

Analysis and simulations of the initial phase in multispecies biofilm formation

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Abstract

The work presents a mathematical modelling approach to study dynamic competition during the attachment phenomena in the initial phase of biofilm growth. Biofilm development is described by a set nonlinear hyperbolic partial differential equations. Diffusion of substrates through biofilm is modeled by a set of semilinear parabolic partial differential equations. The two sets of equations are mutually connected. The resulting mathematical problem is a free boundary value problem, which is essentially hyperbolic. A characteristic-like method is introduced to convert differential equations to integral equations. Fixed-point theorem is used to obtain existence, uniqueness and properties of solutions. The model has been applied to the biological competition of heterotrophic-autotrophic bacteria in a multi-specie biofilm. The effects of different attachment rates on the biofilm dynamic performances predicting biofilm thickness, volume fractions of bacterial species and substrate concentration trends have been investigated. The simulations show that the different attachment rates influence biofilm thickness, of course. However, the volume fractions of bacterial species mainly depend on biofilm internal dynamics and substrate concentration trends. The bulk concentrations of microbial species play a relative important role only in the outermost layers of biofilm.

Keywords: biofilm, nonlinear hyperbolic and parabolic partial differen-

tial equations, free boundary problems, mathematical modelling.

AMS subject classification: 92Bxx, 35Lxx, 35Kxx, 35R35, 93A30.

1. Introduction.

Biofilms are commonly defined layer like aggregations of microorganisms and are involved in a variety of scenarios including pollution, corrosion, biofouling, biomedical applications, bacterial growth in water distribution systems, attached growth systems for wastewater treatment. Biofilm metabolism is characterized by some inherent features that provide several advantages and some challenges for applications. In effect, a single biofilm can exhibit varying environmental and kinetic characteristics, that is, it can include a variety of microbial groups contributing to the conversion of different organic and inorganic substrates; biofilm cells are at least 500 times more resistant to antimicrobial agents [1] and benefit from interspecies cooperation.

Biofilm development is determined by “positive” processes, like cell attachment, cell division, and polymer production, which leads to biofilm volume expansion, and “negative” processes, like cell detachment and cell death, which contribute to biofilm shrinking. The main biofilm expansion is due to bacterial growth and extracellular polymer production. The nutrients necessary for biofilm growth are dissolved in the bulk liquid and are transported by molecular diffusion first through the boundary layer, where the external mass transfer resistance is concentrated, and then through the biofilm matrix. The external fluid flow regulates biofilm growth by establishing the concentration of substrates and products at the liquid-solid interface. At the same time the fluid flow shears the biofilm surface, eroding the protuberances. So biofilm structure results from the interplay of different interactions, such as mass transfer, conversion rates and detachment forces. An accurate modeling of such a system have to take into account all of these factors, since these factors strongly affect the overall performance of biofilm-based systems [2].

The transport of microorganisms to and from the biofilm (attachment and detachment) is particularly important since it defines the microbial ecology of the biofilm and plays a crucial role in the start up of biofilm reactors. In particular, biofilm formation occurs when specified bacterial species, able to make the first colonization of substratum surface, attach to the surface and start producing an extrapolymeric matrix that will allow the attachment of other microbial species [3]. This bacterial adhesion can be performed only by few microbial species and it is especially rapid and specific if the surface in question is itself a nutrient. After the first

colonization, a thin layer of biofilm constitutes and the flux of micro organisms from bulk liquid to biofilm continues playing a crucial role in both biofilm development and in competition of microbial species for substrates and place.

The attachment process is influenced by both physical and biological factors, such as bulk liquid characteristics in terms of kind and concentration of microbial species, flow velocity and turbulence, geometry of the substratum. Therefore, the attachment flux can assume different values determining different biofilm development pathways. At the same time the growth of the existing thin layer of biofilm is influenced by substrate concentrations and coupled with attachment flux determines the overall biomass volume increase.

Mathematical modelling of biofilm attachment process represents a useful tool to evaluate this phenomenon and to predict the structural biofilm development. Many of biofilm models developed during the last decades are able to reproduce the complex interactions existing between the main processes, including cell attachment, involved in the formation of biofilm structure. Biofilm models have undergone a temporary evolution and mostly differ on the way biomass spreading is treated. Continuum models [4–8] consider biomass as an unicuum and are based on conservation principles. These studies are mostly centered on the biofilm growth dynamics including the biofilm thickness and spatial distribution of microbial species and substrate concentration. These continuum models can be related to the underlying description offered by models at the microscopic scale as documented in [9]. Later, discrete models have been developed to reproduce biofilm spatial heterogeneities by using simple rules [10,11]. These models can capture the various biofilm growth patterns observed in experiments and strongly suggest that the biofilm structure is largely determined by the surrounding substrate concentration. In this work a mathematical model based on a continuum approach and able to describe the attachment process during biofilm growth is presented. In particular, the objectives of this study include:

- to propose a mathematical modelling approach to study population dynamics competition during the attachment phenomena in the initial phase of biofilm growth;
- to provide a qualitative analysis to the solutions of the corresponding free boundary value problem;
- to develop numerical simulations to illustrate the model.

2. Initial phase of biofilm formation.

Consider a multispecies biofilm formed by n microbial species. 1D model of biofilm growth, based on the continuum description, is governed by the following equations, [4–8],

$$(1) \quad \frac{\partial X_i}{\partial t} + \frac{\partial}{\partial z}(uX_i) = \rho_i r_{M,i}(z, t, \mathbf{X}, \mathbf{S}), \quad i = 1, \dots, n, \quad 0 \leq z \leq L(t), \quad t > 0,$$

$$(2) \quad \frac{\partial u}{\partial z} = \sum_{i=1}^n r_{M,i} = G(z, t, \mathbf{X}, \mathbf{S}), \quad 0 < z \leq L(t), \quad t > 0,$$

$$(3) \quad \dot{L}(t) = u(L(t), t) + \sigma(t), \quad t > 0,$$

$$(4) \quad \frac{\partial S_j}{\partial t} - \frac{\partial}{\partial z}(D_j \frac{\partial S_j}{\partial z}) = r_{S,j}(z, t, \mathbf{X}, \mathbf{S}), \quad 0 < z < L(t), \quad t > 0, \quad j = 1, \dots, m,$$

where:

- z is the biofilm growth direction, assumed perpendicular to substratum;
- $f_i(z, t)$ is the volume fraction of microbial species i , $\sum_{i=1}^n f_i = 1$;
- ρ_i denotes constant density;
- $X_i(z, t) = \rho_i f_i$ denotes the concentration of microorganism i , $\mathbf{X} = (X_1, \dots, X_n)$;
- $u(z, t)$ is the velocity of the microbial mass;
- $S_j(z, t)$ denotes the concentration of substrate j , $j = 1, \dots, m$, $\mathbf{S} = (S_1, \dots, S_m)$;
- $r_{M,i}(z, t, \mathbf{X}, \mathbf{S})$ is the specific growth rate;
- $L(t)$ denotes biofilm thickness;
- $\sigma(t)$ is the biomass flux from bulk liquid to biofilm;
- D_j denotes the diffusivity coefficient of substrate j ;
- $r_{S,j}(z, t, \mathbf{X}, \mathbf{S})$ is the conversion rate of substrate j .

2.1. Free boundary value problem.

As outlined in Section 1, we want to discuss the free boundary value problem for the initial phase of biofilm development. We consider the situation where a thin layer of biofilm has been already formed. This leads to assume a strictly positive initial thickness for the biofilm: $L(0) > 0$.

