Assessing the Effects of Solids Residence Time and Volatile Fatty Acid Augmentation on Biological Phosphorus Removal Using Real Wastewater

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ABSTRACT: The purpose of the research presented herein was to evaluate the effects of solids residence time (SRT) and organic acid augmentation on biological phosphorus removal (BPR), with a focus on how these operational variables affect key metabolisms and the distribution of the microbial population. Using laboratory-scale sequencing batch reactors seeded with a mixed microbial consortium and fed real wastewater, we observed that longer SRTs can improve BPR performance; organic acid augmentation can stabilize BPR, but it is not necessary for process success; and higher volatile suspended solids concentrations correlate with improved phosphorus removal. The results also suggest that organic acids may not be critical in driving anaerobic phosphorus release, but in driving aerobic growth. Finally, given an observed population similarity across all tested bioreactors, BPR variability appears to be less influenced by the presence of specific microbes and more affected by the induction of critical metabolisms. *Water Environ. Res.*, **82,** 216 (2010).

KEYWORDS: biological phosphorus removal, solids residence time, organic acid augmentation, volatile fatty acid, food-to-microorganism ratio, polymerase chain reaction, denaturing gradient gel electrophoresis.

doi:10.2175/106143009X12487095236559

Introduction

Wastewater treatment practices in the United States have significantly improved since the passage of the Clean Water Act (PL 92-500); the initial regulatory focus on removing organic carbon (i.e., biochemical oxygen demand) and ammonia-nitrogen from wastewater has resulted in more readily ''fishable and swimmable'' surface water bodies. Having addressed these critical yet readily removed pollutants, attention has recently turned to a more challenging macronutrient—phosphorus—which commonly is measured in municipal wastewater effluent at concentrations that could lead to advanced eutrophication of surface water bodies (Lesjean et al., 2003; Mainstone and Parr, 2002; Oehmen et al., 2007). Specifically, the U.S. Environmental Protection Agency (Washington, D.C.) (U.S. EPA) has recently adopted guidelines that, in some ecoregions, potentially would limit wastewater treatment facility (WWTF) effluent to 0.01 to 0.03 mg phosphorus per liter (Lesjean et al., 2003; U.S. EPA, 2003). While the intent of these guidelines is not without merit, the emerging regulations present WWTFs with significant compliance challenges, given that the effluent concentrations are considered to be approaching the limits of technology (Oleszkiewicz and Barnard, 2006).

Phosphorus is removed from wastewater biologically or through chemical precipitation using metal salts. Chemical processes, which are considered universally applicable and reliable, potentially can achieve proposed stringent effluent standards (Kang et al., 2008); however, these processes yield increased operational costs, can adversely affect effluent pH, and increase solids-handling requirements (Kang et al., 2008; U.S. EPA, 2000). Conversely, biological phosphorus removal (BPR) is a less expensive process to operate, generates less solids, and is arguably more environmentally benign (Kang et al., 2008; Oehmen et al., 2007). Further, solids generated in BPR can be used agronomically (Kang et al., 2008; U.S. EPA, 2000). Unfortunately, the BPR process is not sufficiently reliable or stable to achieve increasingly stringent permit limits (Oehmen et al., 2007), principally as the result of a limited understanding of the complex ecology and metabolisms involved in microbial use and management of phosphorus within wastewater treatment environments (Seviour et al., 2003). Nevertheless, considering the potential negative effects of extensive chemical use on the water environment, our challenge is to advance BPR process knowledge, such that effluent limits can be realized with little or no chemicals.

The empirical BPR process, originally proposed by Fuhs and Chen (1975), is centered fundamentally on cycling microbes between anaerobic (no oxygen or nitrate) and aerobic environments, to elicit the required metabolic responses for excess phosphorus removal (Seviour et al., 2003). Anaerobically, microorganisms uptake and store carbon (assumed to be volatile fatty acids [VFAs]) in the form of polyhydroxyalkanoates (PHAs) and hydrolyze internally stored polyphosphate for energy; the energy is used for VFA metabolism to PHA precursors, and the hydrolyzed phosphate molecules are excreted from the cell, producing an increase in bulk aqueous phosphate concentration. In the aerobic zone, these same microorganisms use the stored carbon for growth and maintenance with concurrent uptake and storage of phosphate. This cyclical process yields a significant net removal of phosphate; further, it is broadly assumed that operating in this manner yields a microbial consortium enriched for a

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population referred to as phosphorus-accumulating organisms (PAOs) (Oehmen et al., 2007). With theory driving practice, full-scale BPR facilities are designed with anaerobic–aerobic basins in series and commonly include VFA augmentation (typically through fermenting primary solids) (Metcalf & Eddy, 2003).

