A New Approach in Modelling the Flocs from Biological Wastewater Treatment Bioreactors

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We present a new hybrid technique aimed to model the floc formation, growth and divers distribution. Our approach is adapted from individual based modeling concepts, using the notion of fundamental building bloc – the grid cell, defined as the lowest volume/surface of the grid enclosing the nascent floc for which the continuum properties are still valid, like concentration of microorganisms, ordinary diffusion etc. Then the available and forbidden vicinities are used to guide the growth of microorganisms in clusters (contiguous building blocks). They expand single free vicinity by single free vicinity as they grow, the next location being randomly selected among all the available vicinities. Attachment is another asset of the model – at a given time interval, whose length depends upon the velocity field of the environment, a fundamental building block comes from the environment and gets fixed to the outskirts of the nascent floc. These simple steps are combined with the mass balance equations for the substrates present in the system and the kinetics describing the biological process. The model is capable of qualitatively reproducing the cross section distribution of flocs, as observed through FISH imagery.

Keywords: floc modelling, diffusion, population community, random formation, attachment, detachment

Activated sludge is the most widely spread technology for treating wastewater. It is an aerobic process and requires important amounts of energy to spend, with implication also on the carbon footprint of the process. Nevertheless, replacing this technology will not happen too soon, so finding ways to make it more energy efficient is desirable.

An activated sludge system has several time scales, at least three coexisting at the biological reactor level – the bioreactor residence time, imposed by the external operating conditions, the life-time of a generic floc, imposed by the bioreactor velocity field and the mechanisms of floc formation and destruction, and the doubling time, characteristic for each microorganism response to the local substrate concentrations.

The core of the transformation is represented by the floc, an aggregate of suspended growth microorganisms and abiotic particles. The main biological processes taking place are organic carbon (performed by heterotrophs – HET), ammonia (performed by ammonia – AOB, and nitrate – NOB, oxidizing bacteria) and phosphorus elimination. Many experimental studies for understanding microbial diversity, microbiology and ecology of the bacteria forming the floc have been carried out [1-3], but subsequent inclusion of the information in mathematical models is an ongoing process.

An important problem, still unsolved, regards the mechanisms of floc formation; how the microorganisms came together, which are the processes shaping the population belonging to a floc and why there are local segregations of microorganisms inside the floc.

The activated sludge process is most compressively described in Activated Sludge Models (ASM1, ASM2, ASM3 and versions), for which lots of kinetic data are available and ready to be used. These models, more or less segregated, are oriented towards the biochemical transformations happening at the bioreactor time scale rather than describing the physical environment (the flocs) where the processes actually take place.

For the floc modeling there are different approaches, depending on the goals aimed. Nevertheless, few attempts have been made to model activated sludge floc formation at microscale and subsequent scale up.

In most models, flocs were considered to have a predefined spherical shape and constant diffusion coefficient for substrates. Few attempts were made to start from small microorganisms clusters (even from individual cells) and see how they grow up together as a floc of arbitrary shape.

The continuous representation of the floc is useful when the environmental influence (i.e. concentration at the bioreactor scale) is to be studied, without entering into details regarding the structure of the floc.

Stenstrom and Song [4] developed a pseudo-homogenous model of nitrifying activated sludge process to investigate effects of mass transport resistance and heterotrophic/nitrifier competition on the apparent relationship between dissolved oxygen (DO) concentration and nitrification. Although there is differentiation between kinetics of AOB and NOB, there is no distinction between their positions in the floc. This means that AOB and NOB are uniformly distributed inside the floc, coexisting together in the same space, which is not what the experimental FISH imagery showed. The main conclusion is that nitrification can take place also to low level of DO. The effects of the dissolved oxygen concentration on biological nitrification in suspended biomass processes were investigated [5]. It was used a model similar with the one of Stenstrom, having the same hypothesis: uniform density of biomass, bioflocs of spherical shape, Michaelis–Menten kinetics, and constant parameters inside the biological flow. It was developed a mathematical model for the interactions among filamentous bacteria, floc forming bacteria and protozoa within a completely mixed activated sludge system [6]. The main merit of the paper is to describe the practical coexistence of three micro-organisms in a generic way. It is the "classical" way of modeling, having mass balances for the three species only,

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considering the substrate constant (or with a sinusoidal variation), but not taking into account any limitation introduced by oxygen. In the end, they use the model to simulate different conditions for which the concentration of filamentous bacteria is lower in the system. No differentiation between AOB and NOB and the biological process was supposed to obey Monod kinetics. Wang et al. [7] use ASM1 to argue that activated sludge processes can be operated at low DO concentration to reduce energy consumption and achieve desired nitrification. They considered a spherical floc and a distribution of flocs (based on their radius size) in the reactor. There is no distinction between AOB and NOB in the floc, but they do show that there existed an optimal DO concentration for the operation of nitrogen removal.

