Aerobic granular sludge is a novel compact biological wastewater treatment technology for integrated removal of COD (chemical oxygen demand), nitrogen, and phosphate charges. We present here a multiscale model of aerobic granular sludge sequencing batch reactors (GSBR) describing the complex dynamics of populations and nutrient removal. The macro scale describes bulk concentrations and effluent composition in six solutes (oxygen, acetate, ammonium, nitrite, nitrate, and phosphate). A finer scale, the scale of one granule (1.1 mm in diameter), describes the two-dimensional spatial arrangement of four bacterial groups—heterotrophs, ammonium oxidizers, nitrile oxidizers, and phosphate accumulating organisms (PAO)—using individual-based modeling (IbM) with species-specific kinetic models. The model for PAO includes three internal charges. We present here a multiscale model of aerobic granular sludge sequencing batch reactors (GSBR) that uses macro- and microscale modeling to describe the metabolism of the four microbial groups.

Introduction

Aerobic granular sludge sequencing batch reactors (1–7) are an attractive alternative to conventional activated sludge processes for wastewater treatment thanks to a compact design (8) with a footprint of only 25% relatively to conventional activated sludge systems. This compact design is possible partially due to the high settling velocity of the granular sludge that renders the use of the traditional settler—indispensable in the classical activated sludge process—unnecessary. This process also allows high substrate loading rates (9) thanks to the high biomass concentrations achieved.

The compact design of granule sequencing batch reactors (GSBR) is also due to its integrated N-removal capabilities, possibly occurring through simultaneous nitrification/denitrification (SND). For SND to occur in aerobic granules, both an aerobic zone for nitrification and an anoxic substrate-rich interior for denitrification must exist within the same granule. The coexistence of an outer aerobic shell and an inner anaerobic zone is largely dependent on the bulk concentration of oxygen. However, whereas nitrification is favored by a high oxygen concentration, lower oxygen concentrations increase the size of the inner anaerobic zone where denitrification may occur. A previous one-dimensional modeling study (10) has determined that a DO value of 40% (3.2 mgO₂/L) would be optimal for SND.

GSBR operation was recently improved by introducing an anaerobic feeding phase. This provided selective pressure for slower growing organisms resulting in improved granular sludge stability (11). With this, the anaerobic/aerobic cycle provided conditions for the proliferation of polyphosphate-accumulating organisms (PAO) if enough phosphate was provided and the sludge residence time is suitably controlled. PAO are capable of storing poly phosphate (poly-P) as an energy reserve, a process in which polyhydroxyalkanoates (PHA) and glycogen are also involved (12), generating biological phosphate removal.

Integrated removal of chemical oxygen demand (COD), nitrogen and phosphate is achieved by a combination of several bioconversion carried out by multiple microbial groups coexisting in aerobic granules (13). Understanding how the operating conditions influence the composition of this microbial population and, consequently, the reactor performance is key for process engineering. For this purpose, we introduce a multiscale model the GSBR that uses individual-based modeling (IbM) of the microbial population in the granule integrated here for the first time with reactor-scale sequencing batch dynamics. IbM was introduced to microbial populations by Kreft et al. (14) and, while being particularly suited to address ecological and evolutionary questions (15, 16), was applied previously to several environmental bioprocesses involving biofilms and filamentous sludge (reviewed in ref (17) and anaerobic granules (18). IbM was recently made more accessible by a framework for multispecies biofilm modeling (19) which also facilitates the customized description of diverse microbial species metabolism with structured biomass. This framework was extended here to describe aerobic granular sludge process.

Materials and Methods

Model Description. We consider here three spatial scales (Figure 1). The individual scale describes the metabolism of individual biomass elements. The granule scale describes the spatial structure of the aerobic granule. The reactor scale describes dynamics of the entire reactor (i.e., bulk concentration of all solutes and total amount of biomass). The system modeled here is a lab scale GSBR (3 L volume) used previously in several experimental studies (13).

