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Dynamic Growth Rates of Microbial Populations in Activated Sludge Systems

Peter G. Stroot[1]; Pascal E. Saikaly[2]; and Daniel B. Oerther[3]

Abstract: Results of mathematical modeling and whole cell 16S ribosomal RNA-targeted fluorescence in situ hybridizations challenge the widely held perception that microbial populations in "steady-state" activated sludge systems share a common net growth rate that is proportional to the inverse of the mean cell residence time. Our results are significant because they encourage bioprocess engineers to appreciate the differences in growth physiology among individual microbial populations in complex mixed microbial communities such as suspended growth activated sludge bioreactor systems.

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CE Database subject headings: Activated sludge; Ecology; Microbes; Biological treatment; Biomass.

Introduction

The definition of "steady-state" for a suspended growth activated sludge (AS) bioreactor system is based on a materials balance approach and includes no net accumulation of soluble substrate (S_S), particulate substrate (X_S), nor particulate biomass (X_B) after a period equal to three times the mean cells residence time (MCRT). In practice, steady-state is achieved by maintaining uniform operating conditions resulting in consistent process performance (i.e., the continuous removal of growth limiting resources and a constant level of total biomass). Under the steady-state assumption, all of the individual microbial populations inhabiting an AS system demonstrate the same net growth rate that is proportional to the inverse of the MCRT. This mathematical identity leads to the perception that the structure of the microbial community inhabiting an activated sludge system is unchanging (i.e., "stable"). Recent developments in modeling approaches and molecular biology-based tools to identify and quantify microbial populations provide new opportunities to challenge this perception.

For example, Huisman and Weissing (1999, 2000, 2001) showed that competition for growth limiting nutrients produces oscillations in the abundance of individual bacterial populations carrying out identical functions in chemostat-type bioreactors. Sommer (1985) demonstrated a similar oscillatory behavior in chemostat-type bioreactors treating pulses of influent nutrients. Although uniform operating conditions resulted in consistent process performance including continuous removal of total substrate and a constant level of total biomass, the levels of biomass attributed to individual bacterial populations experienced variation.

The application of 16S ribosomal RNA-targeted tools has been used to qualitatively describe dynamic changes in the composition of microbial communities in AS systems (Kaewplpat and Grady 2002) as well as in anaerobic digester systems (Fernandez et al. 1999; Zumstein et al. 2000). Despite the report of consistent process performance due to uniform operating conditions, the prevalence of individual microbial populations as determined using 16S rRNA fingerprinting techniques demonstrated variation in the structure of the microbial communities over time suggesting dynamic growth rates for individual microbial populations.

In the current study, we used mathematical modeling and quantitative whole cell 16S rRNA-targeted fluorescence in situ hybridizations (FISH) to predict and experimentally determine the in silico and in situ net growth rates of individual microbial populations in AS systems during start-up to steady-state conditions. The results reported herein challenge the perception that the net growth rate of microorganisms in an AS system reach a "steady state" value after three times the MCRT. Furthermore, an alternative approach is suggested to reconcile traditional theory and our results.

Materials and Methods

Model Development

The model developed in this study is based on mass balance equations for growth limiting nutrients and heterotrophic bacterial populations using the conventional, completely mixed AS bioreactor configuration. In developing the model the following assumptions were made: (1) the growth limiting nutrients are consumed by all of the different heterotrophic bacterial populations; (2) competition is only for complementary nutrients (i.e., nutrients that have metabolically independent requirements for growth, such as ammonium ion and phosphate ion); (3) the

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Table 1. Model Parameters

	Half saturation constant, K_s, mg/L			Yield coefficient, Y		
Population	Nutrient 1	Nutrient 2	Nutrient 3	Nutrient 1	Nutrient 2	Nutrient 3
1	12.00	3.60	10.80	0.42	0.21	0.12
2	10.80	12.00	3.60	0.24	0.21	0.17
3	9.24	9.12	12.84	0.83	0.10	0.12

system has an ideal clarifier with assumed zero volume; and (4) the hydraulic retention time (HRT) is constant at 0.5 day.

The model was developed to describe the competition of $N=3$ heterotrophic bacterial populations competing for $K=3$ complementary growth limiting nutrients:

$$\left(\frac{dS_j}{dt}\right)V = Q_{\text{in}}S_{jo} - Q_eS_j - \sum_{i=1}^{N}\frac{\mu_i(S_1,\ldots,S_K)X_iV}{Y_{ji}} \quad \text{for } j=1,\ldots,K \tag{1}$$

$$\left(\frac{dX_i}{dt}\right)V = \mu_i(S_1,\ldots,S_K)VX_i - Q_wX_i - b_iX_iV \quad \text{for } i=1,\ldots,N \tag{2}$$

where j=nutrients; i=microbial populations; S_{jo}=influent concentration of nutrient j; S_j=effluent concentration of nutrient j; Q_{in}=influent flow rate; Q_e=effluent flow rate; Q_w=waste flow rate; Y_{ji}=yield coefficient of bacterial population i for nutrient j; V=volume of the completely mixed reactor; μ_i=specific growth rate of bacterial population i; X_i=concentration of bacterial population i; and b_i=first order decay rate for bacterial population i.

