MATHEMATICAL MODELLING OF ANAEROBIC MULTISPECIES BIOFILM INCLUDING NEW BACTERIAL SPECIES INVASION

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ABSTRACT

A 1-D mathematical model for analysis and prediction of microbial interactions within anaerobic multispecies biofilms for methane production is presented. The model combines the related processes of Hydrolysis, Acidogenesis, Acetogenesis, Methanogenesis and takes into account phenomena of substrate reaction and diffusion, biomass growth, detachment and, in particular, the colonization of new species from bulk liquid to biofilm. The colonization phenomenon is initiated by planktonic cells, present in the bulk liquid but not initially in the biofilm, which thanks to the characteristic porous structure of biofilm matrix, may enter the channels and establish where they find favourable growth conditions. The latter may be related to the accumulation of metabolic waste products, such as the acetate for acetogenic bacteria, that can be used as growth substrates by other microorganisms.

According to D'Acunto *et al*. (2015), the biofilm growth process is governed by nonlinear hyperbolic PDEs and substrate dynamics are dominated by semilinear parabolic PDEs. The transport of colonizing bacteria from the bulk liquid to the biofilm is modelled by using a diffusion-reaction equation, where the reaction term represents the loss of planktonic bacteria due to their establishment within the biofilm. Overall this leads to a complex free boundary value problem essentially hyperbolic. Equations are integrated numerically by using the method of characteristics as strongly suggested by the qualitative analysis of the free boundary value problem. Mass conservation equation plays an important role in checking the accuracy of simulations.

The model is based on the biological framework of ADM1 (Batstone *et al*., 2002) and has been applied to simulate microbial competition and evaluate the influence of substrate diffusion on microbial stratification. A specific scenario has been analysed: it is related to the colonization of methanogenic archea of a biofilm initially constituted only by proprionate degraders. The results reveal that the introduction of motile bacteria into the biofilm allows the colonization by the new species as determined by substrate profiles. As it is reasonable to expect, the archea are found in the inner part of the biofilm where the most favourable conditions for their growth establish. The model predictions will help engineers or operators to have a better insight into biofilm dynamics in order to optimize process design or practical operation.

Keywords: Invasion Model, Biofilm; Mathematical model; Anaerobic digestion.

1. Introduction

Anaerobic digestion (AD) is one of the oldest and well-studied technologies for the effective treatment of organic wastes and wastewaters (Ariunbaatar *et al*., 2014). Interest in anaerobic treatment is increasing over years as it presents some significant advantages, such as limited environmental impacts and high potential for energy recovery, when compared to the alternative aerobic treatments.

The AD of complex organic macromolecules is generally achieved through the sequential and coordinated activity of various microbial (bacterial and archaeal) trophic groups, which catalyze: the three main reactions occuring during the entire process of anaerobic transformation to methane: hydrolysis, acid forming and methanogenesis (McKeown *et al*., 2012). These microbial groups establish syntrophic relationships where the later members of the food chain depend on the previous for their substrates, but also they may have significant influence on the earlier members in the chain by removing the metabolic products (Garcia *et al*., 2000). As widely demonstrated by experimental activity, the microbial community interactions are strongly affected by various operating and designing parameters, such as the pH value, the operating temperature, the composition of the feedstock, the loading rate and the retention time. For instance, different microbial communities develop in digesters operating on different retention times as the choice of a shorter value may even lead to the washout of the slow growing methanogens, which on their count require large reactors and longer retention time to ensure complete degradation of organic matter in conventional anaerobic digesters (Poh & Chong, 2009).

In this context, considerable attention has been recently paid towards the development of high rate reactors in order to decrease reactor volume or retention time and, on the same time, maximize community functions and the related methane production (Rajeshwari *et al*., 2000). Even though there are different kinds of high rate digesters, one of the most promising technologies is represented by the anaerobic biofilm digesters. In these systems, microorganisms are attached to a solid surface and/or each other forming micro colonies or biofilms. The adhesion of microorganisms over solid carriers with large specific surface areas, leads to high biomass concentration and high reaction rates, thus reducing the reactor volume needed. (Wang *et al*., 2010; Gong *et al*., 2011). Moreover, the grow of bacteria in the sessile state makes possible decoupling the hydraulic retention time from the residence time of the biomass. Overall, these systems are characterized by long start-up periods mainly related to the slow formation of an active biofilm.