The kinetic model for the bioconversions is based on the activated sludge model no. 1 (ASM1) with the addition of metabolic description of PAO and separate description of ammonium and nitrite oxidizing bacteria (20). The kinetic model is composed by the 18 bioreactions (described in tables T1–T4 of the Supporting Information). Figure 2 represents the metabolism of the four microbial groups.
We consider six solute species: oxygen ($\text{O}_2$), acetate (substrate, $\text{S}_{\text{Ac}}$), ammonium ($\text{S}_{\text{NH4}}$), nitrite ($\text{S}_{\text{NO2}}$), nitrate ($\text{S}_{\text{NO3}}$), and phosphate ($\text{S}_{\text{PO4}}$). A seventh species, dinitrogen gas ($\text{N}_2$) is produced by denitrification reactions and considered for mass balances purposes. $\text{N}_2$ does not influence any of the reaction kinetics because it is an inert end-product of ammonia oxidation released to the atmosphere. We consider eight particulate species: active mass of ammonium oxidizing bacteria ($X_{\text{NH}}$), active mass of nitrite oxidizing bacteria ($X_{\text{NO}}$), active mass of heterotrophic bacteria ($X_{\text{H}}$), active mass of phosphate accumulating organisms ($X_{\text{PAO}}$), the internal storage polymers PHA (polyhydroxyalkanoates, $X_{\text{PHA}}$), poly phosphate (poly-P, $X_{\text{PP}}$) and glycogen ($X_{\text{GLY}}$), and inerts ($X_i$). Individual biomass elements belong to one of the following four bacteria groups: ammonia oxidizing bacteria (NH), nitrite oxidizing bacteria (NO), nonphosphate accumulating heterotrophs (H), and phosphate accumulating organisms (PAO). The active species present in one individual defines its bacterial group. In addition, individuals of all four species may have a fraction of inerts resulting from the decay.

The 3 L lab-scale reactor is operated in a 3 h cycle. At the end of each cycle the reactor is half-emptied, following typical operation (13). A volume of 1.5 L of fresh feed is then added to refill the reactor before a new cycle begins. Both the emptying and refilling operations are assumed to be instantaneous; i.e. substrate concentration becomes the volume weighted average from the substrate concentrations in influent and that remaining in the reactor. Dynamics of bulk concentrations of each solute during each 3 h cycle are described generally as

$$\frac{dS_{\text{bulk}}}{dt} = n_g R_i(t) [M L^{-3}T^{-1}]$$

(1)

where $n_g$ is the number of granules in the reactor, and $R_i$ is the global conversion rate of the solute $i$ by a single granule. Conversions by nongranular biomass suspended in bulk liquid are here neglected (see below).

The dynamics of dissolved oxygen is not modeled by eq 1. Instead, three aeration scenarios are considered. The first assumes a constant DO during the entire 3 h cycle, such as

![FIGURE 1. The spatial and temporal scales of the multi-scale model. (a) Spatial scales range from individual biomass elements, at a scale of a few micrometers to dynamics of multiple bacterial groups in the "aerobic granule scale", in millimeter scale, to the full system (a 3 L lab scale GSBR). The 2-D computational domain represents a small subvolume inside the reactor containing a single granule. The number of granules in the reactor ($n_g$) is a multiplier used for scaling-up the granule scale mass balances to the bulk concentrations dynamics (eq 1). The computational domain is given a depth, $\Delta z$, for mass balancing purposes, as previously described for 2-D biofilm simulations (19). (b) Temporal scales range from "short-term dynamics" describing each 3-h cycle with precision, to "long-term dynamics" in which GSBR operation is simulated over 2 years.](image)
that carried out in earlier lab-scale studies ([10], [21]). The second
regime consists of aerobic/anoxic phases during the cycle.
Here, bulk concentration of oxygen is set to zero in the first
hour, and to a value $\delta_{O_2,aeration}$ during the following 2 h, as
carried out in more recent studies ([11], [13]). The third regime
is a variation of the later aeration strategy where the aerobic
phase will be subject to on/off control dependent on the
concentration of ammonia ([20]).

