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Multi-scale modelling of bacterial communities.

Arturo Alvarez-Arenas Alcami.

Department of Computer Science

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Newcastle University

ABSTRACT

Motivation: The aim of this project is to get a full understanding of the behaviour of the bacterial communities and the biological processes which take place in a bioreactor during waste-water treatment. A multi-scale mathematical modelling connecting the different scales will be reproduced in this paper. This model encompass scales from single bacteria, to bacterial flocs, to the physical plants.

Concentrations of the soluble components, mass of the different type of cells and the shape of the floc are analysed and discussed in this paper.

Contact: a.alvarezarenas-alcami@newcastle.ac.uk

1 INTRODUCTION

Nowadays, working in waste-water techniques is of huge importance. These treatment systems are key nodes in the network of environmental services that civil engineers provide for the cities. Biological waste treatment is one of the core technologies in environmental engineering. In addition to the importance of cleaning the water, the understanding of the behaviour of the bacterial communities when they interact through competition and commensalism can be extrapolated to many other systems that occur in nature. For these reasons there is an ever increasing interest in studying said techniques.

To address such an important topic this paper will use computational and mathematical modelling. Biological systems are very complex, from how a single cells spreads to the interaction of many cells. Using mathematical and computational systems is a powerful approach to understanding and predicting the behaviour of the different biological systems (Meng *et al.*, 2004). Before applying these techniques to the biological systems, the experiments were previously done in a biological laboratory. For example, in order to calculate the reproduction of a type of cell under different concentrations, a new cell culture medium had to be prepared and scientists had to wait weeks until the cells spread. This process was very laborious, aswell as both money and time consuming. With the use of mathematical models, the process is much simpler. If it is possible to associate a mathematical model to the biological system from some data collected in laboratory experiments, it will be very easy to simulate that model under other conditions. In order to simulate it, a widespread technique is to implement that model with a computer. The advantages are clear: the process of simulating the model under different conditions is much easier, the costs are much less and the time spent is also significantly reduced (some computational programs are very time consuming). On the other hand, real laboratory experiments give exacts results, while its computational model gives only an approximation.

In a waste-water plant, there are many factors to take into account. In a bioreactor, there are different types of organisms which can grow or die depending on the concentrations of the nutrients in the water. Those organisms interact between each other, forming flocs, which are aggregates of different bacteria (Oilghux *et al.*, 2011). Some of them form also small clusters inside the floc. Initial concentrations of nutrients in the water, initial concentrations of bacterium, bioreactor size, properties of initial bacterium can result in very different systems after only a few days . Trying to simulate different environments to extract some conclusions will be very hard, expensive and time consuming, being almost impossible to reach a conclusion about how an initial amount of a nutrient is decisive in the final concentrations of the bacteria, or how the size of the bioreactor affects the system.

With this in mind, an ambitious and necessary approach is to create a mathematical model undertaking all the processes happening in the entire bioreactor, and a computational model able to reproduce and modify it easily. The creation of that model will be crucial in obtaining a full understanding of all events happening in a waste-water treatment plant, allowing us to determine which processes or concentrations are more or less decisive to the final results. There is not only one way to create a mathematical model of the entire systems. Delving into the literature it is possible to find different papers on modelling the process of waste-water treatment. Some of them use individual base modelling in planar microbial colonies(Kreft *et al.*, 1998), some of them use biofilms (Alpkvist *et al.*, 2006) (Kreft *et al.*, 2001) (Picioreanu *et al.*, 2004) while other papers focus the attention in a granular sludge.

The goal of this paper is to reproduce the theoretical model of a two dimensional activated sludge floc present in the paper (Ofieru *et al.*, 2014) and to get a full understanding of the complications and difficulties that this complex model may have. Given that it is impossible to simulate the entire bioreactor, two main scales are considered. How to connect the micro-scale and the macro-scale will be studied and analyzed. Small changes in the micro-scale will cause big changes at the macro-scale. The shape of the floc, and the concentrations of the biomass and the soluble components in the bioreactor will be studied and analysed. In addition, how the cells spread will be explained in detail in this paper.

2 METHODS

Simulating the entire bioreactor would be impossible in terms of time and computational costs. Therefore, a simulation connecting different scales is needed. In this model, we consider that the biomass in the bioreactor is formed only by flocs. Given that it is impossible to simulate all the flocs that are in the bioreactor, in the micro-scale model only one floc will be studied. The other flocs will not be simulated, and we assume that the changes in these flocs are the same as the floc studied. In addition, the number of flocs present in the bioreactor will be recalculated over time, in order to calculate the biomass and the concentrations of the soluble components in the bioreactor.

The scheme used in the mathematical model of the paper can be seen in figure 1. The bioreactor is supposed to be in the place where the flocs are being developed. Water with constant concentrations of soluble components is entering in the bioreactor through the inlet constantly. In addition, the purge is eliminating some biomass of the bioreactor according to the values solid dilution time (SDT) and hydraulic retention time (HRT). A change in those values will change the speed in which the biomass is being eliminated by the purge.

Flocs are considered as aggregates or clusters of microbes which are stuck together. Microbes need flocculating agents to be settled in flocs. These flocculating agents can be made and secreted in water by some microbes.

Figure 2 is an example of an easier scheme of the model, showing the two spatial-scales. In a big bioreactor there are many flocs. In order to follow the development of one generical floc, we consider a small aerea where that floc is being developed. That space is called the separator, but it is just a theoretical space, there is not a real separation in the bioreactor plant. A separator is a square of size L (chosen), in which just one floc is developed, starting in the center of the square. Therefore, in the entire bioreactor there are as many separator as number of flocs.

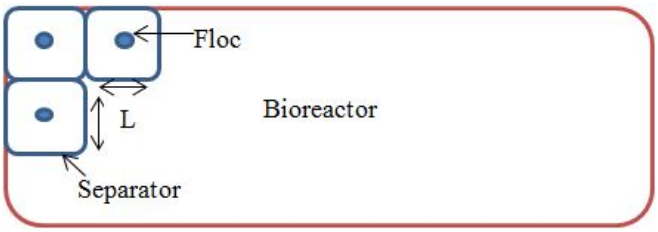

Fig. 2. Distribution of the flocs within the bioreactor

In addition to the two spatial scales, in this model there are three time scales:

- Fastest time-scale, the diffusion of soluble components and metabolic activities (seconds).
- Middle time-scale, the time that the bacterium takes to double (minutes to hours).
- Slowest time-scale, total time spent by a floc in the bioreactor (hours and days).

In this model, there are two main types of elements whose concentrations are studied.

- **Soluble components:** The soluble components are oxygen (O2), ammonia (NH4), nitrite (NO2), nitrate (NO3) and substrate (S). Their concentrations will be crucial to the way the microorganims grow and die.
- **Particulate components:** The particulate components are the microorganisms conforming the biomass in the bioreactor. In this model the microorganisms involved in the floc are heterotrophs (HET), ammonia oxidizers (AOB), and nitrite oxidizers (NOB). In addition, another particulate component, the extracellular polymeric substances (EPSs) which are excreted by heterotrophs, form part of this model. It is important to know that both AOB and NOB bacteria are always present in clusters of the same species.

The objective of this model is to calculate the concentrations in the bioreactor giving some initial values. Those values are:

Inputs of the model:

- Size of bioreactor and separator.
- Initial concentrations of soluble and particulate components in the floc.
- Initial concentrations of soluble and particulate components in the bioreactor.
- Other values: diffusion coefficientes, decay constants,kinetic parameters, yields,initial concentrations in the inlet. These values have been taken from different papers.

And with these inputs, we aim to get the following outputs.

Outputs of the model:

- Biomass in the bioreactor (concentrations of particulate components)
- Concentrations of soluble components in the bioreactor.
- Shape of the floc.

