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A CELLULAR AUTOMATA MODEL FOR BIOFILM GROWTH

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Abstract. *Biofilms are aggregates of bacteria attached to surfaces, which are very adaptable to changes in the environment and survive under extreme conditions. These living structures are behind many problems in research and industry. Therefore there is an increasing interest in improving our understanding on biofilms to be able to control them, either designing protocols to destroy them when harmful or promoting their growth when beneficial.*

A bidimensional cellular automata model for biofilm development is proposed to study the biofilm behaviour as its key growth parameters vary. The model includes several metabolic and spreading mechanisms typical of bacteria: cell division and spreading, detachment mechanisms adapted to the flow and probabilistic rules for EPS matrix generation.

Numerical simulations of the model reproduce a number of biofilm patterns observed in real experiments: ripples, streamers, mushroom networks and patches. The influence of the nutrient concentration and the type of flow on the evolution of the bacterial community is monitorized.

Biofilm tends to cover the whole surface when enough nutrients are available. Erosion enhances the creation of holes in this cover and promotes a variety of geometric patterns. The survival of the colony and its final shape will depend on the balance between the main growth parameters.

Large Reynolds numbers and poor nutrient sources promote the formation of flat, and thin biofilms. Decreasing the Reynolds number or increasing the nutrient and oxygen concentration enhance pattern formation.

Keywords: *Biofilms, cellular automata, probabilistic models, erosion, EPS Matrix.*

1. INTRODUCTION

Nowadays, the study of biofilms is a promising area of research because its presence in many industrial and medical problems contexts. They can be defined as an aggregate of bacteria embedded into a self-generated polymeric substance which can attach onto a wide variety of supports, from any kind of inorganic surfaces until living tissues, being extremely

resistant to physical or chemical aggressions. They develop and reproduce trying to spread into the environment using many different strategies [11] that are not fully understood.

These living organisms take part in many critical processes of great importance in their respective fields of study. Examples are the biofilms formed in the lungs, which are responsible for deadly cystic fibrosis, or biofilm impact on surgery, when pacemakers and artificial joints are implanted and may induce chronic and acute infections [20,3]. However, biofilms can be used to our advantage to achieve different purposes if they are properly controlled. Biofilms that become a problem in drinking water systems [13] can be exploited for biodegradation of pollutants [20] in soil or water. They can also be used to detect chemicals and pathogens, or to measure variables in MEMS [20,8]. For technological applications, a precise control of growth processes is required to produce biofilms with a specific structure.

In order to achieve this last objective, a deep understanding of biofilm development and spreading mechanisms is essential. A good biofilm model should take into account the nature of the considered bacteria, the environment in which the biofilm is formed, the parameters that have to be fitted with experiments, and the predictions to be made [23,12,10].

There are many of models trying to explain how biofilms behave under different external and internal variables which affect them. These models can be classified into three different categories [23,12,10]: continuum, individual based (IbM) or cellular automata (CA) models.

Continuum models treat the biofilm as a continuum solid with similar properties, typically a gel, polymer or viscous fluid [9]. They are often two-phase models which consider a fluid phase containing a mixture of nutrients and the biofilm phase [4]. IbM models are a discrete approach in which microbes are seen as spherical particles which evolve according to reaction-diffusion equations for nutrients and oxygen coupled with bacterial growth and spreading of biomass [15]. There are several variants which try to include a greater degree of complexity observed in real biofilms, like the EPS matrix [1]. CA models propose a simple set of evolution rules [7,16] in a bounded discrete domain for a system filled with biomass and fluid which will evolve in time. They can include basic metabolic mechanisms easily, but more complex behaviours such as microbe attachment to surfaces [6], quorum sensing to form biofilms [2,25], generation of EPS matrix [22,24] or interaction with the surrounding flow [21,5,14] are more difficult to implement. The stochastic approach of these type of models allow them to reduce assumptions and hypothesis about the dynamic of the system to a minimum. CA models are usually less costly than solving classical PDE models.

