Is the whole the sum of its parts? Agent-based modelling of wastewater treatment systems

A. J. Schuler, N. Majed, V. Bucci, F. L. Hellweger, Y. Tu and A. Z. Gu

ABSTRACT

Agent-based models (ABMs) simulate individual units within a system, such as the bacteria in a biological wastewater treatment system. This paper outlines past, current and potential future applications of ABMs to wastewater treatment. ABMs track heterogeneities within microbial populations, and this has been demonstrated to yield different predictions of bulk behaviors than the conventional, ''lumped'' approaches for enhanced biological phosphorus removal (EBPR) completely mixed reactors systems. Current work included the application of the ABM approach to bacterial adaptation/evolution, using the model system of individual EBPR bacteria that are allowed to evolve a kinetic parameter (maximum glycogen storage) in a competitive environment. The ABM approach was successfully implemented to a simple anaerobic-aerobic system and it was found the differing initial states converged to the same optimal solution under uncertain hydraulic residence times associated with completely mixed hydraulics. In another study, an ABM was developed and applied to simulate the heterogeneity in intracellular polymer storage compounds, including polyphosphate (PP), in functional microbial populations in enhanced biological phosphorus removal (EBPR) process. The simulation results were compared to the experimental measurements of single-cell abundance of PP in polyphosphate accumulating organisms (PAOs), performed using Raman microscopy. The model-predicted heterogeneity was generally consistent with observations, and it was used to investigate the relative contribution of external (different life histories) and internal (biological) mechanisms leading to heterogeneity. In the future, ABMs could be combined with computational fluid dynamics (CFD) models to understand incomplete mixing, more intracellular states and mechanisms can be incorporated, and additional experimental verification is needed.

Key words | agent-based modelling, cellular automata, distributed states, enhanced biological phosphorus removal, heterogeneity, intracellular polymer, Raman microscopy, wastewater treatment

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INTRODUCTION

Agent-based (also called distributed state, individual-based and micro-scale) models are different from traditional population-level (also called lumped, population-level and macro-scale) models in that they model autonomous, independently-acting individuals within a system. As such, ABMs explicitly simulate population heterogeneity or intra-population variability, which is lost in the lumped approach. This modeling approach has been applied to a variety of systems, including human and animal migration, traffic, stock trading, and land development. In the biological sciences, ABMs have traditionally been applied to higher-level organisms (e.g. fish, wolves), but there is a recent trend to simulate microbes (see review in Hellweger and Bucci 2009), such as the cell quota distribution in phytoplankton (Hellweger and Kianirad 2007). More recently, agent-based models (ABMs) have been applied to biological wastewater treatment systems.

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There, the agents represent individual bacteria that take up, metabolize and excrete nutrients, and the population-level behavior (i.e. concentration of various constituents) emerges as a result of the cummulative action of these agents. These applications have revealed potential deficiencies in conventional lumped-typed approaches and provided new insights. This paper provides a review of previous work on ABMs in wastewater treatment, current research in two U.S. laboratories active in this area, and outlines promising areas for future research.

PAST WORK

In cases where ABMs have been applied to microbial systems, the individual units are generally bacteria or groups of bacteria (also refererred to as ensembles or super-individuals). In wastewater treatment, ABMs have been applied to attached and suspended growth processes. For the attached-growth process, ABMs have been extensively used to model the develoment of physical structures in biofilms/flocs (Picioreanu *et al.* 1998, 2005, Chang *et al.* 2003, Martins *et al.* 2004, Batstone *et al.* 2006, Xavier *et al.* 2007).