Knowing this, it is time to explain in detail both scales, the micro spatial-scale, which undertakes all the processes happening within the floc and the macro spatial-scale, which undertakes the processes happening in the bioreactor. How to connect both models will be also explained

Micro-scale model (the separator)

First of all, the separator is going to be divided in different theoretical regions. The separator is divided into three sub-domains in order to take into account the external and internal mass transfer resistances. In figure 3 it is possible to see both the floc Ω_f, the boundary layer Ω_b and the bulk liquid Ω_r. The boundary layer, which surrounds all the floc biomass, is considered as a circle whose centre is the centre of the separator.

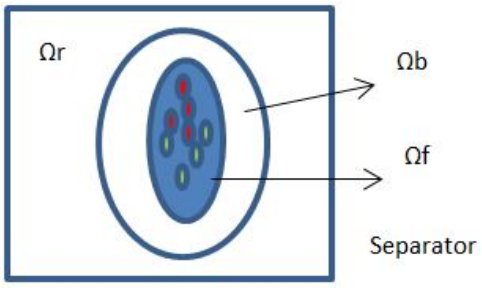

Fig. 3. Scheme of a separator

The aim of this micro-scale model is to calculate the development of one floc over time. In order to calculate the growth and the decay of the cells in the floc, it is necessary to calculate the concentration of the soluble components. Given its very quick timescale when compared to the other processes of the floc which are much slower, the soluble components are assumed to

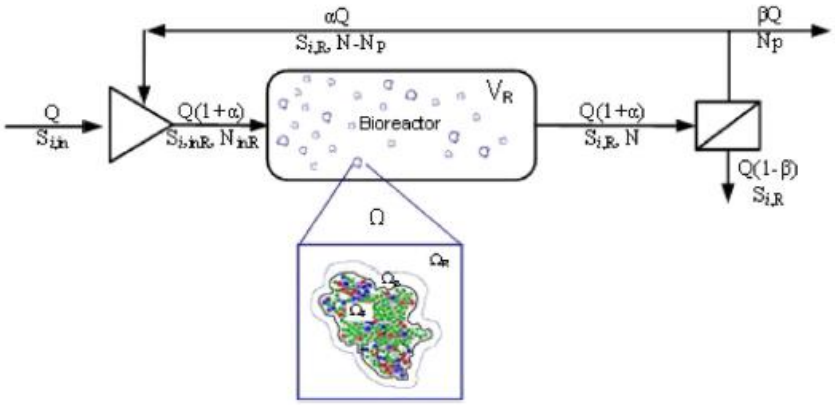

Fig. 1. The scheme of the reactor-separator system. Picture taken from the paper (Irina D.Ofiteru et al., 2013)

be in a pseudo-steady state. The local concentrations of each soluble component depend on its position and are calculated in a different way depending on the region.

- **In Ω_f:** The concentrations ($S_{i,F}$) are calculated solving the following systems of partial differential equations (Eq 1). Each equation represents one type of the soluble components. This partial differential equation representing a diffusion-reaction system is an elliptic equation, which describes a steady-state phenomenon, as explained above. In this equation, $r_i(x, y)$ is a function which is made up of the sum of all the rates of the processes in which our soluble component i is involved in the position (x,y).

$$D_i(\frac{\partial S_{i,F}}{\partial x^2}+\frac{\partial S_{i,F}}{\partial y^2})+r_i(x,y)=0 \quad i \epsilon \{O2, NH4, NO2, NO3, S\} \tag{1}$$

  D_i is the diffusion coefficient for each type of soluble component.
- **In Ω_b:** The local concentrations in the boundary layer are calculated with a similar equation, but in this region no reactions take place because there are no organisms, therefore the function $r_i(x, y) = 0 \quad \forall (x, y) \epsilon \Omega_b$.
- **In Ω_r:** It is assumed that the bulk liquid is perfectly mixed, so the concentrations of the soluble components in this region are the same as in the bulk liquid in the bioreactor.

After calculating the concentrations of soluble components in the separator the growth and decay of all cells can be calculated. Furthermore, other processes that occur in the bioreactor will be simulated, to get a more realistic shape of the floc, and a better approximation of the mass of the particulate components. These events can be grouped into two categories, the discrete processes and the continuous processes.

Discrete processes.

- **Attachment of a new cell.** This process simulates the attachment of a single cell floating in the bioreactor to the floc. Each τ_c period of time, an attachment of a new cell is done, where τ_c is the mean time between two attachments of one cell to the floc. As an attachment is a frequent event, in our simulations $\tau_c = 1$, which means that in each iteration a new cell is attached. The random process is carried out in the following way. First of all, a random angle is generated so a new position is established out of the circle that surrounds the floc (boundary layer). That position is attracted to the centre until it is close enough to the floc. In that position a new cell is generated with the following probabilities: HET(0.6), AOB(0.2), NOB(0.2). The code used is reproduced in Algorithm 1.

Algorithm 1: Attachment of a single cell to the main floc.

```
φ = random angle ∈ (0, 2π)
R-atta = maximum-radio +5 ∗ generic-radio
current-position = (φ ,R-atta)
while TRUE do
    R-atta = R-atta - generic-radio
    for cell ∈ floc do
        if distance(cell,current-position) ≤ 3∗cell-radio then
            final-position = current-position
            break If
            break While
        end
    end
end
```

- **Attachment of a group of cells.** This process aims to simulate the attachment of a group of cells or micro-floc from the bioreactor. This is the only way to attach new cell colonies of AOB and NOB to our floc. When a micro-floc(from the bioreactor) is attached to our floc, consequently the number of flocs in the bioreactor decreases, as will be explained in the macro-scale model. Each τ_{mf} period of time, in this model $\tau_{mf} = 2$, an attachment of a micro-floc is carried out. Firstly, a file with a group of cells from a directory is selected. That file is a simulation of the floc during 1.5 days. In addition, a random angle to attach both the main floc and the micro-floc is generated. That angle can be changed by up to ten degrees, in order to select the angle that gives the shortest distance between the two groups. The micro-floc is being attracted with that angle until it is close enough to the main floc. This event is implemented following the structure of Algorithm 2.
- **Detachment of a group of cells.** This event tries to emulate the separation of a part of the floc from the main floc. In theory, that group of cells detached from the floc will be released in the bioreactor. Therefore, when a detachment event occurs, the number of flocs in the bioreactor increases. Each τ_k period of time, in this model $\tau_k = 5$, a detachment of a micro-floc is carried out. As before, a random angle

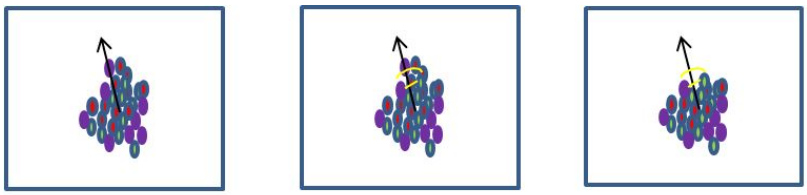

Fig. 4. Structure of a detachment process

is selected. That angle is modified by up to ten degrees and the angle with the maximum radium is selected. In addition, a random number between 0.5 and 1 is selected. All the cells that are in an interval of ten degrees with that angle and with a radio larger than the random number by the maximum radio are removed from the main floc. If some of the AOB and NOB cells that are outside of the region form part of a cluster, the entire cluster is removed, even if some parts of that cluster are inside the region. Figure 4 will help to understand this process.