In this paper we develop a cellular automata model to obtain a qualitative understanding of biofilm evolution in a rectangular pipe at increasing Reynolds numbers. The paper is organized as follows. Section 2 and 3 describes the model and the probabilistic rules for all metabolic processes that participate in biofilm dynamics. An asymmetric erosion mechanism adapted to flows moving parallel to the surface is proposed, together with simple probabilistic rules accounting for EPS matrix generation and its influence on biofilm cohesion. Section 4 illustrates several results found in the performed simulations. Finally, section 5 summarizes the conclusions.

2. OVERALL APPROACH

3. Overall approach

Our model attempts to describe the formation and evolution of a biofilm, which is believed to be as follows [11]. Once planktonic cells have reach the substratum, they attach to it and start reproducing if conditions are favorable (enough nutrients, not very agressive external conditions, etc.). First, microcolonies are formed as a prelude of an exponential growth. During the growth fase, the bacteria forming the biofilm suffer physiological changes like the loss of their flagella, promoting the expression of new metabolic behaviours (EPS secretation). EPS generation enhances biofilm growth on vertical direction, looking for richer carbon and oxygen concentrations. Macrocolonies are formed. These mature objects will be eroded by the external hydrodynamic forces, resulting on a rich variety of morphologies [17] (mushrooms, ripples, streamers, etc.). After some time and once a critical bacterial population has been reached, quorum sensing mechanisms activate spreading mechanisms (rolling, rippling, darting, etc., see figure 2). The main objective of these mechanisms is to colonize new surface and ensure the survival of the colony. The biofilm life cycle is closed then. A schematic figure can be seen in Figure 1.

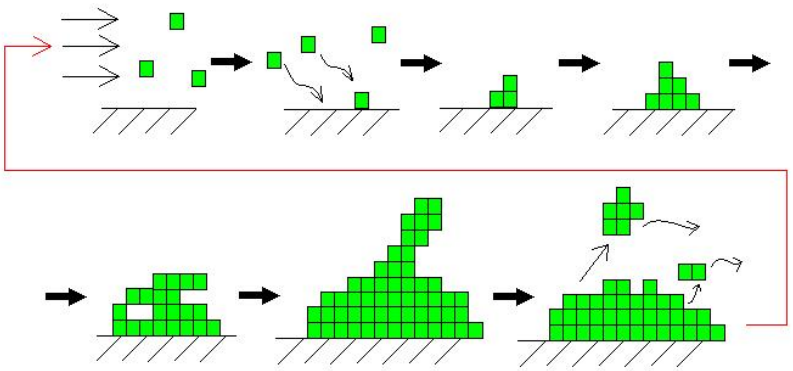

Figure 1. Schematic development of a biofilm.

The CA model will be developed in a 2D grid (see [7,16] and references therein), corresponding to a longitudinal section of a rectangular pipe (x=length,z=height), see Figure 3. Grid tiles can be occupied by water, biomass or substratum material (three possible states), and have the size of one bacteria in order to consider them as individual units.

The pipe is filled with a mixture of water and nutrients that flows at a certain velocity. The Reynolds number (computed using the hydraulic diameter) will be used as a measurement of the shear stress produced by the flow. A biofilm seed is already attached to the bottom of the pipe at the beginning of each simulation.

The model will evolve with time, simulating a dynamic system where grid tiles change their state according to a set of sub-models which govern the metabolic activities associated to

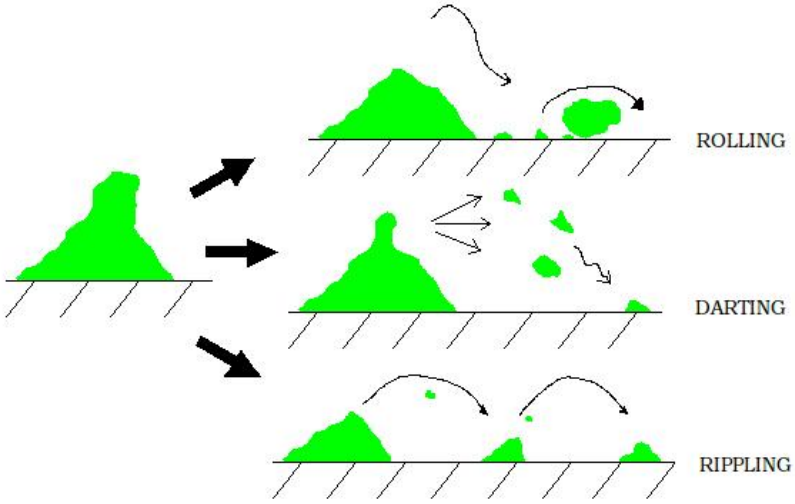

Figure 2. Examples of spreading mechanisms used by biofilms.