A combination of integrated modelling and microelectrode techniques to investigate the microbial processes, the DO and the redox (OPR) status inside sludge flocs in different DO and COD concentrated wastewater treatment was used [8]. The proposed model takes into consideration the mass transfer of substrate and oxygen, the biological reactions, nitrification, the biomass hydrolysis and the endogenous respiration inside activated sludge flocs and is based on ASM3. The data acquired from experiments are from full-scale aeration tanks. The activated sludge flocs are spherical in shape and symmetry having constant diffusion coefficient throughout the matrix. The model simulation and the experimental microelectrode profiles indicate the microenvironment of activated sludge floc; the outer layer efficiently participates in the metabolism of pollutants, while the interior of the floc rapidly decreases in activity. The model predicts that cell hydrolysis, aerobic/anoxic substrate storage and diffusion resistance inside the sludge lead to zones of increased substrate concentration inside the floc, in which the rate of biodegradation would de exceeded by the rate of substrate inlet. With pollutants removed from the system, oxygen could penetrate deeper in the floc, resulting in a decrease of the zone with increased substrate concentration and the enlargement of the aerobic region.

With the availability of the advanced imaging techniques, the internal structure of flocs was deciphered and the modeling moved towards more sophisticated approaches like cell automata, individual based and population balance – from outside-in to inside-out, from a predefined shape filled with uniformly distributed non-clustered microorganisms to shapes built (quasi)randomly as growth, attachment, detachment and interactions among species develop.

The researchers suggested that the floc has three levels of structuration [9]:

-2.5μm which is bacteria, embedded in a gel-like matrix of exopolymers;

-13μm size microcolonies of embedded bacteria, which are also linked together with the exopolymers;

-125μm size represented by aggregates of micro-colonies.

A different fractal dimension was calculated both for microcolonies and microflocs, showing that the aggregation mechanisms are not the same: cell-division for microcolonies and diffusion limited particle-cluster for microflocs. Stoll and Buffle [10] present computer simulation of the flocculation processes. Two- and three dimensional models for bridging flocculation between large polymer chains and comparatively small colloidal particles were developed. The floc structures were investigated as a function of chain/particle concentration ration, chain conformation, and space dimension and the results suggested that the floc morphology is strongly dependent on the chain conformation and to a slight extent to the chain/particle concentration ratio. The examples presented are generic and not specifically related to wastewater treatment flocs.

Several papers on individual based model, cell automata, use of AQUASIM, analytical solution of reaction-diffusion equation, most of them developed for biofilms, were published by Delft modeling group which has an important contribution in this field [11-13]. To simulate three-dimensional formation of activated sludge flocs it was adapted an individual based model originally developed for a biofilm system [14]. There were considered two types of bacteria: floc-forming and filamentous. AOB and NOB are "lumped" together and presented in competition with filamentous (heterotrophous) bacteria, but their distribution in the floc was not evaluated. Kinetics was assumed to be similar for both bacterial morphotypes under consideration and the competition between them was based on morphology and on microgradients of substrate concentration. A combination of continuous and discrete modelling was realized by [15] who used continuum representation of EPS and discrete individual description of microbial cells. The transport of individual cells within the biofilm is assumed to be by advection and individual shoving. The processes within the biofilm take place at different time scales: transport and reaction of soluble components is very fast (seconds) and biomass balances much slower (order of hours to days). One case study is with a biofilm containing AOB, NOB and heterotrophs. AOB and NOB were considered not to produce EPC and tending to grow in compact clusters. Previous to this paper, there were two publications on a continuous model for biofilms [16, 17], where the geometry of the biofilm is described by the interface between the biomass and the surrounding liquid. An innovative approach [18] was to characterize the biofilm mechanics using immersed boundary method. There is no differentiation between the cells which form the biofilm – i.e. does not answer the question how is the floc forming? The main focus is on mechanical stress in biofilms and subsequent induced detachment. The biofilm is seen as a viscoelastic fluid which acts as an elastic solid on short time scales and as a viscous fluid on longer time scales. The behaviour of biofilm results by solving Navier-Stokes equation for a mixed material domain. The biofilm region is discretized into a number of nodes connected by springs. However, the approach is to model interaction between fluid flow and biomass, without considering how the biofilm/floc is formed.

An example of population balances model for dynamics of flocculation is the one which presents an experimental technique to monitor activated sludge flocculation [19]. Even though activated sludge is a heterogeneous biological system dynamics of flocculation were found to follow the principles of inorganic shear-induced flocculation. Activated sludge flocculation was successfully described using population dynamics, although the value of collision efficiency was significantly less than found in inorganic systems.

Abstraction of the floc and simplifying hypothesis

Next we will develop a 2D model of the floc, using some simplifying hypothesis. Our model is hybrid, since it uses concepts from individual based models, as developed by Delft school, together with classical ones. Based on experimental evidences, collected with FISH based techniques, we assume that the developing floc will have the generic dimensions L_x x L_y. These dimensions designate a rectangle on which we draw a grid of *Nr* x *Nc*

elements – the grid cells, which represent the smallest area (volume, in a 3D model) for which properties assigned to continuous space are still valid, like temperature, pressure, concentration etc. Each grid cell could be free of microorganisms, or have a given number of heterotrophs, ammonia oxidizing bacteria or nitrite oxidizing bacteria (fig. 1). Since the goal of the present study is to develop a floc model including its growth, we assume that the process of growth and formation of the floc starts from few randomly distributed clusters of microorganisms, each of a grid cell size. The initial number and distribution could be linked to the local state of turbulence. It must be emphasized that in a grid cell there cannot be different types of bacteria. More, based also on experimental evidence, there always will be a space of at least a grid cell between clusters of different types of bacteria (fig. 1, the space between HET, AOB and NOB clusters). The microorganisms in a grid cell will grow and divide according to the substrate concentration in that particular area, which is subjected to diffusion limitations biological process consumption and/or production.