We assumed that the specific growth rate is given by the noninteractive Monod model for growth on complementary nutrients. The use of the noninteractive Monod model to describe bacterial growth is well established (Tilman 1977; Baltzis and Fredrickson 1988; Huisman and Weissing 1999). The noniteractive form of the Monod model is based on the assumption that the specific growth rate of each bacterial population is only limited by a single growth-limiting nutrient (i.e., the noninteractive Monod is a switching function where the identity of the growth-limiting nutrient might change as environmental conditions $[S_1,\ldots,S_K]$ change). Therefore μ_i will be equal to the lowest value in Eq. (3)

$$\mu_i(S_1,\ldots,S_K) = \min\left(\frac{\hat{\mu}_iS_1}{K_{1i}+S_1}\cdots\frac{\hat{\mu}_iS_K}{K_{Ki}+S_K}\right) \quad \text{for } i=1,\ldots,K \tag{3}$$

where $\hat{\mu}_i$=maximum specific growth rate for bacterial population i; K_{1i}=half saturation constant of bacterial population i on growth-limiting nutrient 1; and K_{Ki}=half saturation constant of bacterial population i on growth-limiting nutrient K. All simulations were performed using *Berkeley Madonna Version 8.0.1* (Macey et al. 2000) employing a fourth-order Runge-Kutta numerical approximation with a fixed time step of 0.0625 day.

The kinetic parameters (half saturation constant, K_s, maximum specific growth rate, μ_i, decay rate, b, and yield coefficient, Y) were selected to be similar to values reported in the literature (Henze et al. 1987, 1988, 1995; Gujer et al. 1998). For simplification, we assumed a constant maximum specific growth rate of 24 day^{-1} for all populations. However, the half saturation constants and the yield coefficients are different for various combinations of bacterial populations and growth-limiting nutrient (Table 1). A first order decay rate of 0.25 day^{-1} was chosen for all heterotrophic bacterial populations.

Experimental Methods

Two, 4-L sequencing batch reactors were operated for a minimum of three MCRT. The reactors were seeded with mixed liquor collected from the Mill Creek Wastewater Treatment Plant, Metropolitan Sewer District of Greater Cincinnati that treats approximately 130 million gallons of municipal sewage per day using two parallel conventional activated sludge systems. Reactor A was operated with three cycles per day, while Reactor B was operated with two cycles per day. The reactors differed in target MCRT, feed strategy, idle phase, and HRT as shown in Table 2. Both reactors were fed a fresh acetate minimal media prepared daily. The feed for the reactors contained per liter of water: 0.85 g $CH_3COONa\cdot 3\ H_2O$, 0.15 g NH_4Cl, 0.066 g KH_2PO_4, 0.09 g $MgSO_4\cdot 7\ H_2O$, 0.01 g $CaCl_2\cdot 2\ H_2O$, 0.01 g $Na_2S_2O_3\cdot 5\ H_2O$, 0.001 g yeast extract, 5 g $NaHCO_3$, and 0.3 mL nutrient solution (van Groenestijn et al. 1989). Samples were taken from the reactors for physical and microbiological analyses. Suspended solids (SS) and volatile suspended solids (VSS) were determined according to standard methods (APHA/AWWA/WEF 1998). For the microbiological analysis, 2 mL-samples were removed, centrifuged at 12,200×g, and the supernatant was decanted. The FISH samples were fixed for 24 h at room temperature in 4% (w/v) paraformaldehyde prepared in 1×PBS (130 mM NaCl and 10 mM sodium phosphate buffer adjusted to pH 7.0). Subsequently, the samples were stored in a 50% (V/V) mixture of ethanol and 1×PBS (pH 7.0) at −20°C (Oerther et al. 2000).

The oligonucleotide probe for FISH was conjugated with the cyanine dye, Cy3, before purification with an oligonucleotide probe purification cartridges (MegaBases, Inc., Evanston, Ill.). The fluorescently labeled probe, S-G-Acin-0659-a-A-24 (5′ CTGGAATTCTACCATCCTCTCCCA), was diluted to 50 mg L^{-1} with H_2O, and stored in 50-μL aliquots at −20°C in the dark (Oerther et al., unpublished).

Prior to FISH, all fixed samples were sonicated for floc disaggregation and homogenization. The samples were sonicated three times for 1 min each at the maximum setting on a

Table 2. Operating Conditions for Laboratory-Scale Reactors A and B

	Reactor	
	A	B
Reactor volume (L)	3.00	3.000
HRT (day)	0.50	0.625
Target MCRT (day)	2.00	4.000
Settle time (h)	0.75	0.750
Decant time (h)	0.25	0.250
React time including feed time (h)	7.00	4.000
Feed time (h)	0.17	3.750
Idle time (h)	0.00	7.000
Wasting frequency using Garrett strategy (day^{-1})	1.00	1.000