For suspended-growth wastewater processes, ABMs have been applied to study microbial diversity within given populations. Traditionally, wastewater microbes are conceptualized as chemical entities that react with nutrients (i.e. DOC) to form more microbes. This view is reflected in conventional methods of measuring microbes (e.g. as volatile suspended solids, VSS concentrations) and in units used in models (e.g. mg C/L). Models with a more "structured" approach to biomass can include explicit modeling of microbial storage products. Gujer (2002) demonstrated the potential usefulness of the ABM approach for a bacterium with a single storage product in a system with completely mixed flow reactors (CMFRs). The key feature of such systems is that the bacteria with microbial storage products cycle through "feast-famine" conditions as they travel through different CMFRs in an activated sludge system. Completely mixed hydraulics dictate these bacteria experience variable hydraulic residence times (HRTs) as they do so. This variability in HRT in turn leads to heterogeneity in storage product content in individual bacteria, which strongly influences their functional behavior in ways not predicted by a lumped model approach.

The ABM approach was demonstrated to be critical to the modeling of enhanced biological phosphorus removal (EBPR), which relies on the storage products polyphosphate, polyhydroxyalkanoates (PHAs), and glycogen in completely mixed reactor systems (Schuler 2005). In systems with non-variable hydraulic residence times, such as plug flow reactors (PFRs) or sequencing batch reactors (SBRs), heterogeniety (distributed bacterial states) is generally not expected, although it could be introduced by incomplete mixing and biological factors, like phylogenetic and/or phenotype differences among the same functional group of microoganisms such as PAOs. Schuler (2006) demonstrated that population heterogeneity (distributed states) decreased as the number of completely mixed reactors in series increased (because flow hydraulics approached those in a PFR), and the "error" associated with the lumped modeling approach decreases. Schuler and Jassby (2007) demonstrated that endogenous consumption of microbial storage products tends to increase the difference between lumped and ABM predictions, since the ABM approach accounts for the phenomenon that bacteria with longer than average hydraulic residence times in a given reactor can experience decreased activity as their energy reserves are depleted.

The importance of these findings is that populations with distributed states tend to perform more poorly than predicted by lumped models (which assume uniform states across each population modeled in each biological reactor) with respect to phosphorus removal and overall survivability of the PAOs. This is important because all currently-available commercial models utilize lumped approaches. One reason for this is that polyphosphate (and glycogen and PHA) storage by PAOs requires adequate residence times in anaerobic (for PHA synthesis) and aerobic (for polyphosphate and glycogen synthesis, and for microbial growth) reactors. However, the existence of hydraulic residence time distributions in completely mixed flow reactors leads to some PAOs experiencing with much lower residence times, and therefore much lower storage product accumulation, within a given reactor, which tends to lower their effectiveness for phosphorus removal and their competitive advantage in EBPR systems.

CURRENT WORK

ABMs for modeling evolution/adaptation in wastewater treatment systems

An interesting metabolic challenge confronting PAOs (and also glycogen accumulating organisms, or GAOs) in a given EBPR system is optimizing how much polyphosphate and glycogen should be stored for substrate uptake and growth. The optimal solution (with respect to maximizing growth rates) would appear to be to store just enough polyphosphate and glycogen during the aerobic phase required for rapid

uptake of acetate throughout the anaerobic phase. If more than this amount is stored, an unnecessary metabolic burden is carried by a given PAO, which would place it at a competitive disadvantage to other PAOs, while if less is stored, the cell will take up less acetate than would be possible otherwise, which would also place the cell at a competitive disadvantage (Figure 1).

In an EBPR sequencing batch reactor, which is operated on a sequential anaerobic, aerobic, settling, draw, fill, cycle, the optimal-storage solution is relatively straightforward: with regular and predicable anaerobic and aerobic retention times, PAOs may adapt to a condition that provides rapid uptake of acetate without excessive storage. (Note that this does not necessarily mean glycogen and polyphosphate are completely depleted at the end of each anaerobic phase, as rates of acetate uptake may slow down at lower concentrations of these storage products - this is dependent on half-saturation constant values in saturation kinetics). However, when completely-mixed reactor hydraulics are considered, the problem is more complex: in this case, residence times of individuals are variable, and the "optimum" amount of each storage product is a function not only of the average HRTs, but also the variability in residence times, which introduces a large amount of uncertainty to PAO (and GAO) experiences as they cycle through the anaerobic and aerobic reactors.

