Modeling and Analysis of Biofilms Formation and Evolution in Wastewater Treatment Processes using Multi-Agent Systems

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Abstract: Biofilms are complex but omnipresent and industrially important structures. Many studies were devoted to build mathematical models for biofilm analysis but most of them were based on classical continuous approaches. On the other side, only very few studies were directly based on the functional mechanisms to obtain virtual systems which mimic the descriptive observations and allow to predict the state of a biofilm and its possible evolution. This paper will present a non-classical approach based on multi-agent systems for the analysis of biofilm formation. This model shows the spatial repartition of biomass within the biofilm and the impact of the biofilm activity on its environment. Many kind of biofilms can be simulated with the model ; it has been qualitatively validated with the case of the biofilm of an anaerobic wastewater treatment plant. The biomass is modeled by virtual agents that represent micro-colonies of identical bacteria. Each agent follows a mass balance law while its growth is described by a Monod law and its detachment is calculated as a function of its depth in the biofilm and of the level of hydrodynamic forces. The agents interact then with each other to find equilibrium between density and spatial growth. The diffusion-reaction problem has been solved in two dimensions by the Alternating Directions Implicit method. Several simulations are performed to analyze influence of characteristics of the influent liquid phase, for the molecules diffusion in the biofilm and for the biochemical reaction.

Keywords : Biofilm, Modeling, Multi Agent System, Anaerobic Digestion

1 INTRODUCTION

Biofilms are very common biological structures and are met on every solid support in contact with water. They can be defined as matrix-enclosed bacterial populations adherent to each others. In some cases, biofilms are a costly problem because of their presence in pipes or on chirurgical instruments. However, biofilms are also industrially useful, especially in biological processes such as wastewater treatment plants (WWTPs).

In anaerobic or aerobic WWTPs, microorganisms are used to remove organic and nutrient pollution. The performances of the treatment plants can be considerably increased by favoring the fixation of the biomass in the reactor within biofilms.

Biofilms are very hard to investigate but recent tools like confocal microscopy and micro-sensors have increased our knowledge of their three dimensional structure. The both have shown indeed that biofilms are very heterogeneous and dynamic structures. In particular, use of microelectrodes showed that wastewater biofilms are highly stratified systems where components concentrations, density and biomass activity evolve as a function of the biofilm depth [Zhang *et al*. 1994]. Molecular methods like Fluorescence In Situ Hybridization (FISH) or immunolabeling also brings information to the analyze of biofilms in terms of community structure and colonies [Winpenny *et al*. 2000]. The FISH method applied to anaerobic sludge granules showed a zonation of different microorganisms and it appears that this zonation depends on the wastewater composition [Santegoeds *et al.* 1999]

2 BIOFILM MODELING

The studies about biofilm resulted in descriptive knowledge (*e.g.,* evolution of properties of the biofilm in space and time) and functional knowledge (*e.g.,* cell-cell communication) of biofilms. Reliable biofilm models are useful to increase the performances of plants and

particularly to reduce the startup duration of a plant. Furthermore, biofilms modeling allows once to check hypothesis on biofilm mechanisms and to predict its evolution with respect to time.
Many biofilm models have been built but they are often based on classical continuous methods and cannot take into account all the available biological knowledge. In particular, during the initiation phase, the biofilm is not a continuous structure but isolated growing colonies. The classical models are then not suitable for the representation of the whole complexity and heterogeneity of the biofilms.
Other approaches based on cellular automata [Picioreanu *et al.* 1998a] or multi-agent systems [Kreft *et al.* 1998] aim to model the spatial and time variations of biofilms. Compared to a cellular automata, one of the main advantages of a multi-agent system is the possibility to model a higher level of interactions between elements and to represent more explicitly the individuals.

3 DESCRIPTION OF THE MODEL

The objective of this studies is to build a multi-agent biofilm model, based on a limited number of basic interactions between bacteria but able to represent the spatial and temporal heterogeneity of a biofilm. One of the constraint was to build a model as generic as possible, without hypothesis about the nature of the biofilm or the kind of reaction.
Schematically, two dynamics contribute to the evolution of a biofilm as shown in figure 1 : adsorption, division, erosion and decay of microorganisms (X) contribute to the bacterial dynamic, diffusion, convection and reaction of substrate (*S*) and products (*P*) molecules contribute to the chemical dynamic [Wik 1999].