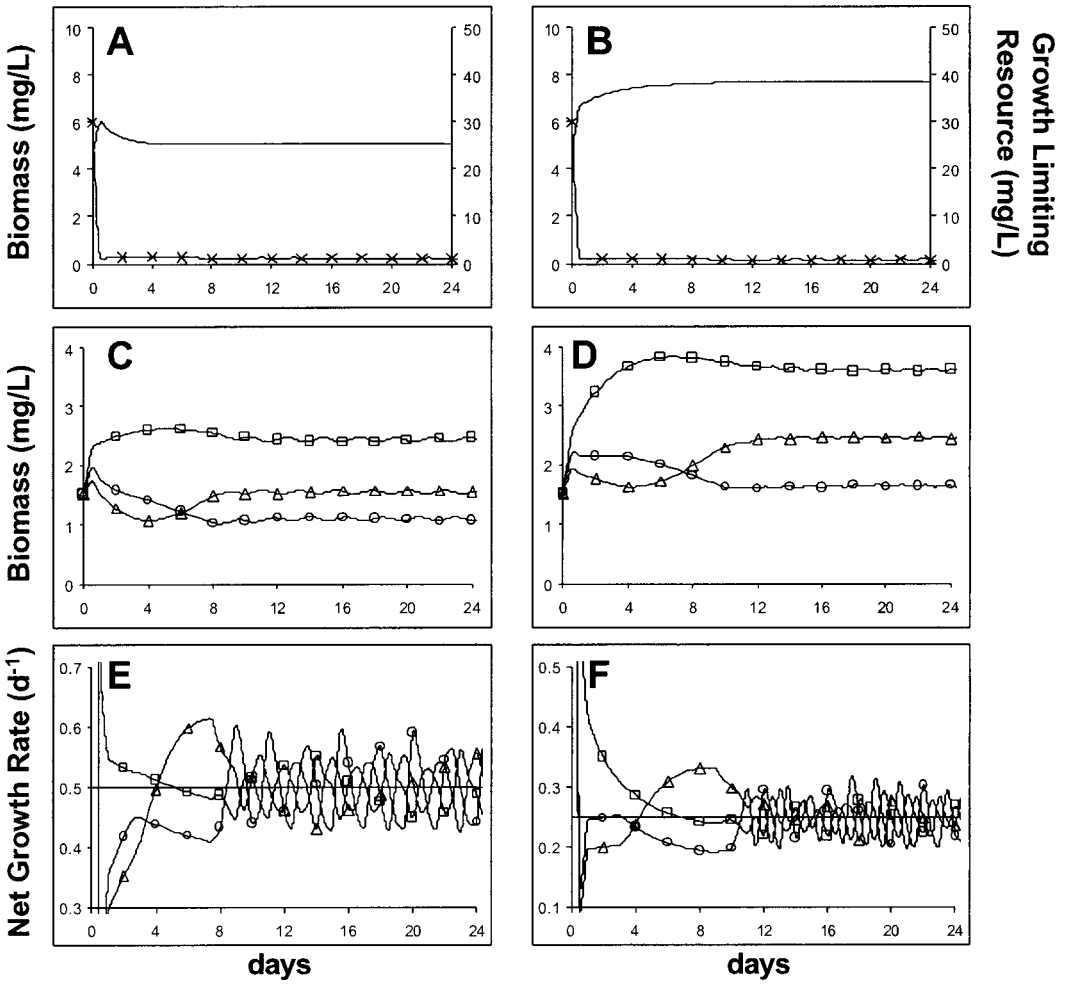

Fig. 1. Results of model simulations. Total biomass (solid line) and total effluent growth limiting substrate (×) for a reactor operated at a MCRT of 2 days (A) and 4 days (B). Biomass levels for Population 1 (?), Population 2 (○), and Population 3 (?) for a reactor operated at a MCRT of 2 days (C) and 4 days (D). Comparing net growth rates for Population 1 (?), Population 2 (○), and Population 3 (?) with the inverse of the MCRT (solid line) for a reactor operated at a MCRT of 2 days (E) and 4 days (F).

Tekmar-Dohrmann model TM 130 PB sonicator and then cooled to −20°C after each sonication. These samples were applied in a sample well on a heavy Teflon coated (HTC) microscope slide (Cel-Line Associates, New Field, N.J.) and air-dried. After dehydration with an increasing ethanol series [50, 80, 95% (v/v) ethanol, 1 min each], each sample well was covered with 16 μL of hybridization buffer [20% (v/v) formamide, 0.9 M NaCl, 100 mM Tris HCl (pH 7.0), 0.1% sodium dodecyl sulfate (SDS)]. Fluorescently labeled oligonucleotide probe, 2 μL (100 ng), was added to each well of the microscope slide. Hybridizations were conducted in a moisture chamber for 2 h, in the dark, at 46°C. The slides were washed for 30 min at 48°C with 50 mL of pre-warmed wash solution [215 mM NaCl, 20 mM Tris HCl (pH 7.0), 0.1% SDS, and 5 mM ethylenediaminetetraacetic acid (EDTA)]. Samples were counterstained with ice-cold, fresh 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) staining solution (0.2 mg DAPIL−1) for 1 min, rinsed with water, and rapidly air-dried. Fixed, hybridized cells were mounted with Cargille immersion oil (Type FF, Cedar Grove, N.J.) and a cover slip.

Probe conferred fluorescence was visualized with a Nikon Microphot epifluorescence microscope (Eclipse E600), and digital images were captured using a Spot-2 charge coupled device (CCD) camera. Digital images were manipulated using *MetaMorph* (version 4.6, Universal Imaging Corp., Downington, Pa.) imaging software.

Cells identified by probe-conferred fluorescence were counted relative to DAPI stained cells in the following manner. For each sample, *Acinetobacter* spp. cells were counted within 10 clusters of approximately 100 cells each. Thus a total of approximately 1,000 DAPI stained cells were counted for each sample.

