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Multi-scale modeling of activated sludge floc structure formation in wastewater bioreactors

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Abstract

A multi-scale computational model was created for the formation of activated sludge floc structure. The model couples mass balances for substrates and biomass at reactor scale with an individual-based approach for the floc morphology, shape and micro-colony development. Among the novel model processes included are the group attachment of micro-flocs to the core structure and the clustering of nitrifiers. Simulation results qualitatively describe the formation of globular colonies of ammonia and nitrite oxidizers in the extracellular polymeric substance produced by heterotrophic microorganisms, as also observed in fluorescence in situ hybridization images. These results are the first step towards a multi-scale model of the activated sludge wastewater treatment systems, which could also be extended to other engineered biological systems.

Keywords: activated sludge floc, nitrification, individual-based model, colony size

1. Introduction

All open engineered biological communities comprise biologically and physically complex environments in which the macro-level performance is a function of the emergent properties of micro-level changes in composition and activity. The single most important such system is arguably activated sludge. Good mathematical models are important for improved design and operation, starting with the bioreactor and the separation unit, and including the floc/biofilm, the main processing units for nutrients removal. The processes imply different scales, all reflected in the modeling approaches. At macro-scale (reactor), the floc is seen most often as a simple pseudo-homogeneous sphere, with no clusters of species. When modeled as an entity at the micro-scale, the floc is a structured unit, where relations develop among different microbial species. Despite the increasing number of experimental studies on microbial diversity and ecology of nitrifying bacteria (Maixner et al., 2006), there is no unifying approach to predict the flocs characteristics based on their environment. Modeling how heterogeneity of flocs structure, distribution and dynamics concur to the system performance is challenging. Current models do not aim at linking micro-scale floc formation with the main contributors to the biological processes and bioreactor performance.
The goals of this modeling study are: (i) to reproduce the observed floc-like structures; (ii) to integrate the micro-scale model for the floc with the bioreactor operation. The bottom-up approach gives the advantage of retaining important biological information about the floc, seen as an aggregate of microorganisms and abiotic particles.

2. Methods

2.1. Experimental

The microbial community structure within activated sludge flocs was analyzed in samples from a municipal wastewater treatment plant (Spenneymoor, County Durham, UK) by the combined use of fluorescence *in situ* hybridisation (FISH) and confocal laser scanning microscopy (Maixner et al., 2006). The images obtained showed different shapes and dimensions of the flocs, all having common characteristics, which were further abstracted into the model features. In all images obtained, ammonia oxidizing bacteria (AOB) and nitrate oxidizing bacteria (NOB) were forming compact globular micro-colonies. A typical example is presented in Figure 1.

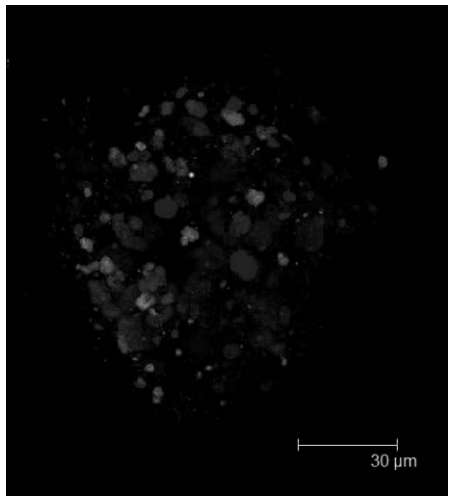

Figure 1. Fluorescence *in situ* hybridization of an activated sludge floc, observed by confocal laser scanning microscopy. Green – heterotrophic bacteria; blue – ammonia oxidizing bacteria (AOB); yellow – nitrite oxidizing bacteria (NOB).

