Project report UP-09
Simulation of interactions within a bacterial biofilm.

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1 Introduction

Since the existence of modern science, model building has been a fundamental goal. These models serve as essential tools for understanding complex phenomena, allowing us to dissect entangled systems and sometimes predict their behavior. In the context of microbial communities, biofilms stand out as captivating examples of collective microbial life. These biofilms, which consist of densely packed microorganisms adhering to surfaces, exhibit remarkable resilience and adaptability. They thrive in diverse environments, from natural ecosystems to industrial settings, and play pivotal roles in processes such as biodegradation, wastewater treatment, and medical infections [1].

Complexity of Biofilms

Biofilms are not simple assemblages of individual microbes; rather, they represent complex ecosystems with emergent properties. Within these microbial communities, complex interactions occur—ranging from cooperative symbiosis to competitive antagonism. The biofilm matrix, composed of extracellular polymeric substances (EPS), provides structural integrity and facilitates communication between resident microorganisms. This matrix acts as a protective shield, protecting inhabitants from external threats such as antibiotics, immune responses and environmental fluctuations. As a result, biofilms exhibit resilience against adverse conditions, making them difficult to eradicate [1],[2].

Microbial Interactions

Understanding the dynamics of microbial interactions within biofilms is crucial. These interactions can be cooperative, where microorganisms work together to exploit resources or improve survival. Conversely, they can be competitive, leading to competition for resources, predation or the production of inhibitory compounds. The balance between cooperative and competitive interactions shapes biofilm behavior, influencing critical aspects such as growth rates, spatial distribution and community stability. Deciphering these complex relationships is essential for developing effective biofilm management strategies.

Research Challenges

Traditional methods for studying biofilms have limitations. Experimental approaches, such as culture and microscopy, provide valuable insights but often lack scalability and fail to capture the full complexity of biofilm dynamics. Additionally, the heterogeneity of biofilms - both spatially and temporally - poses challenges for precise characterization. It is for these reasons that for several decades, a whole section of biofilm research has focused on the creation of models allowing the explanation of the formation but also of the spatio-temporal evolution of biofilms.

Biofilm modeling

The creation of biofilm models is complex since the very nature of these communities is complex and our knowledge about them, although growing, still leaves something to be desired. When constructing such modeling tools, a large number of phenomena can be taken into account: chemical phenomena, biological phenomena (growth, competition, cooperation, cellular movement) and physical ones (diffusion, structural impact).

These various phenomena are difficult to model in the case of biofilms because they are all largely interdependent. Indeed, the formation of a bacterial biofilm is highly dependent on the environmental conditions and the bacterial strain(s) present. The activity of each cell impacts the shape, state and evolution of the biofilm. Thus one of the most widespread approaches consists of the creation of a cellular automaton whose rules depend on laws approximating the phenomena that we wish to incorporate into the model. This approach is particularly suitable because by defining rules on a unit scale, numerous phenomena can emerge. In addition, it is relatively simple to implement, which makes it a very relevant and widely modular tool. It is this approach that I chose to pursue for this project.

2 Model and Methods

First of all I would like to remind you what a cellular automaton is. It is an algorithm in which each box of the space or grid used respects a set of rules which dictate its evolution. These rules are calculated from the neighborhood of each cell to be processed. The type of neighborhood used in the model is defined beforehand and two types of neighborhoods are classically used, namely the VonNeumman neighborhood or the Moore neighborhood, a representation of which is shown in [**Figure 1**].

I wanted to carry out this project on this subject because I was able to work on this theme on various occasions with Mr Romain Briandet and Virgile Guéneau from the B3D team at the Micalis Institute. More particularly, beyond modeling the formation of biofilms, the interest here is to build a model capable of accounting for interactions between bacteria of different strains. To do this, I relied heavily on the work presented in [3].

Model used

The previously cited article presents a cellular automaton method for simulating biofilm formation by incorporating nutrient diffusion and consumption, cell division and biomass sharing between neighboring cells. To model diffusion phenomena, [3] proposes Fick's Law, of which here is the partial differential equation followed by its numerical approximation:

$$\frac{\partial P_X}{\partial t} = D_X \left(\frac{\partial^2 P_X}{\partial x^2} + \frac{\partial^2 P_X}{\partial y^2} + \frac{\partial^2 P_X}{\partial z^2} \right)$$

$$\frac{P_X(i,j)_{t+\Delta t} - P_X(i,j)_t}{\Delta t} = D_X \left(\sum_{m=1}^{M} P_X(m)_t - M P_X(i,j)_t \right)$$

- P_X : Nutrient X concentration $[kg.m^{-3}]$
- D_X :Diffusion constant of nutrient X $[m^2.s^{-1}]$
- M : Total number of neighbors, m is the m-th neighbor.

For this model, the authors make the hypothesis that the part of the environment whose diffusion is simulated is far enough from the edges to simplify the estimation of diffusion in space-time. However, it is important to note that the simulations will be carried out here with a Neumman condition on all the borders of the simulation domain (zero flow on all borders which corresponds to real walls). This contradiction, however, has little impact on the results, which justifies why we keep these boundary conditions. Regarding the phenomena of cell growth and consumption, these are modeled by the Monod equation which accounts