Results and Discussion

Model Results

Using the model described above, we considered three heterotrophic bacteria populations competing for three growth-limiting nutrients in AS bioreactors operated with an MCRT of 2 or 4 days. For each in silico experiment, representative results of model simulations are reported in Fig. 1. Uniform operating conditions resulted in consistent process performance with a continuous removal of nutrients and a constant level of total biomass for both MCRT [Figs. 1(a and b)]. Therefore the in silico systems met the traditional definition of "steady-state" for a suspended growth AS bioreactor. Despite

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"steady-state" conditions, the levels of biomass attributed to individual bacterial populations experienced variation for both MCRT [Figs. 1(c and d)]. The oscillatory behavior observed for the individual bacterial populations are the result of disequilibrium conditions that are generated by the competition process itself (i.e., no external disturbance). A sensitivity analysis of the in silico results including changing the time step for numerical integration demonstrated that the results reported in Fig. 1 are not due to an unstable numerical approach. For each in silico experiment, the results of the net growth rate for each bacterial population at the two MCRT are shown in Figs. 1(e and f). The net growth rate of each population oscillates in a range of values that have a mean equal to the inverse of the MCRT. All representative results reported in Fig. 1 demonstrated a consistent pattern of oscillations for a period of 10,000 days (results not shown), despite the fact that the values of $\hat{\mu}_i$ used in this study are not typical of activated sludge models. Nevertheless, values of maximum specific growth rate between 17 and 100 day^{-1} were reported in the literature for some organisms (Madigan et al. 2003). Moreover, we did run the simulations for values of $\hat{\mu}_i$ between 5 and 24 day^{-1} and the results showed similar trends as in Fig. 1 (data not shown).

To our knowledge, this is the first model that demonstrates that the structure of the bacterial community in a suspended growth AS bioreactor system can demonstrate dynamic growth rates despite maintaining uniform operating conditions and demonstrating consistent process performance. The validity of the results of our in silico experiments are supported by several recent studies of laboratory-scale bioreactors, which show experimentally that the bacterial community composition is dynamic in bioreactors operated to encourage stable conditions (Fernandez et al. 1999; Kaewplplat and Grady 2002).

For example, Kaewplpat and Grady (2002) used denaturing gradient gel electrophoresis (DGGE) targeting 16S rDNA to document changes in the structure of the bacterial community inhabiting a laboratory-scale activated sludge system operated using uniform conditions. The results of their DGGE analyses showed that the structure of the bacterial community within parallel treatment systems was highly dynamic despite efforts to operate the parallel systems using identical uniform conditions. In similar work, Fernandez and co-workers (1999) reported fluctuations in the structure of the microbial community in functionally stable methanogenic bioreactors operated for a period of 605 days. The experimental results reported in these two studies suggest that the structure of the microbial community can be dynamic even when consistent process performance is observed and uniform operating conditions are employed.

We suggest that our model offers an explanation for these observations. According to our simulations, the total community biomass is constant even though the levels of individual bacterial populations are dynamic. This constant total community biomass could explain consistent process performance even though the levels of individual bacterial populations are dynamic. Despite the fact that our model addresses an important concept, namely competition and its significant role in dynamic growth rates of individual microbial populations, it has at least two limitations that should be considered. First, the model is developed to study competition of bacterial populations within one functional group of microorganisms, namely, "aerobic heterotrophs." However, activated sludge systems often support the growth of multiple functional groups including denitrifying heterotrophs, nitrifying autotrophs, and phosphorous accumulating organisms. Therefore the model presented herein should be expanded to consider competition of bacterial populations among different functional groups. Second, this study focused on the effects of competition within a single trophic level (bacteria). Effects of competition in the presence of higher trophic levels, ciliates (predators), and carnivores (top predators) should also be considered. Third, the composition of the influent wastewater as described in the current model is composed of complementary nutrients. However, wastewater treatment systems typically contain a variety of growth limiting resources including substitutable and complementary nutrients as well as energy substrates such as organics and dissolved oxygen. Examples of substitutable resources are the ammonium ion and amino acids. Both can be used as a nutrient source for nitrogen. Examples of complementary resources are nitrogen and phosphorus. To our knowledge there is no theoretical or experimental study that describes simultaneous growth limitation by substitutable or complementary nutrients as well as energy substrates. In the future, the model presented herein should be expanded to examine such combinations of growth limiting resources.

Experimental Results

The proportion of individual cells of *Acinetobacter* spp. was measured in two reactors using FISH with an oligonucleotide probe as compared to total cells visualized with DAPI stain. Representative images of FISH with samples from Reactor B are shown in Fig. 2. The number of cells of *Acinetobacter* spp. in Reactor B was higher on Day 12 (Fig. 2, C) as compared to the number observed on Days 8 and 10 (Figs. 2, A and B, respectively). Occasionally flocs with high levels of *Acinetobacter* spp. cells that appeared to be microcolonies of a single cell type were observed on Day 6 for Reactor A (not shown) and Day 12 for Reactor B (Fig. 2, D). *Acinetobacter* cells were enumerated in samples removed from both reactors, and their abundance throughout the experiment is shown in Fig. 3. Steady state operation, defined as constant operating conditions for a period of three times the MCRT, was achieved on Day 6 for Reactor A and Day 12 for Reactor B. Despite consistent process performance, large shifts in the abundance of *Acinetobacter* cells were observed on Days 5 and 6 for Reactor A and on Day 12 for Reactor B.