Due to the way we defined the grid cells, the bacteria from a cluster of several grid cells (down to a single one, when the process of floc formation starts) grow till they could occupy, through division, another grid cell. The growth of the floc inside the grid is done grid cell by grid cell. Consequently, we introduce the concept of vicinity, meaning the grid cells surrounding a cluster, which are divided into two groups – available or forbidden. In available vicinity a cluster could grow, while forbidden vicinity is that particular grid cell which belongs to the vicinities of at least two different bacteria clusters (see Figure 1 for details). If any of the two clusters would grow occupying any of the forbidden vicinities, it would touch another cluster of different bacteria, violating the conditions we imposed upon floc growth.

Our conceptual model is completed with two important processes, attachment and detachment. The former is a random process, meaning that, due to the nearby environment full of bacteria, clusters of the size of a grid cell could occupy any of the free grid cells surrounding the nascent floc. The latter is a pseudo-random process since it means that a cluster of the size of a grid cell could be removed from a larger bacteria cluster and taken into the environment. The detachment condition is that the removed grid cell should have only one side in contact with the larger cluster (fig. 1, HET and NOB could have only two grid cells detached, while AOB could have three). The frequencies of attachments and detachments are function of the local state of turbulence, compactness of the floc and position of the cluster inside the floc.

The mathematical model

The grid, of L_x x L_y dimension, is filled with liquid with a generic substrate, S, linked to the environment through some convenient boundary conditions. In the present case, the assumption is that the environment surrounding the floc is perfectly mixed, hypothesis currently accepted for the activated sludge wastewater treatment bioreactors. Further, let's assume that inside the floc / grid the mass transfer mechanism is by diffusion only, so that a mass balance for the substrate could be written, for the free of microorganisms grid cells, as:

$$\frac{\partial S}{\partial t} = \mathrm{D}_x \frac{\partial^2 S}{\partial x^2} + \mathrm{D}_y \frac{\partial^2 S}{\partial y^2} \quad (1)$$

In equation (1), $\mathcal{D}_x$ and $\mathcal{D}_y$ are the diffusion coefficients, particular for the designated substrate or product, effective in the grid cells free of microorganisms. When the grid cells are occupied by clusters of microorganisms, the substrate concentration is affected by reaction-diffusion (HET, AOB and NOB grid cells) and equation (1) changes to:

$$\frac{\partial S}{\partial t} = \mathrm{D}_x^* \frac{\partial^2 S}{\partial x^2} + \mathrm{D}_y^* \frac{\partial^2 S}{\partial y^2} - R_b \quad (2)$$

In equation $\mathcal{D}_x$ and $\mathcal{D}_y$ are the diffusion coefficients corresponding to the space occupied by microorganisms, much lower than $\mathcal{D}_x$ and $\mathcal{D}_Y$, R_b is the substrate consumption rate, corresponding to the microorganism type present in the microcolonies within the grid elements, while S is the generic substrate concentration in the cluster with microorganisms.

The initial and boundary conditions for equation are (fig.1):

Initial Condition (we assume that, at the very beginning of the growth process, the grid is filled with the same liquid phase as the environment, S_o):

$$t = 0, \forall x \in [0, L_x], y \in [0, L_y] \quad S = S_0 \quad (3)$$

Boundary Conditions (we assume the environment is perfectly mixed):

$$\forall t > 0$$

$$\forall y, x = 0, \quad S = S_0; \quad x = L_x, \quad \frac{\partial S}{\partial x} = 0 \quad (4)$$

$$\forall x, y = 0, \quad S = S_0; \quad y = L_y, \quad \frac{\partial S}{\partial y} = 0 \quad (5)$$

The initial and boundary conditions for equation (2) are (fig. 1):

Initial Condition (we assume that when the microorganisms are randomly placed onto the grid, the latter is filled with the same liquid phase as the environment):

$$t = 0, \forall x \in [0, L_x], \forall y \in [0, L_y] \quad S = S_0 \quad (6)$$

Boundary Conditions (we assume that the cluster position onto the grid determines the boundary condition type):

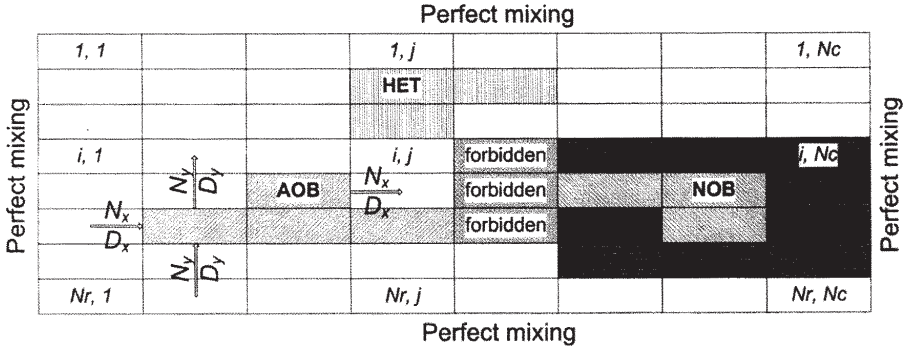

Fig. 1. Sketch of the floc grid cells, introducing the main concepts: HET – heterotrophs cluster, AOB – ammonia oxidizing bacteria cluster, NOB – nitrite oxidizing bacteria cluster, parallel stripes – available vicinities, crossed stripes – forbidden vicinities

$$\forall t > 0$$

-the leftist grid cell of the bacteria cluster is at the left grid boundary

$$x = 0, \forall y \quad S = S_0 \tag{7}$$

-the leftist grid cell of the bacteria cluster is in contact with a free grid cell (we assume flux conservation)

$$x = x_L, \forall y \quad -D_x \left.\frac{\partial S}{\partial x}\right|_{x_L^-} = -D_x^* \left.\frac{\partial S}{\partial x}\right|_{x_L^+} \tag{8}$$