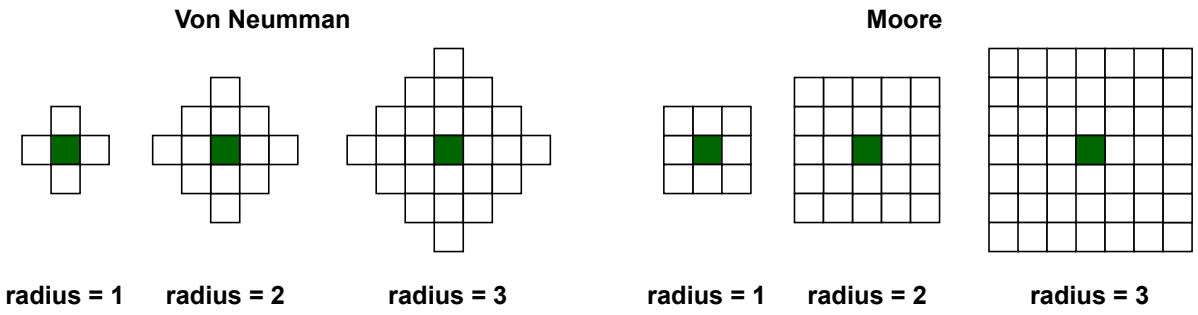

Figure 1: Classic types of neighborhood. Figure created using draw.io software.

for bacterial growth in an environment where the substrate is limiting. In the original article, an extension of this model is chosen to take into account the impact of several substrates when calculating the specific growth rate:

$$\gamma_o[i,j] = \frac{P_O[i,j]}{(K_O + P_O[i,j])}$$

$$\mu_{o,n,c}[i,j] = \mu_{\text{max,souche}} \cdot \gamma_o[i,j] \cdot \gamma_n[i,j] \cdot \gamma_c[i,j]$$

- μ_{max} : Maximum specific growth rate $[h^{-1}]$
- K_X :Half saturation constant of nutrient X. $[kg.m^{-3}]$

Thanks to this model, [3] provides a model capable of accounting for the link between the structure of the biofilm and the nutrient concentrations of the environment, which makes it a solid foundation for the construction of a new model. However, the work in this article did not take into account cell death, an essential component of biofilms since the presence of dead cells can serve both as a support but also as a protective barrier [4],[5] . Furthermore, the objective here being to add interactions within the model, the addition of death conditions would make it possible to account for a certain type of interactions, namely, bacterial antagonism.

Indeed, biofilms secrete a set of molecules including, for example, toxins, a means of combating macro-colonies in situations of nutritional or spatial competition. Nutrients being limiting, space being finite, all conditions are met for both types of competition to take place. Thus the secretion of toxins by the different strains makes it possible to model this aspect of bacterial interactions. So the model that I present here incorporates toxin production, the equation of which is:

$$[Toxin]_{\text{cellule}_i} = \frac{n^2_{\text{other}} \cdot \alpha_{\text{cellule}_i} \cdot 1}{\exp(n_{\text{same}} + 1)}$$

- $[Toxin]_{\text{cell}_i}$: Toxin production from the cell $[kg.m^{-3}]$
- n_{other} : Number of living cells of foreign strains in the vicinity of the cell
- n_{same} : Number of living cells in the vicinity being of the same strain as the cell
- α_{cell_i} : Toxin production rate $[kg.m^{-3}.h^{-1}]$

(The multiplication by 1 is explicitly shown here to specify that we are multiplying the hourly production by a duration of one hour, duration corresponding to the time unit of this project.) The equation that I propose aims to account for the production of toxin (parameter α_{cell_i}) but also to condition this production. Indeed, the bacteria will only produce toxins if there is a foreign

strain in its vicinity ($n_{\text{other}} > 0$), this coefficient is squared to accentuate the importance of the presence of these foreign bacteria and to introduce non-linearity in the response of the bacteria to this foreign presence. This is weighted by the exponential of the number of neighboring cells of the same strain as the cell concerned plus 1 to count the cell itself. The effect of this denominator is to limit individual toxin production when the bacteria is surrounded by cells from its population.

In fact this was chosen because within biofilms, thanks to quorum sensing among other things, the cells are 'aware' of their environment, the production of toxin being a significant metabolic effort, reducing individual production when it is in group saves resources individually while ensuring high production by the entire cluster. Furthermore, to account for the fact that this toxin production corresponds to a significant metabolic effort, I propose to link the growth rate to toxin production. Here is the new writing:

$$\mu[i,j] = \mu_{o,n,c}[i,j] \exp(-\omega_{\text{strain}}[Toxin]_{\text{cell}_i})$$

- ω_{strain} : Toxin production penalty coefficient. Set to 1 by default.

With this model, the more toxin the cell produces, the more its growth rate decreases. The quantities produced individually being absolutely small, $\exp(-\omega_{\text{strain}}[Toxin]_{\text{cell}_i})$ is close to 1. The parameter ω_{strain}, depending on the strain, allows us to translate to what extent the production of toxin impacts the growth of the strain; the higher the latter, the more a high production will reduce the rate. of growth. As you can see, these additions greatly enrich the model by taking into account crucial phenomena in the life of biofilms. The power of this model is also to be able to simulate complex relationships between bacterial strains with varied characteristics. More precisely, here is the current exhaustive list of parameters allowing the definition of a strain:

- μ_X : maximum specific consumption of nutrient X $[h^{-1}]$
- μ_{max} : maximum specific growth rate $[h^{-1}]$
- mobility : proportion of mobile bacteria
- O_status : affinity for oxygen (0 for anaerobic and 1 for aerobic)
- $X_{threshold}$: Threshold concentration for death for nutrient or toxin $[kg.m^{-3}]$
- alpha_TOX : toxin production rate $[kg.m^{-3}.h^{-1}]$
- tox_prod_penalty : ω_{strain}
- tox_+_id : Type of toxin produced by the strain
- tox_-_id : Type of toxin that inhibits this strain
- C_{div} : minimum biomass needed for a cell to divide $[kg]$
- Shared_portion : Proportion of biomass to share if division and movement are impossible

Now that we have defined cell growth, toxin production, and strain parameters, let's define nutrient consumption. [3] provides a way to calculate bacterial consumption using the following equation for a nutrient X:

$$J_X[i,j] = \mu_X[i,j] \cdot \frac{P_X[i,j]}{(K_X + P_X[i,j])}$$

- $J_X[i,j]$: Consumption rate (h^{-1}) of nutrient X.