The case where $L(0) = 0$ was discussed in [12]. In addition, it is assumed that there is no biomass flux at the support and this implies $u(0, t) = 0$. Therefore, the following initial conditions will be associated to equations (1)-(3)

$$(5) \quad X_i(z, 0) = \varphi_i(z), \quad i = 1, \dots, n, \quad 0 \leq z \leq L(t),$$

$$(6) \quad u(0, t) = 0, \quad t > 0,$$

$$(7) \quad L(0) = L_0 > 0,$$

where L_0 denotes the initial thickness of biofilm and $\varphi_i(z)$, $i = 1, \dots, n$, the initial concentrations of microbial species.

The initial phase of biofilm growth is strongly influenced by the attachment. This process can be defined as the immobilization of cells suspended in the bulk liquid to biofilm or substratum. Mathematically, the attachment is modelled as a flux, usually denoted by σ . Moreover, the biofilm development in this phase also depends on substrate availability, since a thin layer of biofilm has been already constituted. For mature biofilms, the most significant biomass flux occurs from biofilm to bulk liquid. This biological process, known as detachment, mostly depends on hydrodynamic conditions and biofilm thickness. However, in the initial phase, attachment is the prevailing process. So, σ is assumed to be a strictly positive function of time in this work. The attachment affects microbial species distribution into biofilms and can even introduce new species existing in the bulk liquid in the already developed biofilm structure. So, the following boundary condition is needed, [6],

$$(8) \quad X_i(L(t), t) = \psi_i(t), \quad i = 1, \dots, n, \quad t > 0,$$

where $\psi_i(t)$ denotes the concentration of the microbial species i in the bulk liquid.

Finally, we introduce the initial-boundary conditions for equations (4). The initial conditions are quite general

$$(9) \quad S_j(z, 0) = S_{j0}(z), \quad 0 \leq z \leq L_0, \quad j = 1, \dots, m.$$

Moreover, no flux boundary conditions are assumed at the biofilm support and prescribed boundary condition on the free boundary

$$(10) \quad \frac{\partial S_j}{\partial z}(0, t) = 0, \quad S_j(L(t), t) = S_{jL}(t), \quad t > 0, \quad j = 1, \dots, m,$$

$$(11) \quad S_{j0}(L_0) = S_{jL}(0), \quad S_{j0}(0) = 0.$$

The value assumed by substrate concentrations at biofilm/bulk-liquid interface take into account the effect of mass-transport resistance. The second equation in (10) determines the substrate concentration trends into biofilm and reflect the bulk liquid substrate availability. Microbial growth rate depends on substrate concentration so the boundary conditions strongly affect bacterial species distribution, determining the predominance of some bacterial species over others.

The free boundary problem considered in this work is summarized by equations (1)-(4) with initial-boundary conditions (5)-(11).

3. Characteristic-like method.

When $0 \leq z_0 \leq L_0$, we consider the characteristic-like lines for system (1), Figure 1,

$$(12) \quad z = z(z_0, t), \quad 0 \leq z_0 \leq L_0, \quad t > 0,$$

defined by

$$(13) \quad \frac{\partial z}{\partial t}(z_0, t) = u(z(z_0, t), t), \quad z(z_0, 0) = z_0, \quad 0 \leq z_0 \leq L_0, \quad t > 0.$$

When $z_0 = L(t_0) (> L_0)$, $0 < t_0 \leq t$, we consider the characteristic-like lines, Figure 1,

$$(14) \quad z = z(L(t_0), t) = c(t_0, t), \quad 0 < t_0 \leq t, \quad t > 0,$$

defined by

$$(15) \quad \frac{\partial c}{\partial t}(t_0, t) = u(c(t_0, t), t), \quad c(t_0, t_0) = L(t_0), \quad 0 < t_0 \leq t, \quad t > 0.$$

Using (13) in (1) gives the following system of nonlinear differential equations

$$(16) \quad \frac{d}{dt} X_i(z(z_0, t), t) = F_i(z(z_0, t), t, \mathbf{X}, \mathbf{S}), \quad i = 1, \dots, n, \quad 0 \leq z_0 \leq L_0, \quad t > 0,$$

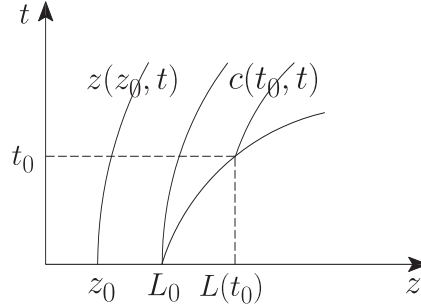


Figure 1. Characteristic-like lines.

with initial conditions

$$(17) \quad X_i(z(z_0, 0), 0) = X_i(z_0, 0) = \varphi_i(z_0), \quad 0 \leq z_0 \leq L_0, \quad i = 1, \dots, n,$$

where

$$(18) \quad F_i = \rho_i r_{M,i} - X_i \sum_{h=1}^n r_{M,h} = F_i(z, t, \mathbf{X}, \mathbf{S}), \quad i = 1, \dots, n.$$

Using (15) in (1) gives the following system of nonlinear differential equations

$$(19) \quad \frac{d}{dt} X_i(c(t_0, t), t) = F_i(c(t_0, t), t, \mathbf{X}, \mathbf{S}), \quad i = 1, \dots, n, \quad 0 < t_0 \leq t, \quad t > 0,$$

with initial conditions

$$(20) \quad X_i(c(t_0, t_0), 0) = X_i(L(t_0), t_0) = \psi_i(t_0), \quad 0 < t_0 \leq t, \quad t > 0, \quad i = 1, \dots, n.$$

Differential system (16)-(17) is equivalent to the integral system

$$(21) \quad \begin{cases} X_i(z(z_0, t), t) = \varphi_i(z_0) + \int_0^t F_i(z(z_0, \tau), \tau, \mathbf{X}(z(z_0, \tau), \tau), \mathbf{S}(z(z_0, \tau), \tau))) d\tau, \\ i = 1, \dots, n, \quad 0 \leq z_0 \leq L_0, \quad t > 0, \end{cases}$$

which incorporates the initial conditions (17).

Differential system (19)-(20) is equivalent to the integral system

$$(22) \quad \begin{cases} X_i(c(t_0, t), t) = \psi_i(t_0) + \int_{t_0}^t F_i(c(t_0, \tau), \tau, \mathbf{X}(c(t_0, \tau), \tau), \mathbf{S}(c(t_0, \tau), \tau))) d\tau, \\ i = 1, \dots, n, \quad 0 < t_0 \leq t, \quad t > 0, \end{cases}$$

which incorporates the initial conditions (20).