While the empirical BPR process involves induction of a complex and interdependent sequence of metabolisms, from a macro-perspective, the concept of induced microbial famine is a singular overarching theme. *Microbial famine* can be defined generally as the coupling of an imposed environmental condition (i.e., wherein microbes are deprived of one or more critical macronutrients required for growth, which creates stress and further enriches for a certain population) and the associated metabolic responses; within the context of BPR theory, baseline famine is created by exposing microbes to cyclical anaerobic–aerobic conditions. However, famine can be enhanced operationally and modulated by varying the solids residence time (SRT) and the quantity of macronutrients (e.g., VFAs) provided. Through the SRT, WWTF operators can increase the concentration of microbes exposed to the cyclical anaerobic–aerobic conditions; augmenting influent raw wastewater with a VFA-rich waste stream similarly allows operators to increase the concentration of microbes exposed to the anaerobic environment, but also facilitates more growth aerobically, which could increase aerobic phosphorus uptake and removal. The effects of these operational parameters on BPR have not been fully elucidated. Regarding SRT, Metcalf & Eddy (2003) suggest that, as the SRT increases, microbes aerobically deplete intracellular storage reserves, resulting in less efficient acetate uptake and PHA synthesis anaerobically and thus less efficient BPR; Brdjanovic et al. (1998) and Wang and Park (2001) drew similar conclusions. Smolders et al. (1995) suggested that there is a minimum SRT based on polyphosphate reserves and also determined that BPR is successful at a 20-day SRT, while Lee et al. (2007) suggested that phosphorus removal is improved at longer SRTs, because the biomass implicitly contains more phosphorus. Research on VFA effects has been focused principally on process biochemistry or on how the carbon source affects the microbial population (Oehmen et al., 2007).

To evaluate the potential effect of these operational parameters on BPR, we tested a matrix of laboratory-scale sequencing batch reactors (SBRs), each fed real wastewater and seeded with a mixed microbial consortium from a full-scale WWTF. The principle objectives of this research were to

(1) Interrogate the effect of SRT on BPR,
(2) Quantitatively evaluate the effect of VFA augmentation on BPR, and
(3) Qualitatively assess and compare the mixed microbial consortia across the matrix of bioreactors.

Central to this research was the use of real wastewater; the majority of BPR research has been conducted using synthetic wastewater.

Materials and Methods

Source of Wastewater. Raw wastewater was obtained from the Moscow, Idaho, WWTF; the Moscow WWTF treats approximately 7600 to 15 000 m^3/d (2 to 4 mgd) in an aerobic–anoxic (A^2/O) oxidation ditch process (Metcalf & Eddy, 2003) with no primary solids fermentation. Fermenter liquor was recovered from a laboratory bench-top fermenter fed thickened primary solids from the Pullman, Washington, WWTF. Pullman operates a conventional modified Ludzack-Ettinger (MLE) process with a high-rate, completely mixed, mesophilic anaerobic digester (Metcalf & Eddy, 2003).