Experimental results for both reactors are reported in Fig. 3. Uniform operating conditions resulted in consistent process performance with a continuous removal of nutrients and an approximately constant level of total biomass for both MCRT [Figs. 3(a and b)]. Despite "steady-state" conditions, the levels of biomass attributed to individual bacterial populations experienced variation for both MCRT [Figs. 3(c and d)]. The product of the proportion of the number of individual cells of *Acinetobacter* spp. to the number of total cells visualized with DAPI stain and the VSS of samples from both reactors is shown in Figs. 3(c and d). Reactor A demonstrated a 300% increase in the level of biomass attributed to *Acinetobacter* cells from Day 5 to 6 followed by a 90% decrease from Day 6 to 7. Reactor B had a 75% decrease from Day 4 to 5 and a 540% increase from Day 10 to 12. This increase does not take into account the high levels of *Acinetobacter* spp. observed in occasional flocs (Fig. 2, D). The presence of these microcolonies suggests that the proportion of *Acinetobacter* spp. may be even greater in Reactor B at Day 12.

The level of *Acinetobacter* spp. biomass in the reactors was used to calculate the net growth rate of *Acinetobacter* spp., and these in situ growth rates are compared to the inverse of the MCRT in Figs. 3(e and f). According to the Garret strategy, the MCRT was calculated using the level of VSS from the previous sampling time as the concentration of the biomass in the wasted

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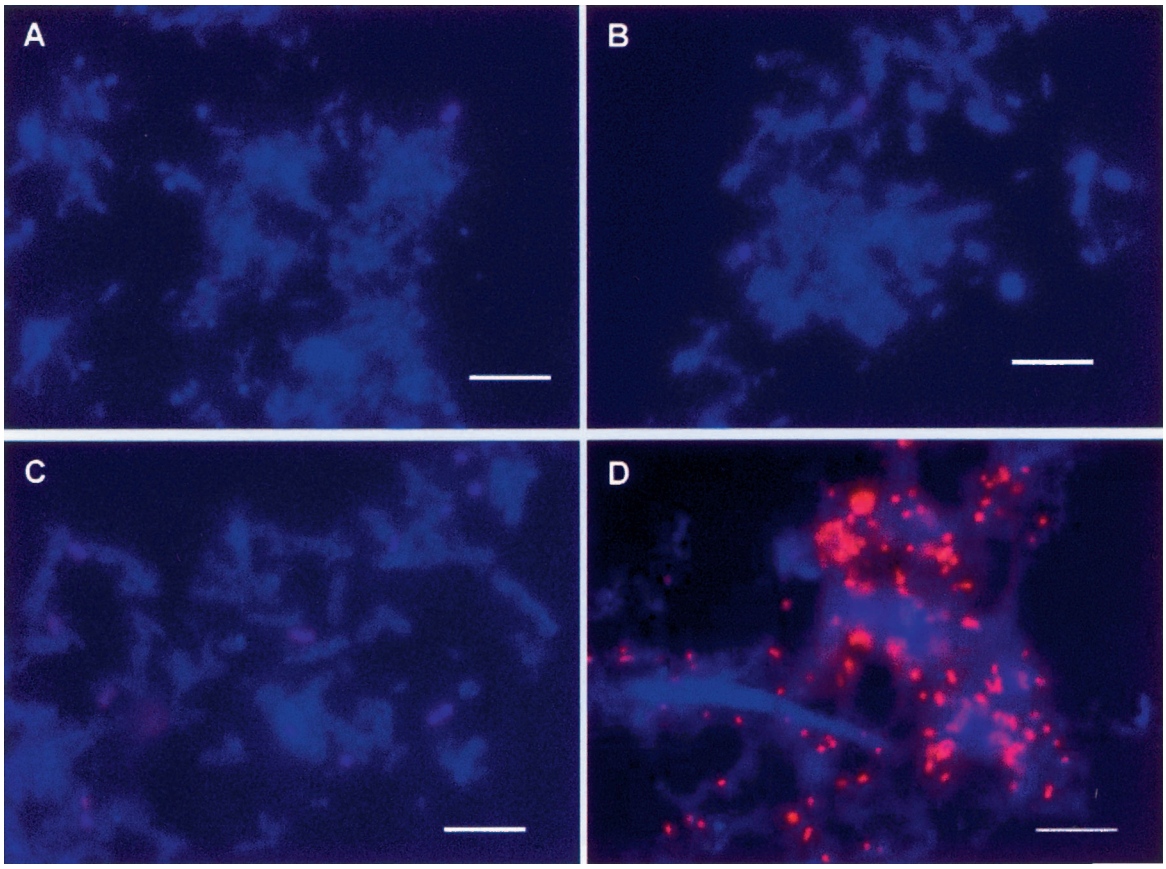

Fig. 2. (Color) Representative images of whole cell FISH using a Cy3 labeled probe designed to target the genus *Acinetobacter* (red) and DAPI stain (blue) for Reactor B for Day 8 (A), Day 10 (B), and Day 12 (C and D). The size bar for images A, B, and C is 5 μm. The size bar for image D is 20 μm.

sludge whereas sludge loss through the effluent was calculated using an average effluent biomass concentration of 20 mg L^{-1}. Net growth rates of *Acinetobacter* spp. biomass which are lower than the inverse of the MCRT were observed for multiple sampling points. This result indicated that *Acinetobacter* spp. were growing slowly or not at all and should eventually wash out according to traditional AS theory. Although the in situ net growth rate of *Acinetobacter* spp. in Reactor A was often below the growth rate predicted by the inverse of the MCRT for Days 7–20, they were never washed out of the system. Reactor B often demonstrated a net growth rate for *Acinetobacter* spp. below the growth rate predicted by the inverse of the MCRT from Days 1–10. At these low and even negative net growth rates, washout or loss of a microbial population due to decay is expected, but the *Acinetobacter* spp. biomass net growth rate increased substantially on Day 12. Net growth rates that exceed the values predicted from the inverse of the MCRT are not easily explained by traditional AS theory. The net growth rate of *Acinetobacter* spp. cells significantly exceeded the growth rates predicted by the inverse of the MCRT on Days 5, 6, 20, and 22 in Reactor A and on Day 12 in Reactor B.