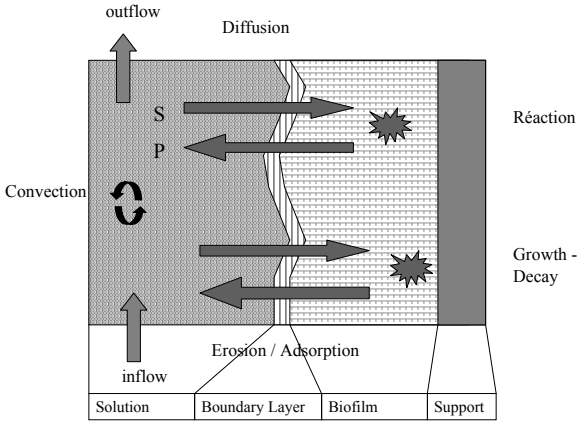

Figure 1. Schematic representation of a biofilm

According to the classical architecture of a multi-agent system, the presented model describes the system in terms of agents situated in an environment. Logically, the agents should be the bacteria themselves. The multi-agent model developed by Kreft simulates each bacteria. However due to computation limits and the chosen simulation scale, the very high concentrations met in biofilms do not allow us to model individually each bacteria. The agents are then defined as bacterial colonies which mimic the behavior of individual bacteria. Each agent is characterized by its species, its mass and a set of kinetic parameters. It is able to catalyze chemical reactions, to produce biomass and to create new agents.
The kinetics for chemical reactions and for biomass formation are very different [Wik 1999]; this difference allows us to model independently both phenomena. Dynamic of chemical species belongs to the spatial dynamic while the dynamic of biomass belongs to agent's dynamic.

3.1 Dynamic of the agents

One modeling step for the agents corresponds to the growth and decay phenomena and to the spreading of the biomass.
One of the key parameter is the activity of each bacterial agent. The activity is a function of environmental conditions and endogenous parameters genetically determined. The activity μ of each agent is computed at each time step and is used to determine its own biomass creation *dX*. The activity computation can be based on different models : zero or first order kinetic, Monod law, Haldane law. In our study, different laws could be chosen without any modification of the muti-agent system architecture.

$$\left(\frac{dX}{dt}\right)_{growth} = \mu X$$

In parallel, each agent looses a quantity γ of biomass. In the following, it is assumed that this quantity is determined as a function of its position x in the biofilm, the level of hydrodynamic pressure estimated by the hydraulic retention time (*HRT*) of the plant and a constant γ°. This constant is identified to fit with the global detachment determined by a global mass-balance model.

$$\gamma(x) = \gamma^\circ \frac{x}{HRT} \qquad (1)$$

The sum of biomass production and biomass detachment is the net production of biomass as shown by this equation :

$$\frac{dX}{dt} = (\mu - \gamma)X \qquad (2).$$

The destination of the net biomass production is determined with respect to a set of limitations on the mass of an agent, the concentration of

biomass in the biofilm and the number of colonies of a grid cell :

- if the created biomass cannot be allocated to the agent itself, it is transferred to its neighbours,
- if there is no neighbour, the agent creates a new agent on a free cell,
- if the neighbours are too saturated, this biomass is ignored by the model.

This set of rules is the consequence of the hypothesis that the agents are fixed. Then the dynamic of the biofilm is modeled by the creation or the disparition of agents.

3.2 Spatial Dynamic

One modeling step for the environment simulates the diffusion of molecules through the biofilm, the chemical reactions and the mixing with the feeding flow.

The chemical reaction are catalyzed by the microorganisms, so the reaction rate for substrate (S) degradation is proportional to the agent's activity μ. And the yield coefficient k.

$$\left(\frac{dS}{dt}\right)_{x,y}^{Reac} = k_S \mu X_{x,y} \qquad (3)$$

The diffusion of the molecules is simulated only within the biofilm and the boundary layer. So the part of the environment submitted to the diffusion is determined by the presence of agents, which defines the biofilm. The bulk phase is supposed to be homogenous. The diffusion of a molecule (S) is locally described by the Fick law as a function of its spatial gradient and its diffusivity D_S.

$$\left(\frac{dS}{dt}\right)_{x,y}^{Diff} = D_S\left(\frac{d^2S}{dx^2} + \frac{d^2S}{dy^2}\right) \qquad (4).$$

The two terms of local biomass variation (diffusion and reaction) define a partial differential system with moving boundaries. It has been solved by a spatial discretisation in two dimensions computed using the Alternating Direction Implicit Method (ADI) which combines unconditional stability with calculational simplicity [Picioreanu *et al.* 1998a].