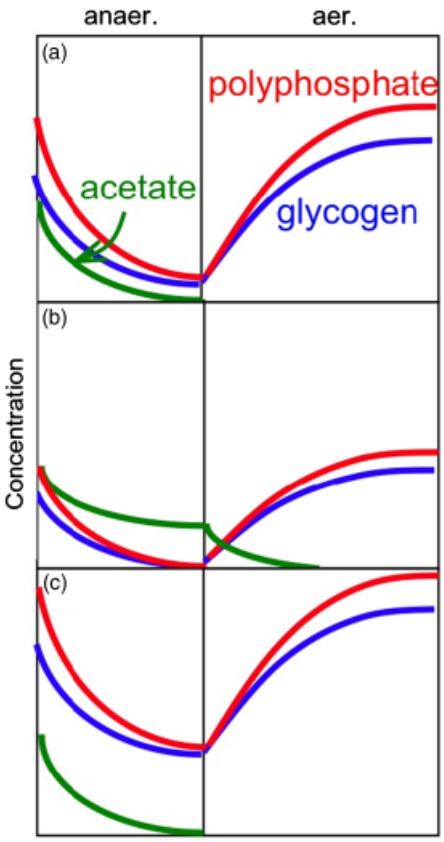

Figure 1 | Consequences of too little or too much glycogen and polyphosphate storage by a PAO in a sequencing batch reactor with anaerobic/ aerobic cycling: (a) ''optimal'' solution, (b) insufficient glycogen storage, resulting in lower anaerobic acetate uptake, and (c) excessive glycogen storage, resulting in wasted metabolic effort and reduced carbon usage.

Adaptation/evolution ABM objectives. The ABM approach was applied to this problem with the objectives of developing what is, to the best of our knowledge, the first application of ABMs to study microbial adaptation/evolution in wastewater treatment processes, to apply this approach to storage product accumulation in EBPR systems, and to study how unpredictability in completely mixed reactor systems affects adaptation and optimal (or most "fit") solutions to the storage product problem faced by PAOs.

Adaptation/evolution ABM Methods. Agent-based modeling of an A/O EBPR system was used to explore individual adaptation in the dynamic and somewhat unpredictable conditions inherent in completely mixed reactors. Modeling was conducted using DisSimulator, a Matlab-based program previously described (Schuler 2005)). The system studied was an acetate-fed system with biokinetic equations and parameter values generally as listed in Schuler and Jassby (2007) with the following exceptions: the decay rates for glycogen, polyphosphate, and PHA were 0.4/d, 0.2/d, and 0.2/d, respectively, The solids residence time was 5 days, the total anaerobic and aerobic HRTs were 1.5 and 4 hours, respectively, and the data presented herein addressed a system with 2 anaerobic reactors and 2 aerobic reactors in series. Adaptation / evolution was incorporated to the program by, at each cell division, adjusting the maximum glycogen storage constant (glyfracmax) value in the biokinetic equation for glycogen storage of each daughter cell. Glyfracmax was adjusted a random amount linearly distributed over the range $\pm 25\%$ of the parent cell value. The value of this parameter was tracked with individual cell states (along with glycogen, polyphosphate, and PHA storage), and was therefore allowed to vary amongst individuals. While any one (or more) of several different paremeters (e.g., maximum PHA storage and polyphosphate storage) could have been allowed to evolve, maximum glycogen storage was selected because it lies, in a sense, at the center of the bacterial "challenge" of allocating carbon usage, since in the aerobic phase stored PHA carbon is diverted primarily to either growth (for a shorter term benefit) or to glycogen (for a potential longer term benefit).