-the rightist grid cell of the bacteria cluster is in contact with a free grid cell (we assume flux conservation)

$$x = x_R, \forall y \quad -D_x^* \left.\frac{\partial S}{\partial x}\right|_{x_R^-} = -D_x \left.\frac{\partial S}{\partial x}\right|_{x_R^+} \tag{9}$$

-the rightist grid cell of the bacteria cluster is at the right grid boundary (the finiteness of the substrate variation)

$$x = L_x, \forall y \quad \frac{\partial S}{\partial x} = 0 \tag{10}$$

-the bottommost grid cell of the bacteria cluster is at the bottom grid boundary

$$y = 0, \forall x \quad S = S_0 \tag{11}$$

-the bottommost grid cell of the bacteria cluster is in contact with a free grid cell (we assume flux conservation)

$$y = y_D, \forall x \quad -D_y \left.\frac{\partial S}{\partial y}\right|_{y_D^-} = -D_y^* \left.\frac{\partial S}{\partial y}\right|_{y_D^+} \tag{12}$$

-the uppermost grid cell of the bacteria cluster is in contact with a free grid cell (we assume flux conservation)

$$y = y_U, \forall x \quad -D_y^* \left.\frac{\partial S}{\partial y}\right|_{y_U^-} = -D_y \left.\frac{\partial S}{\partial y}\right|_{y_U^+} \tag{13}$$

-the uppermost grid cell of the bacteria cluster is at the upper boundary

$$y = L_y, \forall x \quad \frac{\partial S}{\partial y} = 0 \tag{14}$$

The substrate mass balance is rendered dimensionless using the following notations for the generic substrate concentration, σ, length and width, ξ and η, time, τ:

$$\sigma = \frac{S}{S_0}, \xi = \frac{x}{L_x}, \eta = \frac{y}{L_y}, \tau = \frac{t}{t_0}, t_0 = \min\left(t_x = \frac{L_x^2}{D_x}, t_y = \frac{L_y^2}{D_y}\right) \tag{15}$$

Applying (15) to the free of microorganisms' grid cells, we get:

$$\frac{\partial \sigma}{\partial \tau} = \frac{t_0}{t_x}\frac{\partial^2 \sigma}{\partial \xi^2} + \frac{t_0}{t_y}\frac{\partial^2 \sigma}{\partial \eta^2} \tag{16}$$

The substrate mass balance equation could be rendered dimensionless for the clusters with microorganisms too ($\rho_b = t_o \cdot R_b$):

$$\frac{\partial \sigma}{\partial \tau} = \frac{t_0}{t_x}\frac{D_x^*}{D_x}\frac{\partial^2 \sigma}{\partial \xi^2} + \frac{t_0}{t_y}\frac{D_y^*}{D_y}\frac{\partial^2 \sigma}{\partial \eta^2} - \rho_b \tag{17}$$

Dimensionless initial and boundary conditions for equation are:

$$\text{IC: } \tau = 0, \forall \xi, \eta \in [0,1] \quad \sigma = 1 \tag{18}$$

$$\text{BCs: } \forall \tau > 0$$

$$\forall \eta \in [0,1], \xi = 0 \quad \sigma = 1; \xi = 1 \quad \frac{\partial \sigma}{\partial \xi} = 0 \tag{19}$$

$$\forall \xi \in [0,1], \eta = 0, \quad \sigma = 1; \eta = 1, \quad \frac{\partial \sigma}{\partial \eta} = 0 \tag{20}$$

Boundary conditions for equation – IC is the same as (18):

$$\forall \eta \in [0,1]$$

$$\xi = 0, \sigma = 1; \xi = \xi_L, D_x \left.\frac{\partial \sigma}{\partial \xi}\right|_{\xi=\xi_L^-} = D_x^* \left.\frac{\partial \sigma}{\partial \xi}\right|_{\xi=\xi_L^+};$$

$$\xi = \xi_R, D_x^* \left.\frac{\partial \sigma}{\partial \xi}\right|_{\xi=\xi_R^-} = D_x \left.\frac{\partial \sigma}{\partial \xi}\right|_{\xi=\xi_R^+}; \xi = 1, \frac{\partial \sigma}{\partial \xi} = 0 \tag{21}$$

$$\forall \xi \in [0,1]$$

$$\eta = 0, \sigma = 1; \eta = \eta_D, D_y \left.\frac{\partial \sigma}{\partial \eta}\right|_{\eta=\eta_D^-} = D_y^* \left.\frac{\partial \sigma}{\partial \eta}\right|_{\eta=\eta_D^+};$$

$$\eta = \eta_U, D_y^* \left.\frac{\partial \sigma}{\partial \eta}\right|_{\eta=\eta_U^-} = D_y \left.\frac{\partial \sigma}{\partial \eta}\right|_{\eta=\eta_U^+}; \eta = 1, \frac{\partial \sigma}{\partial \eta} = 0 \tag{22}$$