- **Removing islands.** . This process tries to eliminate from the main floc some cells or small group of cells that may not be next to the floc. The processes of detachment of a group of cells and decay of cells (to be explained below) can generate islands within the floc. In order to remove them, the centre of the square is considered as the main island. For each cell, if the square 5x5 that surrounds the cell overlaps with the main island, that cell is considered part of the main island. This process is repeated until there are no changes, and all of the cells that are not in the main region are removed. Once those cells have been removed (released into the bioreactor) the number of flocs decreases slightly. This process follows the structure of Algorithm 3:

Algorithm 2: Attachment of a group of cells to the main floc.

```
Initialize radio-refer = 0
Continue = TRUE
while Continue do
    φ = random angle ∈ (0, 2π)
    for cell ∈ main floc do
        if |cell-angle - φ |≤ π/10 and cell-radio ≥ radio-refer
        then
            radio-refer = radio-cell
            Continue = FALSE
            closest-cell = cell
        end
    end
end
R = group-attach radio
while TRUE do
    new-center = (φ,R)
    (dxatt,dyatt) = new-center - previous-center
    floc-positions = floc-positions + (dxatt,dyatt)
    for cell ∈ group cells do
        if distance(cell,closest-cell)< 3 * radio closest-cell
        then
            positions group-attach = current-position
            break For
            break While
        end
    end
    R = R - radio closest-cell
end
positions-floc = positions-floc + positions group-attach
```

Algorithm 3: Removing islands.

```
// Initializing a matrix of the size of the
   separator whose elements are "nothing"
initialize M = matrix(size separator,"nothing")
// marking the position of the cells in the
   matrix
for cell ∈ floc do
    Matrix(cell-position) = "cell"
end
// marking the 6x6 center of the matrix as main
   island
M(6x6 center) = " main island"
// for each position, if the square 5x5 that
   surrounds that position intersects the main
   island, that position is considered part of
   the main island
for position ∈ matrix do
    if M(position) = "cell" and 5x5 neighbourhood position ∩
    main island ≠ ϕ then
        M(position) = "main island"
    end
end
// removing all the cells that are not in the
   main island
for cell ∈ floc do
    if M(cell-position)≠ "main island" then
        eliminate cell
    end
end
```

Continuous processes.

- **Decay of HET, AOB, NOB.** This process simulates the process of the decay of the cells, after this process some cells will be considered dead. First of all, the mass of the virtual dead cells is calculated solving the following equation. For each cell of each type,

$$\frac{dMassDecay_i}{dt} = RateDecay_i \quad i \epsilon \{HET, NOB, AOB, EPS\} \tag{2}$$

  Afterwards, the mass decay of the cells of the same group is added, giving as a result three values, MassDecayHET, MassDecayAOB and MassDecayNOB. For each type of cell, as long as the total mass of the decayed cells exceeds the mass of a single cell, a random cell of that type is removed. The mass of that cell is divided with proportions Y_I and $(1 - Y_I)$ between the mass of the solid debries which remain in the floc and the mass of the substrate to be released in the bioreactor respectively. This process stops when the total mass of the virtual dead cells no longer exceeds that value.

- **Growth.** All cells are growing continuously over time. In order to calculate how much they have grown by, for every cell of each type, the new mass is calculated by solving the following equation,

$$\frac{dX_i(x,y)}{dt} = r_{i,X}(x,y) \quad i \epsilon \{HET, NOB, AOB\} \tag{3}$$

  where $r_{i,X}(x,y)$ is the net production rate, calculated by adding all the rates of the processes in which the particulate component i is involved in the position (x,y). This process is clearly affected by the concentrations of the soluble components in each position. The higher the concentration of the soluble components, the faster the growth of the cells. Therefore, in the zones of the floc where there are more cells, the growth rate is slower.

- **Division:**

  1. Excretion of EPS: This process is carried out for all the HET cells within the floc, and it depends on its volume. If the radium of the HET cell is larger than a constant value, an angle is randomly chosen and an EPS cell is excreted.
  2. Division of HET, AOB, NOB: For each of those cells, if their masses exceed the maximum possible mass, a random angle is selected and a new cell of the same type is generated. When the division takes places, the mass of the two cells is randomly distributed between (0.45-0.55) of the total mass.

Macro-scale model (the bioreactor)

Once the micro-scale model has been simulated, it is then necessary to use that simulation with its values to convert them to the macro-scale model, in order to get the desired outputs. The first thing to be calculated is the number of flocs that are present in the bioreactor, given by the equation 4.

That is a hybrid equation, the first part is continuous and the second one is discrete. The first one represents the mass which is being depurated by the purge, and the second one stands for all the changes that affect the floc in the micro-scale model. As previously stated, the process of attachment a single cell and attachment a group of cells affect the number of flocs by decreasing it, while the process of detachment result in a growth of the number of flocs. Both m_f, m_c and m_{mf} are the mass of the floc, cells and micro-floc respectively, so they are not spatially dependent. This equation is actually a set of differential equations, depending on time. In each tc, tmf and td periods of time, the solution of this equation changes slightly. The generic solution is given by:

$$N(t) = e^{-\frac{t}{SDT}} + k_i \tag{5}$$

But the k_i changes for each interval. For example, $k_0 = 10^{14} - 1$. That means that in certain times the model jumps from one step to another, meaning that the number of flocs in the bioreactor is given by a family of exponential equations in which the constant k_i changes across time.

In addition, it is important to calculate the concentration of soluble components. The bioreactor is considered perfectly mixed. Therefore, the mass balance of each soluble component takes into account the amount of water that is coming into the bioreactor through the inlet ($S_{i,in}$) and the amount that is being depurated by the purge according to the time that a soluble component remains in the bioreactor (HRT). The processes in which the soluble components are involved are also taken into account in ($r_{i,R}$), which is calculated by multiplying the rates in one floc by the number of flocs in the bioreactor.

$$\frac{dS_{i,R}}{dt} = \frac{S_{i,in} - S_{i,R}}{HRT} - r_{i,R} \quad with\ S_{i,R}(t=0) = S_{i,in} \tag{6}$$

In addition, for the concentrations of the substrate in the bioreactor, $S_{S,R}$ it is important to take into account the mass of the substrate released in the bioreactor after the decay process,(MassSubstrate). Thus after solving the differential equation we must recalculate it.

$$S_{S,R} = S_{S,R} + \frac{MassSubstrate * Nflocs}{VolumeReactor} \tag{7}$$

Furthermore, the concentrations of the particulate components within the bioreactor are made up by multiplying the concentrations of each organism in the floc by the number of flocs and divided by the volume of the reactor.

$$CReactor_i = \frac{CFloc_i * NFlocs}{VolumeReactor} \quad i \epsilon \{HET, NOB, AOB, EPS\} \tag{8}$$

Connecting micro-scale and macro-scale

Processes happening within the floc directly affect the total mass balance of both soluble and particulate components in the bioreactor. All activities inside the floc (attachment, detachment) change the mass of the main floc, which is used to estimate the number of flocs within the bioreactor.
In order to calculate the concentration of soluble components, the rate $r_{i,R}$ is needed, and that rate is calculated multiplying the rate in one floc (micro-scale) by the number of flocs. In addition, the concentration of particulate components is made up by multiplying the mass in one floc (micro-scale) by the number of flocs. So it is clear that changes in the micro-scale model may affect considerably the results in the macro-scale model.

Computational model

This model has been implemented in C++, and R has been used to plot the graphics. This code is a reproduction of the MatLab code used for the paper(Ofieru *et al.*, 2014). In this model, the bioreactor is made up by replicates of the generic separator. This separator is a square,which is divided in 450 *dx and 450*dy (being $dx = dy = 1.1 * 10^-6$), conforming a grid in which the particulate and soluble components will be put (Figure 5). That grid will be used to solve the PDE for the soluble components, having as a result a concentration of each soluble component for each point in the grid. With regards to the particulate components, the bacterium will be able to be in a position different to that of one of the nodes in the grid, and in order to calculate some rates, the concentrations of the soluble components of the closest point in the grid will be used.
The code is divided in different sections:

Setting parameters as inputs

In this section the main parameters are fixed.