Our experimental results document variable in situ net growth rates for *Acinetobacter* spp. in laboratory-scale AS bioreactor systems demonstrating consistent process performance using uniform operating conditions. Although our experimental results and the results of our model simulations are in agreement with the observations previously reported in the literature (Fernandez et al. 1999; Kaewplplat and Grady 2002), our experimental results suffer from at least three limitations. First, the experimental procedure used to enumerate individual cells of *Acinetobacter* spp. was only based upon counting approximately 1,000 cells for each sampling point. Although this number represents a practical upper limit to the throughput possible using conventional epifluorescence microscopy, the number of cells present in the AS systems was significantly larger. Therefore sampling error due to small sample size is a problem in our study as it is often a problem in studies using FISH to enumerate individual cells of a phylogenetically defined microbial population. Second, it is unclear if the group of bacteria that are identified by the 16S rRNA-targeted oligonucleotide hybridization probe represent a "bacterial population" as envisioned for the model. The definition of bacterial population for the model is a group of metabolically identical microorganisms sharing the same kinetic parameters; whereas 16S rRNA-targeted oligonucleotide hybridization probes target groups of phylogenetically related microorganisms. Because 16S rRNA phylogeny and kinetic parameters are not matched on a one-to-one basis, we would suggest that it is infeasible to develop a 16S rRNA-targeted oligonucleotide hybridization probe to track a single "bacterial population" as described in the model. Third, even the simple laboratory-scale activated sludge bioreactor systems experimentally tested in this study are significantly more complex when compared to a model containing three bacterial populations competing for three growth limiting nutrients. Therefore we suggest that the comparison between the experimental system and the model should be viewed as an analogy instead of an identity (i.e., the trends observed in the model are similar and

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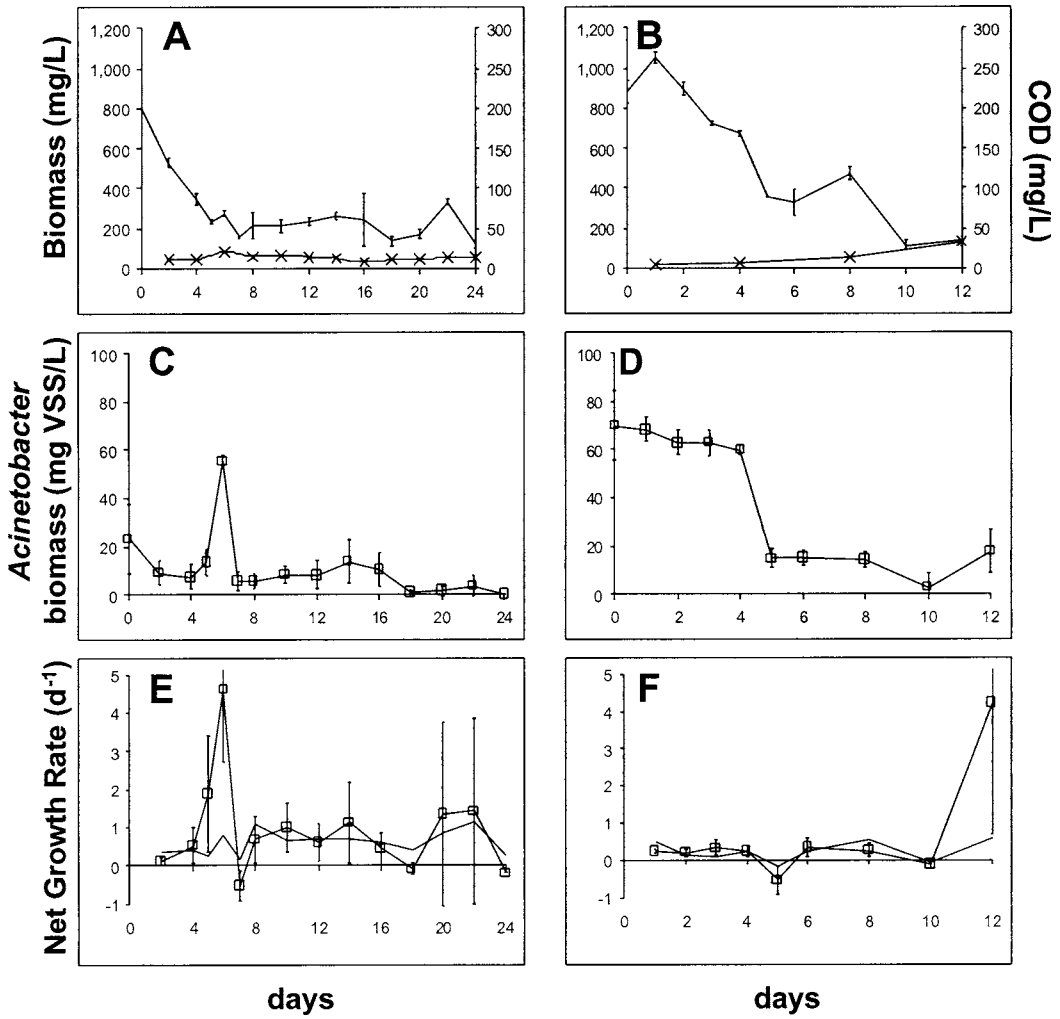