Bioreactor Operational Conditions. Six laboratory-scale SBRs were operated and tested, each at two different SRTs (Table 1). The SBR operation consisted of four sequential steps: (1) fill, (2) react, (3) settle, and (4) decant. During the fill period, wastewater was added to the reactor, while anaerobic and aerobic conditions were imposed during the react period to facilitate wastewater treatment. Following treatment, biomass was allowed to settle to the bottom of the tank, then treated effluent was withdrawn from the top of the reactor. Reactors were operated at SRTs of 10 and 20 days, with total hydraulic residence times (HRTs) of 12 and 18 hours. All reactors were seeded with a mixed microbial consortium obtained from the aerobic oxidation ditch at the Moscow, Idaho, WWTF. Reactors were fed either 100% raw wastewater or 90% raw wastewater plus 10% fermenter liquor (v:v; referred to as ''90:10''). Nitrification was prevented in all reactors by inhibiting the ammonia monooxygenase enzyme through the addition of thiourea (1 mL thiourea/L feedstock) (Hyman et al., 1988). Anaerobic conditions were created by diffusing nitrogen gas into each bioreactor for the first 8 minutes of the anaerobic period (to achieve a dissolved oxygen concentration $<$0.2 mg/L). Compressed air was diffused through the reactor to maintain a dissolved oxygen level greater than 2 mg/L during the aerobic period. The reactors were completely mixed, except during the settle and decant periods. For each operational cycle, the settling period in all reactors was followed by a 2-minute decant period and an 8-minute feed period; to maintain the target HRTs, 400 mL were decanted and fed each cycle using Watson-Marlow peristaltic pumps (Watson-Marlow Bredel Inc., Wilmington, Massachusetts). The 10- and 20-day SRTs were maintained by wasting 10 and 5%, respectively, of the completely mixed total reactor volume (i.e., reactor liquid and biomass) at the end of one aerobic cycle each day. Bioreactor operations were controlled with digital timers. The SBR naming scheme (Table 1) was as follows: the ''F'' or ''NF'' designation represents ''fermenter'' or ''non-fermenter'' fed (i.e., 90:10 feedstock versus 100% raw wastewater); the first number is SRT (days), the second number is overall HRT (hours); and the last two numbers are anaerobic and aerobic cycle times (hours), respectively.

Fermenter liquor was produced in a 12-L completely mixed primary solids fermenter, operated as an SBR, with an SRT and HRT of 4 days. The daily decant was centrifuged at approximately 10 000 rpm, with the supernatant (i.e., fermenter liquor) recovered and stored at 4°C.

Analytical Techniques. Samples were collected from each bioreactor to measure the following parameters: soluble reactive phosphate (SRP), total suspended solids (TSS), mixed-liquor volatile suspended solids (VSS), organic acids, chemical oxygen demand (COD), pH, and dissolved oxygen. All samples were centrifuged to remove biomass and then filtered through a 0.22-μm syringe filter (Millipore Corporation, Billerica, Massachusetts) before testing.

The SRP concentration was determined in accordance with Hach (Hach Company, Loveland, Colorado) method 8048 (equivalent to method 4500-PE of *Standard Methods* [APHA et

Table 1—Summary description of bioreactor operational configuration. For the "feedstock", 100% raw (identified as NF reactors) indicates that the reactor only received raw wastewater, while 90:10 (identified as F reactors) indicates that the reactor received 90% raw wastewater and 10% primary solids fermenter liquor (v:v).

Reactor identification	Reactor volume (L)	SRT (days)	HRT (hours)	Cycles per day	Feedstock	Cycle times (hours)		
						Anaerobic	Aerobic	Settle
NF.10/20.12.1.3	1.2	10/20	12	6	100% raw	1	2.5	0.5
NF.10/20.18.1.3	1.8	10/20	18	6	100% raw	1	2.5	0.5
F.10/20.12.1.3	1.2	10/20	12	6	90:10	1	2.5	0.5
F.10/20.18.1.3	1.8	10/20	18	6	90:10	1	2.5	0.5
F.10/20.12.1.5	0.8	10/20	12	4	90:10	1	4.5	0.5
F.10/20.18.1.5	1.2	10/20	18	4	90:10	1	4.5	0.5

al., 1995]). A Spectronic 20 Genesys spectrophotometer (Thermo Scientific Corporation, Waltham, Massachusetts) was used to measure the absorbance of the reacted sample at a wavelength of 890 nm. Phosphate concentrations were determined using a standard curve (R^2>0.99). The TSS and VSS were measured in accordance with *Standard Methods* 2540 D and 2540 E (APHA et al., 1995). The pH was measured using a Thermo Scientific Corporation Accumet AP85 waterproof pH/conductivity meter. Dissolved oxygen measurements were collected using a Hach HQ30d meter with an LDO101 dissolved oxygen probe.