bacterial behaviour and external factors affecting biofilm cells: cellular division and spreading to neighboring tiles, generation of EPS matrix and biomass detachment. Each of these events will be assigned a probability that depends on the concentration of oxygen and substrate, the type of fluid flow or the number of neighboring cells containing biomass and their location. The final behavior of the system will also depend on the bacterial strain and its affinity to the nutrient source. The sub-models will cover the following aspects of biofilm formation and evolution:

- dynamics of dissolved components (nutrient, oxygen) outside the biofilm and inside. Reaction-diffusion equations govern concentration fields inside the domain, but a quasi-steady approximation is performed because diffusion and reaction processes of dissolved components are likely to be faster than biological processes rates [1].

- Biofilm erosion. Each biofilm cell has a probability to be carried by the flow depending on its location, the number and location of neighboring bacteria, the hydrodynamic shear stress and the cohesion of the biofilm. Biofilm fragments attached to the rest of the film by a few cells must also be considered.

- EPS matrix generation [22,24]. Each bacterium has a probability to produce EPS matrix depending on its location, the concentration of substrate and oxygen and the hydrodynamic shear stress. The cohesion of the biofilm will be enhaced by EPS formation, which will affect at the same time detachment processes.

- Reproduction and spreading. Each bacteria has a probability to reproduce depending on the availability of oxygen and nutrients [7]. New bacteria will fill neighboring empty tiles or shift existing bacteria with a certain probability.

We describe below our approach to incorporate the above mentioned mechanisms in the model.

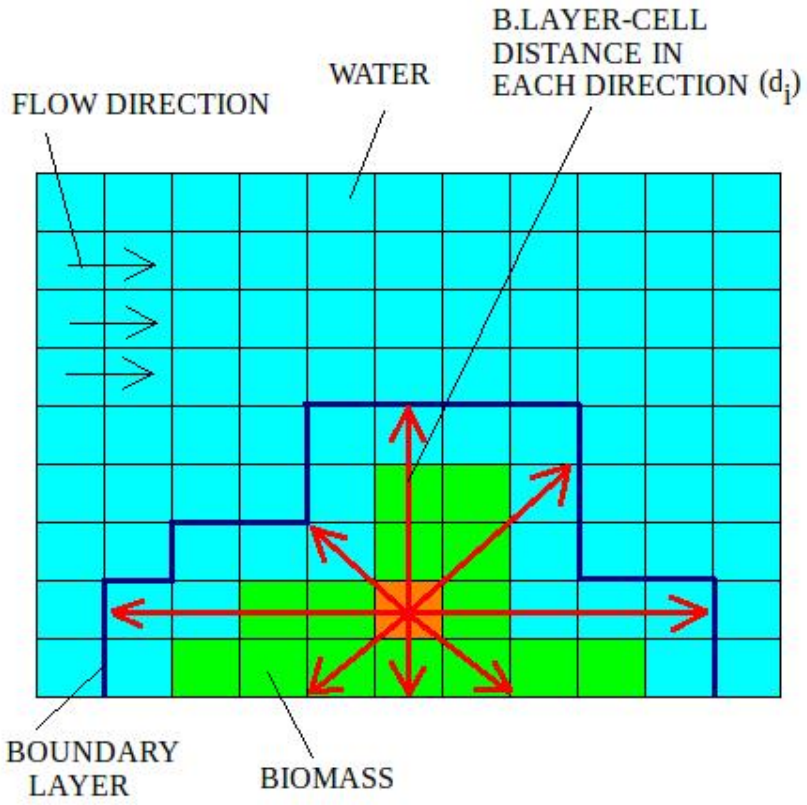

Figure 3. Geometry for the cellular automata description.

4. Implemented metabolic mechanisms

Our CA model defines certain rules for the basic metabolic activities of bacteria. As said in the previous section, the bacterial behaviours taken into account are: biofilm surface erosion, limiting concentration mass transfer, cellular division, spreading and generation of EPS matrix.