- Bioreactor and separator sizes and other constants.

$$\frac{dN(t)}{dt} = -\frac{N(t)}{SDT} \cdot \frac{m_f}{m_f + m_c \cdot \delta(t - i \cdot \tau_c) + m_{mf}(\emptyset) \cdot \delta(t - j \cdot \tau_{mf}) - m_c \cdot \varphi \cdot \delta(t - k \cdot \tau_d)} \tag{4}$$

- Properties of initial bacterium
- Diffusion coefficients in water
- Kinetic parameters
- Decay constants
- Initial concentrations

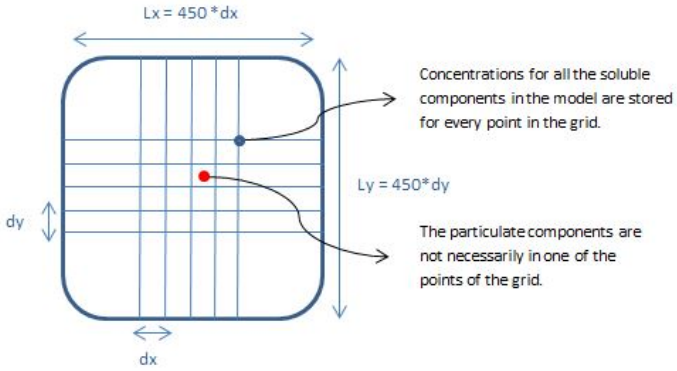

Fig. 5. Structure of the Separator

Main loop

Each step in this loop stands for an interval time of 0.1 days. In the code is represented by the name "iterGrowth". The code is implemented with the following processes. The separation between the micro-scale and the macro-scale is very clear, calculating before all the micro-scale events and using some values to calculate de macro-scale model afterwards.

Micro-scale

1. Calculate soluble components concentrations in the separator.
   The PDE is solved in C++ using a program of finite differences.
2. Single attach.
   This process happens if $iterGrowth \equiv 0 mod(\tau_c)$
3. Group attach.
   This process happens if $iterGrowth \equiv 0 mod(\tau_{mf})$
4. Group detach.
   This process happens if $iterGrowth \equiv 0 mod(\tau_k)$
5. Removing Islands.
   This process happens if $iterGrowth \equiv 1 mod(\tau_k)$
6. Growth and Decay.
   The ODEs are solved using the Euler method.
7. Division and EPS excretion.

Macro-scale

8. Calculate the number of flocs.
   The differential equation is solved by the Euler Method.
9. Calculate soluble components concentrations in the bioreactor.
   The differential equation is solved by the Euler Method.
10. Calculate particulate components concentrations in the bioreactor.

3 RESULTS

During the computational simulation of this model some problems arose. Due to the size of the separator (450x450) it was impossible to simulate the model solving the PDEs. Even decreasing the size to 50x50, the space and the time required to do a simulation of ten days exceeded our limitations, as we were only able to simulate for 3.5 days. In order to simulate the model and to get a general understanding but knowing that this model will not reproduce the exacts results, the model was run without solving the PDE. Instead of that, in the model at different times the concentrations of the soluble components are changed following the results in the paper (Ofieru *et al.*, 2014). With this modification, it is possible to run the model and interpret the results, but without forgetting that those results will be different in a model where the concentrations of the soluble components would be given by solving the PDEs.

The development of the flocs are different in each simulation, so in order to analyse the results of the concentrations a mean of a number of simulations will be used. On the other hand, the plots related with the shape of the floc are done with just one simulation.

In figure 6 it is possible to appreciate how the cells are growing and dying and how they spread. After one day the floc starts to spread within the separator, and the concentrations of each type of particulate component are almost the same. After two days it is clear that some new groups of cells have been attached to the main floc, and the density of cells in the floc has slightly increased. After four days it is possible to appreciate that some groups of cells have been detached, and how the HET cells (black colour) start to dominate with regards to the other cells. It is also possible to see some dead cells. After six days the concentrations of HET starts to be large, due to its growth is faster than the other cells so many division takes place. The shape is also changing due to the attachment and detachment processes. After eight days the concentration of HET cell is still increasing, and after ten days it is possible to see how the most of the cells in the floc are HET. Given that in this model there is not a shoving process many cells can be in the same position, which means that there are more AOB, NOB, EPS cells than are shown in the picture. A shoving routine should be included in further versions of this model.

The floc's shape is changing over the time, and its shape is directly related with some random processes, such attachment and detachment of a group of cells. That means that it is possible to get very different outputs with the same initial values. In figure 7 there are some of the possible outputs after a simulation of ten days with the same initial conditions. Those four pictures differ greatly, but with one thing in common, the HET cells predominate over the others.

In Table 1 the concentrations of each type of particulate component and the concentration of dead cells in the bioreactor with different values of the SDT are present. Given that the randomness of the model clearly affects those concentrations, the model has been

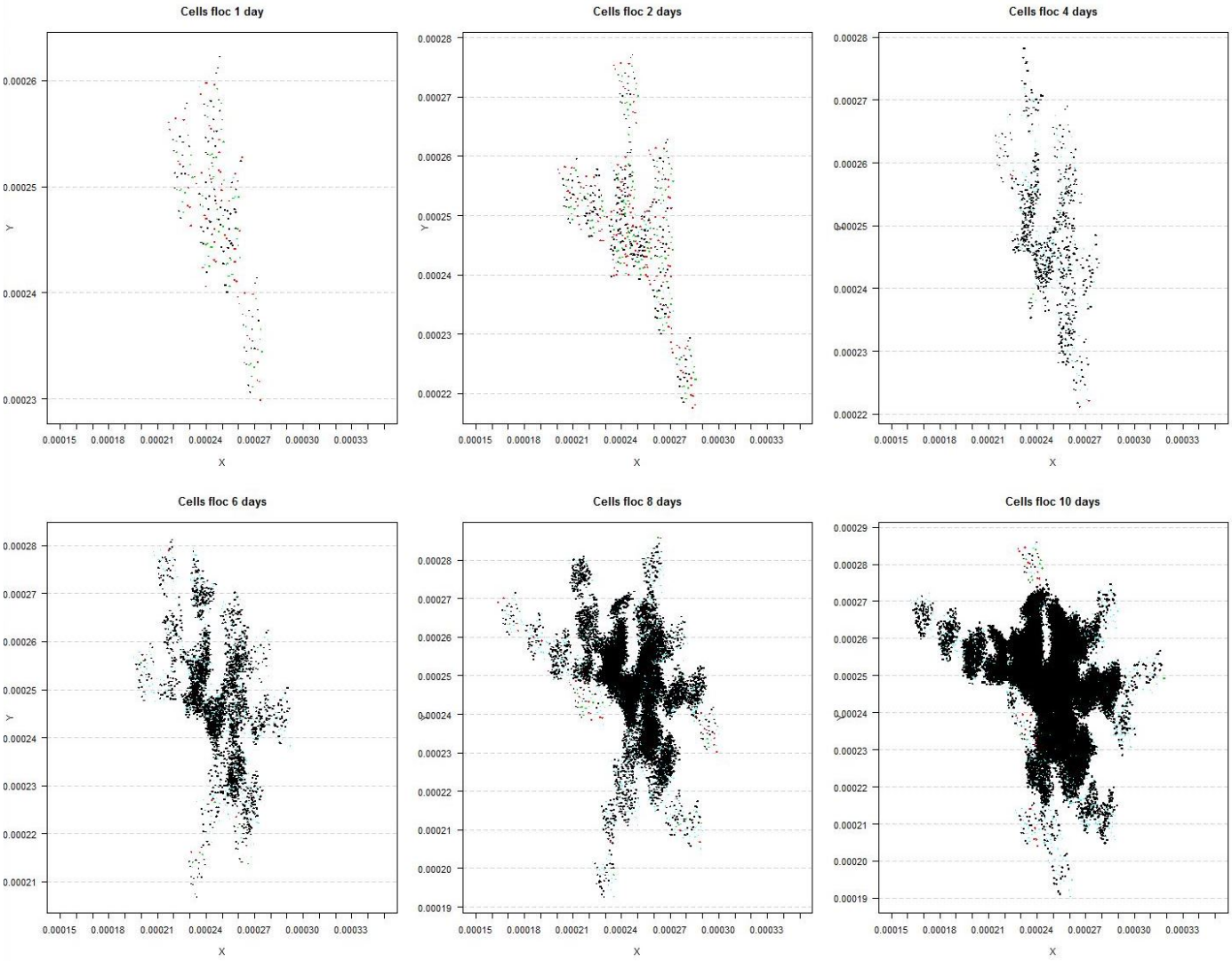

Fig. 6. Simulation floc over ten days. Black-HET , Red-AOB, Green-NOB, Dark Blue-EPS, Light Blue-DEAD. SDT = 5.1

Table 1. Concentration of particulate components in the bioreactor

SDT	HET	AOB	NOB	EPS	DEAD
5.1	217.3786	0.0746	0.0616	0.2193	3.1021
9.5	514.3124	0.0889	0.0958	0.5166	7.4884
11.5	541.6493	0.1489	0.1379	0.5971	7.7718
13.5	604.8681	0.2549	0.2071	0.6894	8.7554

Concentrations are measured in $kg * m^-2$

run ten times and the values of the concentrations are the mean of all those values.