The concentrations in the liquid phase are influenced by the feeding of the tank and by the exchanges between the biofilm and the bulk. Assuming that the biochemical reactions are localized only in the biofilm, the exchanges between the two compartments 'biofilm' and 'liquid phase' can be modeled. This equation shows the variation of the concentration in the liquid phase $S°$ in function of the retention time HRT, the feed concentration Sin, the specific surface σ and the flow J between the biofilm and the free phase.

$$\frac{dS°}{dt} = \frac{S_{in} - S°}{HRT} + J\sigma \qquad (5)$$

3.3 Model architecture

The model is implemented into the software CORMAS which is an agent-based simulation framework initially dedicated to the natural-resources management [Bousquet. *et al* 1998].

The figure 2 shows the architecture of the model implemented from the predefined objects (SpatialEntity, AgentLocation and CormaModel) of the CORMAS framework. The definition of generic classes allows us to add to the model new kinds of agents or new chemical reactions.

At each time step, each agent computes its activity as a function of the substrate concentrations. Then, the global activity of each grid's cell is calculated and used by the algorithm of diffusion-reaction.

It is important to notice that every calculated variable is stocked into a buffer variable ; the variable is updated at the end of a step, when every computation has been made. Such a parallel schedulling seems to create the minimal modelling bias for this case.

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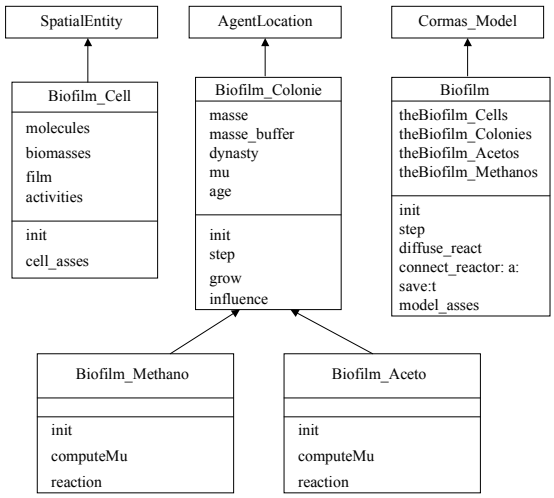

Figure 2. Model Architecture

4 RESULTS

This generic model has been applied to a particular case to simulate the biofilm dynamics of a 1 cubic meter anaerobic digestion upflow fixed-bed reactor [Steyer *et al.* 2001].

A mass-balance model was successfully validated on this plant but without modeling any spatial biofilm structure [Bernard *et al.* 2001]. This model described the anaerobic fermentation in two main steps, an acetogenis step which transforms the organic carbon in Volatile Fatty Acids (VFA) and a methanogenis step which

transforms the VFAs in biogazes, essentially composed of CH_4 and CO_2.
So two kinds of agents have been defined, 'Biofilm_Aceto' and 'Biofilm_Methano', and the dynamic of three molecules has been followed : complex substrate, volatile fatty acid and methane.
In accordance with [Bernard *et al.*], the chosen relations to describe the activity of agents are a Monod law for the acetogenesis and a Haldane relation for the methanogenesis.

$$\begin{cases} \mu_1 = \mu_1^{\max} \dfrac{S_1}{S_1 + K_1^s} \\ \mu_2 = \mu_2^{\max} \dfrac{S_2}{S_2 + K_2^s + \left(S_2 / K_2^I \right)^2} \end{cases} \quad (6)$$

The Monod law models the limitation by the complex substrate (S_1), the Haldane law models the limitation and the inhibition phenomena by VFAs (S_2).

4.1 Simulation results

The spatial 2-dimensionnal grid is generally from 750 to 1500 µm large with a spatial step of 50 µm. A higher resolution was not possible for computational reasons. The figure 3 shows the model graphical interface. The first window is the control panel where agents and simulation properties can be defined, the second window is the spatial grid where agents are represented as triangles, the background color illustrates the local concentration. The support is supposed to be at the right side of the grid, the bulk at the left side.