The computational domain represents a 2 d median
cross-section of a single granule. Simulations start with 10
equally sized particles of each of the four microbial groups,
constituting a granule of 120 $\mu$m. As in previous models ([19]),
spatial solute distributions can be calculated from a steady-
state diffusion–reaction equation for each of the solutes since
diffusion is much faster than microbial growth:

$$D_i \left( \frac{\partial^2 S}{\partial x^2} + \frac{\partial^2 S}{\partial y^2} \right) + r_i = 0 \ [M L^{-3} T^{-1}]$$

(2)

$D_i$ is the diffusivity of species $i$ and $r_i$ is the local conversion
rate of $i$. Equation 2 is solved in the granule and in the
surrounding concentration boundary layer. Complete mixing
rate of $i$. Equation 2 is solved in the granule and in the

Results

Figure 1b shows the short term and long-term dynamics of
the bulk concentrations of solutes in one cycle and in the
effluent respectively. More detailed results for the time
courses of the granule composition and effluent concentra-
tions for the five simulations may be found in Figure F1 of
the Supporting Information. Figure F2 (Supporting Informa-
tion) shows results from typical 3 h cycles from the mature
stages of GSBR operation. Mature stage is here defined as the
period when both granule composition and effluent con-
centrations become stationary. Next, we comment on the
results from case no. 1. Finally, the remaining simulation
results are briefly described, referring to the differences from
case no. 1.

Initial Development Stage. This case describes a con-
tinuously aerated system at a high dissolved oxygen con-
centration, mimicking the conditions of experiments reported in
ref 4. Initial development stage is the period in which the
granule grows up to the maximum diameter of 1100 $\mu$m,
which here lasts 30 days. During this period, the non-PAO
heterotrophic population ([1]) grow very rapidly quickly
becoming the dominant bacterial group in the granule (see
Figure 3a–f). Complete acetate removal is achieved in the
second cycle, dividing the cycle into a period when acetate
is present, called feast phase, and famine phase ([10]). The
feast phase is characterized by strong oxygen gradients throughout the granule, with the outer layers having a high oxygen concentration and the core of the granule experiencing anaerobic conditions. Gradients are caused by the high rate of oxygen consumption by heterotrophic bacteria consuming acetate aerobically. In contrast, in the famine phase is characterized by strong oxygen gradients throughout the granule, with the outer layers having a high oxygen concentration and the core of the granule experiencing anaerobic conditions. Gradients are caused by the high rate of oxygen consumption by heterotrophic bacteria consuming acetate aerobically. In contrast, in the famine

FIGURE 2. Schematic representation of the bioreactions carried out in individuals of the four microbial groups. Bioreactions are detailed in tables T1 and T2 (Supporting Information). Not represented here, but implemented in the model, is the capability of PAO carrying out PHA consumption, phosphate ("PO₄³⁻") uptake and growth using nitrite ("NO₂⁻") and/or nitrate ("NO₃⁻") in the absence of O₂. "Ac" represents acetate.

FIGURE 3. Spatial distributions of microbial populations and inert materials in aerobic granules. (a–h) Granule development for simulated case no. 1. Initially, fast growing heterotrophic organisms (in blue) constitute the majority of the granule biomass at day 26. In later stages, phosphate accumulating organisms (in red) dominate in spite of growing at a slower rate, since they make a more efficient use of the substrate consumed. (i) Fluorescence in-situ hybridization (FISH) of microbial groups in the granule visualized using confocal laser scanning microscopy (green is ammonium oxidizing bacteria; blue is eubacteria; red is PAO, reproduced with authorization from ref 13); (j) Mature granule from lab scale GSBR stained with live/dead kit (Molecular Probes, Eugene, OR), showing accumulation of inert material, stained red by propidium iodide, in the granule core, whereas live bacteria, stained green with Cyto-9, are located in the outer layers, similarly to what was obtained from simulations (i.e., panels g and h).
Phase oxygen penetrates well into the granule. Growth of ammonia oxidizers is only possible in famine phase when competition for oxygen is lower. Growth of ammonium oxidizers is low in the initial period but sufficient for complete NH$_4^{+}$ removal to be achieved by day 3. Any NO$_2^-$ produced by ammonium oxidizers is rapidly converted to NO$_3^-$ by nitrite oxidizers. Growth of PAO is low and phosphate removal at day 30 is only 10%. By day 30, inerts already constitute more than 50% of the granule mass, originated mostly from the decay of heterotrophs.