4.1. Dynamics of concentration fields

The evolution of a biofilm depends on the availability of carbon sources and oxygen, which will be governed by how concentration fields change in both phases. One of them will become the limiting concentration, that is, the one that penetrates less deeper into the biofilm. In the fluid phase, the concentration field is governed by convection-diffusion equations coupled to the fluid. Inside the biofilm, we have a reaction-diffusion equation. The model is completed with boundary conditions at the walls of the pipe and the fluid/biofilm interface. As said previously, diffusion and reaction rates are faster than biological processes rates [1], allowing to simplify the complete set of diffusion-convection-reaction equations to a quasi-steady approximation. In experiments, the concentration is usually kept almost constant within the fluid. We approximate the solution of the outer convection-diffusion equations by a constant outside a boundary layer of thickness d_B. In practice, this thickness is controlled by the velocity of the flow. In our framework, it becomes a model parameter, following [7]. Choosing the concentration value at the bulk/boundary layer interface C as control parame-

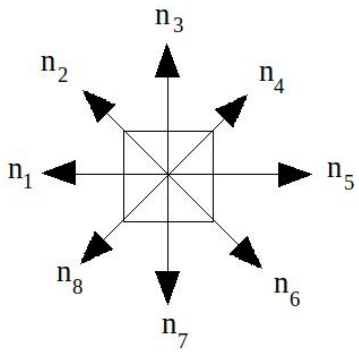

Figure 4. Neighbor location in the cellular automata description.

ters, the concentrations inside the region containing the biofilm and the boundary layer are governed by:

$$-D\Delta^2 c = k\frac{c}{c+K}, \tag{1}$$

with zero flux conditions at the substratum. The diffusion constants are assumed to be the same for the biomass and the boundary layer. The right hand side in the equation represents the nutrient uptake kinetics. Here, k is the uptake rate for the limiting concentration and K its Monod half-saturation coefficient. The values of all these parameters depend on the bacteria species forming the biofilm.

Performing a zero-order uptake kinetics approximation (the right hand side is replaced by just a constant k) an explicit approximation to the solution is found [7]:

$$c(cell) = \left(C^{1/2} - \sqrt{\frac{k}{2D}\left[\frac{1}{8}\sum_{i=1}^{8}\frac{1}{d_i(cell)^2}\right]^{-1}} \right)^2, \tag{2}$$

where $d_i(cell)$ are distances between the cell and the bulk/boundary layer interface in the directions joining the cell with its eight neighbors.

4.2. Erosion and detachment

Surface cells are exposed to shear forces exerted by the flow, straining them and finally leading them to fracture into small pieces of biofilm which will be carried away by the flow. Cells covered by other cells are assumed to be protected from erosion. Exposed cells will detach with a probability which depends on the number and location of their neighbors relative to the motion of the fluid, the biofilm cohesion (which is controlled by EPS matrix generation) and the hydrodynamic shear stress (which is a function of the Reynolds number and the position of the cell).

The hydrodynamic stress on a cell acts in the x direction (see Figure 3). In each cell, a balance between this stress and mechanical support against the flow given by its neighbours will be performed. This mechanical support will depend on factors like the number

of neighbours or their specific spatial distribution around the considered cell. Notice that each neighbour will contribute with a specific support. Let us number the eight neighboring tiles around the considered biomass tile clockwise, starting with the western direction, so that $n_1, n_2, n_3, n_4, n_5, n_6, n_7, n_8$ denote the neighbors located to the west, northwest, north, northeast, east, southeast, south, and southwest, respectively. Figure 4 illustrates the location of possible neighbours. There are several considerations about them: If cell n_1 is occupied, considered cell is unlikely to be carried away. The strongest support against the flow is exerted by the neighbor in position n_5. Next in magnitude are positions n_4 and n_6, and next n_3, n_7. Neighbors n_2, n_8 add a small contribution.