2.2. Model description

To describe the observed floc morphology the system was represented at two scales. First, at micro-scale we developed an individual-based model for microbial growth and spreading, considering the main guilds of microorganisms implied (heterotrophs – consuming carbon source and oxygen, ammonia oxidizing bacteria – consuming ammonia and oxygen producing nitrite and nitrite oxidizing bacteria – consuming nitrite and oxygen), based on Martins et al. (2004). The steps considered in the floc evolution are: microbial growth and spreading, attachment of individual cells and attachment of groups of cells. Only the heterotrophs are producing extracellular polymeric substances (EPS), which surrounds and separates the cells. In contrast, the AOB and NOB are not producing EPS, therefore growing in distinct clusters. Together with the three types of microorganisms, EPS is also represented in this model by particulate entities. Microbial processes (see Table 1) are driven by the local concentrations of substrates (carbon source C_S, ammonium C_{NH4}, nitrite C_{NO2}, and oxygen C_{O2}). The two-dimensional substrate fields are found by solving diffusion-reaction mass balances for substrates in the floc and its surroundings. A constant-thickness (10 μm) mass transfer boundary layer follows the floc margins.

Second, for the reactor scale, the mass balances for the substrates are constructed by considering that the floc developed is representative for the whole biomass growing inside a continuous reactor with recycle and purge. Biomass balance over the reactor-separator system includes formation in the flocs and elimination by the purge.

2.3. Parameters

Values of yields and kinetic parameters were chosen according to established activated sludge and biofilm models (Wanner et al., 2006), namely: $Y_{HET} = 0.61$ $gCOD_X/gCOD_S$; $Y_{AOB} = 0.33$ $gCOD_X/gN$; $Y_{NOB} = 0.08$ $gCOD_X/gN$, $Y_{EPS} = 0.18$ $gCOD/gCOD_S$; $\mu_{m,HET} = 3$ d^{-1}; $\mu_{m,AOB} = 0.76$ d^{-1}; $\mu_{m,NOB} = 1.1$ d^{-1}. Influent flowrate was 3.43 m^3/d, with concentra-

tions $C_{in,S}$ = 0.04 kgCOD/m^3, $C_{in,NH4}$ = 0.04 kgN/m^3, $C_{in,NO2}$= 0.001 kgN/m^3, and $C_{in,O2}$ = 0.005 kg/m^3. A recycle/influent ratio of 0.2 and a purge fraction 0.01 were considered, which, for a reactor volume of 1 m^3, result in HRT = 0.3 d and SRT = 5.1 d. Oxygen was supplied by aeration with a specific flow of 12 kg d^{-1}m^{-3} and oxygen saturation concentration was 0.009 kg/m^3. Diffusion coefficients in the floc were assigned equal values with those in bulk water, namely: $D_{O2} = 1.73\cdot10^{-4}$ m^2/d; $D_{NH4} = 1.21\cdot10^{-4}$ m^2/d; $D_{NO2} = 1.03\cdot10^{-4}$ m^2/d; $D_S = 4.32\cdot10^{-5}$ m^2/d. The total biomass for inoculum was 0.06 kg, resulting in an initial number of flocs of 10^{14}.

Table 1. Stoichiometric matrix and processes rates for growth of heterotrophs, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). COD means "chemical oxygen demand".

Process	**Particulate components**				**Soluble components**			
	X_{HET} $\frac{kg_{COD}}{m^3}$	X_{AOB} $\frac{kg_{COD}}{m^3}$	X_{NOB} $\frac{kg_{COD}}{m^3}$	X_{EPS} $\frac{kg_{COD}}{m^3}$	C_S $\frac{kg_{COD}}{m^3}$	C_{O2} $\frac{kg}{m^3}$	C_{NH4} $\frac{kg_N}{m^3}$	C_{NO2} $\frac{kg_N}{m^3}$
Growth heterotrophs	1			$\frac{Y_{EPS}}{Y_{HET}}$	$-\frac{1}{Y_{HET}}$	$-\frac{1-Y_{HET}-Y_{EPS}}{Y_{HET}}$		
Growth AOB		1				$-\frac{3.42-Y_{AOB}}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}}$	$\frac{1}{Y_{AOB}}$
Growth NOB			1			$-\frac{1.15-Y_{NOB}}{Y_{NOB}}$		$-\frac{1}{Y_{NOB}}$