Adaptation/evolution ABM Results. A series of modeling runs were conducted to explore microbial adaptation in EBPR systems. Figure 2 shows an example, with competition

between a heterotrophic and a PAO population with two scenarios of different initial PAO glycogen storage capacities: 2a and 2b are with an initial glyfracmax value of 0.4 mg/mg, and 2c and 2d are with an initial glyfracmax value of 0.07 mg/mg. Figure 2c shows that when the PAO population was initially set to a glyfracmax value of 0.07 mg/mg, the PAO population was initially out-competed, but began to gain in population around Day 40, when the average glyfracmax had increased to about 0.12 mg/mg. This indicated that the "fittest" PAOs, which had randomly evolved to higher amounts of glycogen storage, had out-competed low-glycogen PAOs (indicated by increasing glycogen with time, Figure 2d). The reverse trend occurred in Figure 2b. A comparison of the two scenarios shows that the final states were the same in each, indicating that the initial conditions did not affect the final "optimum" target state. The Darwinian principle of survival of the fittest dictated that cells with sub-optimal glyfracmax values were out-competed by cells with glyfracmax values better adapted to the reactor system modeled, resulting in convergence to an average glyfracmax value of 0.18 mg/mg in both cases.

Figures 2b, d show the average glycogen storage only; the predicted glycogen (as well as PHA and polyphosphate) contents onf individual PAOs in the anaerobic and aerobic phases are shown in Figure 3. The data in Figure 3 has been sorted by glycogen storage, and so glycogen content appears as continuous lines, while PHA and polyphosphate are scattered. Although hetergenous, the PAO population had generally lower glycogen and polyphosphate storage and higher PHA storage in the final anaerobic reactor (Figure 3a), relative to the final aerobic reactor (Figure 3b), consistent with the EBPR model.

An important consideration in the construction of this model was constraining the evolutionary rate so that the bacteria did not evolve so rapidly that they quickly reached non-viable states (e.g, uncompetitively high or low glycogen storage values) and were washed out of the system (an example of the so-called "Darwinian Demon" problem). In this study, the evolutionary rate was set to a maximum of ± 25% change in the maximum glycogen storage at each cell division, which was determined to be adequate to prevent such runaway evolution. Very little is known about actual rates of evolution in wastewater treatment systems, and this should be a focus of future research.

Because of variability in reactor residence times and system complexity, the analytical determination of "optimal" amounts of storage may be not be possible, but agent-based modeling provides a means of estimating such values, in addition to revealing emergent behaviors about the systems. Agent-based models appear to be excellent tools for studying adaptation/evolution in biological systems. To our knowledge this is their first application for this purpose in biological treatment systems, and possibly in microbial systems in general.

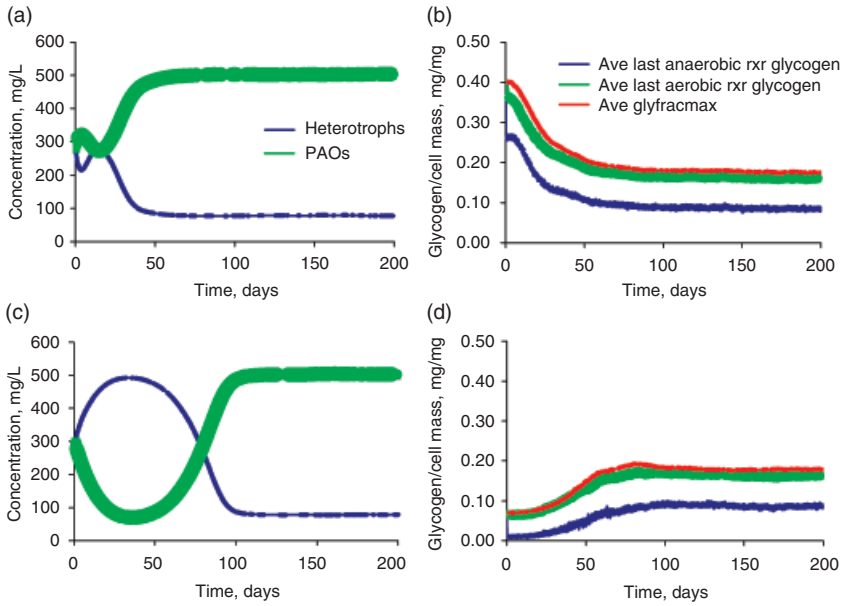

Figure 2 | Agent-based modeling of PAO adaptation and competition with higher initial glycogen storage (2a and 2b) and lower initial glycogen storage (2c and 2d) in an EBPR system with 2 anaerobic and 2 aerobic reactors. Subscribers to the online version of Water Science and Technology can access the colour version of this figure from http://www.iwaponline.com/wst