Solving systems (21) and (22) gives X_i along the lines (12) and (14), respectively. Therefore, the complete resolution requires the knowledge of those lines. Now, the integral equations for lines (12) and (14) are derived. From (13)

$$(23) \quad z(z_0, t) = z_0 + \int_0^t u(z(z_0, \tau), \tau) d\tau, \quad 0 \leq z_0 \leq L_0, \quad t > 0,$$

From (2)

$$(24) \quad u(z(z_0, t), t) = \int_0^{z(z_0, t)} G(\zeta, t, \mathbf{X}(\zeta, t), \mathbf{S}(\zeta, t)) d\zeta,$$

where equation (6) has been employed. By considering the change of variable $\zeta = z(\zeta_0, t)$, equation (24) becomes

$$(25) \quad u(z(z_0, t), t) = \int_0^{z_0} G(z(\zeta_0, t), t, \mathbf{x}(\zeta_0, t), \mathbf{s}(\zeta_0, t)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, t) d\zeta_0,$$

where the following notations have been used

$$(26) \quad \mathbf{x}(\zeta_0, t) = \mathbf{X}(z(\zeta_0, t), t), \quad \mathbf{s}(\zeta_0, t) = \mathbf{S}(z(\zeta_0, t), t).$$

Inserting equation (25) into (23) gives the integral equation for $z(z_0, t)$

$$(27) \quad \begin{cases} z(z_0, t) = z_0 + \int_0^t d\tau \int_0^{z_0} G(z(\zeta_0, \tau), \tau, \mathbf{x}(\zeta_0, \tau), \mathbf{s}(\zeta_0, \tau)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \tau) d\zeta_0, \\ 0 \leq z_0 \leq L_0, \quad t > 0, \end{cases}$$

which incorporates the initial condition $z(z_0, 0) = z_0$. Moreover, since $\partial z / \partial z_0$ is involved, we also need

$$(28) \quad \frac{\partial z}{\partial z_0}(z_0, t) = 1 + \int_0^t G(z(z_0, \tau), \tau, \mathbf{x}(z_0, \tau), \mathbf{s}(z_0, \tau)) \frac{\partial z}{\partial z_0}(z_0, \tau) d\tau,$$

which follows easily from (27).

Consider the characteristic-like lines when $z_0 > L_0$. From (15)

$$(29) \quad c(t_0, t) = L(t_0) + \int_{t_0}^t u(c(t_0, \tau), \tau) d\tau, \quad 0 < t_0 \leq t, \quad t > 0.$$

From (2)

$$(30) \quad u(c(t_0, t), t) = \int_0^{z(L_0, t)} G(\zeta, t, \mathbf{X}(\zeta, t), \mathbf{S}(\zeta, t)) d\zeta$$

$$+ \int_{z(L_0, t)}^{c(t_0, t)} G(\zeta, t, \mathbf{X}(\zeta, t), \mathbf{S}(\zeta, t)) d\zeta,$$

By considering the change of variable $\zeta = z(\zeta_0, t)$ in the first integral and $\zeta = c(\tau_0, t)$ in the second one

$$(31) \quad u(c(t_0, t), t) = \int_0^{L_0} G(z(\zeta_0, t), t, \mathbf{x}(\zeta_0, t), \mathbf{s}(\zeta_0, t)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, t) d\zeta_0 \\ + \int_0^{t_0} G(c(\tau_0, t), t, \mathbf{x}(\tau_0, t), \mathbf{s}(\tau_0, t)) \frac{\partial c}{\partial \tau_0}(\tau_0, t) d\tau_0,$$

where notations (26) have been used, and in addition

$$(32) \quad \mathbf{x}(\tau_0, t) = \mathbf{X}(c(\tau_0, t), t), \quad \mathbf{s}(\tau_0, t) = \mathbf{S}(c(\tau_0, t), t).$$

Inserting equation (31) into (29) gives the integral equation for $c(t_0, t)$

$$(33) \quad c(t_0, t) = L(t_0) + \int_{t_0}^t d\tau \int_0^{L_0} G(z(\zeta_0, \tau), \tau, \mathbf{x}(\zeta_0, \tau), \mathbf{s}(\zeta_0, \tau)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \tau) d\zeta_0 \\ + \int_{t_0}^t d\tau \int_0^{t_0} G(c(\tau_0, \tau), \tau, \mathbf{x}(\tau_0, \tau), \mathbf{s}(\tau_0, \tau)) \frac{\partial c}{\partial \tau_0}(\tau_0, \tau) d\tau_0, \quad 0 < t_0 \leq t, \quad t > 0,$$

which incorporates the initial condition $c(t_0, t_0) = L(t_0)$. Another version of this equation will be provided in Section 4, where also $\partial c / \partial t_0$ will be derived.

Finally, by using notations (26) and (32), equations (21) and (22) are rewritten as

$$(34) \quad \begin{cases} x_i(z_0, t) = \varphi_i(z_0) + \int_0^t F_i(z(z_0, \tau), \tau, \mathbf{x}(z_0, \tau), \mathbf{s}(z_0, \tau)) d\tau, \\ i = 1, \dots, n, \quad 0 \leq z_0 \leq L_0, \quad t > 0, \end{cases}$$

$$(35) \quad \begin{cases} x_i(t_0, t) = \psi_i(t_0) + \int_{t_0}^t F_i(c(t_0, \tau), \tau, \mathbf{x}(t_0, \tau), \mathbf{s}(t_0, \tau)) d\tau, \\ i = 1, \dots, n, \quad 0 < t_0 \leq t, \quad t > 0. \end{cases}$$

4. Free boundary.

Consider free boundary equation (3), rewritten as

$$(36) \quad \dot{L}(t_0) = u(L(t_0), t_0) + \sigma(t_0) = u(c(t_0, t_0), t_0) + \sigma(t_0).$$

Use equation (31)

$$(37) \quad \dot{L}(t_0) = \sigma(t_0) + \int_0^{L_0} G(z(\zeta_0, t_0), t_0, \mathbf{x}(\zeta_0, t_0), \mathbf{s}(\zeta_0, t_0)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, t_0) d\zeta_0 \\ + \int_0^{t_0} G(c(\tau_0, t_0), t_0, \mathbf{x}(\tau_0, t_0), \mathbf{s}(\tau_0, t_0)) \frac{\partial c}{\partial \tau_0}(\tau_0, t_0) d\tau_0.$$

Hence,

$$(38) \quad L(t_0) = L_0 + \int_0^{t_0} \sigma(\theta) d\theta \\ + \int_0^{t_0} d\theta \int_0^{L_0} G(z(\zeta_0, \theta), \theta, \mathbf{x}(\zeta_0, \theta), \mathbf{s}(\zeta_0, \theta)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \theta) d\zeta_0 \\ + \int_0^{t_0} d\theta \int_0^\theta G(c(\tau_0, \theta), \theta, \mathbf{x}(\tau_0, \theta), \mathbf{s}(\tau_0, \theta)) \frac{\partial c}{\partial \tau_0}(\tau_0, \theta) d\tau_0, \quad 0 < t_0 \leq t.$$

Insert the expression above into equation (33) and obtain

$$(39) \quad c(t_0, t) = L_0 + \int_0^t d\theta \int_0^{L_0} G(z(\zeta_0, \theta), \theta, \mathbf{x}(\zeta_0, \theta), \mathbf{s}(\zeta_0, \theta)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \theta) d\zeta_0 \\ + \int_0^{t_0} \sigma(\theta) d\theta + \int_{t_0}^t d\theta \int_0^{t_0} G(c(\tau_0, \theta), \theta, \mathbf{x}(\tau_0, \theta), \mathbf{s}(\tau_0, \theta)) \frac{\partial c}{\partial \tau_0}(\tau_0, \theta) d\tau_0 \\ + \int_0^{t_0} d\theta \int_0^\theta G(c(\tau_0, \theta), \theta, \mathbf{x}(\tau_0, \theta), \mathbf{s}(\tau_0, \theta)) \frac{\partial c}{\partial \tau_0}(\tau_0, \theta) d\tau_0, \quad 0 < t_0 \leq t, \quad t > 0.$$

Finally, we easily derive the equation for $\partial c / \partial t_0$ from (39)

$$(40) \quad \frac{\partial c}{\partial t_0}(t_0, t) = \sigma(t_0) + \int_{t_0}^t G(c(t_0, \tau), \tau, \mathbf{x}(t_0, \tau), \mathbf{s}(t_0, \tau)) \frac{\partial c}{\partial t_0}(t_0, \tau) d\tau.$$

5. Special problem.

Equations (34), (35), (27), (28), (38), (39) and (40), which describe the free boundary problem, are mutually connected. In addition, they depend on substrates S_j , $j = 1, \dots, m$. So, also diffusion equations (4) must be involved. In this section we discuss a special mathematical problem by neglecting the dependence on substrates. The general situation will be considered in Section 6.