It is clear that the higher the value of SDT, the higher the concentrations of all particulate component. This fact is not surprising. The solid dilution time is the time that a floc spent in the bioreactor before it is eliminated through the purge, so that means that the number of flocs in the bioreactor is larger as the value of SDT increases. The number of flocs directly affects the concentrations in the bioreactor, so the fact that the concentrations increase was expected. The surprising data is the concentration of the HET cells. While the concentrations of the other types are in normal values, the concentration of the HET cells is far larger than the others. One possibility for that result is the fact that the PDE are not being solved. The cells in the separator are consuming the soluble components in order to grow, so in those places of the separator where there are more cells, the concentrations of the soluble components would be lower than the values that are being used in this model. That means that the cells can grow and grow and the soluble components are not being consumed. In order to get a more realistic result an implementation with a PDE will be done.

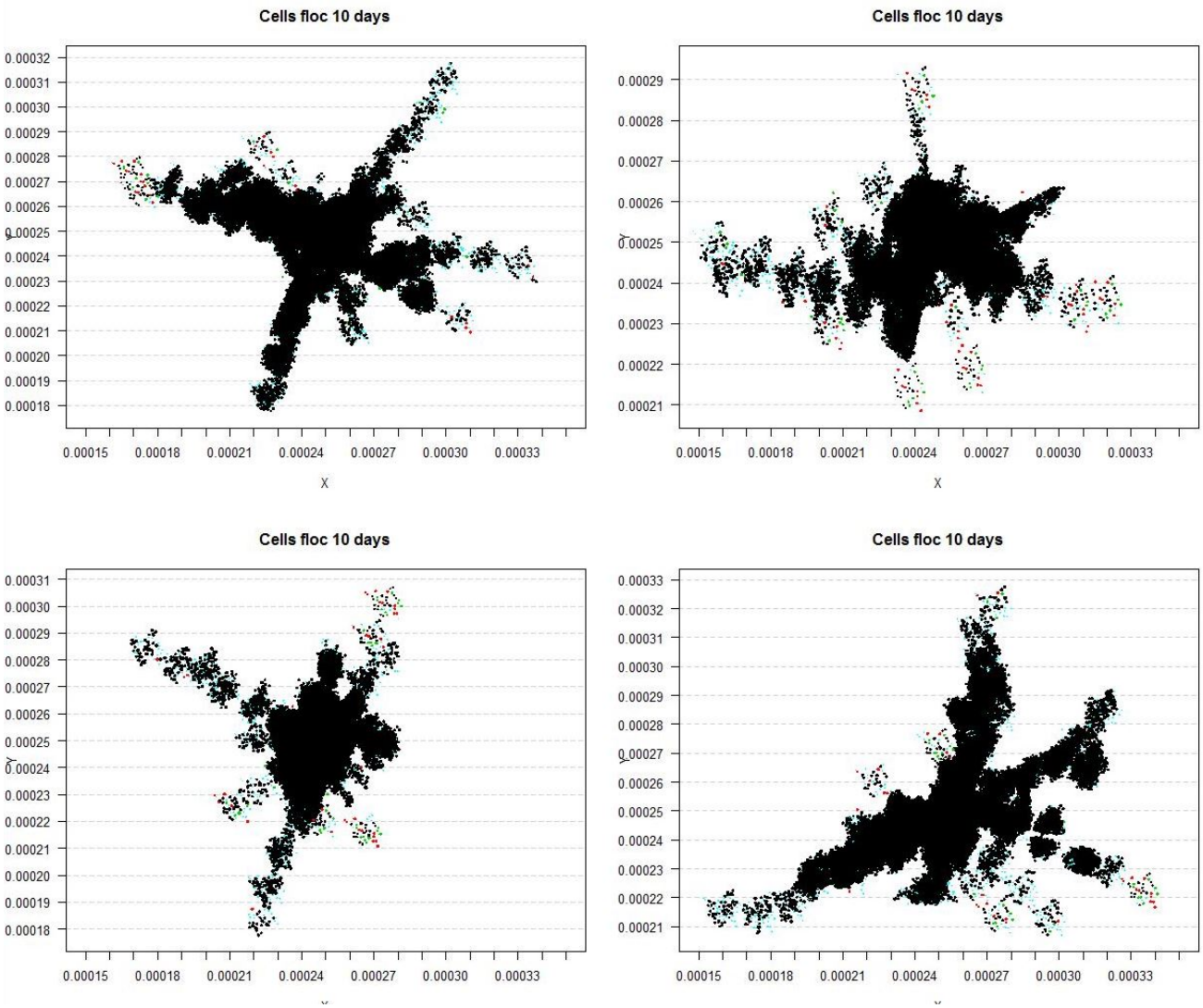

Fig. 7. Different flocs after a ten days simulation. Black-HET , Red-AOB, Green-NOB, Dark Blue-EPS, Light Blue-DEAD. SDT = 5.1

Moreover, it is also important to know how the concentrations of each microorganism are changing over time. Figure 8 shows the progression of each particulate component with a SDT of 5.1. As expected, the HET concentration starts to grow quickly, its growth being exponential. On the other hand, AOB and NOB cells are suffering a lot of small changes in their concentrations. They are growing in a "continuous" way but they undergo big changes at some points, their concentrations being notoriously diminished. This is due to the randomness of the detachment process. Furthermore, the EPS concentration behaves in a smoother way. Its concentration is slightly increasing over the ten days. Moreover, the DEAD cells have an interesting behaviour. Until the fifth day, the concentration is close to zero, but at that point more and more cells start to die, increasing the concentration in an exponential way.

Figure 9 shows the numbers of flocs in the bioreactor with different values of SDT. The number of flocs is decreasing over time for all the different values of SDT, but with a different speed. As

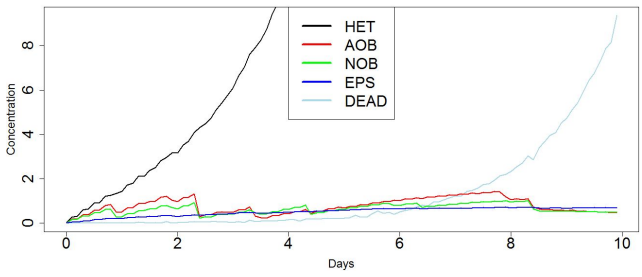

Fig. 8. Concentration of particulate components in the bioreactor. SDT = 5.1. Concentrations are measured in $kg * m^-2$

explained above, a lower SDT means that a floc remains less time in the bioreactor before being eliminated through the purge. Therefore, the number of flocs in a system with a lower SDT will decrease

faster. But this is not the only fact to take into account with regards the number of flocs. The number of flocs is also decreasing due to the attachment of a single and a group of cell processes, processes that are happening often during the simulation. When a detachment event takes place, the number of flocs at that time decreases a bit less than when it does not occur.

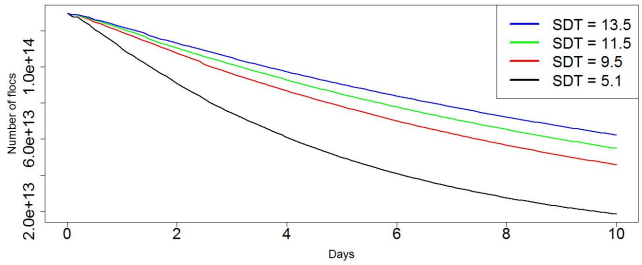

Fig. 9. Number of flocs in the bioreactor with differents SDT

The concentration of the soluble components in the reactor changes greatly between two different simulations. To get a general idea 15 simulations have been run and the mean of the concentrations was calculated. Results are shown in figure 10. During the first four days the concentrations of O2, S and NH4 are increasing over time. After that period, those concentrations start to decrease faster. This has a logical reason, the larger the number of cells in the floc, the higher the concentration that is consumed by the cells. Concentrations of NO2 and NO3 are close to zero all the time.