According to (Férnandez *et al*., 2008), the development of an anaerobic multispecies biofilm can be divided into three stages: an initial attachment phase characterized by random adhesion of the cells to the surface; a consolidation phase defined by the appearance of microcolonies; and a maturation phase. The complexity of the microbial ecosystem has been found to increase over time due to the appearance of new community members. This may be related to the accumulation of metabolic waste products, such as the acetate for acetogenic bacteria, that can be used as growth substrates by methanogenic bacteria. The latter show a reduced capability of colonizing the surface but their establishment within the biofilm is strongly affected by the formation of favourable environmental conditions for their growth. The presence of relatively large channels and pores within the matrix structure might allow the entry of these colonizing cells which may abandon the planktonic state and start to grow as biofilm.

In parallel to experimental investigations, complex mathematical models and numerical simulations have been proposed to investigate development, structures, and ecological interactions of anaerobic biofilms. However, little attention has been directed towards successional invasion in anaerobic biofilm. Here a mathematical model for anaerobic multispecies biofilm formation and development based on the biological framework of ADM1 is presented.

2. Mathematical model

In this section we briefly recall the free boundary value problem which describes the invasion of new bacterial species into biofilms introduced in (D'Acunto *et al*, 2015). The mathematical model takes into account the dynamics of resident species, which are supposed to dominate initially within the biofilm, and invading species. The latter are not present initially in the biofilm but their growth depends on the diffusion of motile cells from the bulk liquid into the biofilm and their further establishment within specific environmental niches. The biofilm growth is governed by the following equations (D'Acunto *et al*, 2015)

$$\frac{\partial X_i}{\partial t}+\frac{\partial}{\partial z}\left(uX_i\right)=\rho_i r_{Mi}(z,t,\mathbf{X},\mathbf{S})+\rho_i r_i(z,t,\mathbf{\Psi},\mathbf{S}),$$
$$0\le z\le L(t),\ \mathrm{t}>0,\ i=1-7, \tag{1}$$

$$\frac{\partial u}{\partial z}=\sum_{i=1}^{7}(r_{Mi}+r_i),\qquad 0<z\le L(t),\ \mathrm{t}\ge 0, \tag{2}$$

$$\frac{\partial \Psi_i}{\partial t}-D_{Mi}\frac{\partial^2\Psi_i}{\partial z^2}=r_{\Psi,i}(z,t,\mathbf{X},\mathbf{\Psi},\mathbf{S}),$$
$$0<z<L(t),\ \mathrm{t}>0,\ i=1-7. \tag{3}$$

While the diffusion of substrates is governed by the equations

$$\frac{\partial S_j}{\partial t}-D_i\frac{\partial^2 S_j}{\partial z^2}=r_{S,j}(z,t,\mathbf{X},\mathbf{S}),$$
$$0<z<L(t),\ \mathrm{t}>0,\ j=1-12. \tag{4}$$

The free boundary evolution is governed by the following ordinary differential equation

$$\dot{L}(t)=u(L(t),t)+\sigma(t),\qquad \mathrm{t}>0, \tag{5}$$

where:
$X_i(z,t)=\rho_i f_i$ denotes the concentration of the microbial species i $X=(X_1,\ldots,X_7)$; ρ_i is the constant density; f_i denotes the volume fraction of microbial species i=1-7; $S_j(z,t)$ is the concentration of substrate j=1-12; $\Psi_i(z,t)$ represents the concentration of planktonic species diffusing from bulk liquid to biofilm, $\Psi=(\Psi_1,\ldots,\Psi_7)$; u(z,t) is the velocity of the microbial mass displacement with respect to the biofilm support interface; D_j denotes the diffusivity coefficient of substrate j; D_{Mi} denotes the diffusivity coefficient of planktonic species i; $r_i(z,t,\Psi,S)$ is the specific growth rate; $r_{\Psi,i}(z,t,\Psi,X,S)$ is the specific growth rate due to the colonization phenomenon; $r_{S,j}(z,t,X,S)$ is the conversion rate of substrate j; σ(t) is the exchange flux between biofilm and bulk liquid.
The following initial-boundary conditions have been considered for Eqs 1-5

$$X_i(z,0)=\begin{cases}\varphi_i(z), i=1,..,n_1\\ \varphi_i(z)=0, i=n_1+1,..,n\end{cases};\quad u(0,t)=0,\quad 0\le z\le L_0,\ t\ge 0,\quad i=1-7,$$
$$\frac{\partial\Psi_i}{\partial z}(0,t)=0,\ \Psi_i(z,0)=\Psi_{0i}(z)=0,\ \Psi_i(L(t),t)=\Psi_{iL}(t),\qquad 0\le z\le L_0,\ t\ge 0,\ i=1-7,$$
$$S_j(z,0)=S_{0j}(z),\qquad 0\le z\le L_0,\ j=1-12, \tag{6}$$
$$\frac{\partial S_j}{\partial z}(0,t)=0,\quad \frac{\partial S_j}{\partial z}(L(t),t)=G_j,\quad t>0;\ j=1-12,$$
$$L(0)=L_0.$$