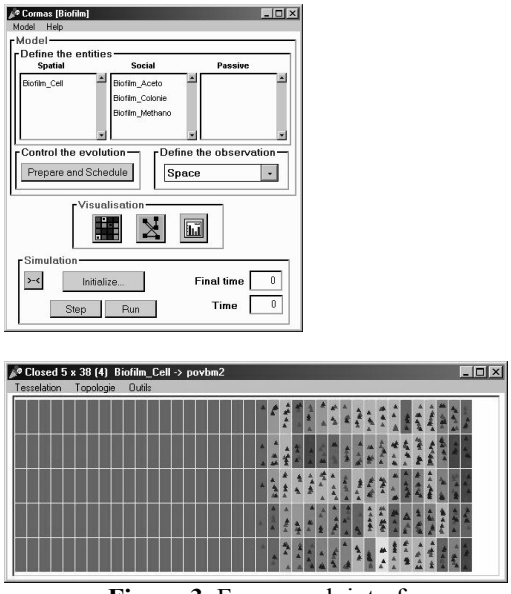

Figure 3. Framework interface

The results of a 2 weeks long simulation, initialized with a thick (750 µm) but homogenous biofilm are presented in the figures 4 and 5.The influent of the reactor is only composed of complex substrate. In Figure 4, the two first rows show the evolution during the simulations of the biomass concentration profiles in the biofilm (denoted X1 for acetogenous and X2 for methanogenous). The apparition of biomass gradients is easily explainable by the erosion strength. The third row shows the apparition of preferential zones for acetogenous and methanogenous microorganisms ; the biomass ratio (X1/(X1+X2)) profile, which was flat at the initialization, shows a higher representation of methanogenous colonies in the deepest layers of the biofilm. Nevertheless it is not possible to separate a methanogenous layer and an acetogenous layer, the evolution of properties is continuous. The chemical concentrations profiles are in accordance with the biomass profiles.
The Figure 5 shows the evolution during the same simulation of global concentrations, as they could be measured in the bulk phase. A first part from t=0 to t=6 days shows a washing of the colonies, which goes with the augmentation of substrate concentrations in the free phases. After one week, the biofilm reaches a steady-state and the concentrations in the liquid phase are stabilized.
Other simulations have shown that the evolution of biomass ratio was not so strong when the concentration of volatile fatty acid in the influent was higher [Lardon 2001]. It means that the apparition of a zonation within the biofilm is determined by the influent composition ; the presence in the liquid phase with a high concentration of the intermediate product (Volatile Fatty Acids in our case) seems to be the determining factor of the biofilm zonation illustrated here by the agent succession.

4.2 Sensitivity analysis

It is not convenient to manage the validation of a multi-agent model because of the virtual aspect of the agents themselves.
However, a good way to partially validate such a model is the sensitivity analysis. The sensitivity of the model to a set of parameters variations has been tested : it is expected that some parameters have no effect on the result, and for others the variation of the simulation results need to be explainable. Each parameter sensitivity has been tested independently (Lardon 2001).
The division threshold, which fixes the minimal mass of an agent for its division, appears as a tuning between density growth and thickness growth. The $\gamma°$ term introduced in the equation (1) fixes the abrasion force. For low values of $\gamma°$,

the biofilm growth is quicker. Then, the substrate concentration is very low and the biomass activity too.

The maximal number of agents by grid cell has no significant effect. Simulations have been done with 5, 10 and 20 agents by grid cell. The case 1 agent by grid cell corresponds to a cellular automata.

There is no detectable time discretisation bias. Simulations with time step from 1 to 15 minutes do not show significant differences. This demonstrates that the hypothesis of quasi independence between the bacterial dynamic and the chemical dynamic was correct.

However, there is a bias on space discretisation. In fact this bias probably comes from the spreading biomass rules but other rules with moving colonies could solve this bias. Nevertheless, the sensitivity to the spatial step does not modify the trends of the model but only the numerical values.

Some computation sequences and alternative diffusion algorithms have been tested and the method presented appears to be the fastest without any difference on the results.