This equation makes it possible to establish a link between the consumption of the cells and the current levels of surrounding nutrients, which is indeed important since when the concentrations decrease substantially, the bacterial cells adapt their consumption. This allows them to survive for a certain time in sometimes unfavorable conditions until environmental conditions improve or, on the contrary, until their death. It is important to note that in the original article, the authors chose to multiply the nitrogen and carbon consumption

rate by the Monod coefficient for oxygen. They justify this choice by arguing that almost all biological processes involve oxygen in one form or another. Although this hypothesis is interesting, I decided not to apply the oxygen coefficient for nitrogen and carbon.

I felt it was better to use the default Monod equation for numerical reasons. Indeed, multiplication by the oxygen coefficient makes consumption and, to some extent, growth, much lower, but, as we will see later, de novo growth of cells is already difficult to achieve, so not doing this multiplication has little impact in terms of modeling but also in practice but will still make de novo growth more frequent.

The last point to address regarding the constructed model corresponds to the concrete definition of bacterial growth. Calculating the growth rate is one thing, but using it to change the biomass is another. Once again my thinking started from the work of [3], in the latter, the biomass is updated in the following way:

$$Biomasse[i,j]_t = Biomasse[i,j]_{t-1} \cdot (\mu[i,j]_t + 1) \cdot 1$$

This model should be interpreted as an accumulation of biomass over a period of one hour which corresponds to $Biomass[i,j]_{t-1} \cdot \mu[i,j]_t$. We will talk about biomass concentration ($kg.m^{-3}$). However, this will not be the model adopted here. Indeed the defect observed by this model is the following: when using realistic values for the simulation, in particular for the initialization of the biomass (a bacterium has an average mass of $1 * 10^{-12} kg$) but also regarding the nutrient concentrations in $kg.m^{-3}$, the growth rate μ is obviously very low. Multiplying the already very small biomass value by this growth rate results in a truly tiny accumulation of biomass, so close to 0 that the new biomass value is essentially unchanged from the first.

Beyond that, it must be remembered that with each division, the biomass is divided by two: the effect of a term of accumulation of tiny biomass coupled with conservation of the biomass during the division leads to an impossibility of observe de novo growth (i.e. growth due to accumulation and not simply by the initial biomass value) when realistic values are used. To overcome this problem I thought about another way to see bacterial growth. Every process in the world is subject to the conservation equation, with processes allowing the growth of a living being as well. Every living being uses energy and matter to grow, so the consumption of nutrients by a bacteria can be seen as the source of matter that it can use for its growth. There is still a link between the current size of an individual and their growth, a link exploited in the model cited above. Taking all of this into consideration, ultimately here is the growth model:

$$\beta_O[i,j]_t = J_O[i,j]_t \cdot P_{O,diff}[i,j]_t \cdot 1$$

$$\kappa[i,j]_t = \beta_O[i,j]_t + \beta_N[i,j]_t + \beta_C[i,j]_t$$

$$B[i,j]_t = B[i,j]_{t-1} + \mu[i,j]_t \cdot (\kappa[i,j]_t + B[i,j]_{t-1}) \cdot 1$$

Where :

- $\beta_X[i,j]_t$ is the consumption of nutrient X by the cell (i,j) at time t expressed in $[kg.m^{-3}]$
- $B[i,j]_t$ is the biomass concentration corresponding to the cell (i,j) at time t expressed in $[kg.m^{-3}]$

Finally, based on the equations provided in [3] and those added, we can now define our update system as follows:

$$\begin{cases} P_O[i,j]_t = P_{O,diff}[i,j]_t - J_O[i,j]_t P_{O,diff}[i,j]_t \cdot 1 \\ P_C[i,j]_t = P_{C,diff}[i,j]_t - J_C[i,j]_t P_{C,diff}[i,j]_t \cdot 1 \\ P_N[i,j]_t = P_{N,diff}[i,j]_t - J_N[i,j]_t P_{N,diff}[i,j]_t \cdot 1 \\ Toxin[i,j]_t = Toxin[i,j]_{t-1} + Toxin_{prod}[i,j]_t \\ B[i,j]_t = B[i,j]_{t-1} + \mu[i,j]_t (\kappa[i,j]_t + B[i,j]_{t-1}) \cdot 1 \end{cases} \quad (1)$$

Other features

With this set of equations governing the environment and biomass evolution, I decided to add more complexity to the model with respect to biological

processes. Division and sharing of resources was already present in [3] and I already mentioned adding death conditions in the simulation. However, there is one last phenomenon that I have not yet addressed: cellular movement. In biofilms, it has been shown that some bacteria are still mobile within the biofilm formation [6], [7]. It depends on the bacterial community forming the biofilm and environmental conditions. It has also been observed that during the life cycle of a biofilm, bacterial cells can be ejected from the original biofilm. So I added the possibility for each cell to move and the possibility of creating strains with different mobility (the "mobility" parameter introduced earlier).

Thus, cell movement will be possible in the vicinity of the bacteria. In one iteration, and therefore after one hour, the bacteria will be able to move a maximum of r microns, r being the neighborhood radius. These speeds correspond well to the order of magnitude that has already been observed in certain [6] biofilms. However, it is necessary to realize that this motility is highly strain specific and could therefore vary widely depending on the bacteria considered.

Since reproduction is the primary goal of any living thing, I decided that reproduction would be the first event considered when processing cells. Thus, only if a cell cannot divide will it attempt to move into its neighborhood.
I also added the ability for dead matter to decompose (if the corresponding parameter is set to True, dead matter will eventually disappear), the ability to initialize the environment with non-homogeneous concentrations of nutrients and different initialization states namely 'floor', 'surface', 'on_cluster', 'separate' and 'random'.