Organic acid (e.g., acetic, butyric, and propionic) concentrations in the respective wastewaters and reactors were measured using a Hewlett-Packard (Palo Alto, California) 6890 series gas chromatograph with a flame-ionization detector. The temperature of the column (Alltech Heliflex AT-wax column, length 30 m, internal diameter 0.32 mm; Grace Davison Discovery Sciences, Deerfield, Ilinois) was held constant at 150°C; the injector was maintained at 250°C, and the detector was operated at 250°C. Helium was used as the carrier gas at a flowrate of 1.2 mL/min. Samples were acidified to a pH of 2 before injection. Approximately 0.5 μL of sample was injected in a 20:1 split mode for analysis. The respective sample organic acid concentrations were confirmed by a retention time matching with known standards and quantified using a standard curve (R^2>0.99). Soluble COD tests were performed in accordance with method 5220-D of *Standard Methods* (APHA et al., 1995) using Hach high-range ampules with a Hach COD reactor and a Spectronic 20 Genesys spectrophotometer (Thermo Scientific Corporation). The COD concentrations were determined using a standard curve (R^2>0.99).

Polymerase Chain Reaction for DNA Amplification. DNA from each reactor was extracted using an UltraClean soil DNA extraction kit (MoBio Laboratories, Inc., Solana Beach, California). Samples were stored at −20°C. DNA fragments containing bacterial 16S rRNA genes (560 bp) obtained from the UltraClean soil DNA kit were amplified in polymerase chain reaction (PCR) mixtures containing approximately 100 ng of template DNA; 2.5 U Taq polymerase; 0.4 μM of each of the primers 341F-GC (*CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC* TAC GGG AGG CAG CAG), containing a 40-bp GC-clamp to enhance separation in denaturing gradient gel electrophoresis (DGGE) (clamp sequence in italics), and 907R (CCG TCA ATT CMT TTG AGT TT); 1× polymerase buffer; 0.4 mg/mL bovine serum albumin; and 1.5 mM magnesium chloride ($MgCl^2$); in a final volume of 50 μL. The primers were selected because they have been shown to cover a highly conserved region within the domain Bacteria (Baker et al., 2003). The PCR reaction was performed with 5 minutes of initial denaturation at 94°C and 30 cycles of 94°C for 1 minute, 55°C annealing for 1 minute, 72°C extension for 2 minutes, and a 72°C final extension for 5 minutes. To confirm successful amplification, DNA was resolved by 1.5% agarose gel electrophoresis using 1× TAE buffer (40 mM Tris base, 20 mM acetic acid, and 1 mM 0.5 M EDTA [pH 8.0]) for 1 hour at 6 V/cm. The gel was stained in 1× TAE containing 1X SYBR Gold (Invitrogen, Eugene, Oregon) for 30 minutes and destained with deionized water for 10 minutes, visualized with a UV transilluminator, and photographed using Kodak 1D Image Analysis Software (Eastman Kodak Company, Rochester, New York).

Denaturing Gradient Gel Electrophoresis Analysis. The DGGE was performed with the Bio-Rad DCode System (Bio-Rad Laboratories, Inc., Hercules, California). Approximately 200 ng of amplified DNA were applied directly onto a 6% (w/v) parallel denaturing polyacrylamide gel in 1× TAE (40 mM Tris base, 20 mM acetic acid, and 1 mM 0.5 M EDTA [pH 8.0]) with gradients that were formed with 40% (w/v) acrylamide/bis stock solution (acrylamide and N,N'-methylene-bis-acrylamide, 37.5:1) and contained 20% denaturant (8% formamide [v/v], 0.14 M urea, 1× TAE, 6% [final v/v] of 40% acrylamide/Bis, 0.09% [v/v] ammonium persulfate, and 0.09% TEMED) to 70% denaturant (28% formamide [v/v], 0.49 M urea, 1x TAE, 6% [final v/v] 40% acrylamide/Bis, 0.09% [v/v] ammonium persulfate, and 0.09% TEMED). Electrophoresis was performed at a constant voltage of 200 V and a temperature of 60°C for 18 hours. After electrophoresis, the gel was stained in 1× TAE containing 1× SYBR Gold (Invitrogen) for 30 minutes and destained with deionized water for 10 minutes, visualized with a UV transilluminator, and photographed using Kodak 1D Image Analysis Software (Eastman Kodak Company).

Results and Discussion

The results presented and discussed herein represent the performance for each independent SBR monitored 3 times over approximately 1 week after each bioreactor had reached steady-state operation. Each reactor was first operated at a 10-day SRT; steady-state conditions were assumed to predominate following an operational period equal to 3 SRTs. When the SRT was shifted to 20 days, steady-state conditions were assessed through the mixed-liquor suspended solids concentration (variance ±10%). All reactors were operated at room temperature (approximately 20 to 21°C), and the research laboratory was temperature-controlled at approximately 20°C.