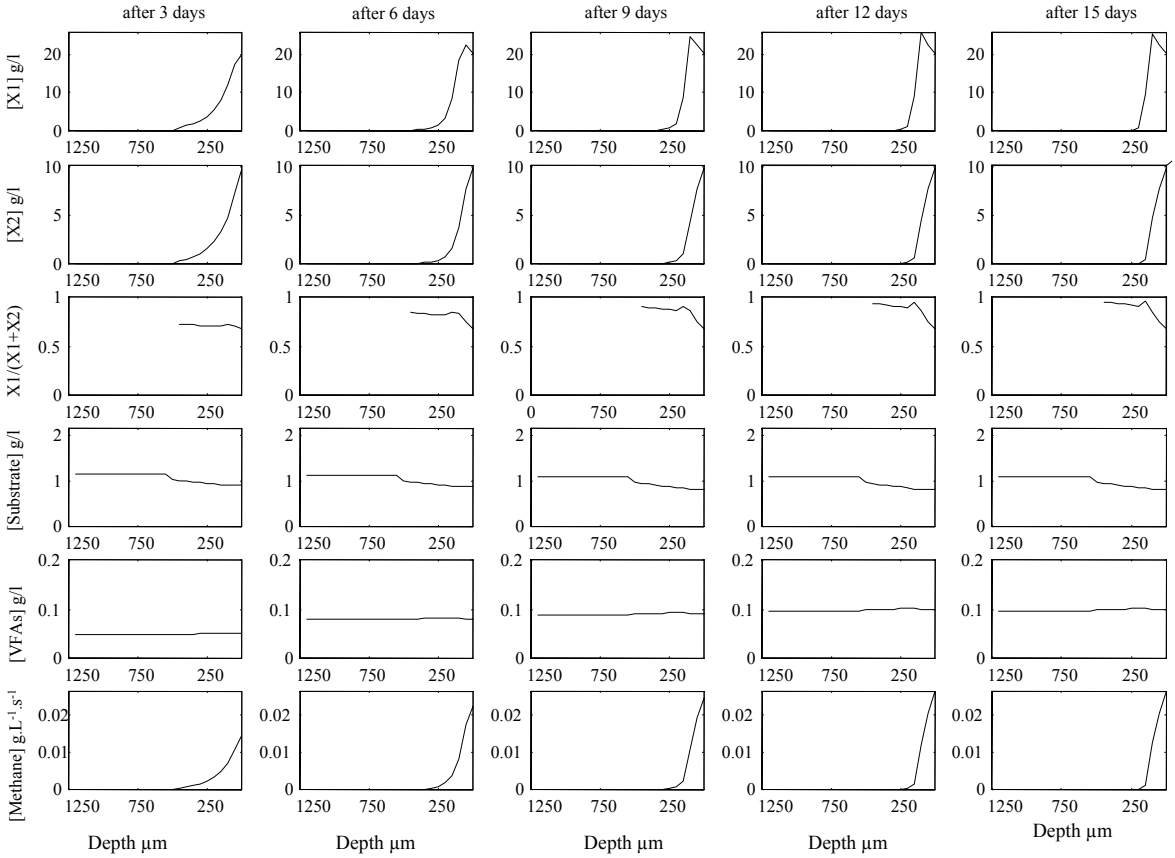

Figure 4. Profiles evolution in the biofilm
[X1] : acetogenous concentration in g/L, [X2] : methnogenous concentration in g/L
[VFA] : Volatil Fatty Acids in g/L

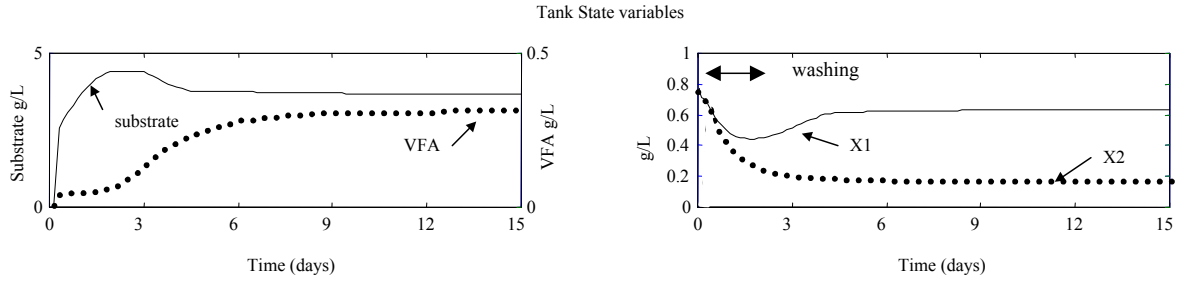

Figure 5. Concentrations evolution at tank scale.

5 CONCLUSION

A generic multi-agent model for biofilm dynamic was developed. This model has been applied for validation to the case of the biofilm of an anaerobic Waste Water Treatment Plant. The simulations have reproduced the trends experimentally observed with the microelectrodes or the *in situ* hybridation method, *i.e.* the formation of gradients within the biofilm. Simulations have also isolated favorable conditions for the spontaneous apparition of such a spatial distribution of the trophic chain. This model is still only qualitatively validated but future work will allow us to validate it quantitatively. Particularly, the introduction in the model of the extra-cellular matrix and more interactions between agents should improve the simulation of the biomass spreading and detachment phenomena.

The introduction of agents in biofilm modeling seems to be very useful for the understanding of fundamental mechanisms of biofilms. Individual phenomena like cell-cell communications or mutations are natural and easy to implement on such a model and their effect on the macroscopic structure can be checked without too much difficulty. This model offers an adaptive framework for the verification of biological hypothesis. Questions like the minimum biodiversity necessary to the stability of a biofilm, or the coexistence criteria between different microorganisms populations could find interesting answers with this multi-agent framework. Furthermore the understanding and a clear representation of the biological mechanisms should allow one to give good estimations of the state of a biofilm in a biological reactor and to improve the control policy.

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