0 - \gamma_2 t} \leq 2L_{n+1} \|(\mathbf{x}, c, c_{t_0}) - (\tilde{\mathbf{x}}, \tilde{c}, \tilde{c}_{t_0})\| / (\gamma_1 \gamma_2). \tag{3.16}$$

Consider Eq. (3.14)

$$|C_{t_0}(t_0, t) - \tilde{C}_{t_0}(t_0, t)| e^{-\gamma_1 t_0 - \gamma_2 t} \leq L_{n+2} \int_0^{t_0} \left[\sum_{h=1}^n |x_h(t_0, \theta) - \tilde{x}_h(t_0, \theta)| + |c(t_0, \theta) - \tilde{c}(t_0, \theta)| + |c_{t_0}(t_0, \theta) - \tilde{c}_{t_0}(t_0, \theta)| \right] e^{-\gamma_1 t_0 - \gamma_2 \theta} e^{-\gamma_2(t-\theta)} d\theta.$$

Hence,

$$|C_{t_0}(t_0, t) - \tilde{C}_{t_0}(t_0, t)| e^{-\gamma_1 t_0 - \gamma_2 t} \leq L_{n+2} \|(\mathbf{x}, c, c_{t_0}) - (\tilde{\mathbf{x}}, \tilde{c}, \tilde{c}_{t_0})\| / \gamma_2. \tag{3.17}$$

From (3.15)–(3.17) it follows that

$$\|(\mathbf{y}, C, C_{t_0}) - (\tilde{\mathbf{y}}, \tilde{C}, \tilde{C}_{t_0})\| \leq p \|(\mathbf{x}, c, c_{t_0}) - (\tilde{\mathbf{x}}, \tilde{c}, \tilde{c}_{t_0})\|,$$

where

$$p = \frac{1}{\gamma_2} \sum_{i=1}^n L_i + \frac{2}{\gamma_1 \gamma_2} L_{n+1} + \frac{1}{\gamma_2} L_{n+2}.$$

If the positive constants γ_1, γ_2 are chosen large enough, then $p < 1$ and A is a contractive map. So, the theorem is proved. \square

Corollary 1. Under the same hypotheses as Theorem 1 the function $L \in C([0, T])$.

Proof. See Eq. (3.8). \square

4. Solution to free boundary problem

The integral system in Section 3 provides the solution for the biomass concentrations in the form

$$X_i = X_i(c(t_0, t), t), \quad i = 1, \dots, n, \tag{4.1}$$

whereas the original problem requires the solution in the form

$$X_i = X_i(z, t), \quad i = 1, \dots, n. \tag{4.2}$$

Since

$$z = c(t_0, t), \tag{4.3}$$

we immediately realize that a problem of inversion of the function c with respect to t_0 arises. This issue is analysed in the following:

Theorem 2. Under the same hypotheses as Theorem 1, if

$$\sigma(t_0) \geq \sigma_L > 0, \quad G(c(t_0, t), t, \mathbf{x}(c(t_0, t), t)) \geq 0, \quad 0 \leq t_0 \leq t \leq T, \tag{4.4}$$

then

$$c(t_0, t) > 0, \quad c_{t_0}(t_0, t) > 0, \quad 0 \leq t_0 \leq t \leq T. \tag{4.5}$$

In addition,

$$L(t_0) > 0, \quad 0 < t_0 \leq t \leq T, \quad \dot{L}(t_0) > 0, \quad 0 \leq t_0 \leq t \leq T. \tag{4.6}$$

Proof. Inequalities (4.5) follow from the application of Picard's process of successive approximations to integral equations (3.6)–(3.7). Estimates (4.6) are easily derived from (2.12) and (3.8).

Now, the function c can be inverted and the function (4.2) is obtained. \square

Theorem 3. Under the same hypotheses as Theorem 1, if

$$\psi_i(t_0) \geq 0, \quad F_i(c(t_0, t), t, \mathbf{x}(c(t_0, t), t)) \geq 0, \quad 0 \leq t_0 \leq t \leq T, \tag{4.7}$$

$i = 1, \dots, n$, then

$$x_i(t_0, t) \geq 0, \quad 0 \leq t_0 \leq t \leq T, \quad i = 1, \dots, n. \tag{4.8}$$

Proof. Inequality (4.7) follows from the application of Picard's process of successive approximations to integral equations (3.5). \square

Consider integral equation (2.8) rewritten in terms of fraction volumes $f_i = X_i/\rho_i$

$$f_i(c(t_0, t), t) = \psi_i(t_0)/\rho_i + \int_{t_0}^t (r_{M,i} - f_i G) d\tau, \quad i = 1, \dots, n. \tag{4.9}$$

Since $\psi_i(t_0)/\rho_i, i = 1, \dots, n$, represent the initial fraction volumes, we must assume

$$\sum_{i=1}^n \psi_i(t_0)/\rho_i = 1. \tag{4.10}$$

Now, we expect that the same condition is satisfied by the sum of functions f_i at any time

$$f(c(t_0, t), t) = \sum_{i=1}^n f_i = 1 \quad \forall t. \tag{4.11}$$

This result will be proved in Theorem 4.

Theorem 4. Under the same hypotheses as Theorem 1, if hypothesis (4.10) holds, then condition (4.11) is satisfied.

Proof. Summing (4.9) on i and using (4.10) yields

$$f(c(t_0, t), t) = 1 + \int_{t_0}^t G(1 - f) d\tau, \quad 0 \leq t_0 \leq t \leq T, \tag{4.12}$$

with initial condition

$$f(c(t_0, t_0), t_0) = 1. \tag{4.13}$$

The initial value problem (4.12)–(4.13) has the unique solution $f = 1$. \square

5. Conclusions

A free boundary problem for the attached cell layer in multispecies biofilm formation has been studied in this paper. Assumptions on fictitious initial biomass concentrations and biofilm thickness are not required. The main result is an existence and uniqueness theorem for the biomass concentrations $X_i = X_i(z, t)$, $i = 1, \dots, n$, and thickness $L = L(t)$ in the space of continuous functions for $0 < t \leq T$, $\forall T > 0$. The functions X_i and L can be differentiated to satisfy the original differential problem. We proved that the concentrations are positive ($X_i \geq 0$) and the fraction volumes $f_i = X_i/\rho_i$ satisfy the relationship $\sum_{i=1}^n f_i = 1$. Furthermore, it was proved that L is an increasing function of time ($\dot{L} > 0$).

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