3 Algorithm

To implement this biofilm simulator, I wrote a script in Python that defines a class called 'GRID' which contains all the necessary methods from initialization methods to update rules, plots and the execution function. The main difficulties encountered during the realization of this Python project were the optimization and visualization of the simulation due to very spread concentration scales and biomass values close to 0. Small tricks made it possible to solve most visualization problems. As for optimization, I tried to use vector operations with the numpy library as often as possible, which significantly improved the calculation time. However, the execution time remains slow depending on the simulation parameters chosen. Factors that greatly affect performance are:

- Grid size (especially for 3D simulations)
- Division threshold and initial biomass values: the smaller the division threshold compared to the initial biomass value, the longer the cells will be able to divide. If many cells survive each iteration, growth increases the number of cells exponentially, slowing down the calculation time.
- Neighborhood radius: users have the possibility to choose the parameter of the neighborhood radius, the larger this radius, the more costly each iteration is in calculation time due to the fact that the number of boxes associated with each cell is a non-binding function. -linear of the radius ($r^2 + (r+1)^2$ for the Von Neumman neighborhood and $2(r+2)^2$ for that of Moore).

However, this is mainly due to the nature of the project, since it is a cellular automaton with many rules based on equations, the number of operations does not adapt well to increases in scale due to neighborhood considerations and the different calculations carried out within all the simulated processes. A representation of the algorithm is presented in [**Figure 2**].

This algorithm begins by initializing all the grids useful for the current simulation, sets the initial concentrations then inoculates the first bacterial cells. After these initialization steps, for each iteration, the diffusion of environmental molecules

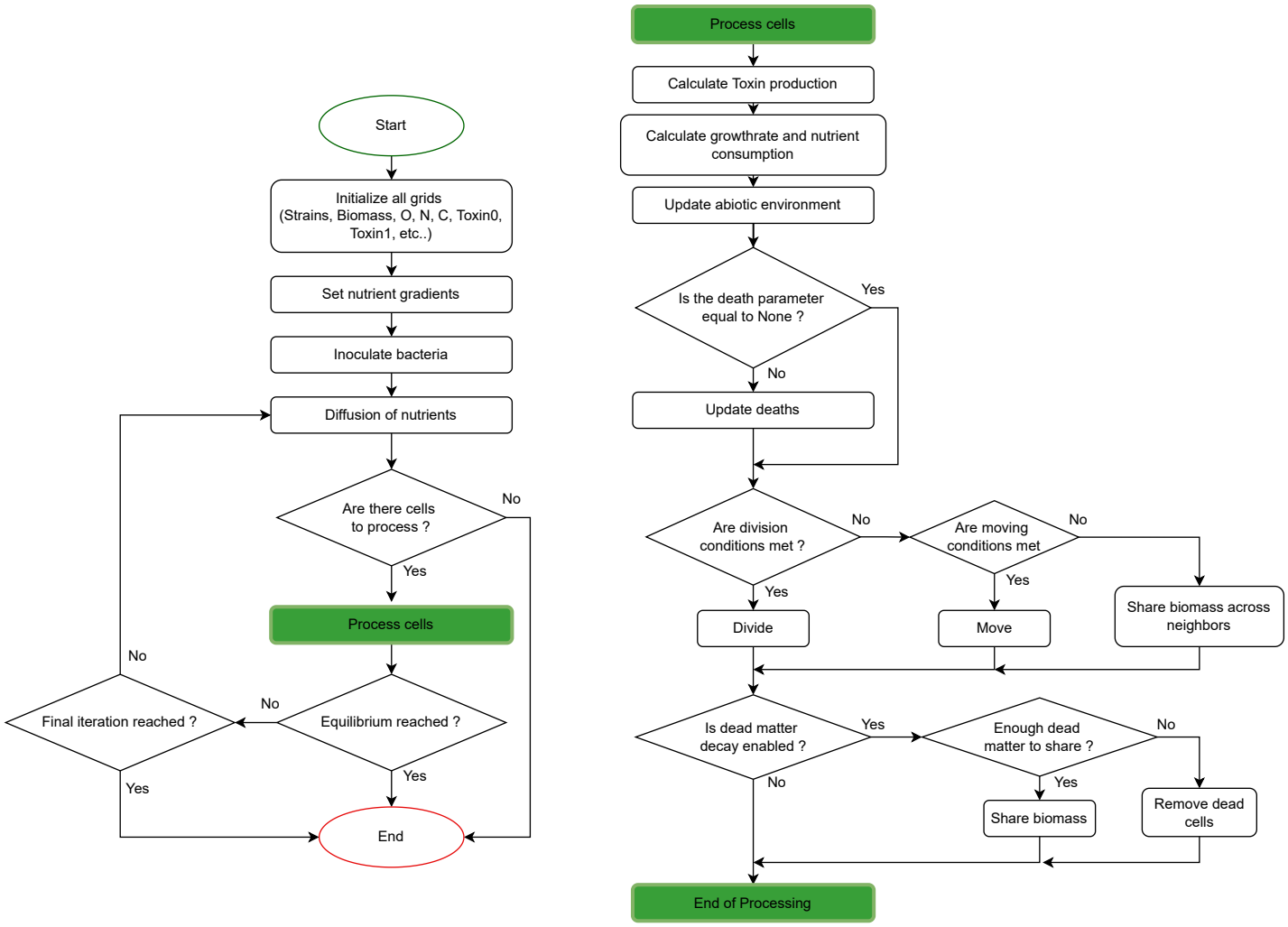

Figure 2: Algorithm. Figure created using draw.io software.

is calculated then, for each cell to be treated, we calculate the production of toxins, the consumption of nutrients and update the growth rate on the Based on the calculations carried out, we finally update the abiotic environment (concentrations of nutrients and toxins).
After that, we update deaths if possible, then we look at conditions for reproduction, movement and resource sharing. Deaths are updated before reproduction to account for the fact that in any population of living beings, only individuals living in sufficiently good conditions and whose fitness is sufficiently strong reproduce. Thus by calculating deaths before reproductions a form of selection is made, although it is very environment dependent and not very fitness dependent.