Precisely, in this section we analyze the free boundary problem governed by the following system of integral equations

$$(41) \quad x_i(z_0, t) = \varphi_i(z_0) + \int_0^t F_i(z(z_0, \tau), \tau, \mathbf{x}(z_0, \tau)) d\tau, \quad i = 1, \dots, n,$$

$$(42) \quad z(z_0, t) = z_0 + \int_0^t d\tau \int_0^{z_0} G(z(\zeta_0, \tau), \tau, \mathbf{x}(\zeta_0, \tau)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \tau) d\zeta_0,$$

$$(43) \quad \frac{\partial z}{\partial z_0}(z_0, t) = 1 + \int_0^t G(z(z_0, \tau), \tau, \mathbf{x}(z_0, \tau)) \frac{\partial z}{\partial z_0}(z_0, \tau) d\tau,$$

when

$$(44) \quad 0 \leq z_0 \leq L_0, \quad 0 < t \leq T, \quad T > 0,$$

and

$$(45) \quad x_i(t_0, t) = \psi_i(t_0) + \int_{t_0}^t F_i(c(t_0, \tau), \tau, \mathbf{x}(t_0, \tau)) d\tau, \quad i = 1, \dots, n,$$

$$(46) \quad c(t_0, t) = \int_0^t d\theta \int_0^{L_0} G(z(\zeta_0, \theta), \theta, \mathbf{x}(\zeta_0, \theta)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \theta) d\zeta_0 \\ + L_0 + \int_0^{t_0} \sigma(\theta) d\theta + \int_{t_0}^t d\tau \int_0^{t_0} G(c(\tau_0, \tau), \tau, \mathbf{x}(\tau_0, \tau)) \frac{\partial c}{\partial \tau_0}(\tau_0, \tau) d\tau_0 \\ + \int_0^{t_0} d\tau \int_0^\tau G(c(\tau_0, \tau), \tau, \mathbf{x}(\tau_0, \tau)) \frac{\partial c}{\partial \tau_0}(\tau_0, \tau) d\tau_0,$$

$$(47) \quad \frac{\partial c}{\partial t_0}(t_0, t) = \sigma(t_0) + \int_{t_0}^t G(c(t_0, \tau), \tau, \mathbf{x}(t_0, \tau)) \frac{\partial c}{\partial t_0}(t_0, \tau) d\tau,$$

$$(48) \quad L(t_0) = \int_0^{t_0} d\theta \int_0^{L_0} G(z(\zeta_0, \theta), \theta, \mathbf{x}(\zeta_0, \theta)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \theta) d\zeta_0 \\ + L_0 + \int_0^{t_0} \sigma(\theta) d\theta + \int_0^{t_0} d\theta \int_0^\theta G(c(\tau_0, \theta), \theta, \mathbf{x}(\tau_0, \theta)) \frac{\partial c}{\partial \tau_0}(\tau_0, \theta) d\tau_0,$$

when $z_0 \geq L_0$ and

$$(49) \quad 0 < t_0 \leq t, \quad 0 < t \leq T, \quad T > 0.$$

In this situation systems (41)-(43) and (45)-(47) and equation (48) can be solved in series as shown in the following theorems.

Consider system (41)-(43) and introduce some new notations. Firstly, let us redefine the vector \mathbf{x}

$$(50) \quad \mathbf{x} = (x_1, \dots, x_n, x_{n+1}, x_{n+2})$$

where

$$(51) \quad x_{n+1}(z_0, t) = z(z_0, t), \quad x_{n+2}(z_0, t) = \frac{\partial z}{\partial z_0}(z_0, t).$$

In addition, let

$$(52) \quad \varphi_{n+1}(z_0) = z_0, \quad F_{n+1}(\tau, \mathbf{x}(\zeta_0, \tau)) = G(z(\zeta_0, \tau), \tau, x_1, \dots, x_n) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \tau),$$

$$(53) \quad \varphi_{n+2}(z_0) = 1, \quad F_{n+2}(\tau, \mathbf{x}(z_0, \tau)) = G(z(z_0, \tau), \tau, x_1, \dots, x_n) \frac{\partial z}{\partial z_0}(z_0, \tau).$$

Assume F_i continuous and bounded

$$(54) \quad M_i = \max |F_i|, \quad 0 < \tau \leq t, \quad 0 \leq z_0 < L_0, \quad |x_i - \varphi_i| < \rho_i, \quad i = 1, \dots, n+2,$$

where ρ_i are positive constants. Setting

$$(55) \quad T = \min\{\rho_1/M_1, \dots, \rho_n/M_n, \rho_{n+1}/(M_{n+1}L_0), \rho_{n+2}/M_{n+2}\},$$

suppose that F_i satisfy the Lipschitz condition

$$(56) \quad |F_i(\tau, \mathbf{x}) - F_i(\tau, \tilde{\mathbf{x}})| < \lambda_i \sum_{h=1}^{n+2} |x_h - \tilde{x}_h|, \quad \lambda_i > 0, \quad i = 1, \dots, n+2,$$

on

$$(57) \quad D_1 = \{0 < \tau \leq t < T, \quad 0 < \zeta_0 \leq z_0 < L_0, \quad |x_i - \varphi_i| < \rho_i, \quad i = 1, \dots, n+2\}.$$

Theorem 5.1. *If hypotheses (54)-(57) hold and $\varphi_i \in C(0, L_0)$, then there exists a unique continuous solution to system (41)-(43), $x_i \in C((0, L_0) \times (0, T))$.*

Proof. Denote by Σ_1 the space of continuous vectors \mathbf{x} which satisfy the inequalities

$$(58) \quad |x_i - \varphi_i| < \rho_i, \quad i = 1, \dots, n+2, \quad 0 \leq z_0 \leq L_0, \quad 0 \leq t \leq T,$$

and consider the norm

$$(59) \quad \|\mathbf{x}\| = \sum_{i=1}^{n+2} \max \exp(-\gamma_1 z_0 - \gamma_2 t) |x_i(z_0, t)|, \quad 0 \leq z_0 \leq L_0, \quad 0 \leq t \leq T,$$

where γ_1 and γ_2 are positive constants that will be specified later on.

