Supplementary information for

Modeling nutrient removal in the aerobic granular sludge system by connecting reactor- and granule-scale models

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This file includes:

- **Supplementary texts with references (SI Text A and B)**
- **Legends for Video 1**
- **Supplementary tables (Supplementary Table I, II, III, IV, and V)**
- **Legends for Supplementary figures (Supplementary Figure 1 and 2)**

SI Text A. Reactor-scale model

Model development

System definition

Sludge for wastewater treatment is assumed to be a collection of granules of various sizes, at any moment in time. Consider wastewater with volume V, in which N_{chem} chemical species are dissolved. Suppose that n_g granules, which are composed of at most N_{part} particulate species, are mixed with the wastewater. Assume that concentrations of chemical species k = 1, 2, ..., N_{chem}, and particulate species j = 1, 2, ..., N_{part} are S_k and X_j, respectively. For the particulate matter alone, assume the mass of granule i = 1, 2, ..., n_g to be m_i; then, the mass of the particulate species j in the granule i is $m_i^{(j)}$. The concentration of biomass in the reactor, X_j, is therefore

$$X_j = \frac{1}{V}\sum_{i=1}^{n_g} m_i^{(j)}. \quad (1)$$

If the granules are assumed to be spheres, then the total volume V_i for granule i and its effective diameter D_i are given by

$$V_i = \sum_{j=1}^{N_{part}} \frac{m_i^{(j)}}{\rho_j}, \quad D_i = \left(\frac{6V_i}{\pi}\right)^{1/3}, \quad (2)$$

where ρ_j denotes the density of particulate species j.

Concentrations in the reactor over time

The changes in the concentrations S_k and X_j with time are governed by the following ordinary differential equations (mass balances over the reactor):

$$\frac{dS_k}{dt} = \sum_{l=1}^{N_{react}} Y_{lk}\, R_l, \quad (3)$$

$$\frac{dX_j}{dt} = \sum_{l=1}^{N_{react}} Y_{lj}\, R_l, \quad (4)$$

where l = 1, 2, ..., N_{react} denotes each microbiological reaction. Y_{lj} and Y_{lk} are the yields of component j or k as a result of reaction l. R_l is the rate of reaction l, defined generally by Eq. 5:

$$R_l = q_l^{max} \prod_r \varphi_r \cdot X_{J(l)}, \quad (5)$$

where q_l^{max} denotes the maximum specific rate of reaction l and ϕ_r is a Monod saturation function that controls the rate depending on S_k or X_j. $J(l)$ denotes the particulate species that mediates the reaction l.

The yields Y_{lj} and Y_{lk} in all microbiological reactions are defined in the stoichiometric matrix in Supplementary Table I, the specific reaction rates R_l are in Supplementary Table II, and the parameter values are presented in Supplementary Tables III and IV.

Irrespective of the above dynamics, the changes in the dissolved oxygen concentration S_{O2} was set to zero during the anaerobic and anoxic periods of the SBR cycles, and was

set to 1 [g/m^3] during the aerobic period unless otherwise noted.

Update of granule mass in time

The change of mass $m_i^{(j)}$ of particulate species j in granule i is expressed as

$$\frac{dm_i^{(j)}}{dt} = \sum_l Y_{lj}\left(\frac{R_l}{X_{J(l)}}\right) m_i^{(J(l))}. \quad (6)$$

This rate equation corresponds to a reaction in which all components of each granule are well mixed. However, solving $N_{part} \times n_g$ equations for all $m_i^{(j)}$ (as in Eq. 6) and N_{chem} equations for S_k (as in Eq. 3) simultaneously is unrealistic since n_g is a very large number (on the order of thousands or more).