Finally, if dead matter decomposition is enabled, we update the dead matter states. This process is repeated the desired number of iterations, unless there are no more living cells to treat or the strain matrix no longer changes (equilibrium). Note that this equilibrium state with living cells still present can only be achieved if movement is disabled and under certain conditions. The model now known, the algorithm described, it is now time to observe the simulation results.

4 Numerical experiment

As I have already announced, the main interest of this project is to be able to simulate bacterial interactions. The possibility of defining the various characteristics of each strain makes it possible to represent different bacterial strains on many points. Thus, I wanted to take up one of the conditions experimented by [8]: the co-inoculation of *E.coli* and bacteria of the genus *Bacillus*. The aim of this experiment is to quantify the antagonistic or non-antagonistic effect of the presence of *Bacillus* bacteria on the development of the pathogenic *E.coli* biofilm. With the help of different sources cited below, I was able to find out in particular about the bacteria *B. velezensis*. So, let's imagine two strains: one of *E.coli*, not producing a "toxin" affecting *B.velezensis* and a second strain of *B.velezensis* producing a "toxin" affecting against *E.coli*. Here are some characteristics of the bacteria that we will put in the simulation:

- *E. coli* : Aerobic, Highest known maximum specific growth rate (3 h^{-1}), Very Mobile. [9], [10], [7].
- *B. velezensis* : Aerobic, High growth rate, Production of bacillunoic acid (considered here as a "toxin" secreted in the event of competition). [11], [12], [13], [14].

The parameterization of the two bacteria is presented in [**Table 1**]. As announced earlier, I assume here that the possible toxin secreted by *E.coli* does not affect *B.velezensis* and for this I set a zero toxin production for *E .coli* with respect to *B.velezensis*. This simplifying hypothesis is useful to further distinguish these two bacteria. Indeed as they are represented, *E.coli* will present extremely rapid growth, a considerable advantage in this finite space with limited resources, however *B.velezensis* will be able to use the production of " toxin" to try to win this competition. As a reference for this interaction I took the results presented by [8], although our strains of *E.coli* and *B.velezensis* are hypothetical and therefore do not correspond absolutely not to those used in these experiments, these results allow me to have a comparison with an example of a real mixed biofilm of these two bacteria. These results show after 24 hours a very weak antagonism between the bacteria *B. velezensis* and *E.coli* whose interaction score is 0.39 (score between 0 and 1, below 0.5 we can consider that there is no or very little antagonism). The iteration unit being the hour, we will simulate for 24 iterations in order to correspond to 24 hours. The result obtained is presented in [**Figure 3**].

First of all, the simulation parameters which made it possible to obtain its results are:

- Grid size: 160x160 (one box corresponding to

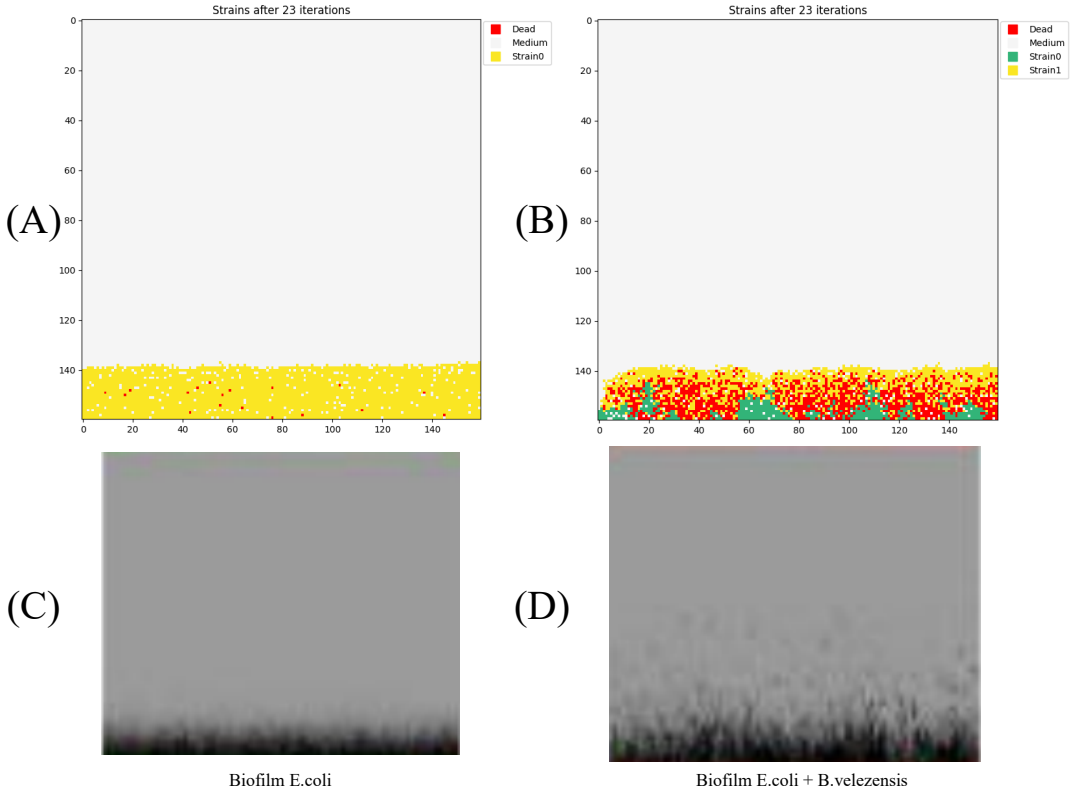

Figure 3: (A) Slice of the simulated *E.coli* biofilm (Strain0 in yellow) after 24 hours. (B) Slice of the simulated mixed biofilm of *E.coli* (Strain1, in yellow) and *B.velezensis* (Strain0, in green) after 24 h. (C) Slice of the *E.coli* biofilm after 24 hours from [8]. (D) Slice of the mixed biofilm of *E.coli* and *B.velezensis* after 24 hours from [8]. (C) and (D) were obtained by [8] using confocal microscopy and the BiofilmQ software allowing the reconstruction of the images obtained. These images correspond to squares with a side of 160 microns, the same size was used for the simulation.

Parameter	***B.velezensis***	***E.coli***
mu_O $[h^{-1}]$	0.3	0.3
mu_N $[h^{-1}]$	0.15	0.15
mu_C $[h^{-1}]$	1.5	1.5
mu_max $[h^{-1}]$	0.95	3
mobility	0.095	1
O_status	1	1
O_threshold $[kg.m^{-3}]$	2e-10	2e-10
N_threshold $[kg.m^{-3}]$	5e-7	5e-7
C_threshold $[kg.m^{-3}]$	0.2e-7	0.2e-7
alpha_TOX $[kg.m^{-3}.h^{-1}]$	0.8e-15	0
TOX_threshold $[kg.m^{-3}]$	7.1e-6	0.1e-13
tox_prod_penalty	1	1
tox_+_id	0	1
tox_-_id	1	0
Cdiv $[kg.m^{-3}]$	8.1e-13	8.1e-12
Shared_portion	0.5	0.5

Table 1: Parameters characterizing *B.velezensis* and *E.coli*.

a bacteria, this therefore corresponds to a grid of 160 μm side)

- Initial number of cells: 32 for each strain (20% of length)
- Initial Biomass : $1 * 10^{-12}$ $[kg.m^{-3}]$
- Inoculation: 'floor' with 'thickness' = 1 =¿ A cell carpet is inoculated on the bottom.
- Moore neighborhood with radius 1
- Toxin diffusion constant: [15] estimated the diffusion in the periplasm between 3 and 6 $\mu m^2.s^{-1}$. The periplasm being less concentrated in molecules than the cytoplasm, I hypothesize here that we can compare the diffusion of proteins and metabolites within the periplasm to that observed in the aquatic environment surrounding the biofilm. So $D_{tox} = 4.5 * 10^{-6} \mu m^2.s^{-1}$
- Initial nutrient values (in $[kg.m^{-3}]$) are $7*10^{-3}$ for Oxygen, $0.7 * 10^{-3}$ for Nitrogen , $25 * 10^{-3}$. Values used by [3]. A linear variation is applied from top to bottom for Oxygen (highest concentration at the top of the grid) and from bottom to top for Carbon and Nitrogen.
- All death conditions are activated, no renewal of dead matter.

By comparing the simulated biofilm of *E.coli* (**Figure3A**) with the image of the real biofilm of *E.coli* (**Figure3C**), we see that the latter is quite flat with little thickness and that these characteristics are also found with the simulated biofilm. Then by observing the simulated biofilms of *E.coli* and that of the two species mixed after 24 hours (**Figure3B**), we observe that the height of the two is similar and that *E.coli* developed well in both cases. However, we can clearly see the effect of the *B.velezensis* toxins, many *E.coli* bacteria are dead. This is expected given the parameters used. We can still see that this was not enough to have an antagonistic effect on the development of *E.coli* since it still manages to gain the upper hand and largely colonize the bottom of the grid. So I judge these results to be satisfactory in terms of modeling the interaction of interest since the comparison with the real images can be made quite accurately and the conclusions drawn from them are the same.

One of the perspectives of [8] is the use of a consortium of "positive" strains in order to stem the development of pathogenic biofilms. The first *B.velezensis* strain previously used in the simulation did not achieve this objective, but what about a combination of two *B.velezensis* strains? So let's add a strain *B.velezensis* which differs slightly from the previous one in that it has a slightly lower growth rate (let's set it at 0.85 h^{-1}), a production of toxin 2 times weaker than the first, but greater mobility (0.3). This strain will consume slightly more carbon due to its increased mobility ($\mu_C = 2\ h^{-1}$) and slightly less nitrogen for an arbitrary reason ($\mu_N = 0.1\ h^{-1}$). All other settings are the same. Thus, we will have two similar strains in the environment, but with slightly different characteristics which will produce a toxin impacting *E.coli*.

But before seeing the result by inoculating these 3 strains, let's see if the second strain of *B.velezensis* leads to the same conclusions or not as the first. The results of this new simulation are presented in [**Figure 4**].
In this figure, we can see that if we only observe the biofilm at 24 hours (**Figure4A**), we do not know if there is an antagonistic effect or not, the two strains seem to occupy the space quite well. This observation is supported by the analysis of the structure of the population over time up to 24 hours (**Figure4C**) where we can deduce that the 2 strains are neck and neck with superiority passages for both, however, at 24 hours, the dynamic clearly does not seem to have taken its final trend. By simulating the evolution of the biofilm over a longer period, we can finally conclude on the antagonistic effect or not of this second strain: whether on the state of the biofilm at 48 hours (**Figure4B**) or on the evolution of the structure of the population over time, we see that it is indeed the pathogen *E.coli* which emerges victorious in this competition, thus the second strain has also not succeeded in stemming the development of pathogenic biofilm. However, it should be noted that at 24 hours, the presence of this strain slows down the growth of the pathogenic biofilm more than the first strain of *B.velezensis* tested.