The kinetics of the biological process

The activated sludge treatment of wastewater is a complex biological process, involving – in the present approach – three clusters of microorganisms (heterotrophs, ammonia and nitrite oxidizing bacteria) and four substrates (oxygen, ammonia, nitrite and carbon based). Consequently, the process is described by three growth rates, one for each cluster of microorganism, and four consumption rates, for all substrates. For the growth rates we adopted a simple Monod-derived kinetic, since the goal of the present paper is to focus on the floc modelling, keeping the rest as simple as possible, but still reliable [20].

The growth rates

$$R_{HET} = \mu_{\max}^{HET} \cdot X_{HET} \frac{S}{K_S^{HET} + S} \frac{O_2}{K_{O_2}^{HET} + O_2} \tag{23}$$

$$R_{AOB} = \mu_{\max}^{AOB} \cdot X_{AOB} \frac{NH_4}{K_{NH_4}^{AOB} + NH_4} \frac{O_2}{K_{O_2}^{AOB} + O_2} \tag{24}$$

$$R_{NOB} = \mu_{\max}^{NOB} \cdot X_{NOB} \frac{NO_2}{K_{NO_2}^{NOB} + NO_2} \frac{O_2}{K_{O_2}^{NOB} + O_2} \tag{25}$$

The consumption rates

$$R_S = -Y_S^{HET} \cdot R_{HET} \tag{26}$$

$$R_{O_2} = -Y_{O_2}^{HET} \cdot R_{HET} - Y_{O_2}^{AOB} \cdot R_{AOB} - Y_{O_2}^{NOB} \cdot R_{NOB} \tag{27}$$

$$R_{NH_4} = -Y_{NH_4}^{AOB} \cdot R_{AOB} \tag{28}$$

$$R_{NO_2} = Y_{NO_2}^{AOB} \cdot R_{AOB} - Y_{NO_2}^{NOB} \cdot R_{NOB} \tag{29}$$

In the kinetic model the notations S, O_2, NH_4 and NO_2 stand for the concentrations of carbon based, oxygen, ammonia and nitrite substrates, while μ_{max} and K_χ denote the Monod constants – maximum specific growth rate and affinity constant – particular to each process and substrate,

Item	Value	Item	Value	Item	Value	Item	Value
$\mu_{\max}^{HET}$	4.0	$K_{O_2}^{HET}$	10^{-3}	Y_{HET}	0.5	$Y_{O_2}^{NOB}$	13.4
		K_S^{HET}	10^{-2}	Y_S^{HET}	2.0	$Y_{NO_2}^{NOB}$	12.5
$\mu_{\max}^{AOB}$	1.5	$K_{O_2}^{AOB}$	$0.3 \cdot 10^{-3}$	$Y_{O_2}^{HET}$	0.4	S_0	$40 \cdot 10^{-3}$
		$K_{O_2}^{NOB}$	$0.5 \cdot 10^{-3}$	$Y_{O_2}^{AOB}$	9.4	$O_{2,0}$	$4 \cdot 10^{-3}$
$\mu_{\max}^{NOB}$	1.5	$K_{NH_4}^{NOB}$	10^{-3}	$Y_{NH_4}^{AOB}$	3.0	$NO_{2,0}$	10^{-3}
		$K_{NO_2}^{NOB}$	$1.3 \cdot 10^{-3}$	$Y_{NO_2}^{AOB}$	3.0	$NH_{4,0}$	$20 \cdot 10^{-3}$

Table 1
KINETIC AND ENVIRONMENT DATA (ALL MAXIMUM SPECIFIC GROWTH RATES ARE IN DAY^{-1}, CONCENTRATIONS IN kg/m^3 AND YIELDS IN kg/kg)

respectively. The particular values used in this study are given in table 1, together with the environment concentrations surrounding the grid where the floc is forming and growing.

Results and discussions

The aforementioned mathematical model permits the study of floc formation and growth starting from few clusters of heterotrophs, ammonia and nitrite oxidizing bacteria, each of the size of a grid cell only, whose number and position onto the grid is randomly generated. Apart from the initial distribution, several other phenomena should shape the final position and distribution of the clusters of microorganisms, namely diffusion, attachment, detachment and NOB growth dependency upon AOB production of nitrite. The current implementation disregards the effects of detachment, since the information in literature about this matter are very scarce and only at qualitative level. No attempt was made to rigorously study the detachment and put it into a relationship with the operating parameters of the bioreactor or the local conditions of the environment – shear stresses, turbulence level, position into the floc of the detached clusters etc. The attachment can happen individually, with clusters the size of a grid cell, or with already structured micro-flocs, either in incipient growing, or resulted from a break of a bigger floc. The latter seldom happens, since the floc once grown to a certain level, doesn't break easily. Currently, we implemented only the attachments at individual cluster level, at random positions upon the grid, but in the vicinity of the growing floc. The attachments happen inside a time window of 3 h, with a probability of 75%, irrespective of the microorganism type. This could be customized, according to the type of bacteria and their concentrations in the environment, but our intention was to keep this simpler, so we can focus on the main aspects regarding the modelling of the floc.