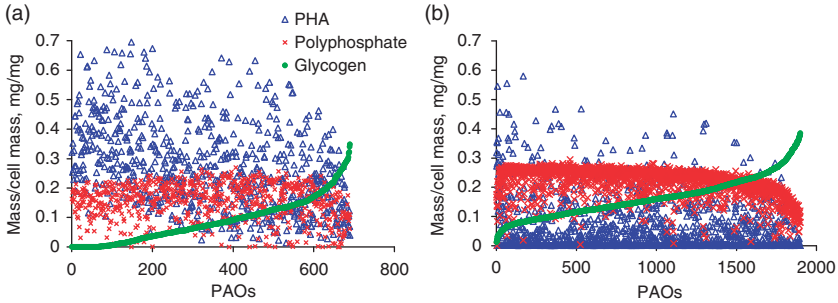

Figure 3 | Agent-based model predictions of population state heterogeneity for Day 200 of the model run shown in Figures 2c, d. The PAO population in the downstrean anaerobic reactor (3a) and the downstream aerobic reactor (3b) are shown, and the population has been sorted from low to high glycogen content. Subscribers to the online version of Water Science and Technology can access the colour version of this figure from http://www.iwaponline.com/wst

Model/data comparison of heterogeneity

Despite the increased use of agent-based modeling to predict heterogeneity and its effect on the wastewater treatment process performance, there have been no direct validations of these predictions. Observation and evaluation of factors that may lead to the heterogeneity of microbial states among cells has been very difficult, if not impossible, due to the lack of analytical methods that allow for cellular level quantification of state properties. Recently, we have developed a Raman microscopic method for cellular-level quantification and evaluation of functionally-relevant intracellular polymers dynamics in microbial populations during the EBPR process (Majed *et al.* 2009). Raman measurements showed that intracellular polyphosphate (PP), polyhydroxyalkanoates eg. polyhydroxybutyrates (PHB) and glycogen are distributed across the population (i.e. not all cells have the same amount) in both continuous flow as well as SBR systems.

In addition to the lack of validation, there is a question about the source of the variability. Is it external, due to different life histories (i.e. hydraulic residence time in different reactors, Guijer 2002, Schuler and Jassby 2007)? Or, is it internal, due to the fact that two daughter cells are not exactly identical in size and other properties (Kreft *et al.* 1998)? The model of Schuler and Jassby (2007) predicts distribution of storage states in EBPR populations based on non-uniform hydraulic residence times. In this study, we developed an agent-based model for simulating an EBPR process, particularly focusing on the distribution of PP. The model simulation results were compared to the results experimentally obtained in the lab-scale EBPR system using Raman Microscopy. A series of simulations utilizing different methods of introducing heterogeneity were performed to evaluate and identify the processes that most likely contribute to the observed patterns of heterogeneity. An overview of the methods and results are presented below and the details will be provided in a forthcoming publication (Bucci *et al.* 2010).