A dimensionless normalized parameter representing the total force on each cell ranging from zero to one is introduced:

$$\tau(cell) = R(Re)(1 - \chi_1(cell))(1 - \sum_{i=2}^{8} e_i \chi_i(cell)). \tag{3}$$

$R(Re) \in (0, 1)$ is an increasing function of the Reynolds number. The functions $\chi_i(cell)$ take the value one whenever the cell has a neighbor located at the position n_i, and vanish otherwise. The weights and other parameters have been chosen taking into account that the fluid flows in the x direction and satisfy $e_i \in (0, 1)$, $\sum_{i=2}^{8} e_i = 1$. They represent the added resistance against the flow due to neighboring cells depending on their position.

A probability law for cell erosion can be defined following [7]:

$$P_e(cell) = \frac{1}{1 + \frac{\sigma(cell)}{\tau(cell)}}. \tag{4}$$

Whenever $\tau(cell) = 0$, we set $P_e(cell) = 0$. Here, $\tau(cell)$ is given by (3), and $\sigma(cell)$ represents the biofilm strength. In practice, the cohesion of a biofilm is governed by the generation of EPS matrix. A cohesion parameter $\sigma(cell)$ varying in accordance with the local EPS generation production is introduced in Section 4.3.

At each time step and for each cell, we generate a random number $r \in (0, 1)$. When $r < P_e(cell)$, the cell detaches from the biofilm. Erosion due to the flow may occur as detachment of single cells or of whole fragments of biofilm, depending on the geometry.

4.3. EPS matrix generation

In small colonies, bacteria try to reproduce at the maximum possible rate by consuming all available resources in the environment. As the size and thickness of the biofilm grow, nutrients and oxygen become scarce inside due to the high bacterial population. A strategy developed by bacteria to keep a constant supply of nutrients and oxygen is to invest part of their energy on generating EPS matrix, allowing the bacterial colony to expand their mass transfer surface with the environment by growing mainly in vertical direction. This grants an easier access to nutrients and oxygen supply [22,24]. In our model, each cell has a probability to produce EPS matrix [11], see Figure 1. The EPS matrix also spreads over the neighbouring bacteria making their reproduction harder. As bacteria fall deeper in the biofilm, their chances to produce EPS increase.

EPS generation is also affected by the shear stress acting on biofilm surface. High shear forces will lead to stronger EPS matrix structures. Biofilms grown at low Reynolds numbers tend to be carried away with the flow as the Reynolds number is increased. However, biofilms grown at large Reynolds numbers in turbulent regimes are difficult to detach from surfaces, and expand easily when hydrodynamic conditions become less agressive with biofilm surface or the availability of carbon improves (switching to a richer source or increasing the nutrient concentration) [3].

In our model, EPS matrix production has a probability of happening that depends on the availability of nutrients and oxygen at the cell position and the shear exerted by the flow. The EPS matrix is generated with the following probability law:

$$P_{eps}(cell) = R(Re)\left(1 - \frac{c(cell)}{c(cell) + K}\right), \tag{5}$$

where c represents the limiting concentration (nutrient or oxygen) computed as described in Section 4.1. The parameter $R(Re) \in (0, 1)$ has been introduced in section 4.2.

At each time step and for each cell, a random number $r \in (0, 1)$ is generated: If $r < P_{eps}(cell)$, the cell will generate EPS instead of reproducing, as it requires less external chemicals and energy than reproduction.

The amount of EPS matrix produced controls the cohesion (strength) of the biofilm. We propose a local measure σ of the biofilm cohesion which takes into account the number of neighbors and whether they generate EPS matrix or not:

$$\sigma(cell) = \frac{\sigma_0}{8}\sum_{i=1}^{8}\sigma_i(cell), \tag{6}$$

where

$$\sigma_i(cell) = \begin{cases} 0 & \text{if cell } n_i \text{ is not present} \\ \alpha & \text{if cell } n_i \text{ is present, but does} \\ & \text{not produce EPS matrix} \\ 1 & \text{if cell } n_i \text{ produces EPS matrix} \end{cases} \tag{7}$$

and $n_1(cell)$, $n_2(cell)$,...,$n_8(cell)$ denote the eight neighbour locations for the cell under study (see Figure 4) and $\sigma_0, \alpha \in (0, 1)$. These parameters represent the strength of the EPS matrix generated by the bacteria and the strength of the attachment between standard bacteria. We have selected $\sigma_0 = 1$, $\alpha = \frac{1}{2}$ in our computer experiments, but they should be fitted according with the type of bacteria considered in the simulation, being necessary real experiments with selected bacteria. Cohesion increases with the Reynolds number, since the probability to generate EPS matrix is larger for large Reynolds number.