Only the *B.velezensis* strains fail to prevent the growth of *E.coli*, let us now look at the effect of co-inoculation of the 3 strains, results presented in [**Figure 5**].

We then observe a clear difference with respect to the development of *E.coli* with a much greater proportion of dead *E.coli* cells visible in the biofilm as well as a slightly lower thickness of biofilm compared to previous simulations. Thus we observe a synergistic effect of the association of the two strains which results in a real antagonism against *E.coli*.

Through this experiment, we can therefore see that the model is useful for trying to understand various interactions within microbial biofilms and is capable of accounting for phenomena observed in the study of biofilms. However, this simulator has a certain number of limitations that are important to highlight.

5 Discussion

Indeed, we can begin this criticism of the simulator with the fact that no implemented process really accounts for the production of exo polysaccharides (EPS) which is nevertheless necessary for the formation of any biofilm and being the source of many of the mechanical, chemical and biological resistance properties of biofilms. Our current model can be seen as completely ignoring the EPS or only considering the latter forms an infinitesimally small layer around the bacteria. In both cases, the problem remains the same: the properties of this EPS layer are neglected, preventing our model from accounting for certain key processes such as antibiotic resistance of cells in the center of the biofilm, because diffusion through the EPS layer is largely slowed down, the nature of certain structures such as goblet biofilms

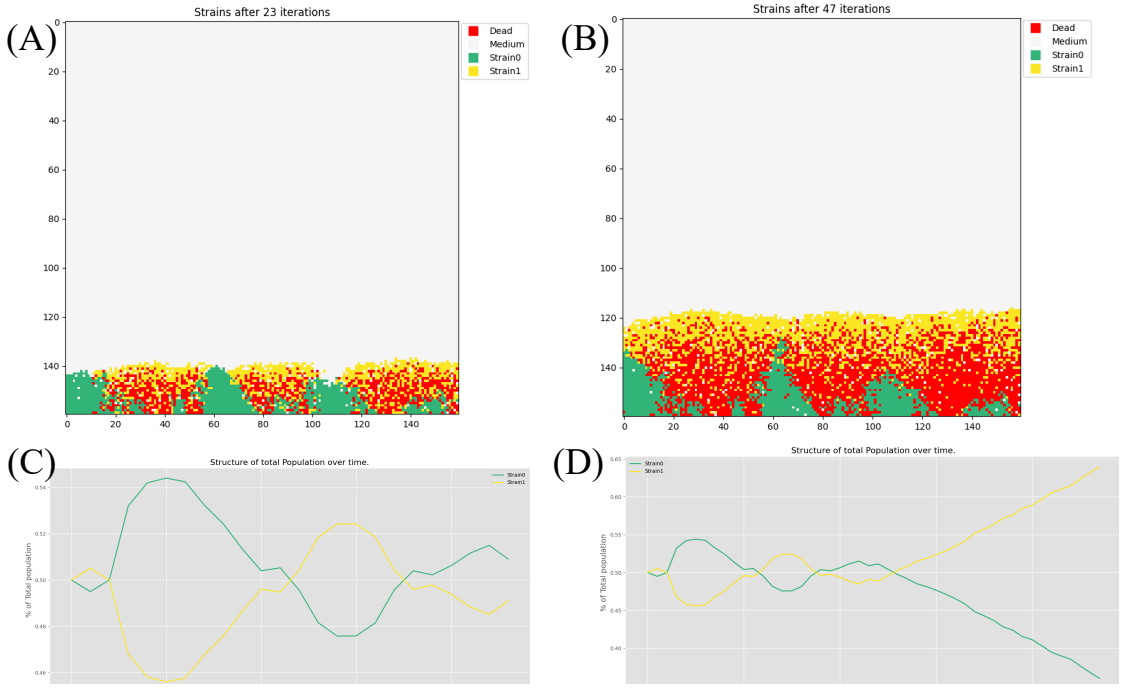

Figure 4: (A) Slice of the simulated mixed biofilm of *E.coli* (Strain1, in yellow) and the second strain of *B.velezensis* (Strain0, in green) after 24 hours. (B) State of the biofilm after 48 hours. (C) and (D) Structure of the total population over time up to 24 and 48 hours respectively.

or others which are partly possible thanks to the mechanical support that the EPS layer constitutes. Another widely studied phenomenon in bacterial biofilms corresponds to the possibility of expelling bacteria from the biofilm once the latter has reached an advanced stage of maturity in order to colonize another part of the environment. This phenomenon is crucial for many types of biofilm and is not reproducible with the constructed model.

Regarding the model itself, we can also criticize my choice of equation for toxin production, the link between toxin production and growth rate and finally the calculation of the biomass at each iteration. Indeed these equations are based on my vision of important phenomena and my way of translating them into equations, however they are absolutely not supported by data, both because we lack data for this type of phenomena in particular under the form of biofilm but also because my approach was not to adapt these equations to any series of data, making these choices very open to criticism. However, these equations still make it possible to account for the desired phenomena within the framework of this simulator, which is satisfactory for the modeling aspect for educational purposes and not for predictive purposes.

6 Conclusion

To conclude on this project, during these 2 weeks, I was able to build a biofilm simulator allowing the simulation of various phenomena such as spatial and nutritional competition, the formation of biofilm structures resembling some observed in culture, but also toxin production. Although it has a certain number of flaws, this model can prove interesting in trying to understand bacterial interactions within biofilms and the resulting population dynamics.