Consider the map $\mathbf{y} = A\mathbf{x}$ on Σ_1 defined by

$$(60) \quad y_i(z_0, t) = \varphi_i(z_0) + \int_0^t F_i(\tau, \mathbf{x}(z_0, \tau)) d\tau, \quad i = 1, \dots, n,$$

$$(61) \quad y_{n+1}(z_0, t) = \varphi_{n+1}(z_0) + \int_0^t d\tau \int_0^{z_0} F_{n+1}(\tau, \mathbf{x}(\zeta_0, \tau)) d\zeta_0,$$

$$(62) \quad y_{n+2}(z_0, t) = \varphi_{n+2}(z_0) + \int_0^t F_{n+2}(\tau, \mathbf{x}(z_0, \tau)) d\tau.$$

Map (60)-(62) maps Σ_1 into itself because of hypotheses (54)-(55). In addition, it is a contractive map. Indeed, setting $\tilde{\mathbf{y}} = A\tilde{\mathbf{x}}$ we derive

$$(63) \quad |y_i - \tilde{y}_i| \exp(-\gamma_1 z_0 - \gamma_2 t) \leq$$

$$\lambda_i \int_0^t \exp(-\gamma_2(t-\tau)) \sum_{h=1}^{n+2} \exp(-\gamma_1 z_0 - \gamma_2 \tau) |x_h(z_0, \tau) - \tilde{x}_h(z_0, \tau)| d\tau \leq$$

$$\lambda_i \|\mathbf{x} - \tilde{\mathbf{x}}\| \int_0^t \exp(-\gamma_2(t-\tau)) d\tau \leq \frac{\lambda_i}{\gamma_2} \|\mathbf{x} - \tilde{\mathbf{x}}\|, \quad i = 1, \dots, n,$$

$$(64) \quad |y_{n+1} - \tilde{y}_{n+1}| \exp(-\gamma_1 z_0 - \gamma_2 t) \leq$$

$$\lambda_{n+1} \int_0^t d\tau \int_0^{z_0} e^{-\gamma_1(z_0-\zeta_0) - \gamma_2(t-\tau)} \sum_{h=1}^{n+2} e^{-\gamma_1 \zeta_0 - \gamma_2 \tau} |x_h(\zeta_0, \tau) - \tilde{x}_h(\zeta_0, \tau)| d\zeta_0 \leq$$

$$\lambda_{n+1} \|\mathbf{x} - \tilde{\mathbf{x}}\| \int_0^t d\tau \int_0^{z_0} e^{-\gamma_1(z_0-\zeta_0) - \gamma_2(t-\tau)} d\zeta_0 \leq \frac{\lambda_{n+1}}{\gamma_1 \gamma_2} \|\mathbf{x} - \tilde{\mathbf{x}}\|,$$

$$(65) \quad |y_{n+2} - \tilde{y}_{n+2}| \exp(-\gamma_1 z_0 - \gamma_2 t) \leq$$

$$\lambda_{n+2} \int_0^t e^{-\gamma_2(t-\tau)} \sum_{h=1}^{n+2} e^{-\gamma_1 z_0 - \gamma_2 \tau} |x_h(z_0, \tau), \tau) - \tilde{x}_h(z_0, \tau), \tau)| d\tau \leq$$

$$\lambda_{n+2} \|\mathbf{x} - \tilde{\mathbf{x}}\| \int_0^t e^{-\gamma_2(t-\tau)} d\tau \leq \frac{\lambda_{n+2}}{\gamma_2} \|\mathbf{x} - \tilde{\mathbf{x}}\|.$$

Summing (63)-(65) gives

$$\|\mathbf{y} - \tilde{\mathbf{y}}\| \leq \lambda \|\mathbf{x} - \tilde{\mathbf{x}}\|,$$

where

$$\lambda = \sum_{i=1}^n \frac{\lambda_i}{\gamma_2} + \frac{\lambda_{n+1}}{\gamma_1 \gamma_2} + \frac{\lambda_{n+2}}{\gamma_2}$$

can be made < 1 if γ_1 and γ_2 are selected large enough. Therefore, $y = A\mathbf{x}$ is a contractive map and the theorem is proved. \square

Similar reasonings are now applied to system (45)-(47). In addition, we assume that the functions $x_i(z_0, t)$, $i = 1, \dots, n$, and $z(z_0, t)$ are known, as determined in the previous discussion. In particular, it is assumed to be known the following function

$$(66) \quad \psi_{n+1}(t_0, t) = L_0 + \int_0^{t_0} \sigma(\theta) d\theta + \int_0^t d\theta \int_0^{L_0} G(z(\zeta_0, \theta), \theta, \mathbf{x}(\zeta_0, \theta)) \frac{\partial z}{\partial \zeta_0}(\zeta_0, \theta) d\zeta_0.$$

Define the functions $x_{n+1}(t_0, t)$ and $x_{n+2}(t_0, t)$ as follows

$$(67) \quad x_{n+1}(t_0, t) = c(t_0, t), \quad x_{n+2}(t_0, t) = \frac{\partial c}{\partial t_0}(t_0, t),$$

and introduce the new vector \mathbf{x}

$$(68) \quad \mathbf{x}(t_0, t) = (x_1, \dots, x_n, x_{n+1}, x_{n+2})$$

where x_1, \dots, x_n are the functions in equation (45). Note that the functions x_i defined in (67)-(68) are different from the functions x_i defined in (50)-(51). In addition, let

$$(69) \quad F_{n+1}(\tau, \mathbf{x}(\tau_0, \tau)) = G(c(\tau_0, \tau), \tau, x_1, \dots, x_n) \frac{\partial c}{\partial \tau_0}(\tau_0, \tau),$$

(70)

$$\psi_{n+2}(t_0, t) = \sigma(t_0), \quad F_{n+2}(\tau, \mathbf{x}(t_0, \tau)) = G(c(t_0, \tau), \tau, x_1, \dots, x_n) \frac{\partial c}{\partial t_0}(t_0, \tau).$$

Assume F_i continuous and bounded

$$(71) \quad K_i = \max |F_i|, \quad 0 < \tau < t, \quad 0 < \tau_0 < t, \quad |x_i - \psi_i| < \mu_i, \quad i = 1, \dots, n+2,$$

where μ_i are positive constants. Furthermore,

$$(72) \quad T = \min\{\mu_1/K_1, \dots, \mu_n/K_n, \sqrt{\mu_{n+1}/(2K_{n+1})}, \mu_{n+2}/K_{n+2}\},$$

and F_i satisfy the Lipschitz condition

$$(73) \quad |F_i(\tau, \mathbf{x}) - F_i(\tau, \tilde{\mathbf{x}})| < \lambda_i \sum_{h=1}^{n+2} |x_h - \tilde{x}_h|, \quad \lambda_i > 0, \quad i = 1, \dots, n+2,$$

on

(74)

$$D_2 = \{0 < \tau_0 < t_0, \quad 0 < \tau < t_0 \leq t < T, \quad |x_i - \psi_i| < \mu_i, \quad i = 1, \dots, n+2\}.$$

Theorem 5.2. *If hypotheses (71)-(74) hold and $\psi_i, \sigma \in C(0, T), i = 1, \dots, n$, then there exists a unique continuous solution $x_i \in C((0, T) \times (0, T)), i = 1, \dots, n+2$ to system (46)-(48).*

Proof. Denote by Σ_2 the space of continuous vectors $\mathbf{x}(t_0, t)$ which satisfy the condition

$$(75) \quad |x_i - \psi_i| < \mu_i, \quad i = 1, \dots, n+2, \quad 0 \leq t_0 \leq T, \quad 0 \leq t \leq T,$$

and consider the norm

$$(76) \quad \|\mathbf{x}\| = \sum_{i=1}^{n+2} \max \exp(-\gamma_3 t_0 - \gamma_4 t) |x_i(t_0, t)|, \quad 0 \leq t_0 \leq T, \quad 0 \leq t \leq T,$$

where γ_3 and γ_4 are positive constants that will be specified later on.