4.4. Reproduction

The cell reproduction mechanism is similar to that proposed in [7], but we consider that EPS producers do not participate in the reproduction stage. At each time step, and discarding cells producing EPS matrix, the remaining cells will divide with probability:

$$P_d(cell) = \frac{c(cell)}{c_l(cell) + K}, \tag{8}$$

where c denotes the limiting concentration and K its saturation coefficient in the Monod law. The evolution of the concentration is described in Section 4.1. We are neglecting changes in concentration due to newborn cell consumption or cell switching to EPS generation.

At each time step, and for each cell not generating EPS matrix, we compute a random number $r \in (0,1)$. If $r < P_d(cell)$, the cell will divide. Spreading of newborn cells is performed by considering neighbouring grid tiles: if some of them are empty the daughter cell is placed in any of them with equal probability. If reproducing cell is completely surrounded, newborn cell will shift the neighbour cell which offer the minimal mechanical resistance, considering this criteria as the minimum distance from the considered cell to the biofilm outer border.

4.5. Nondimensionalization and parameters

Once the model has been proposed, nondimensionalization of the variables is performed by identifying the minimum number of independent parameters and considering the different magnitude order between terms. Because of all probabilities and controlling parameters concerning the erosion and cohesion intensities are dimensionless, only concentration fields, length and time scale introduce dimensions in the model:

- Time: In the model, time is not given explicitly. It appears in the number of time steps carried out at each simulation. An upper bound for the time step, which allows to relate computational and experimental times is:

$$t = \frac{\ln(2)}{\mu_{max}},$$

  where μ_{max} is the growth rate.

- Length: The basic distance considered in the model is the size of a bacteria a, about 1 or 2 micrometers.

- Concentration: The concentration field is calculated using expression (2) which depends on distances. It involves a number of constants with their units that must be nondimensionalized. Making the changes of variables:

$$\hat{c}=\frac{c}{K},\ \hat{C}=\frac{C}{K},\ F=\frac{ka^2}{2DK},\ \delta_B=\frac{d_B}{a},\ \delta_i=\frac{d_i}{a},$$

  we get:

$$\hat{c} = \left[\hat{C}^{0.5} - \left(F\left[\frac{1}{8}\sum_{i=1}^{8}\frac{1}{\delta_i^2}\right]^{-1}\right)^{0.5}\right]^2, \tag{9}$$

  which gives a dimensionless expression for the concentration.

The four parameters a, k, D, K are reduced to one: F. The controlling parameters are therefore F, $\hat{C}$ and δ_B for the concentration, plus the additional parameters $R(Re)$, σ_0, α, that appear in the probability laws. Once a specific bacteria species is selected, σ_0, α are fixed. The remaining parameters depend on the type of nutrient and flow.