**Maturation.** After reaching maximum granule size, the maturation phase begins with the internal composition of the granule rearranging itself until it stabilizes at day 150. During this period, heterotrophs start losing their dominance in favor of PAO, which steadily grow from the inside of the granule (see sequence of Figure 3f–h). Enrichment in PAO increases phosphate removal, with cyclic phosphate release/uptake resulting in full phosphate removal at day 90. Ammonium oxidizers remain stable, sustaining complete NH$_4^{+}$ conversion.

The Mature Stage. It is only when PAO biomass is removed from the reactor through the process of detachment that actual phosphate removal from the system begins. Before this, phosphate is simply stored inside the granules in the form of poly-P (23). The mature stage that is characterized by a stable microbial population. The concentrations of storage compounds in PAO (PHA, poly-P, and glycogen) oscillate in each cycle (Supporting Information Figure F2c), with feast-phase accumulation of PHA and poly-P and glycogen consumption followed by famine-phase accumulation of poly-P and glycogen with PHA consumption. The average radial distribution of microbial groups (Supporting Information Figure F2d) is in general agreement with the well-known layering observed in biofilms in the presence of different electron acceptors and the organization observed from imaging of FISH stained granules (Figure 3i). The organisms consuming substrate at the lowest redox state grow in depth of the biofilm. Within each redox zone, the faster growing bacteria will be found more at the outside of the biofilm, where substrates are coming from. At the end of simulations, the major component of the granule is inert material (Figure 5a). Inerts are mostly located in the core, in agreement with microscopy imaging of Live/dead stained granules (Figure 3j) and a previous study by McSwain et al. (24). The latter also showed that this inert core is mostly composed by proteins.

Lower Operation DO, Case No. 2. As in case no. 1, initial development is characterized by predominance of heterotrophs, albeit less pronounced since lower DO is advantageous to PAO due to an increased anaerobic region. Consequently, complete P-removal can be achieved sooner (Figure F1d, Supporting Information). Inert accumulation is also faster, and the mature stage reached earlier at day 110. Composition in ammonia and nitrite oxidizers is practically not altered by the lower DO, but N-removal increases to 11%.

Anaerobic/Aerobic Cycle at DO 40%, Case No. 3. The competitive advantage of PAO is enhanced as soon as the feast phase becomes completely anaerobic. This anticipates slightly the full P-removal (Figure F1f, Supporting Information). However, since ammonium oxidation is only possible in the aeration period, N-conversions take longer to stabilize. The mature stage is characterized by a larger fraction of PAO in the granule (Figure 5b). Phosphate and acetate conversions...
Anaerobic/Aerobic Cycle at DO 20%, Case No. 4. This simulation predicts that very low DO has a negative effect on all bioconversions. Full P-removal is significantly delayed and complete conversion of ammonia to nitrate may never fully achieved (Figure F1h, Supporting Information). Effluent concentrations of N-components oscillate constantly and the net N-removal is 40%.

Introducing On/Off Control of DO, Case No. 5. Operating conditions of this simulation are identical to those of case no. 3, with the additional control of aeration. Aeration is switched off during the aerobic phase once ammonia is completely consumed. This results in an increase of N-removal to 60%, the highest of all cases, in spite of only marginal changes in the composition of the microbial population (Figure 5e).

Further Comparison with Experimental Data. The simulated reactor-scale bioconversion dynamics were compared with the trends observed experimentally for solute species in the 3 h cycles. For the continuous aeration (case no. 1), the model results concerning acetate, \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) show qualitative agreement with trends measured by Beun et al. (25) who operated lab scale reactors in the same conditions. Comparisons with experimental results for the cases using anaerobic feeding phases are more difficult due to the impracticality of measuring concentrations during reactor feeding. Nevertheless, results for the aerated phase agree well (Figure F3, Supporting Information).

Discussion

Our simulations provide detailed insight into dynamics of the GSBR process. The present work focused on the effect of different aeration schemes. Nevertheless, the same model may be used to analyze other operating conditions that influence the microbial community such as the cycle duration (26), the influent composition (9), or the mechanical shear stress affecting granule size (27–31).