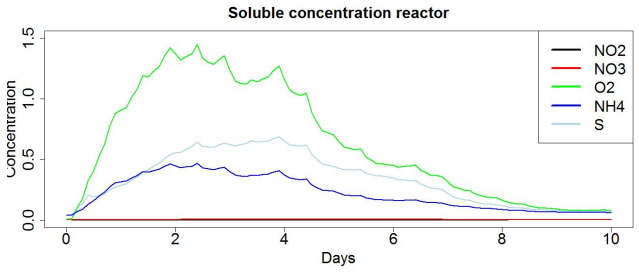

Fig. 10. Concentrations of the soluble components in the bioreactor over ten days. Concentrations are measured in $kg * m^-2$

Comparing PDE solution with no PDE solution

Due to the computational problems it was only possible to simulate 3.5 days of the development of the floc with the concentrations of the soluble component in the bioreactor given by the PDEs. In order to achieve some results, the size of the separator was to be reduced from 450x450 to 50x50. Another problem was that due to the attachment processes and the division, some cells during the simulation laid outside of the separator. When that happened, in other to calculate their growth and decay rates the concentrations of the soluble components used were those ones of the closest point in the border. Even with those modifications, due to a memory problem it was only possible to simulate 3.5 days. But comparing this simulation with another one without using the PDEs solver during the same amount of time and with the same value of SDT is useful to understand the importance of the PDEs solver. The SDT used in the simulations was 5.1 days.

Concentrations of the particulate components are clearly affected by the concentration of the soluble components in the separator. In figures 11 and 12 it is possible to compare both solutions with the PDE version and the no PDE version. First of all it is important to mention that while the results in the no PDE version was made by simulating 15 times the model and calculating the mean of the concentration, due to the time costs with the PDE version the results for this model have been calculated with only one simulation. That means that those results could be different with another simulation, due to the randomness of the model, but it is interesting to compare the graphs. It is clear that both graphs are completely different. In the version with PDE, concentrations start growing during the first 1.2 days, but after the second day the concentrations start to decay, and after the 2.5 days all the concentrations are zero except for EPS. HET concentration reaches a maximum of almost three, much less than its concentration in the model without PDEs. In general, results in the PDE version are more realistic, more than the results in the other model where the concentrations are too high. In addition, in both models the order of the cells with highest concentrations are HET, AOB, NOB, EPS and DEAD. Furthermore, the growth of the concentrations in the PDE version is smoother than in the other one. Attachment and detachment processes affects more considerably in the model without PDEs. The concentration of EPS and dead cells are really similar in both graphs, so even if the no PDE version results in not very real concentrations, it describes well what is happening.

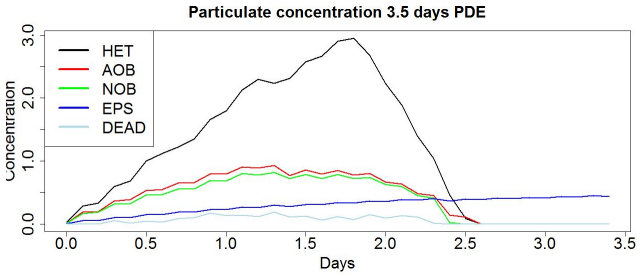

Fig. 11. Concentrations of the particulate components over 3.5 days in the PDE version. Concentrations are measured in $kg * m^-2$

With regards to the concentration of soluble components in both simulations, it is possible to appreciate some similarities and some differences. As before, the PDE graph is the result after only one simulation, while the other one is a mean between 15 simulations. In both the PDE version and the no PDE version the concentrations of NO2 and NH4 are close to zero. Nevertheless, the concentrations of the other three soluble components are slightly different. Due to the soluble concentration in the no PDE version is a mean over 15 simulations,the lines look smother than the PDE version. In the PDE version, concentrations start growing fast, but after the second day, concentrations goes to zero. If the comparative is made with the simulation over ten days, it is possible to appreciate how

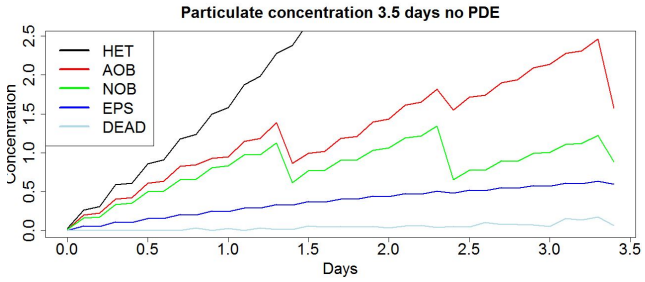

Fig. 12. Concentrations of the particulate components over 3.5 days in the no PDE version. Concentrations are measured in $kg * m^-2$

the behaviour in both simulations is similar, but in the PDE version concentrations reach the zero earlier.

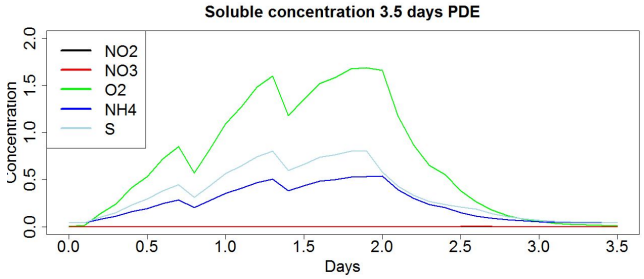

Fig. 13. Concentrations of the soluble components over 3.5 days in the PDE version. Concentrations are measured in $kg * m^-2$

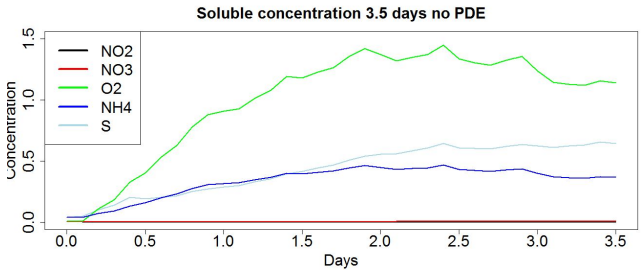

Fig. 14. Concentrations of the soluble components over 3.5 days in the no PDE version. Concentrations are measured in $kg * m^-2$

In figure 15 it possible to see the shape of the floc. Due to at 3.5 days all cells are dead, the plot has been done at 2.4 days. As previously mentioned, the shape of the floc depends directly on the randomness of the processes attachment and detachment, and it is not very related with the concentrations of soluble components in the floc. Therefore the different shapes that both flocs may have are not due to the solving or not of the PDEs. But in this graph, the cells predominating are the dead cells, instead of the HET cells. Due to the computational problems, the size of the separator has been reduced, and due to the new size some of the cells are outside it.

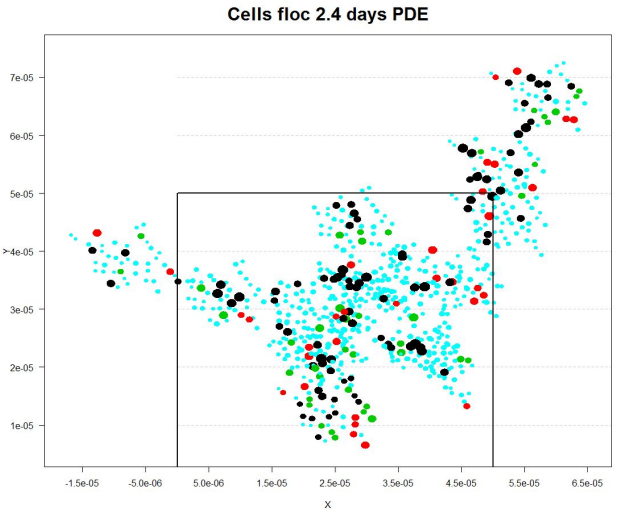

Fig. 15. Shape of the floc in the simulation with the PDEs. The black box is the separator. Black-HET , Red-AOB, Green-NOB, Dark Blue-EPS, Light Blue-DEAD. SDT = 5.1

4 DISCUSSION

In this section the reliability of both the PDE model and the non PDE model is going to be analysed and contrasted with the results of the paper (Ofieru *et al.*, 2014), the paper from which the model was taken. First of all it is important to highlight out the differences between our models and the model in the paper. The structure of the model is the same, but there are some differences in the way some processes are carried out.