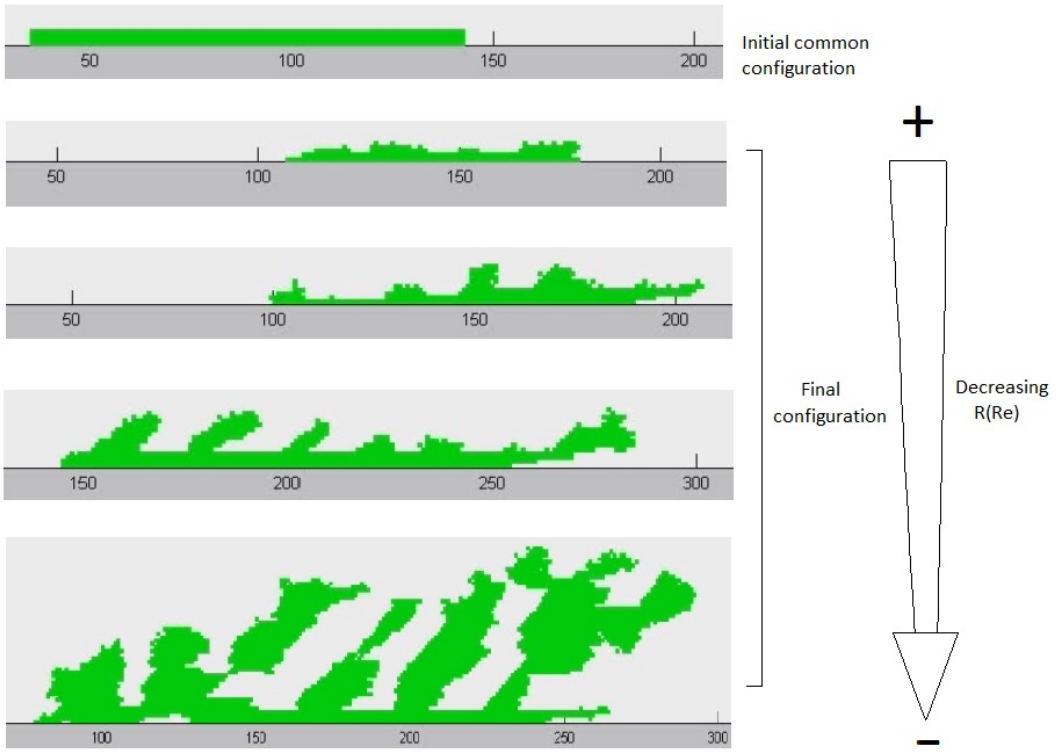

Figure 5. Differences in biofilm geometric patterns if different shear stress values are applied. The same initial conditions for all cases leads to different final morphologies.

5. Numerical results and discussion

In this section, we illustrate the evolution of several initial configurations under different conditions, fixing parameter values that actually do not correspond to any specific bacteria species, since a way to calibrate a number of parameters has yet to be devised.

Figure 5 shows the effect of Reynolds number in the biofilm spreading for two different cases. At low Reynolds numbers, biofilms generate tower-like structures. If the flow strength is increased, this configuration evolves into different shapes which are already reported in the experimental literature: streamers (typical shape in biofilms similar to flags moved by the wind), ripples and finally flat layers.

Figure 6 shows the effect of nutrient concentration in biofilm growth: greater quantities produce an increased growth rate of the biofilm even in high shear stress conditions, because the bacterial colonies sustain the needed growth rate to substitute eroded cells for newborn ones.

In Figure 7, a biofilm patch is eroded by the flow until is completely wiped out. It can be also seen that biofilm initial patch is dragged downstream along the x axis.

Figure 8 shows the effect of the EPS matrix in the biofilm structure. The higher cohesion produced by the effect of the EPS matrix promotes a bigger and more compact biofilm colony if we compare the evolution of the same initial seed using the same Reynolds number.

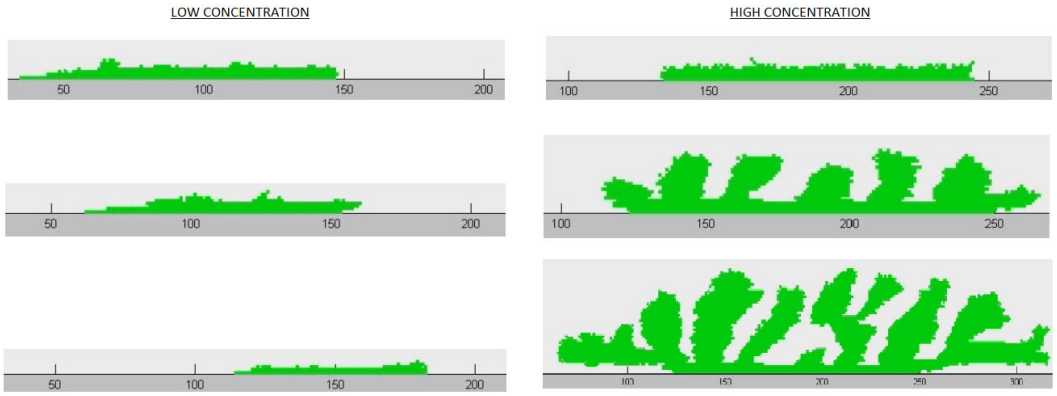

Figure 6. Evolution of a biofilm using a high shear stress flow but different concentration values. High concentration values produce significative growth even though the shear stress is large.