Process	**Rates,** $\frac{kg_{COD}}{m^3 d}$
Growth heterotrophs	$\mu_{m,HET}\frac{C_S}{K_{S,HET}+C_S}\frac{C_{O_2}}{K_{O_2,HET}+C_{O_2}}X_{HET}$
Growth AOB	$\mu_{m,AOB}\frac{C_{NH_4}}{K_{NH_4,AOB}+C_{NH_4}}\frac{C_{O_2}}{K_{O_2,AOB}+C_{O_2}}X_{AOB}$
Growth NOB	$\mu_{m,NOB}\frac{C_{NO_2}}{K_{NO_2,NOB}+C_{NO_2}}\frac{C_{O_2}}{K_{O_2,NOB}+C_{O_2}}X_{NOB}$

2.4. Solution method

The model was implemented in a combination of MATLAB code (ver. 2008b, MathWorks, Natick, MA) as the main algorithm driver, COMSOL Multiphysics (ver. 3.5a, Comsol Inc., Burlington, MA) finite element methods for solving the diffusion-reaction equations and own Java code for the individual-based floc model. Model solution involves a sequence of steps performed in a time loop (time step Δt = 0.002 days). At any time t there are successively solved: (a) the mass balances for substrates at steady state to get the 2-d concentration fields (with COMSOL finite element methods) given the

2-d biomass distribution and given concentrations in the reactor liquid (which are the boundary conditions); (b) biomass growth, division and spreading according to the local substrate concentrations (MATLAB and Java); (c) attachment of individual cells and micro-flocs (from a pool of structures previously created in the same conditions); (d) time evolution of reactor concentrations by coupling the reactor-scale balance with fluxes produced by all the flocs. With the floc geometry and biomass distribution so obtained, a new time step starts. A new model feature is that spreading takes place in two steps: within micro-colonies of nitrifiers, and between these colonies and heterotrophs plus EPS.

3. Results and discussions

In Figure 2 a typical structure obtained for 3.6 days of simulation time is presented. The simulation describes well the microscopy image from Figure 1, having compact and distinct AOB and NOB micro-colonies kept within the HET and EPS matrix. Micro-flocs attachment results in irregular floc shape during its whole development (Figure 2-right). While only small COD, O_2 and ammonium gradients developed in the floc, the local nitrite concentrations reflect the presence of the corresponding bacterial species, being higher around the AOB micro-colonies (arrow a), which are producing it, and decreasing around NOB micro-colonies (arrow b), which are consumers.