Consider the map $\mathbf{y} = B\mathbf{x}$ on Σ_2 defined by

$$(77) \quad y_i(t_0, t) = \psi_i(t_0) + \int_{t_0}^t F_i(\tau, \mathbf{x}(t_0, \tau)) d\tau, \quad i = 1, \dots, n,$$

$$(78) \quad y_{n+1}(t_0, t) = \psi_{n+1}(t_0, t) + \int_{t_0}^t d\tau \int_0^{t_0} F_{n+1}(\tau, \mathbf{x}(\tau_0, \tau)) d\tau_0,$$

$$+ \int_0^{t_0} d\tau \int_0^\tau F_{n+1}(\tau, \mathbf{x}(\tau_0, \tau)) d\tau_0,$$

$$(79) \quad y_{n+2}(t_0, t) = \psi_{n+2}(t_0) + \int_{t_0}^t F_{n+2}(\tau, \mathbf{x}(t_0, \tau)) d\tau.$$

Map (77)-(79) maps Σ_2 into itself because of hypotheses (71)-(74). Moreover, it is a contraction. Indeed, setting $\tilde{\mathbf{y}} = B\tilde{\mathbf{x}}$ we get

$$(80) \quad \begin{aligned} & |y_i(t_0, t) - \tilde{y}_i(t_0, t)| e^{-\gamma_3 t_0 - \gamma_4 t} \\ & \leq \lambda_i \int_{t_0}^t e^{-\gamma_4(t-\tau)} \sum_{h=1}^{n+2} e^{-\gamma_3 t_0 - \gamma_4 \tau} |x_h(t_0, \tau) - \tilde{x}_h(t_0, \tau)| d\tau \\ & \leq \lambda_i \|\mathbf{x} - \tilde{\mathbf{x}}\| / \gamma_4, \quad i = 1, \dots, n, \end{aligned}$$

$$(81) \quad \begin{aligned} & |y_{n+1}(t_0, t) - \tilde{y}_{n+1}(t_0, t)| e^{-\gamma_3 t_0 - \gamma_4 t} \\ & \leq \lambda_{n+1} \int_{t_0}^t d\tau \int_0^{t_0} e^{-\gamma_3(t_0-\tau_0)} e^{-\gamma_4(t-\tau)} \sum_{h=1}^{n+2} e^{-\gamma_3 \tau_0 - \gamma_4 \tau} |x_h(\tau_0, \tau) - \tilde{x}_h(\tau_0, \tau)| d\tau_0 \\ & + \lambda_{n+1} \int_0^{t_0} d\tau \int_0^\tau e^{-\gamma_3(t_0-\tau_0)} e^{-\gamma_4(t-\tau)} \sum_{h=1}^{n+2} e^{-\gamma_3 \tau_0 - \gamma_4 \tau} |x_h(\tau_0, \tau) - \tilde{x}_h(\tau_0, \tau)| d\tau_0 \\ & \leq 2\lambda_{n+1} \|\mathbf{x} - \tilde{\mathbf{x}}\| / (\gamma_3 \gamma_4), \end{aligned}$$

$$(82) \quad \begin{aligned} & |y_{n+2}(t_0, t) - \tilde{y}_{n+2}(t_0, t)| e^{-\gamma_3 t_0 - \gamma_4 t} \\ & \leq \lambda_{n+2} \int_{t_0}^t e^{-\gamma_4(t-\tau)} \sum_{h=1}^{n+2} e^{-\gamma_3 t_0 - \gamma_4 \tau} |x_h(t_0, \tau) - \tilde{x}_h(t_0, \tau)| d\tau. \\ & \leq \lambda_{n+2} \|\mathbf{x} - \tilde{\mathbf{x}}\| / \gamma_4. \end{aligned}$$

Summing (80)-(82) gives

$$\|\mathbf{y} - \tilde{\mathbf{y}}\| \leq \lambda \|\mathbf{x} - \tilde{\mathbf{x}}\|,$$

where

$$\lambda = \sum_{i=1}^n \frac{\lambda_i}{\gamma_4} + \frac{2\lambda_{n+1}}{\gamma_3\gamma_4} + \frac{\lambda_{n+2}}{\gamma_4}$$

can be made < 1 if γ_3 and γ_4 are selected large enough. Therefore, $y = B\mathbf{x}$ is a contractive map and the theorem is proved. \square

Finally, consider equation (48). By using Theorem 5.1 and Theorem 5.2, we immediately obtain the solution for this equation.

Interesting properties of solutions to systems (41)-(43) and (46)-(48) can be proved as in [8,12]. Precisely, $\sum_{i=1}^n f_i = 1$ at any time if it is so initially. In addition, $f_i \geq 0$ at any time.

6. Effect of substrates.

Biofilm development is a complex process strongly influenced by substrates availability. Substrate concentration trends inside biofilm derive from the combination of two processes that take place in the same time within biofilm: microbial conversion of substrates and transport substrate by molecular diffusion. Molecular diffusion of substrates into biofilm is influenced by several factors, such as flux of substrates received from bulk, diffusivity of substrate species, structural characteristics of biofilm. Substrate trends determine spatially heterogeneous growth of microorganism species that contributes to the formation of environmental microniches allowing the coexistence of different microbial species. Distinct chemical niches exist at different depths in biofilms, there are several studies in which chemical gradients have been related to the distribution of specific bacterial species. For example, the stratified distributions of the bacteria that constitute methanogenic consortia have also been described and can be understood in terms of the diffusive exchange of metabolites among species.

In this section the influence of substrates on biofilm growth is considered and it is assumed

$$(83) \quad r_{M,i} = r_{M,i}(z, t, \mathbf{X}, \mathbf{S}).$$

Note that equations (41)-(49) still hold, but F_i and G must be modified according to (83)

$$(84) \quad F_i = F_i(z, \tau, \mathbf{x}, \mathbf{s}), \quad G = G(z, \tau, \mathbf{x}, \mathbf{s}),$$

where notations (26) and (32) have been used.

The diffusion of substrates is governed by equations (4) with initial-boundary conditions (9)-(11). The solution can be expressed in terms of

integral equations by using known results on the heat equation in general regions, e.g. [13]. So, we obtain

$$(85) \quad S_j(z, t) = \int_0^{L_0} S_{j0}(\zeta_0) N_j(z, \zeta_0, t) d\zeta_0 + \int_0^t D_j w_j(\tau) N_j(z, L(\tau), t - \tau) d\tau \\ + \int_0^t S_{jL}(\tau) [N_j(z, L(\tau), t - \tau) \dot{L}(\tau) - D_j N_{j\zeta}(z, L(\tau), t - \tau)] d\tau \\ + \int_0^t d\tau \int_0^{L(\tau)} r_{S,j}(\zeta, \tau, \mathbf{X}(\zeta, \tau), \mathbf{S}(\zeta, \tau)) N_j(z, \zeta, t - \tau) d\zeta, \quad j = 1, \dots, m,$$

$$(86) \quad w_j(t) = 2 \int_0^{L_0} S'_{j0}(\zeta_0) G_j(L(t), \zeta_0, t) d\zeta_0 + 2 \int_0^t \dot{S}_{Lj}(\tau) G_j(L(t), L(\tau), t - \tau) d\tau \\ + 2 \int_0^t d\tau \int_0^{L(\tau)} r_{S,j}(\zeta, \tau, \mathbf{X}(\zeta, \tau), \mathbf{S}(\zeta, \tau)) N_{jz}(L(t), \zeta, t - \tau) d\zeta \\ + 2 \int_0^t D_j w_j(\tau) N_{jz}(L(t), L(\tau), t - \tau) d\tau, \quad j = 1, \dots, m,$$

where the following notations have been used

$$w_j(t) = \frac{\partial S_j}{\partial z}(L(t), t), \quad K_j(z, t) = \frac{\exp(-z^2)/4D_j t}{\sqrt{4\pi D_j t}}, \\ N_j(z, \zeta, t - \tau) = K_j(z - \zeta, t - \tau) + K_j(z + \zeta, t - \tau), \\ G_j(z, \zeta, t - \tau) = K_j(z - \zeta, t - \tau) - K_j(z + \zeta, t - \tau).$$