Therefore, as for numerical convenience, $m_i^{(j)}$ is not directly updated during a small time period Δt. Instead of solving Eqs. 3 and 6 simultaneously for S and m and then calculate X, we chose to solve Eqs. 3 and 4 simultaneously for S and X and then update $m_i^{(j)}$ at the next moment in time (t+Δt) as:

$$m_i^{(j)}(t + \Delta t) = m_i^{(j)}(t) + \Delta m_i^{(j)}(t). \quad (7)$$

Here, the change in mass during the interval of time Δt is

$$\Delta m_i^{(j)}(t) = \Delta X_j(t) \frac{\sum_{l=1}^{N_{react}} Y_{lj}\left(\frac{R_l(t)}{X_{J(l)}(t)}\right) m_i^{(J(l))}(t)}{\sum_{l=1}^{N_{react}} Y_{lj}\, R_l(t)}, \quad (8)$$

with ΔX_j (t) calculated by

$$\Delta X_j(t) = \int_t^{t+\Delta t} dX_j = \int_t^{t+\Delta t} \frac{dX_j}{dt'} dt' = \sum_l \int_t^{t+\Delta t} Y_{lj} R_l(t') dt', \quad (9)$$

when Eq. 4 is used. It should be noted that the relation

$$\Delta X_j(t) = \Delta t \sum_l Y_{lj} R_l(t) \quad (10)$$

is not established. Since

$$\lim_{\Delta t \to 0} \frac{\Delta m_i^{(j)}(t)}{\Delta t} = \frac{dm_i^{(j)}}{dt}(t) \quad (11)$$

is established, the smaller the time step Δt used, the more accurate will be the biomass calculation (as usually the case with explicit discretization of ordinary differential equations).

Granule selection

The terminal settling velocity v_i of granule i in a reactor is given by

$$v_i = \frac{g(m_i/V_i)D_i^2}{18\mu} = \frac{gm_i}{3\pi\mu D_i}, \qquad (12)$$

where g is the gravitational acceleration and μ is the viscosity of wastewater.

Consider the case that all granules in a reactor are well mixed within the wastewater. When the mixing is stopped, each granule begins to settle. The settling velocities are given by v_i if we ignore the accelerating motion occurring for a very short time interval immediately after the initiation of settling. After an interval of time T_{settle} from the start of the settling, granules with wastewater were withdrawn from the upper volume exchange ratio (*VER*, with $0 < VER < 1$) region of the reactor.

As a result, the probability that a granule i remains in the reactor after one cycle of consecutive settling and withdrawing processes is given by

$$P_i^{select} = \begin{cases} \dfrac{H - H \cdot VER}{H - v_i T_{settle}}, & when \quad H \cdot VER > v_i T_{settle} \\ 1, & otherwise \end{cases} \qquad (13)$$

where H denotes the height of the reactor. Note that this probability depends not only on the settling time T_{settle}, but also on the granule size and density through v_i.

Numerical simulation

Implementation

To simulate the system development in time, one time step sequence of calculations includes concentration update, granule mass update, and granule selection, as described above. This sequence is repeated as time advances in steps of Δt. The model solution algorithm was implemented in C++ by using ordinary differential equation (ODE) solvers based on the Bulirsch-Stoer method from Press et al. (2007). To reduce computational load, the computational volume is set to 0.1 mL, which is much smaller than that of the sequencing batch reactor (SBR) used in the experiment (4.5 L).

Initial granule size distribution

In the experiments, inoculated sludge was composed of various sized granules and flocs. For the model, it has been assumed that the cumulative volume of initial granules with diameter between D and $D+\Delta D$ was proportional to $\exp(-(D-D_0)^2/(2\sigma^2_D))\Delta D$, where D_0 and σ^2_D are the parameters related to the mean and variance, respectively, of the initial granule diameter. This assumption was based on the analysis of the sludge images obtained with a bright-field microscope experimentally. The values for D_0 and σ^2_D were determined so as to be consistent with the experimental results of sludge separation based on the granule (floc) size by using several different sieves. The initial number of granules ($n_{g,\mathrm{init}}$) was determined to be consistent with the experimental data of the mixed liquor suspended solids (MLSS). The values D_0 = 137.2 [μm], σ^2_D = 2052 [μm^2], and $n_{g,\mathrm{init}}$ = 6349 (per computational volume of 10^{-7} m^3) were used to generate the initial granules.