Instead of using a program in C++ to calculate the concentration of the soluble components in the separator (PDEs), the model in the paper uses an external tool called COMSOL used in multiphysics which can solve reaction-diffusion systems. Using COMSOL to solve the PDEs would be a good way to improve our code, making it much faster to run and much more precise. In addition, the boundary conditions that are used in their model to solve the PDEs are completely different compared to our model (the initial conditions are the same). They consider that the separator is beside another separator in some of its boundaries, where they assume flux conservation between the boundaries. On the other hand, the other boundaries are consider that are in contact with the bioreactor, and Neumann boundary condition is considered for those boundaries($\frac{\delta S}{\delta x} = 0$ or $\frac{\delta S}{\delta y} = 0$, depending on which boundary) (Alexandru and Lavric, 2010). In our method, the assumption is more simplistic and easier to implement. The bioreactor is considered perfectly mixed, and the concentrations in the bulk liquid in the separator (region Ω_r in figure 3) are the same that the concentrations within the bioreactor. Our separator is considered to be in the middle of the bioreactor, and the concentrations for the soluble components assumed in the boundaries are the same that the concentrations in the entire bioreactor. It is clear that their implementation is more realistic, in a bioreactor the water flow is continuous, so it makes sense that the concentration is being affected by that flow. However, our assumption is good enough for a first approach.

In addition, in each step five PDE in a 50x50 grid have to be solved, one for each soluble component. Therefore, solving the PDEs by

finite difference in C++ it is very time consuming. While the model without the PDEs takes just one or two minutes to be run, the version with the PDE solver takes 15 minutes to run one step, so in order to simulate 100 steps (ten days), 25 hours are needed. However, due to a memory problem, the simulation crashes before it finishes. A computer with a better memory capacity is needed (more than 8GB). Moreover, a parallelization of the systems would reduce the time costs. If in each step each PDE is solved in a different computer (five computers are required), the time costs will be reduced from 25 hours to just five.

Another difference between our models is in the process of attachment of a group of cells. In their model, the group which is going to be attached is randomly selected from a folder with different files. Those files are simulations of the floc during 1-2 days and have a different number and type of cells. In our model, there is a generic file which is attached in every process. Their method simulates the process better, because those groups of cells are in theory mini flocs floating in the bioreactor. Our process is less random, even if the position within the floc where the group of cells is attached is completely random, that group is always the same. That slightly affects the number of flocs, making its behaviour more predictable.

Even if the previous differences can cause slightly different results, the biggest difference between their program and ours is that they include a shoving routine in Java. In that routine, the NOB and AOB cells are grouped in clusters. All the cells belonging to the same cluster are together, and in the process of detachment, when one cell of a cluster is remove, the entire cluster is removed. After collocating the AOB and NOB cells, the rest of cells are being moved to have their own space in the floc. This fact is crucial, because due to the growth and division processes, it could happen that two different cells are occupying the same space. With this routine, those situations are avoided moving the cells in the neighbourhood(cells with less mass are more likely to be moved than the big cells). In our program, that routine has not been implemented, having as result important differences. The shape of the floc is clearly affected. With the routine, cells are well spread, but without it the cells tend to be in the center of the floc. In figure 16 it is possible to see the shape of a floc with that routine, very different with the shapes of the flocs in Figure 7. But not only the shape of the floc is being affected, in addition, the concentration of the particulate components in the floc and therefore in the bioreactor are different. With the shoving routine, AOB and NOB cluster tend to be in an internal position within the floc. Thus, when a detachment event takes place, the micro-floc detached is more likely made up of HET cells. The HET cells grow faster than the AOB and NOB cells, so more division events take place for that kind of cell. This means that the detachment process is the way in which the concentrations of the different cell types are balanced. In our model, without that routine, we have as a result a huge concentration of HET cells, growing exponentially in the no PDE version.

These differences cause different results. First of all the model in the paper and our model without the PDE solver are going to be compared. In addition to the differences in the floc shape, the concentrations of soluble and particulate components in the bioreactor are also different. Comparing the concentrations of particulate components between the two models (Fig 8 and Fig 17) it is clear that our concentrations are higher. In order to come to some conclusions, it is interesting to compare the behaviour of the concentrations in both models. In both models, HET has the highest concentration,

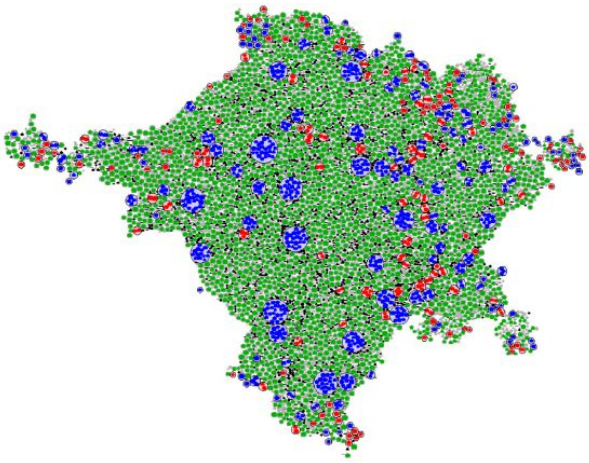

Fig. 16. Shape of a floc simulated in a model with a shoving routine. HET-green, AOB-blue, NOB-red, EPS-grey, inert-black. Picture taken from the paper (Ofieru *et al.*, 2014)

but in our model the growth is exponential, as the difference with the other cells is much larger. Inert cells also finished with the second highest concentration, but in our model it does not start to grow until the fifth day, while in this model it is growing from the beginning and without exponential behaviour. AOB and NOB behave in a similar way, having close concentrations during all the simulation. In addition, EPS concentration in both model have similar behaviour and values. An interesting fact between the two models, it is that in the model with a shoving routine the behaviour of the concentrations is smoother. In our model, the concentrations are directly affected by the processes of attachment and detachment, having as a result a lot of piks and very different concentrations between two successive steps.

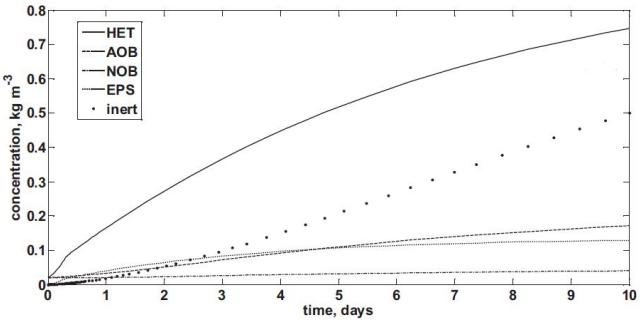

Fig. 17. Concentrations of particulate components in a model including a shoving routine. Picture taken from the paper(Ofieru *et al.*, 2014)

Figure 18 is the graph of the soluble component concentrations in the bioreactor. Results are completely different to ours. The concentrations are close to zero during the ten days, without exceeding 0,08 and reaching a pseudo steady-state since the sixth day. On the other hand, in our simulations, values are much higher, with the concentrations at some points being higher than one for oxygen.