In what follows, we will present three runs which differ to each other only due to the randomness of both the seeding process and the subsequent attachments. It must be emphasized that guiding rule is that the initial number of heterotroph clusters should exceed the number of other two microorganisms clusters and under no circumstances could be lower than it. More, the initial placement should be in the inner half of the grid since, if the clusters are too apart, the external shear forces will break apart the nascent floc.

In figure 2 is presented the evolution of a floc from the initial seeding, with 16 randomly generated and placed clusters: eight for heterotrophs and four for each ammonia

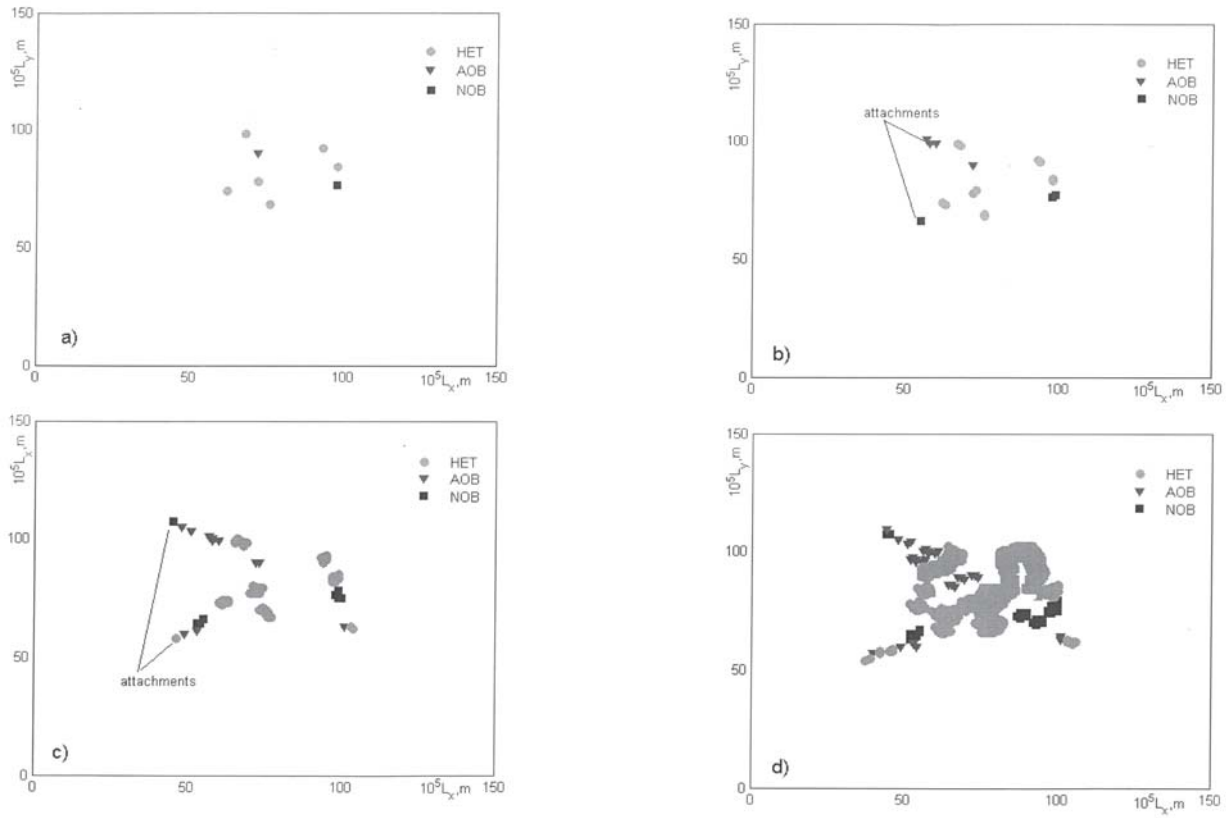

Fig. 2. Random seeding (initial random clusters: 8 HET, 4 AOB and 4 NOB), growth and attachment as main processes of a floc formation (the grid size is 3.10^{-4} x 3.10^{-4} m^2, the kinetic parameters and conditions of the operating environment are given in table 1);
a) initial random distribution of few random clusters, the size of one grid cell each; b) after the first division and three random attachments; c) after thirty divisions – the last five attachments are distinguishable; d) after 2.5 days – two clusters, one of AOBs and the other with NOBs are trapped inside heterotrophs