Methods: Lab-scale continuous flow EBPR system. A laboratory scale continuous flow EBPR system was established that allowed for both A2O (Anaerobic-Anoxic-Oxic) and UCT (University of Cape Town) modes of operation. The HRT and SRT of the system were maintained at 18 hours and 8 days, respectively. The reactor was operated in a controlled temperature room at 20°C. There are two recycle flows in the EBPR system including anoxic sludge recycle from anoxic zone to the anaerobic zone and nitrate recycle from aerobic zone to the anoxic zone. The composition of synthetic wastewater feed was according to Schuler and Jenkins (2003). Phosphorus was added as 45 mg/L sodium phosphate monobasic ($NaH_2PO_4{\bullet}H_2O$) (10 mg-P/L). The organic portion of the feeding consisted of 744 mg/L sodium acetate ($CH_3COONa{\bullet}3H_2O$) (350 mg COD/L) and 15 mg/L of casamino acids. Approximately 50-65% of the microbial cells in the continuous flow reactor were PAOs as confirmed with both Neisser and DAPI staining for polyphosphate. To observe and quantify the dynamics of intracellular polyphosphate, biomass from the continuous flow EBPR process was used for a phosphate release and uptake test that consisted of 180 minutes of anaerobic phase with acetate addition (100 mg COD/L) followed by 180 minutes of aerobic phase. For polyphosphate detection using Raman microscopy, 6 samples were taken throughout the test at 45–90 minutes interval.

Methods: Quantification of intracellular polymers with Raman Microscopy. Raman spectra were acquired using a WITec, Inc. (Ulm, Germany) Model CRM 2000 Confocal Raman Microscope. Excitation (ca. 30 mW at 633 nm) was provided by a Helium/Neon laser (Melles Griot, Carlsbad, CA). More details on data acquisition can be obtained in Majed *et al.* (2009).

Methods: Development of agent-based EBPR model. The agent based model developed was implemented using the iAlgae framework previously described by Hellweger and Kianirad (2007). The model simulates the behavior of PAOs, GAOs and general heterotroph population in an EBPR system. Acetate uptake, PP, PHB, glycogen synthesis, lyses, growth and decay were modeled following the approach from Schuler and Jassby (2007) and Whang *et al*. (2007), although a cell-based approach was used instead of a biomass-based approach. That is, intracellular PP is quantified as gP/cell rather than gP/gCOD. This is necessary to explicitly simulate the cell division event, which is the point when heterogeneity is introduced. Also, a maximum PHB content was introduced. Internal storage compounds were modeled for PAOs and GAOs while general heterotrophs were modeled only with respect to the external nutrients (i.e. Monód kinetic). The model utilized the "deterministic cell size division approach" used by Hellweger and Kianirad (2007). That is, the cells divide once a biomass/size threshold is reached. Biological randomization (internal variability) was simulated with two different approaches and the results were compared with a third simulation where biological randomization was not introduced (external variability due to unequal hydraulic residence times only). The first approach consisted in the randomization at division of the model parameters (e.g. maximum acetate update velocity, $V_{MAX,\ A}$) by drawing values from normal distributions with specified mean and coefficient of variation (CV), truncated at $+/-2$ standard deviations and ≥ 0 (Kreft *et al*. 1998). Note that values were drawn from a "global" distribution, independent of the parameter value of the mother cell. This was done to prevent evolution, which would not be meaningful unless properly constrained ("Darwinian Demon" problem). The second approach included randomization at division of the state variables (biomass and internal stored quotas) by drawing the split fractions for the daughter cells from a normal distribution with mean of 0.5 and specified CV truncated at ± 2 standard deviations and ≥ 0.

Methods: Model application. The model simulated the EBPR process for about 14 days, time needed to remove the effect of the initial condition. For the particular phosphate release and uptake test, the model was used to predict the distributions of PP in PAOs and GAOs which were compared with the measured Raman intensities. Bulk nutrients concentrations were also predicted with the model and compared to measured data.

Results and discussion. 1. EBPR performance: According to the observation of the P release uptake along with the uptake of acetate, the model was able to fit the measured data for bulk orthophosphate and COD well (Figure 4).

2. Dynamics of intracellular polymers: Figure 5 shows the measured and model-predicted distribution of PP at different time points. For example, the observations in Figure 5a indicate that 20% of the population have a PP content of 1.0 10^{-13} gP $cell^{-1}$ or more. Although there are significant discrepancies (e.g. Figure 5a), the model captures much of the observed patterns, including the distribution at several times (e.g. Figure 5c) and the dynamics of how those distributions change over time.