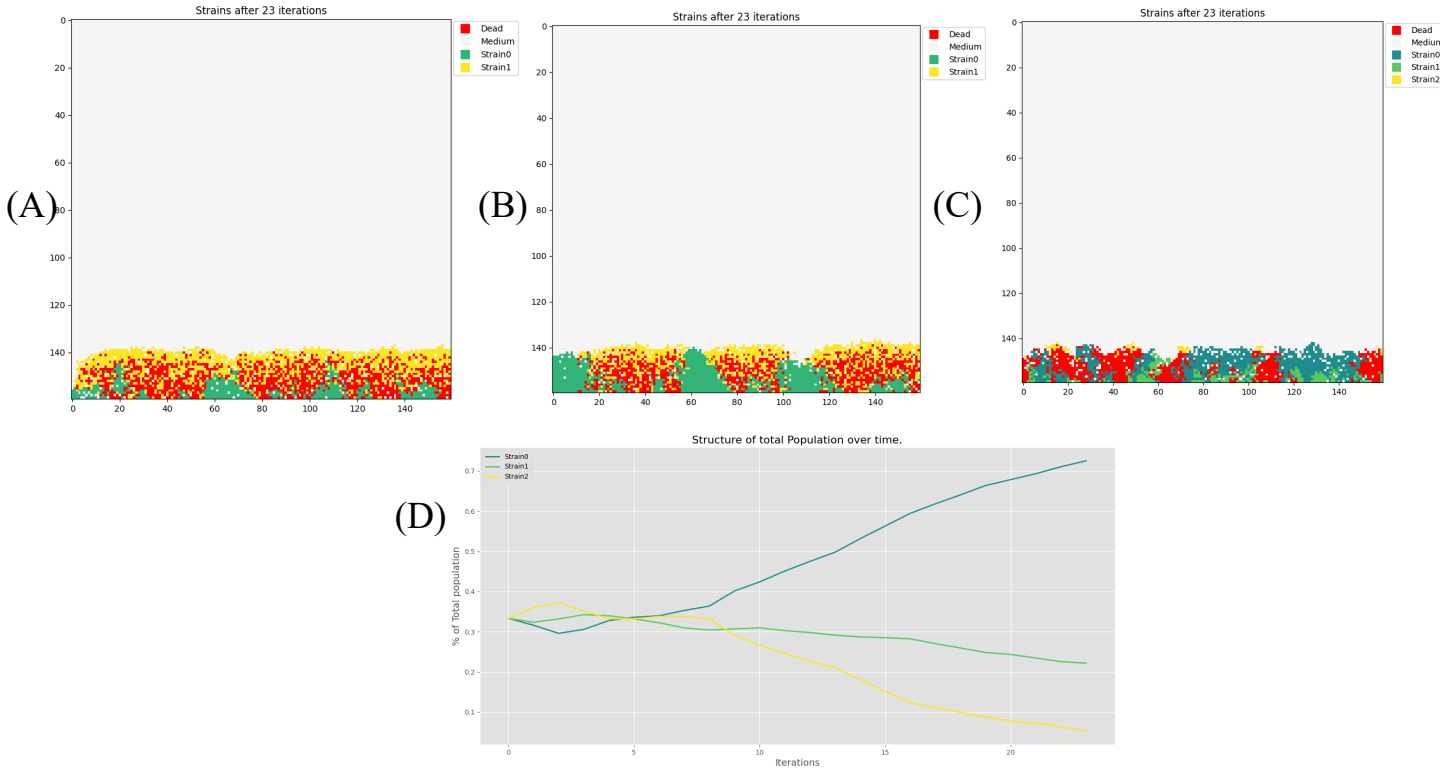

Figure 5: (A) Slice of the simulated mixed biofilm of *E.coli* (Strain1, in yellow) and the first strain *B.velezensis* (Strain0, in green) after 24 hours. (B) Slice of the simulated mixed biofilm of *E.coli* (Strain1, in yellow) and the second strain of *B.velezensis* (Strain0, in green) after 24 h. (C) Slice of the simulated mixed biofilm of *E.coli* (Strain2, in yellow), the 1st strain of *B.velezensis* (Strain1, in green) and the second (Strain0, in blue) after 24 hours. (D) Structure of the total population over time associated with the simulation that produced (C).

However, finding a set of parameters allowing consistent simulations can require some effort. This is why it is therefore advisable to think carefully about all the parameters, in particular to be aware that the model has been designed to use realistic numerical values and therefore very close to 0. 3D simulation is extremely expensive in terms of of operations and this results in an excessively long execution time. We could consider the use of multiprocessing to try to better manage the transition to 3D scaling, and even to better manage larger grid dimensions.
It is obvious that this simulator could be improved by taking into account the production of EPS, by incorporating equations accounting for other types of possible biofilm structures as well as the phenomena of colonization by ejection. In addition, the use of data-driven models for toxin production and growth in the form of biofilm will improve its reliability and perhaps even move this simulator to the predictive side. However, this would require much more time and considerable experimental effort to produce the data necessary for model validation. The source code for this project is available at the following address:
https://github.com/Aelrach/Biofilm-Simulator and whoever you are, if you are interested, do not hesitate to improve it :D!

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8 Bug

The only remaining bug that I'm aware of is an issue with saving toxin graphs. I have not been able to reproduce this bug in other conditions and it has never happened to me at other times. The error in question was: "ValueError: vmin must be less or equal to vmax" raised by the matplotlib module during saving. I haven't been able to find the origin of this bug, especially since during the simulation that produced it, previous iterations had no problems. It seems that this is due to an erroneous automatic setting of vmax and vmin within the matplotlib module when saving. However, in all other cases that I have been able to simulate with the current version, the code behaves correctly when saving Toxins. Due to lack of time, I was not able to explain this unique bug that it is therefore possible to encounter. If you ever encounter it as well, deactivate saving Toxins ('save_TOX' parameters), this will allow you to observe all the other components of the simulation without problems.

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