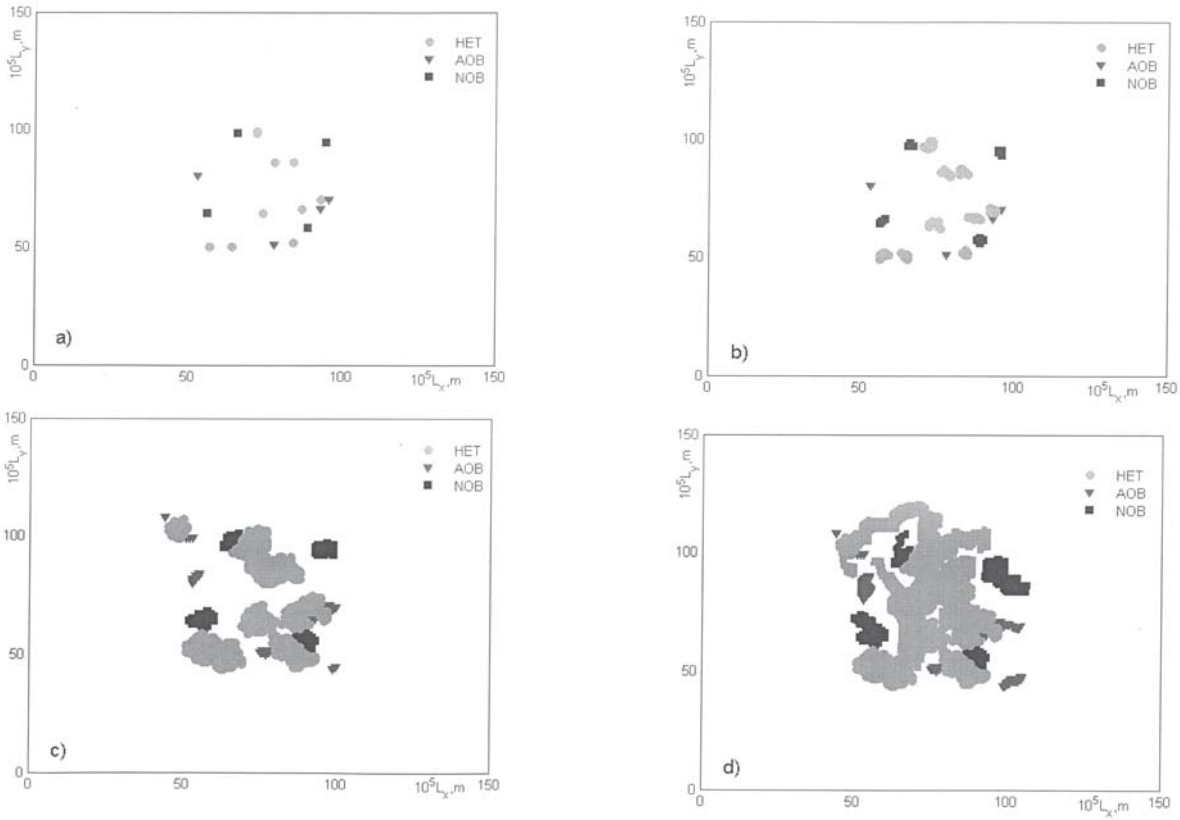

Fig. 3. Random seeding (initial random clusters: 5 HET, 1 AOB and 1 NOB), growth and attachment as main processes of a floc formation (the grid size is 3.10^{-4} x 3.10^{-4} m^2, the kinetic parameters and conditions of the operating environment are given in table 1); *a)* initial random distribution of few random clusters, the size of one grid cell each; *b)* after 0.9 days; *c)* after 1.8 days – the basic structure of the floc starts shaping; *d)* after 2.5 days – several clusters, AOBs and NOBs, are trapped inside heterotrophs

and nitrite oxidizing bacteria (fig. 2a). After the first division (meaning that the bacteria belonging to a cluster grown sufficiently to occupy one nearby grid cell), which happened for the heterotrophs, since they are the fastest growing bacteria among the three species present in the system, we can see how attachment works (fig. 2b). The attached clusters are in the vicinity of the growing floc, since there they cannot be easily removed / detached. After thirty divisions, the floc starts shaping and we can see the fastest growing bacteria will determine the final shape. As can be seen from figure 3c, the attachment is still present, putting new seeds of growing and diversity into the floc structure. After 2.5 days, all the heterotrophs clusters merged into a single cluster, which is the fabric of the mature floc (fig. 2d). We can see, also, that smaller clusters of the other two species could be trapped inside the bigger cluster. The heterogeneous clusters do not touch, although the graphical representation might suggest otherwise. Between them there is still a gap of one grid cell, formed by the forbidden vicinities. This gap ensures, also, the liquid phase through which the substrate could diffuse to inner core of the floc, feeding the bacteria far from the surface of the floc. As the diversity of the floc is higher (meaning many islands of different clusters of the three species are not entrapped), these tiny channels are more and more present, with beneficial effects upon the health of the inner bacteria.

Presently, the model does not cope with the situation of the entrapped clusters, but this will be solved with the introduction of bacteria death as a supplemental mechanism, followed by cellular lysis. The entrapped cells will start lacking substrate so they die, eventually, then their membrane will brake and the debris will be released into the liquid phase.

When the initial floc configuration is strongly imbalanced, like in the figure 3a, the floc growth and structure will be also biased, especially in the absence of death. Still, the random attachments happening each three hours tend to equilibrate the initial distribution, at the expense of latter entrapment of the heterogeneous clusters into the heterotrophs (fig. 3b, 3c and 3d). Again, after 2.5 h, the heterotrophs became a single, large cluster, which surrounded several clusters of AOBs and NOBs.

It could happen that the initial distribution to be formed of the same number of fundamental clusters, like in figure 4a, where the heterotrophs do not surround from the beginning the other clusters of bacteria. This initial distribution, together with random attachment effects, could provide more space for AOBs and NOBs clusters to grow without being entrapped. Figure 4b shows how newly attached clusters become centers from which the floc gains in diversity. As the floc grows, the attachment happens at its outskirts, so the diversity could be preserved, as long as the entrapped clusters are kept as low as possible (fig. 4c). It should be pointed out, again, that even if the heterogeneous clusters appear to touch, there is always the forbidden grid cells separating them (fig. 4b and 4c).