3. Factors that lead to heterogeneity: In order to understand the sources of heterogeneity for the intracellular PP, three different model simulations, utilizing different methods of introducing heterogeneity were performed. The results from those simulations are presented as differently colored lines in Figure 5. The red line corresponds to the simulation where no internal variability is introduced. That is, variability is entirely due to external factors (different residence times). The blue line corresponds to the simulation where model parameters are randomized at division. The green line corresponds to the simulation where model state variables are randomized at division. Variable residence times are included in all simulations, so the variability in the blue and green model simulations includes this effect. All

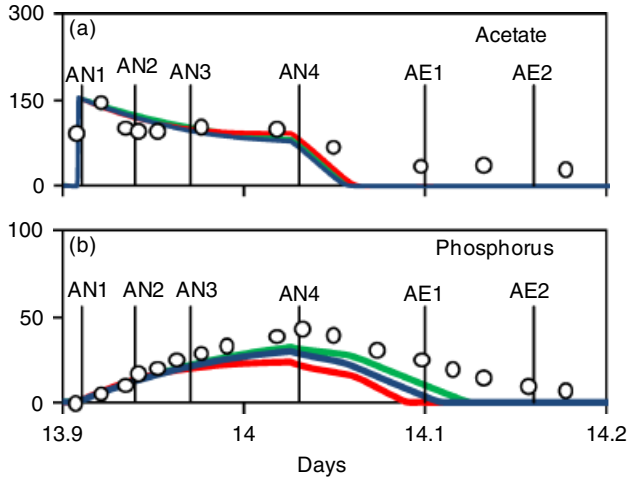

Figure 4 | (a) Extracellular acetate concentration (gCOD m^{-3}) vs. time. (b) Extracellular phosphorus concentration (gP m^{-3}) vs. time. Symbols are data. Lines are model output for three different simulations: blue is the one with all parameter values normally distributed (parameters randomization), green is the one with randomization of the state variables at division following a normal distribution (state variables randomization) and red is the one with no randomization. Black vertical lines indicate the time points at which the Raman intensities where measured (e.g. AN2 corresponds to Figure 5b). Subscribers to the online version of Water Science and Technology can access the colour version of this figure from http://www.iwaponline.com/wst