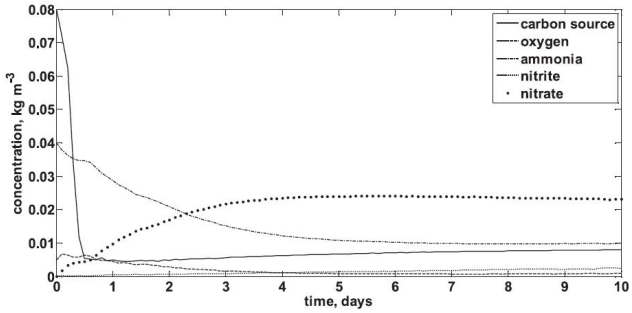

Fig. 18. Concentrations of soluble components in a model including a shoving routine. Picture taken from the paper (Ofieru *et al.*, 2014)

In figure 19 it is possible to see the number of flocs. The behaviour is similar to our model,the number of flocs is decreasing smoothly over time, due to the flocs are being eliminated through the purge. One small difference with our model is that at the beginning, in the first day, the attachment and detachment processes have a greater affect on the number of flocs than in our model. This is because in our model the mass of the group of cells attached is always the same, while in their model that mass is different each time. Therefore, the number of flocs decreases very quickly because the mass of the group attached can be larger than our mass.

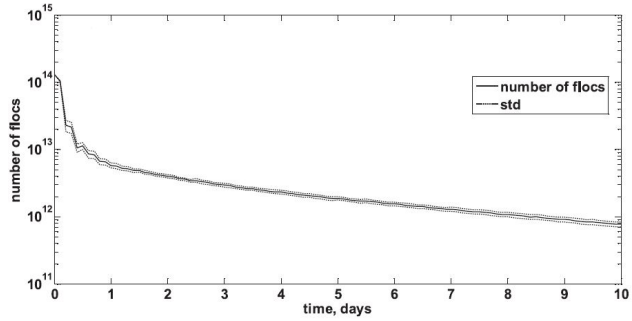

Fig. 19. Number of flocs in a model including a shoving routine. Picture taken from the paper (Ofieru *et al.*, 2014)

Even if the results are slightly higher, that can be because of the differences in the models. But the concentration of HET is completely unexpected. The maximum specific growth rate for HET is six, while the same rate for AOB and NOB are 0.76 and 0.81 respectively. That means that the HET are supposed to grow faster than the other bacteria, but not to the extent in the model. When one cell divides into two new cells, if the average of the cells which survive over the cells dead is higher than one, the number of cells increase exponentially. This is what is happening with the HET cells, and that exponential growth is not balanced with the detachment process and decay as is the case with the other bacteria. Even changing the maximum specific growth rate to two (only to check the results, without a biological meaning) HET cells still grow in an exponential way. This is because of a poor recreation of the soluble components concentrations in the floc, which allows the HET cells to grow a lot without being particularly affected by the decay process.

In our PDE version, values are completely different but have a biological meaning. At the beginning, when there are enough nutrients (soluble components) cells growth faster. But without a shoving routine, many cells are in the same positions. That means, that all those cells are competing for the same nutrients, so they can not grow more, so their concentration are stabilised. After one more day, due to the lack of nutrients, they start to die. They follow the same scheme as figure 20. In addition, as it is possible to see in figure 21 many cells are out of the grid. But in order to take into consideration those cells, it is assumed that those cells are in it closest point in the border. That means that there are more cells in the border, so many of them are competing for the same nutrients. In figure 21 it is also possible to see that many cells are together in the same square in the grid so they are using the same nutrients.

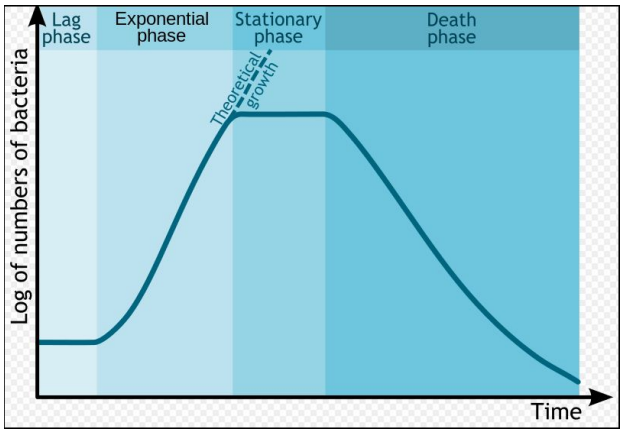

Fig. 20. Phase of microbes under some conditions. Those conditions are similar to our floc conditions.

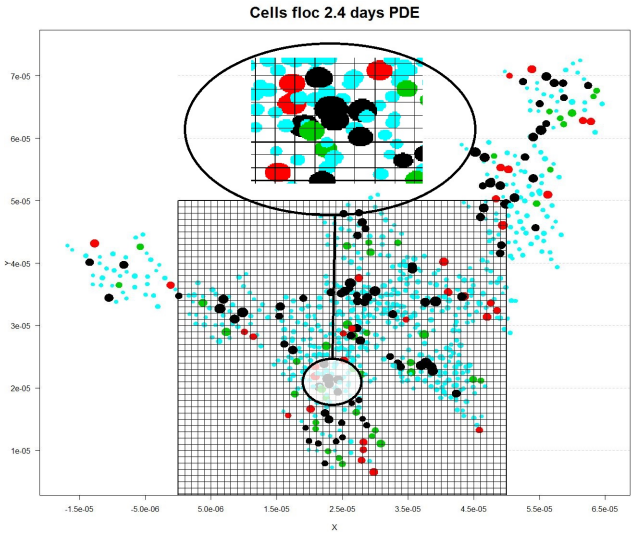

Fig. 21. Ampliation of part of a grid. Many cells are in the same position.

5 CONCLUSION

Mathematical and computational modelling of waste-water treatment processes are very useful in helping to understand all of the complexities that this technique has. There are many events to take into account to reproduce the normal situations that occur in a bioreactor. The mathematical modelling reproducing the growth and decay of the cells, the diffusion of the soluble components in water, and the total numbers of flocs in the bioreactor must be combined with other events to simulate the normal activities that occur in a real bioreactor. As we have seen, implementing all of these processes together is not an easy task, with different complications arising during the simulations. One problem that we found in the process was the computational limitations. Nowadays, there a lot of computers with a huge capacity and a lot of processors that could have solved some of our memory and time problems.

In addition, macro-scale concentrations are clearly affected by the micro-scale properties. It is clear how the processes occuring within the floc affect the total concentration in the bioreactor. In our two models, changing the way in which the concentration of the soluble components in the floc are calculated (firstly with estimated values from a paper and secondly with a PDE solver) leads to get different results in the concentrations of both soluble and particulate components in the bioreactor. Moreover, it is possible to appreciate that when an attachment or a detachment of a group of cells occur, at that point the concentration is being slightly affected. In addition, when comparing our models with the other model from the paper, it is also possible to see how small changes in the procedures have as consequence very different results. Between our models are only three differents, the boundary conditions in the PDEs, the group which is attached to the floc, and the shoving routine. With these three changes in the micro-scale model, the obtained results in the macro-scale model are substantially different.

Understanding the micro-scale model is of vital importance, because small changes in the micro-scale model can result in significant changes in the macro-scale model. Therefore, all of the cells properties must be studied in detail.

Furthermore, the importance of a shoving routine in the model has become clear. Without it, some problems with the detachment process and concentration of cells and soluble components appear. Therefore, the next step to improve this model is to include a shoving routine that is able to create space for a new cell when a division of a cell occurs and to put the AOB and NOB cells into clusters. Once that routine is implemented, the model will produce better results.

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To my family, their support and unconditional love beyond distance provide me the necessary strength to fight for my dreams and achieve my goals.

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6 APPENDIX

Software used:

Windows 7 Enterprise, Processor: Intel(R) Core(TM) i7-3370 CPU @ 3.40 GHz 3.40 GHz. Installed memory (RAM): 8.00 GB. System type: 64-bit Operating System.

Ubuntu 14.04.1 LTS, Workstation 10.0 Virtual Machine. Installed memory (RAM): 8.00 GB, System type: 64-bit, Operating System: Linux.

R 2.15.1. Packages used: plotrix

C++. Compiler gcc 4.8.2. Libraries used: Boost, gsl.