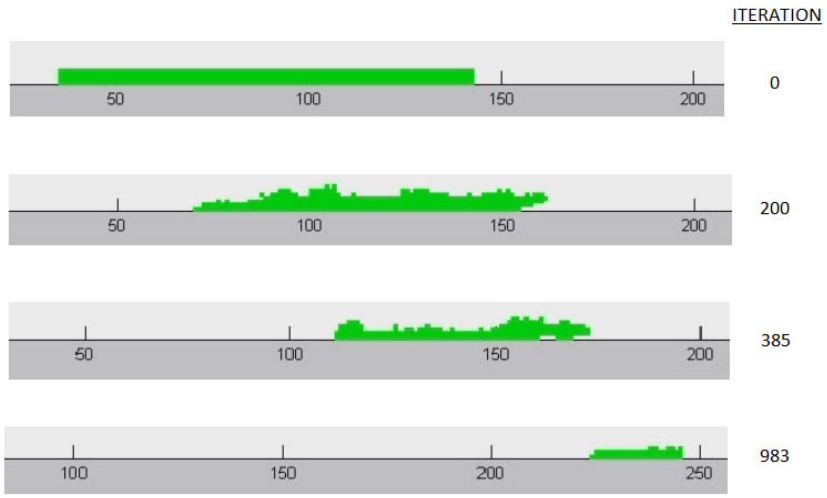

Figure 7. Into a high shear stress conditions, biofilm is dragged downstream and finally being wiped out.

6. Conclusions

We have presented a CA model that reproduces biofilm behavior and patterns experimentally observed. The model describes the evolution of an initial seed of biofilm attached to the surface inside a duct carrying a flow of nutrients and water. Space is discretized in a grid of square tiles. Each tile can have three states: water, biomass or surface. The rules describing numerically reproduction, spreading, EPS matrix generation, erosion and dynamic of dissolved components have been detailed. At each time step, each cell present in the domain has a chance to perform the different programmed metabolic activities, being the system completely stochastic.

The results of the simulations show that the strength of the flow shapes the biofilm structure, giving rise to different morphologies already known in experimental literature:

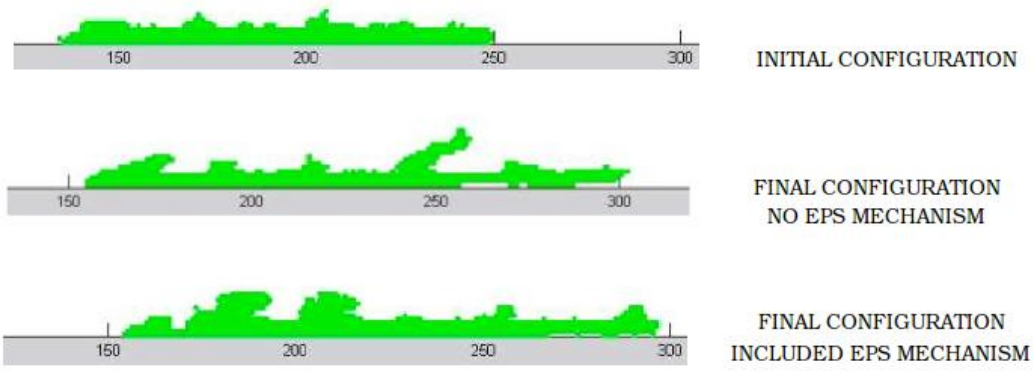

Figure 8. Effect of EPS generation mechanism. EPS promotes a more compact biofilm under the same initial conditions.

mushrooms, towers, streamers, ripples and flat layers. Among all these structures, flat films are specially important for industrial applications. They seem to be formed in regimes of low nutrients and high shear stress (high Reynolds number).

The concentration of nutrients also helps biofilms to grow faster even under high shear stress ambients: reproduction rates can substitute all the eroded cells by the flow increasing biofilm thickness. When the shear is reduced, biofilm growth explodes generating big structures like mushrooms or towers. On the other side, too poor nutrient ambients weaken biofilm structure and its size shrinks.

Acknowledgements

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