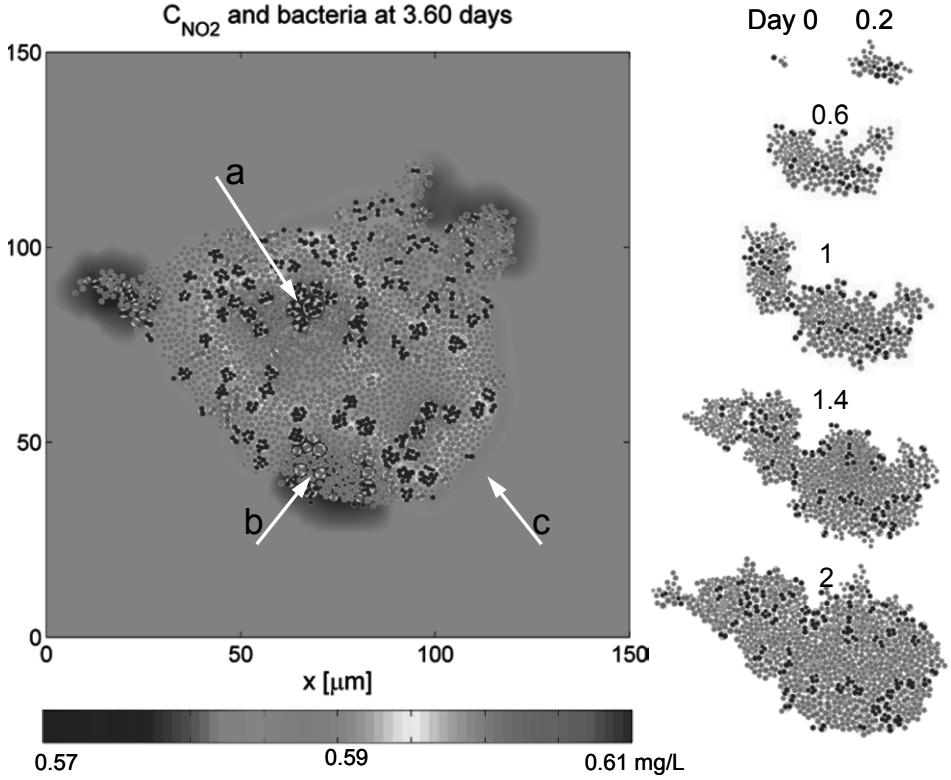

Figure 2. Example of simulated floc development. Left: microbial colonies in the floc (AOB - blue, NOB - red, HET - green, EPS - grey) at 3.6 days and the corresponding nitrite concentration distribution. Arrows point to: (a) nitrite accumulation due to the high density of AOB colonies in that region; (b) nitrite consumption by NOB; (c) concentration boundary layer. Right: different stages of floc development.

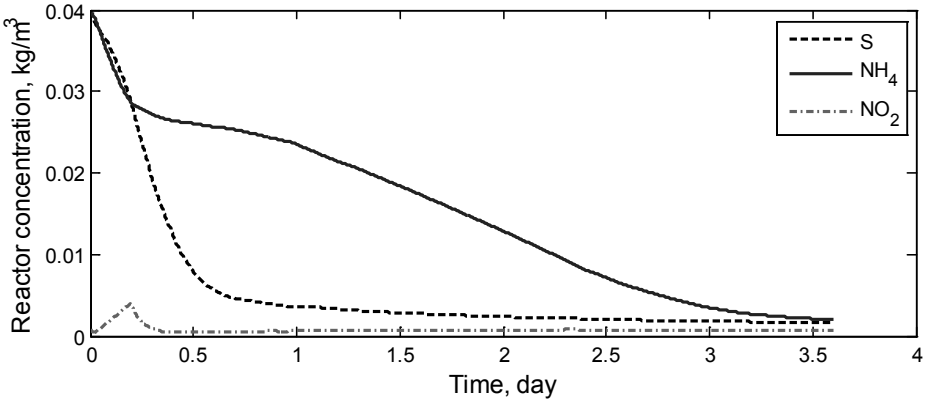

Figure 3. Substrate concentrations in the reactor reaching a quasi-steady state after 3.5 days.

The solution for mass balance of substrates in the reactor (presented in Figure 3) gives their concentrations evolution in time, corresponding with biomass growth. In the initial stage, when there is abundance of ammonium but fewer cells and small flocs, there is an accumulation of nitrite, the intermediate product in nitrification. All substrates approach a pseudo steady-state after 3.5 days, characterized by much lower concentrations of all substrates than in the influent. Heterotrophs, consuming their substrate both for growth and EPS production, lead to a faster decrease of COD than that of ammonium and nitrite (substrates used by nitrifiers). Consequently, while heterotrophs almost stop growing, the nitrifiers continue to grow, divide and increase their colony size within the EPS matrix formed by heterotrophs.

Conclusions

An individual based model was developed for an activated sludge floc inside of a continuous bioreactor. The different scales between mass transport and biomass growth were considered. The main features of the floc, as captured by FISH, e.g., globular colonies of nitrifiers surrounded by heterotrophs and EPS within irregularly-shaped flocs, were qualitatively reproduced by simulation. These results are the first step towards a multiscale model of the activated sludge wastewater treatment systems.

Acknowledgements

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