SI Text B: Improvements in the algorithm of the granule-scale model

The basic algorithm of the individual-based model (IbM) was described in Picioreanu et al. (2004) and used in several other studies (Matsumoto et al. 2010; Xavier et al. 2007). Here, we describe the minor changes made in the algorithm in the calculation of the present granule-scale model.

Growth of single particle

In the two-dimensional IbM, a single granule was described as a collection of growing microbial particles, each particle containing a single microbial species. Although each particle is described as a circle in a two-dimensional space, we can also interpret each particle in three dimensions as a cylinder with height η, and all particles having the same height.

In the original algorithm, a particle described as a cylinder with area α of the top surface and height η (thus having a volume of $\alpha\eta$) grows by increasing its top area while the height remains constant. In a mathematical form, the top surface area changes

from α to $\alpha + \Delta v/\eta$, where Δv is a volume increase.

In the algorithm used in the present granule-scale model, the particle (cylinder) grows by sustaining the ratio of the diameter of the top surface circle to the height of the cylinder. In a mathematical form, the top surface area and the height of the cylinder grow from (α, η) to (α', η'), where α' and η' are the solutions of the following simultaneous equations:

$$\alpha' \eta' = \alpha\eta + \Delta v, \quad (14)$$

$$\sqrt{\alpha'}/\eta' = \sqrt{\alpha}/\eta. \quad (15)$$

This modification was needed to fit the simulation results to the several experimental data simultaneously by reducing the growth rate of the diameter of the granule while that of MLSS was unchanged.

Withdrawing suspended solids from the reactor

In the experiment, excess suspended sludge was withdrawn regularly to maintain an SRT of 30 days. In the present simulation of the granule-scale model, similar sludge withdrawal was effected. In the model, the MLSS was calculated by the biomass of the granule used for the IbM calculation multiplied by the number of granules; therefore, sludge withdrawal was achieved by decreasing the number of granules. n_g/SRT granules were subtracted from the reactor once a day, where *SRT* is the sludge retention time in days.

In addition to the above sludge withdrawal, in real systems, a small amount of sludge flows out from the reactor in the withdrawal period of each SBR cycle. As shown in Fig. 2(B), sludge was suspended in the treated water with a concentration of 0–0.2 kg/m^3. To simulate such outflow of sludge, a small amount of biomass was withdrawn in the withdrawing period of every SBR cycle by decreasing the number of granules. In the present simulation, $n_g \times VER \times 0.005$ granules were subtracted from the reactor at the end of every cycle, where *VER* is the volumetric exchange ratio of the SBR, and the value of 0.005 was related to the ratio of total suspended solids (TSS) of the treated water to MLSS of the reactor given in the experiment (see Fig. 2(B)).

References for the supplementary texts

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Press W, Teukolsky S, Vetterling W, Flannery B. 2007. Numerical Recipes, Third Edition. Cambridge: Cambridge University Press.

Xavier JB, De Kreuk MK, Picioreanu C, Van Loosdrecht MCM. 2007. Multi-scale individual-based model of microbial and byconversion dynamics in aerobic granular sludge. Environmental Science and Technology 41(18):6410-6417.

Legends for videos

Video 1. An example simulation result of the two-dimensional growth of a single microbial granule. Each microbial particle, comprising a single microbial type, was represented by a colored circle. The particles of AOB, NOB, Het, PAO, and GAO were represented by red, green, yellow, blue, and white circles, respectively. The time elapsed from the start of the simulation of the reactor-scale model was printed on the upper left corner.

Supplementary tables

Supplementary Tables I, II, III, IV, and V are provided by a separate PDF file.

Legends for Supplementary figures

Supplementary Figure 1. A photograph of the stirrer used in the present study. The diameter of the impeller is 48 mm.

Supplementary Figure 2. FISH images of the typical granule cross-sections. (A, B) Yellow cells are PAOI (hybridized with both EUB338mix probes; green and Acc-I-444 probes; red). Whole granule section (A) and the magnified image (B) are shown. (C, D) Yellow cells are PAOII (hybridized with both EUB338mix probes; green and Acc-II-444 probes; red). Whole granule section (C) and the magnified image (D) are shown. Scale bars in (A,C) and (B, D) represent 200 and 50 μm, respectively.