The functions $\varphi_i(z)$ represent the initial concentrations of biomass species i. Note that n_1 denotes the number of resident species which constitute the biofilm at t = 0. The initial concentration of these species is supposed different from zero while for the invading species in the biofilm $\varphi_i(z)$ is set to zero as their growth in the non-motile state depends on the diffusion and further attachment of motile cells. The functions $S_{0j}(z)$ represent the initial substrate concentrations within the biofilm and the functions $\Psi_{iL}(t)$ represent the concentration of the invading species in the bulk liquid.

2.1. Numerical methods

In D'Acunto *et al*. (2015), some qualitative properties of the solutions to system (1)-(5) have been proved in order to emphasize model consistency. Apart from being interesting for itself, this analysis represents a discriminator element for the validity of numerical findings. Numerical integration has been performed by applying the method proposed in D'Acunto *et al*. (2011). In particular, the method of characteristics, first introduced in D'Acunto and Frunzo (2011) and particularly suitable to study hyperbolic problems, has been used for the system of non-linear equations (1). The great accuracy of the method is strictly connected to the existence of an invariant for the numerical integration which can be used as a check control for the error. Numerical integration of the system (1)-(5) has been performed using an original software developed in MATLAB® platform.

3. Results and discussion

The model has been applied to simulate microbial competition and to evaluate the influence of substrate diffusion on microbial stratification. Here a specific scenario has been reported. In particular, the coexistence and competition between methanogens and propionate degraders has been studied. The simulation results are shown in Figure 1, where the biomass volume fractions, the substrate profiles and the concentration trend of motile species are reported over time. The model is able to properly reproduce that in the first stages of biofilm development only the propionate degraders can proliferate while the concentration of methanogenic archea stays zero within the biofilm. Only after the formation of an environmental microniche characterized by a higher concentration of acetate, the methanogenic archea start to grow in the inner part of the biofilm where the more appropriate conditions for their growth establish. The concentration of motile cells is found to decrease within the biofilm never reaching zero but as it is reasonable to expect, the invading species establish only in the inner part of the biofilm. As in this simple example, the model can be applied to more generic situations characterized by the contemporary presence of all the microbial species taken into account in ADM1.

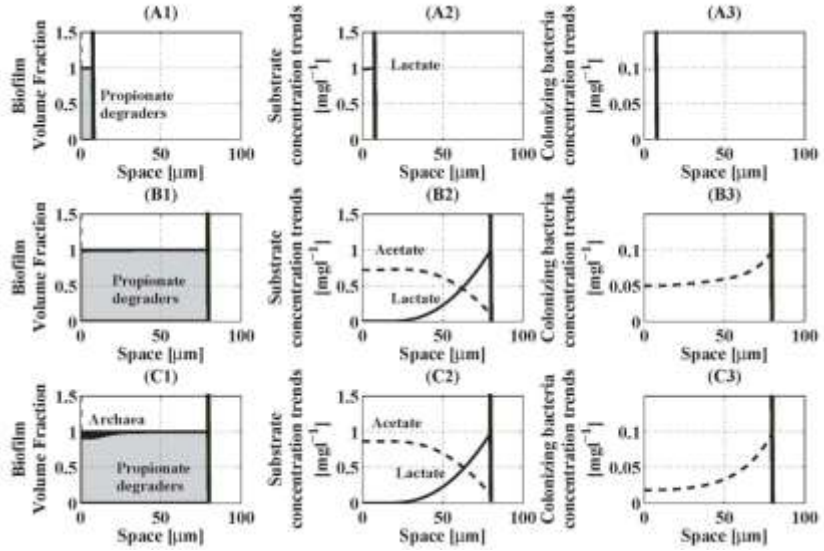

Figure 1: Bacteria volume fractions within biofilm; substrate concentration trends within biofilm, Colonizing bacteria concentration trend within biofilm, after 2 days (A1,A2,A3); 5 days (B1, B2, B3); 20 days (C1,C2,C3).

4. Conclusion

The model predictions will help engineers or operators to have a better insight into biofilm dynamics in order to optimize process design or practical operation.

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