Conclusions

A new hybrid approach was proposed, to model the floc formation, growth and diversity. The model is a combination of classical mass balance equations and some elements from individual based modeling concepts, adapted to cope better with the floc characteristics, as revealed by FISH analysis. From the latter, we adopted the concept of fundamental building bloc, which is the grid cell, defined as the lowest volume/surface of the grid enclosing the nascent floc for which the continuum properties are still valid. Then,

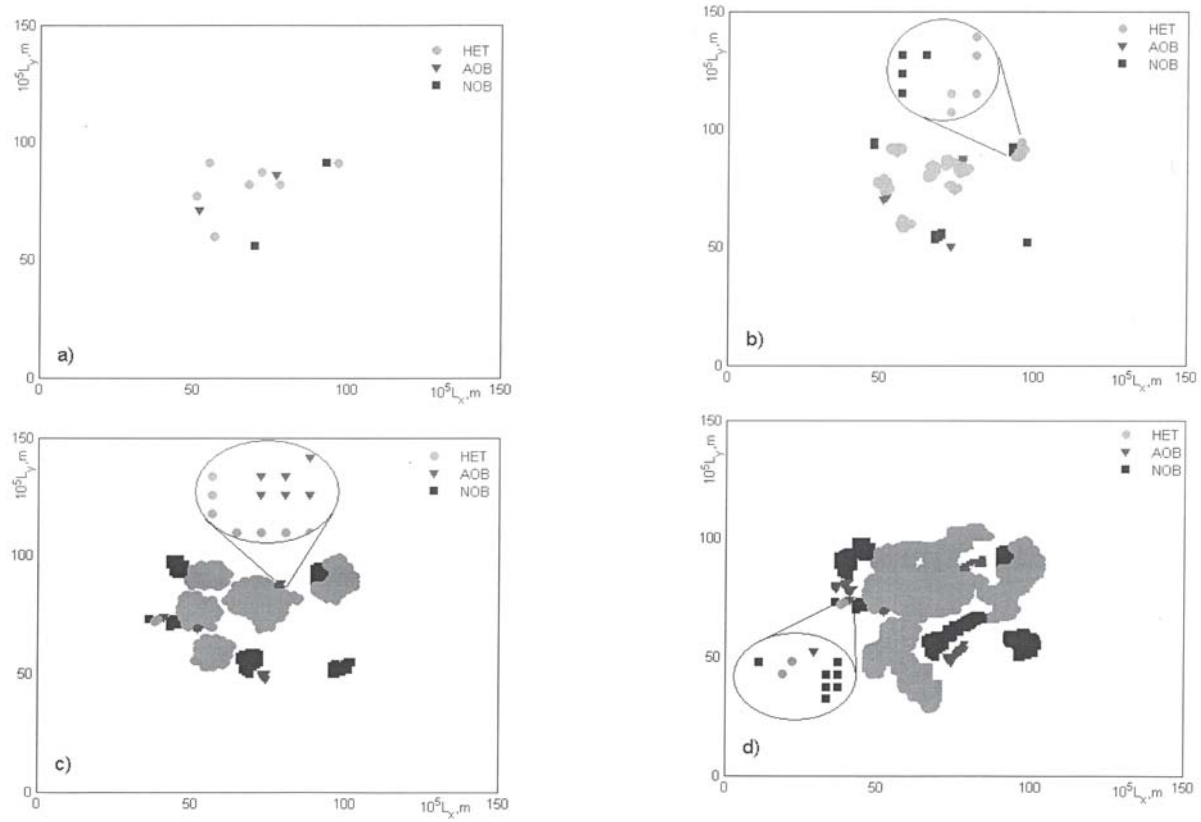

Fig. 4. Random seeding (initial random clusters: 4 HET, 4 AOB and 4 NOB), growth and attachment as main processes of a floc formation (the grid size is 3.10^{-4} x 3.10^{-4} m^2, the kinetic parameters and conditions of the operating environment are given in Table 1); *a)* initial random distribution of few random clusters, the size of one grid cell each; *b)* after 0.9 days; *c)* after 1.8 days – the basic structure of the floc starts shaping; *d)* after 2.5 days – several clusters, AOBs and NOBs, are trapped inside heterotrophs

we introduced the concept of vicinity, as the grid cells surrounding any cluster of microorganisms (which could have multiple contiguous fundamental building blocks); they could be free/available or forbidden. The forbidden grid cells are all included in the non-void set obtained intersecting all the vicinities. Another useful concept is the attachment, which permits increasing the diversity of the final floc distribution.

The floc starts from few randomly seeded building blocks, neither AOBs nor NOBs clusters being allowed to overpass the number of the heterotrophs grid cells. Growth is possible only at a fundamental building block level, occupying a free grid cell belonging to the vicinity.

The model could be used to adjust the microorganisms growth parameters to the floc conditions, meaning much lower levels of substrates than in the bioreactor/ environment, due to the diffusion limitations.

We showed that using these simple elementary steps, as previously defined, the model is capable of qualitatively reproducing the cross section distribution of flocs, as observed through FISH imagery.

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