Now, system (85)-(86) is suitably transformed by using positions (26), (32) and the change of variables $\zeta = z(\zeta_0, t)$, $\zeta = c(\tau_0, t)$ introduced in Section 3. Therefore, for $0 \leq z_0 \leq L_0$ we get

$$(87) \quad s_j(z_0, t) = \int_0^{L_0} S_{j0}(\zeta_0) N_j(z(z_0, t), \zeta_0, t) d\zeta_0 + \int_0^t D_j w_j(\tau) N(z(z_0, t), L(\tau), t - \tau) d\tau \\ + \int_0^t S_{jL}(\tau) [N_j(z(z_0, t), L(\tau), t - \tau) \dot{L}(\tau) - D_j N_{j\zeta}(z(z_0, t), L(\tau), t - \tau)] d\tau \\ + \int_0^t d\tau \int_0^{L_0} r_{S,j}(\zeta(\zeta_0, \tau), \tau, \mathbf{x}(\zeta_0, \tau), \mathbf{s}(\zeta_0, \tau)) N_j(z(z_0, t), \zeta(\zeta_0, \tau), t - \tau) \frac{\partial \zeta}{\partial \zeta_0} d\zeta_0$$

$$+ \int_0^t d\tau \int_0^\tau r_{S,j}(c(\tau_0, \tau), \tau, \mathbf{x}(\tau_0, \tau), \mathbf{s}(\tau_0, \tau)) N_j(z(z_0, t), c(\tau_0, \tau), t - \tau) \frac{\partial c}{\partial \tau_0} d\tau_0.$$

Moreover, for $z_0 > L_0$ and $0 < t_0 \leq t$, system (85) reduces to (88)

$$\begin{aligned} s_j(t_0, t) &= \int_0^{L_0} S_{j0}(\zeta_0) N_j(c(t_0, t), \zeta_0, t) d\zeta_0 + \int_0^t D_j w_j(\tau) N(c(t_0, t), L(\tau), t - \tau) d\tau \\ &+ \int_0^t S_{jL}(\tau) [N_j(c(t_0, t), L(\tau), t - \tau) \dot{L}(\tau) - D_j N_{j\zeta}(c(t_0, t), L(\tau), t - \tau)] d\tau \\ &+ \int_0^t d\tau \int_0^{L_0} r_{S,j}(\zeta(\zeta_0, \tau), \tau, \mathbf{x}(\zeta_0, \tau), \mathbf{s}(\zeta_0, \tau)) N_j(c(t_0, t), \zeta(\zeta_0, \tau), t - \tau) \frac{\partial \zeta}{\partial \zeta_0} d\zeta_0 \\ &+ \int_0^t d\tau \int_0^\tau r_{S,j}(c(\tau_0, \tau), \tau, \mathbf{x}(\tau_0, \tau), \mathbf{s}(\tau_0, \tau)) N_j(c(t_0, t), c(\tau_0, \tau), t - \tau) \frac{\partial c}{\partial \tau_0} d\tau_0. \end{aligned}$$

System (86) can be treated similarly. Notice that integral system (85)-(86) has been widely discussed since it is involved in the well-known Stefan problem. This system can be associated to equations (41)-(49), modified according to (84), and a result of uniqueness and existence to solutions can be deduced.

7. Numerical simulations.

Heterotrophic-autotrophic competition for space with oxygen as common substrate proposed in [5,8] has been used to provide numerical simulations of the free boundary problem introduced in Section 2.

The proposed numerical example, described in details by the equations in Table 1, is based on mass balance equations for substrates, products, and bacterial groups and includes the bio-chemical reactions of heterotrophic-autotrophic competition. The model considers the kinetics of microbial growth and decay and takes into account two groups of bacteria Heterotrophic Bacteria (X_1) and Autotrophic Bacteria (X_2), and three components (substrates), Ammonia (S_1), Organic Carbon (S_2) and Oxygen (S_3). Inert is modelled as another microbial species, whose growth derives from the heterotrophic and autotrophic biomass decay. Oxygen is used for organic carbon oxidation, nitrification and endogenous respiration. Oxidation of ammonia nitrogen to nitrate by the autotrophs provides energy for autotrophic growth.

The differential system (1)-(4) has been integrated numerically [14,15]. An initial biofilm thickness $L_0 = 0.3$ mm has been assumed and Dirichlet-Neumann boundary conditions have been adopted. The oxygen concentration at the interface biofilm/bulk liquid, the values of ammonia and acetate

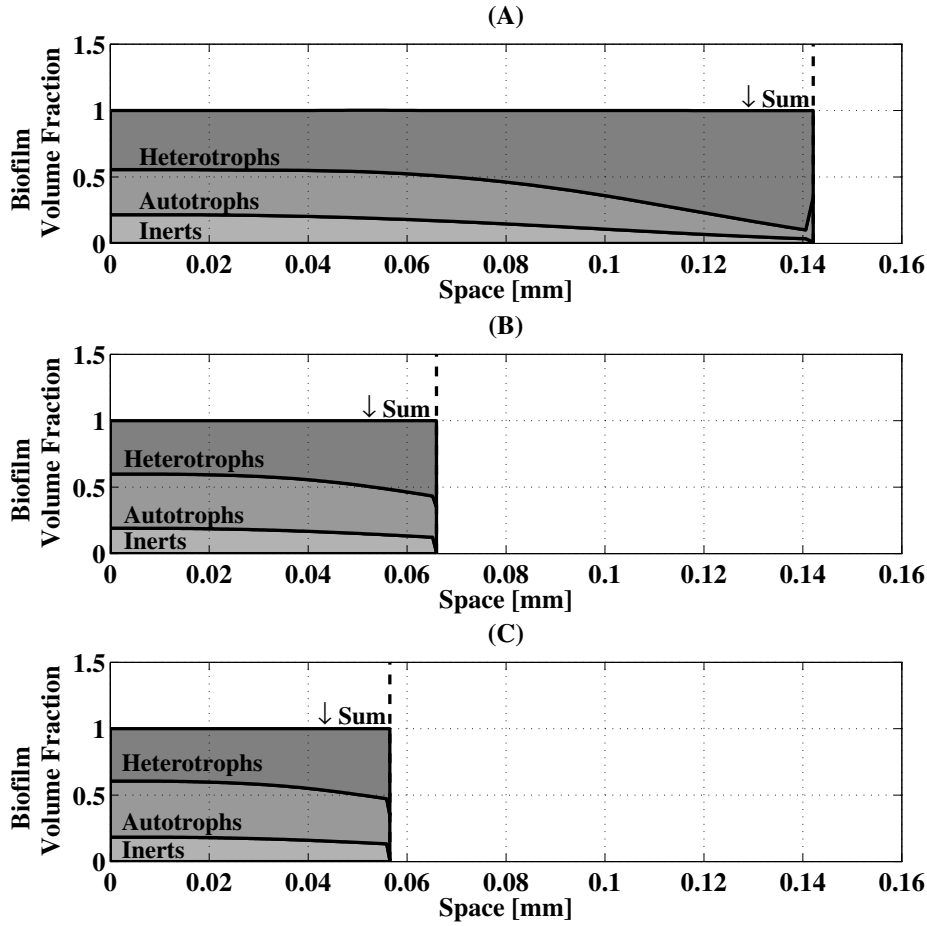


Figure 2. Effect of attachment rate (σ) on the volumetric fraction of the bacterial species in biofilm. A: $\sigma = 5 \text{ mmd}^{-1}$; B: $\sigma = 1 \text{ mmd}^{-1}$; C: $\sigma = 0.5 \text{ mmd}^{-1}$.

fluxes from bulk liquid to biofilm and biological parameters are reported in Table 2.