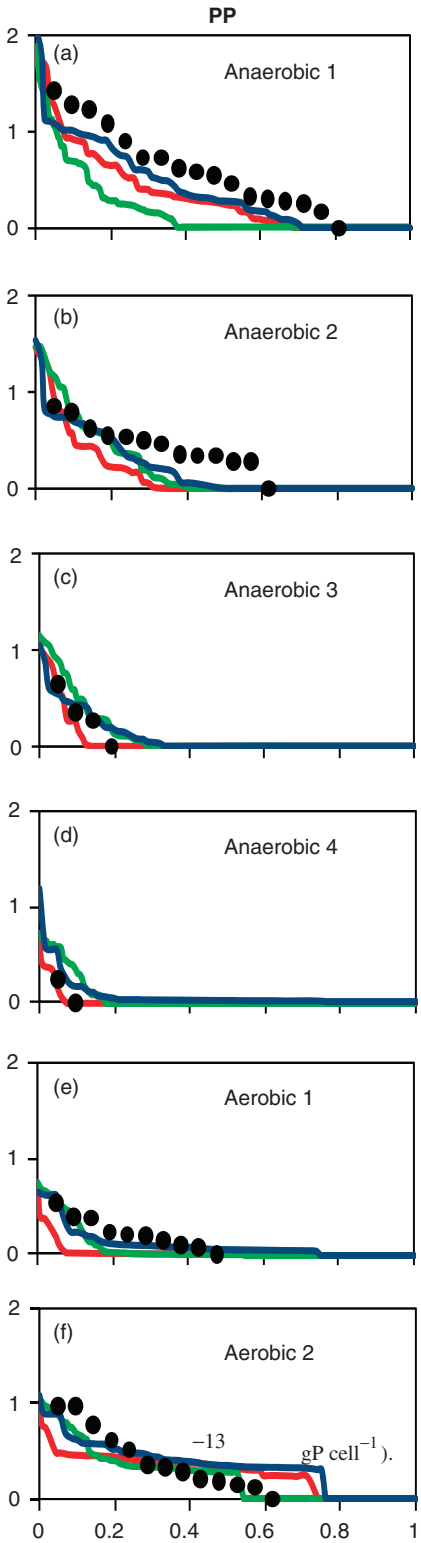

Figure 5 | Distributions for stored PP (10^{-13} gP cell^{-1}). Symbols are laboratory data, lines are the model output for three different simulations (see Figure 4 caption). Subscribers to the online version of Water Science and Technology can access the colour version of this figure from http://www.iwaponline.com/wst

three model simulations produce heterogeneity comparable to observations. However, the simulation with just external variability clearly under-predicts the heterogeneity at times (Figure 5 f).

Next steps. The model application presented above demonstrates the utility of using ABM to investigate heterogeneity in intracellular nutrients in the EBPR process. However, the system includes recycle, which makes it difficult to isolate the effects of internal and external variability, because external variability is always present. To further investigate this, we are now using an SBR system, where the life histories are the same for all cells (Gujer 2002) and the heterogeneity should be entirely due to internal variability. Those results will be presented in a subsequent publication (Bucci *et al*. 2010).

FUTURE DIRECTIONS

Incorporation with CFD modeling. Traditionally wastewater treatment processes are simulated as completely stirred tank reactors (CSTRs), which was also assumed in the previous agent-based models. However, the reality of incomplete mixing is well recognized and computational fluid dynamics (CFD) models are often applied to analyze this. This is a potential application area for ABM, because incomplete mixing within reactors consititutes an additional source of population heterogeneity (even in batch or sequencing batch reactors). Modelers can build on the Lagrangian particle tracking models developed for phytoplankton in natural waters (e.g. Falkowski and Wirick 1981; Nagai *et al*. 2003).

More intracellular states and mechanisms. Past wastewater ABMs included a handful of intracellular states and mechanisms. However, advances in molecular biology and biochemistry are rapidly increasing our mechanistic understanding of bacteria. This knowledge is being assimilated and compiled in "systems biology" models (Ideker *et al*. 2001; Kitano 2002). Numerous systems biology models have been developed (Edwards and Palsson 2000; Hoffmann *et al*. 2002; Lee *et al*. 2002; Castellanos *et al*. 2004; Feist *et al*. 2007; Smallbone *et al*. 2007; Covert *et al*. 2008) and the the field is within reach of dynamically simulating all major networks at a genome scale – a complete cell model. However, those models are limited to predicting the behavior of biological organisms. Many important questions, including those related to wastewater treatment, will require explicit consideration of the interactions of the individual with its environment. The next step is to simulate whole populations of these systems

biology microbes within ecosystems. This can be considered the combination of systems biology and systems ecology – systems bioecology.

Introduce heterogeneity in local-environmental conditions (microbial populations selection relevant conditions). The heterogeneity among microbial populations, even within the same functional group, is ultimately the results of selective local growth condition they are exposed to, which may or may not be the same as overall averaged bulk conditions. A model with compartments representing dominant local conditions (e.g. divided local sections of activated sludge system with different bulk DO and substrate concentrations) may allow the ABM to simulate the stable coexistence of multiple functional groups (and eventually multiple species?) in a wastewater system.

Experimental verification. Most of the work on ABMs in wastewater applications have been modeling studies, and there has been very little experimental verification. The results presented above are an example of one attempt to compare model predictions of individual variability with measurements. With the increasing availability of methods to measure characteristics of single cells, similar approaches may be applied in the future. In addition, more traditional measurements of bulk paramters should be used for verification of the ABMs predictions of system behaviors. The ABM studies of wastewater treatment systems in this and previous papers have been based on modifications of ASM2d models. Many of the parameter values used for PAO processes in ASM2d are not well known, and so experimental verification of ABMs including these processes will also face the challenge of uncertainties in these underlying metabolic models.

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