Fig. 3. Results of experimental studies. Total biomass (solid line) and effluent chemical oxygen demand (×) for a reactor operated at a MCRT of 2 days (A) and 4 days (B) where error bars represent standard deviation of replicate measurements. Biomass levels for *Acinetobacter* spp. (?) for a reactor operated at a MCRT of 2 days (C) and 4 days (D) where error bars represent the combination of standard deviation of cell counts and the variation of VSS measurements following the law of propagation of error. Comparing net growth rates for *Acinetobacter* spp. (?) with the inverse of the MCRT (solid line) for a reactor operated at a MCRT of 2 days (E) and 4 days (F) where error bars represent the combination of standard deviation of cell counts and the variation of VSS measurements following the law of propagation of error.

consistent with the trends observed in the experimental data suggesting a potential correlation that deserves further study). Despite the limitations, we suggest that collectively the in silico and in situ results reported in this paper work towards helping improve our insight that microbial populations inhabiting suspended growth AS bioreactor systems do not share a common net growth rate that is proportional to the inverse of the MCRT.

To accommodate the differences in net growth rates among individual microbial populations, we propose that the relationship among net growth rates of individual microbial populations in activated sludge systems can be described using the following equation:

$$\frac{V}{\Delta t} \cdot \sum_{i=1}^{n} (\Delta X_i) = \sum_{i=1}^{n} (\text{net growth}_i \, X_i) - Q_e \sum_{i=1}^{n} (\Delta X_{i,e}) - Q_w \sum_{i=1}^{n} (\Delta X_{i,w}) \quad \text{for } i = 1, \ldots, N \tag{4}$$

where i=microbial populations; X_i=concentration of bacterial population i; $X_{i,e}$=effluent concentration of bacterial population i; $X_{i,w}$=waste concentration of bacterial population i; Q_e=effluent flow rate, Q_w=waste flow rate; and V=volume of the completely mixed reactor.

For "steady state" conditions, the left-hand side of Eq. (4) goes to zero. Although the bulk biomass levels will not change under nonsteady state conditions, the levels of the individual microbial populations are not assumed to be static. This is important because under nonsteady state conditions or when the reactors are perturbed, the bacterial community structure as well as the performance of the reactors is impacted. In this situation, the overall performance of the reactor will be determined by the redundancy of the species having important stabilizing roles, as well on the ability of the species to respond differently to perturbations. In this situation the change in balance between the microbial populations will influence the overall kinetics in contaminant removal and a modified equation such as the one presented in Eq. (4) that captures this change will better describe the performance of the reactors under perturbed conditions. Thus the requirement for net growth rate to remain proportional to the inverse of the MCRT is

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maintained while the ability to account for changes in the specific growth rate of individual microbial populations is explicitly included.

Summary and Conclusions

The experimental results and the model results are two independent studies that both reveal that the bacterial community structure in activated sludge systems is dynamic. Several investigators (Eichner et al. 1999; Forney et al. 2001; Boon et al. 2002; Kaewpipat and Grady 2002) have reported that the bacterial community structure of laboratory-scale activated sludge reactors seeded with sludge from domestic wastewater treatment plants was not static but constantly changing. This dynamic in the bacterial community structure in activated sludge could be attributed to several biotic and abiotic factors such as resource competition as was shown in the model predictions presented in this study and another study (Saikaly and Oerther 2004), predation, and new selective pressure imposed on domestic sludge (Forney et al. 2001) (in our case synthetic wastewater as opposed to raw sewage).

The significance of population dynamics in natural and engineered systems has been investigated in several theoretical and experimental studies. Fernandez et al. (1999) have shown experimentally that a dynamic microbial community in a continuously stirred tank reactor, operated anaerobically, can maintain a stable ecosystem function. It is already recognized in ecology that nonequilibrium dynamics and oscillations in species abundances favor species coexistence (Huisman and Weissing 1999, 2000, 2001). It is also known that competition for three or more limiting resources may generate oscillations and chaotic fluctuations in species abundances allowing more species to coexist than there are limiting nutrients (Huisman and Weissing 1999, 2000, 2001; Saikaly and Oerther 2004). This is important because both laboratory and field studies showed that diversity (species richness) is positively related to ecosystem stability (Naeem and Li 1997; Tilman 1999). Stability can refer to resistance to disturbance, resilience (rate of recovery after disturbance), and sameness of the identity of community biomass over temporal scales. If the diversity-stability hypothesis developed in these studies of macroecological systems applies to activated sludge systems, then we expect systems with higher diversity to better maintain performance when exposed to environmental perturbations (e.g., toxic shock loads). The importance of diversity in bioreactors was shown in a recent study examining toxic loads of mercury (Canstein et al. 2002). The results of the study showed that diverse communities in packed-bed bioreactors demonstrated enhanced resistance to mercury toxicity as compared to mono culture communities and that the initial diversity was completely recovered after the concentration of mercury was reduced from 10 to 2 mg/L. The results presented in this work are significant because they are relevant to strategies currently used to monitor microbial community structure in AS systems. If the levels of microbial populations in AS bioreactors exhibit a significant degree of variation, then sampling to track the composition of the microbial community structure must be performed on a routine basis to capture the dynamic nature of the community composition. Thus analyses of limited grab samples removed from AS bioreactors may provide an inaccurate "snap shot" of microbial community structure.