The values of kinetic and stoichiometric parameters shown in Table 1 and used in numerical simulation are the following: $\mu_{m,1} = 25$; $\mu_{m,2} = 5$; $b_{m,1} = 1$; $b_{m,2} = 1$; $b_{res,1} = 1$; $b_{res,2} = 0.5$; $Y_1 = 0.8$; $Y_2 = 0.6$; $K_{1,1} = 5$; $K_{2,1} = 1$; $K_{3,1} = 0.1$; $K_{3,2} = 0.1$.

Numerical simulations have been developed in order to predict the biomass distribution and substrate concentration trends over biofilm depth. The results are shown in Figure 2 and Figure 3, respectively. In particular, three sets of simulations at different attachment rates but at the same sim-

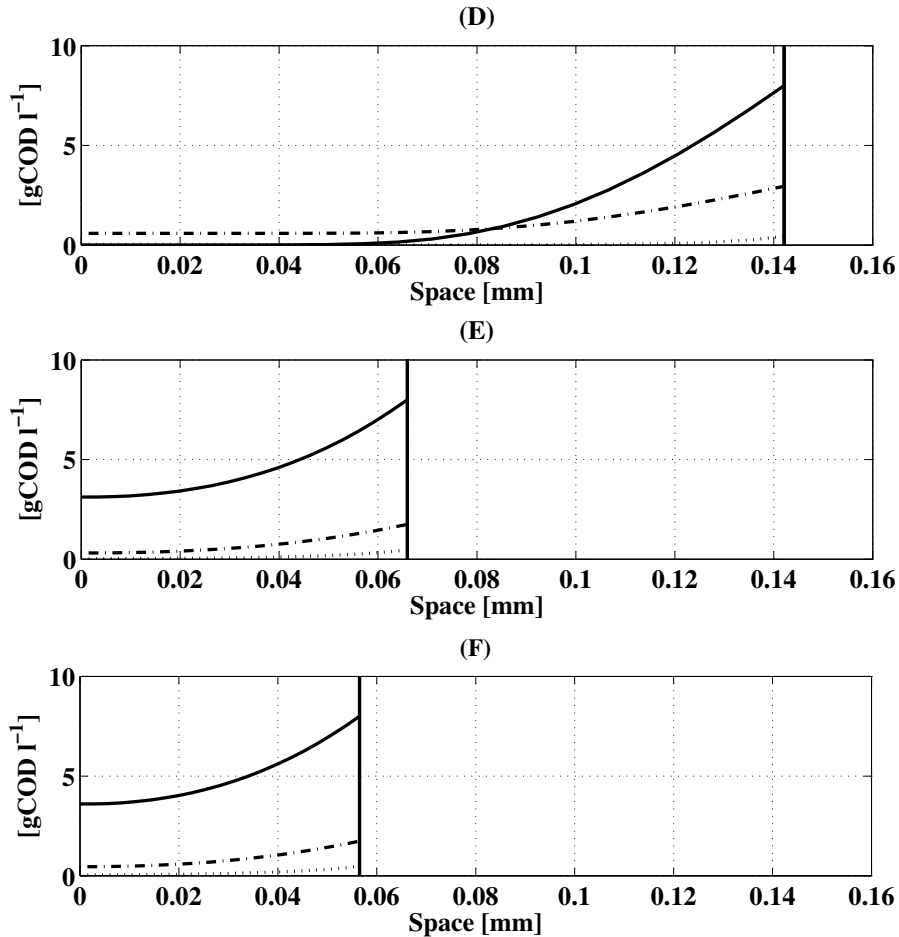


Figure 3. Effect of attachment rate (σ) on the substrate trends in biofilm. Dotted line: ammonia concentration; continuous line: oxygen concentration; dash-dot line: acetate (COD) concentration. A: $\sigma = 5 \text{ mmd}^{-1}$; B: $\sigma = 1 \text{ mmd}^{-1}$; C: $\sigma = 0.5 \text{ mmd}^{-1}$.

ulation time have been performed. The objective is the evaluation of the effects of attachment rate on biofilm growth in terms of biofilm thickness, bacterial species distribution and substrate concentration trends.

When the attachment rates is equal to 5 mmd^{-1} (Figure 2(A)) biofilm thickness is more than two time the biofilm thickness when the attachment rates is less than 1 mmd^{-1} (Figure 2(A,B)). This difference determines different substrate concentration trends into biofilm and, consequently, different biofilm structure occurs. In this case heterotroph bacteria are found to be predominant at the outmost layer of biofilm.

Table 1. Stoichiometry and rate laws for microbial processes. HG = heterotroph growth; AG = autotroph growth; HER = heterotroph endogenous respiration; AER = autotroph endogenous respiration; HD = heterotroph decay; AG = autotroph decay.

Process	X_1	X_2	X_3	S_1	S_2	S_3	Process rate r_j
HG	1	-	-	$-\frac{1}{Y_1}$	-	$-\frac{\alpha_1 - Y_1}{Y_1}$	$\mu_{m,1} X_1 \frac{S_1}{K_{1,1} + S_1} \frac{S_3}{K_{3,1} + S_3}$
AG	-	1	-	-	$-\frac{1}{Y_2}$	$-\frac{\alpha_2 - Y_2}{Y_2}$	$\mu_{m,2} X_2 \frac{S_2}{K_{2,1} + S_2} \frac{S_3}{K_{3,2} + S_3}$
HER	-1	-	-	-	-	-	$b_{res,1} X_1 \frac{S_3}{K_{3,1} + S_3}$
AER	-	-1	-	-	-	-	$b_{res,2} X_2 \frac{S_3}{K_{3,2} + S_3}$
AD	-1	-	1	-	-	-	$b_{m,1} X_1$
HD	-	-1	1	-	-	-	$b_{m,2} X_2$

Table 2. Operational parameters used for model simulations.

Parameter	Unit	Set A	Set B	Set C
COD Flux	gm^{-2}	0,4	0,4	0,4
Ammonia Flux	gm^{-2}	0,8	0,8	0,8
Oxygen Concentration	gl^{-1}	8	8	8
Time Simulation	h	24	24	24
Initial Biofilm thickness	mm	0.03	0.03	0.03
Attachment Rates	mmd^{-1}	5	1	0.5
Initial Volume Fraction of HB	-	0.65	0.65	0.65
Initial Volume Fraction of AB	-	0.34	0.34	0.34
Initial Volume Fraction of Inert	-	0.01	0.01	0.01

It is interesting to note the sharp variation on biofilm volume fraction in the superficial layer of the biofilm. This was expected, since, after the initial phase of attachment, the biofilm volume fraction is mainly determined by internal bacteria metabolism and not by the external biomass flux concentration. The sum equal to one of the three different biofilm volume fractions emphasizes the quality of numerical integration.

The diffuse substrate concentration trends in the biofilm, for three different attachment rate, are shown in Figure 3. The different thickness of biofilm and different kinds of bacterial species growing into biofilm determine different substrate concentration trends. When the attachment rates is equal to 5 mmd^{-1} (Figure 3(A)) there is a sharper decrease of oxygen concentration than attachment rates is less than 1 mmd^{-1} (Figure 3(A,B)). This occurs since a great concentration of heterotrophs at the outmost layer of biofilm implies a greater consumption of oxygen.

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