Attempts to improve N-removal in lab scale reactors have so far consisted in decreasing the operational DO of aerated phases in the GSBR cycle. These strategies are based on the assumption that nitrification and denitrification occur...
simultaneously (13), in which case N-removal is enhanced by decreasing the oxygen penetration in the granule. Results from these simulations describe that simultaneous nitrification/denitrification (SND) is present to some extent (Figure 4). However, they also suggest that SND contribution to high nitrogen removal is limited. Figure 5f shows the relative contribution of SND to the total N-removal in all simulations. In the continuous aeration cases (nos. 1 and 2), total N-removal is low but SND accounts for the largest part. When anaerobic periods are introduced, as in cases 3 and 4, the overall N-removal becomes significantly higher. However, denitrification now occurs mainly during the anaerobic period, when nitrification is absent. In these cases, the nitrate being consumed at the beginning of the 3 h cycle is reminiscent from the previous cycle (see, for example, the black line representing \( S_{\text{NO3}} \) in Figure F2i in the Supporting Information). This means that denitrification and nitrification occur in alternation. Realizing that most N-removal is due to alternating nitrification/denitrification (AND) rather than SND allows designing alternative strategies to further increase N-removal. This is the case of hypothetical on/off strategy proposed in case no. 5, that makes a second anaerobic phase later in the cycle (20). In reality, aeration cannot simply be switched off since it also provides mixing. Recycled off-gas (or \( N_2 \) in lab systems), could be sparged into the reactor to provide mixing but further experiments should evaluate the feasibility of a nonmixed system in the last cycle part.

This study demonstrates application of individual-based modeling (IbM) to the wastewater treatment process of aerobic granular sludge. These simulations provide insight into the many bioconversion processes occurring while describing both short-term dynamics and long-term reactor operation. This novel model integrates for the first time dynamics of microbial metabolisms, granule-scale diffusion-reaction with 2-D spatial organization and larger scale sequencing batch reactor operation. By being computationally demanding, these models are still far from having a disseminated application in process control. Nevertheless, they can already constitute a valuable research tool in academic and industrial research settings to study spatial-temporal dynamics in long-term development of multispecies microbial communities. The use of 2-D and 3-D models can often produce unexpected theoretical results that could not be predicted from 1-D models (16, 18, 32). Nevertheless, simpler 1-D biofilm models, such as the one in the popular AQUASIM software (33) that presently runs effortlessly in off-the-shelf desktop computers, have demonstrated the power of simulation for bioprocess design in environmental technologies. With the foreseeable increase in affordable computing power and efficient numerical methods, we may envision that multiscale individual-based models will be a common tool for the future engineer of biological wastewater treatment processes.

**Nomenclature**

<table>
<thead>
<tr>
<th>AND</th>
<th>alternating nitrification/denitrification</th>
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<tbody>
<tr>
<td>COD</td>
<td>chemical oxygen demand</td>
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<tr>
<td>DO</td>
<td>dissolved oxygen (oxygen concentration as a percentage of its saturation in water)</td>
</tr>
<tr>
<td>GSBR</td>
<td>granular sludge sequencing batch reactor</td>
</tr>
<tr>
<td>IbM</td>
<td>individual-based modeling</td>
</tr>
<tr>
<td>PAO</td>
<td>phosphate accumulating organism</td>
</tr>
<tr>
<td>PHA</td>
<td>polyhydroxyalkanoates</td>
</tr>
<tr>
<td>poly-P</td>
<td>poly phosphate</td>
</tr>
<tr>
<td>( S_i )</td>
<td>concentration of solute species ( i )</td>
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<table>
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<tr>
<th>SND</th>
<th>simultaneous nitrification/denitrification</th>
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**Acknowledgments**

J. B. Xavier was financially supported by the FCT/MCTES, Portugal, through grant SFRH/BPD/11485/2002. Dr. António Maretez’s support as system administrator for the ITQB computational facility is gratefully acknowledged.

**Supporting Information Available**

Additional details are illustrated with five movies, five tables, and four figures. This material is available free of charge via the Internet at http://pubs.acs.org.

**Literature Cited**


Received for review February 2, 2007. Revised manuscript received July 5, 2007. Accepted July 10, 2007. ES070264M