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Notation

The following symbols are used in this paper:

b = biomass decay rate, h^{-1};
K_s = half saturation constant, mg L^{-1};
Q = flow rate, V t^{-1};
S_s = soluble substrate, mg L^{-1};
V = volume;
X = biomass concentration, mg L^{-1};
X_B = particulate biomass, mg L^{-1}
X_s = particulate substrate, mg L^{-1};
Y = yield coefficient g g^{-1}; and
μ = biomass growth rate, h^{-1}; and
θ_c = mean cell residence time, sludge age, or solids retention times.

References

American Public Health Association, American Water Works Association, and Water Environment Federation (APHA/AWWA/WEF). (1998). *Standard methods for the examination of water and wastewater*, 20th Ed., APHA, Washington, D.C.

Baltzis, B. C., and Fredrickson, A. G. (1988). "Limitation of growth rate by two complementary nutrients: Some elementary but neglected considerations." *Biotechnol. Bioeng.*, 31, 75–86.

Boon, N., Goris, J., De Vos, P., Verstraete, W., and Top, E. M. (2000). "Bioaugmentation of activated sludge by an indigenous 3- chloroaniline-degrading Comamonas testosteroni strain, I2gfp." *Appl. Environ. Microbiol.*, 66, 2906–2913.

Canstein, H. V., Kelly, S., Li, Y., and Wagner-Dobler, I. (2002). "Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions." *Appl. Environ. Microbiol.*, 68, 2829–2837.

Eichner, C. A., Erb, R. W., Timmis, K. N., and Wagner-Dobler, I. (1999). "Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community." *Appl. Environ. Microbiol.*, 65, 102–109.

Fernandez, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C., and Tiedje, J. (1999). "How stable is stable? Function versus community composition." *Appl. Environ. Microbiol.*, 65(8), 3697–3704.

Forney, L. J., Liu, W. T., Guckert, J. B., Kumagai, Y., Namkung, E., Nishihara, T., and Larson, R. J. (2001). "Structure of microbial communities in activated sludge: Potential implications for assessing the biodegradability of chemicals." *Ecotoxicol. Environ. Saf.*, 49, 40–53.

Gujer, W., Henze, M., Mino, T., and Loosdrecht, M. V. (1998). "Activated sludge model No. 3." *Scientific and Technical Rep. No. 9*, International Water Association, London.

Henze, M., Grady, C. P. L., Gujer, W., Marais, G. vR., and Matsuo, T. (1987). "A general-model for single-sludge waste-water treatment systems." *Water Res.*, 21(5), 505–515.

Henze, M., Grady, C. P. L., Gujer, W., Marais, G. vR., and Matsuo, T. (1988). "Activated sludge model No. 1." *IAWPRC Scientific and Technical Rep. No. 1*, International Association of Water Pollution Research and Control, London.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M. C., and Marais, G. vR. (1995). "Activated sludge model No. 2." *IAWQ Scientific and*

Technical Rep. No. 3, International Association of Water Quality, London.

Huisman, J., and Weissing, F. (1999). "Biodiversity of plankton by species oscillations and chaos." *Nature (London)*, 402(25), 407–410.

Huisman, J., and Weissing, F. (2000). "Coexistence and resource competition." *Nature (London)*, 407, 694.

Huisman, J., and Weissing, F. (2001). "Fundamental unpredictability in multispecies competition." *Am. Nat.*, 157, 488–494.

Kaewplpat, K., and Grady, C. P. L. (2002). "Microbial population dynamics in laboratory-scale activated sludge reactors." *Water Sci. Technol.*, 46(1–2), 19–27.

Macey, R., Oster, G., and Zahley, T. (2000). *Berkley madonna, version 8.0.1*, ⟨http://www.berkleymadonna.com⟩.

Madigan, M. T., Martinko, J. M., and Parker, J. (2003). *Brock biology of microorganisms*, Prentice-Hall, Upper Saddle River, N.J.

Naeem, S., and Li, S. B. (1997). "Biodiversity enhances ecosystem reliability." *Nature (London)*, 390, 507–509.

Oerther, D. B., Pernthaler, J., Schramm, A., Amann, R., and Raskin, L. (2000). "Monitoring precursor 16S rRNAs of *Acinetobacter* spp. in activated sludge wastewater treatment systems." *Appl. Environ. Microbiol.*, 66, 2154–2165.

Saikaly, P. E., and Oerther, D. B. (2004). "Bacterial competition in activated sludge: Theoretical analysis of varying solids retention times on diversity." *Microb. Ecol.*, 48, 274–284.

Sommer, U. (1985). "Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton." *Limnol. Oceanogr.*, 30(2), 335–346.

Tilman, D. (1977). "Resource competition between planktonic algae: An experimental and theoretical approach." *Ecology*, 58, 338–348.

Tilman, D. (1999). "The ecological consequences of changes in biodiversity: A search for general principles." *Ecology*, 80, 1455–1474.

van Groenestijn, J. W., Zuidema, M., van de Worp, J. J., Deinema, M. H., and Zehnder, A. J. (1989). "Influence of environmental parameters on polyphosphate accumulation in *Acinetobacter* sp." *Antonie van Leeuwenhoek*, 55, 67–82.

Zumstein, E., Moletta, R., and Godon, J. (2000). "Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis." *Environ. Microbiol.*, 2(1), 69–78.