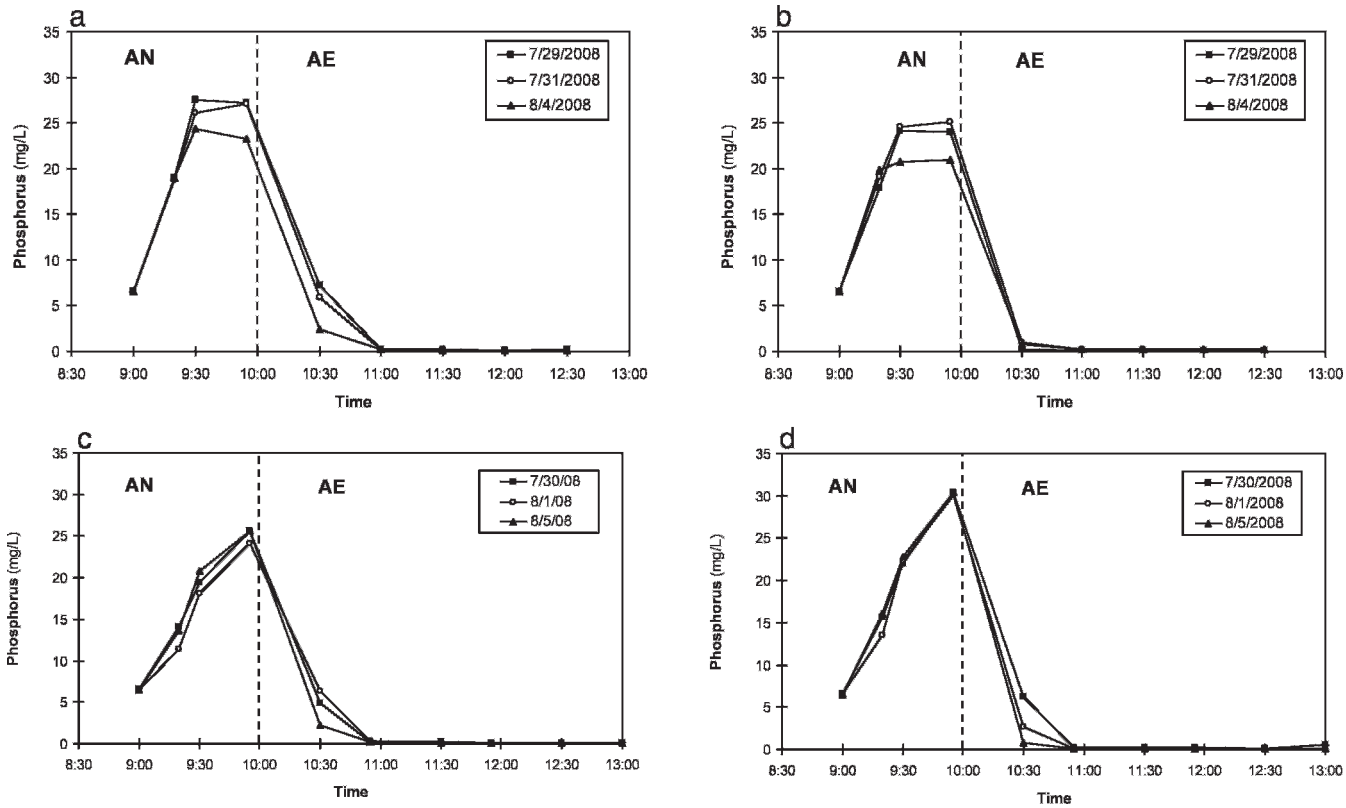

Figure 1—Bulk solution phosphorus profiles for reactors (a) F.10.12.1.3, (b) F.10.18.1.3, (c) F.10.18.1.5, and (d) F.10.12.1.5. Results from each sampling event are shown, with the sampling date noted.

Solids Residence Time Effects on Phosphorus Cycling and Removal. The SRT controls the relative age of the microbes (i.e., how long they remain in the SBRs) and thus the concentration of microbes in the system; longer SRTs result in higher concentrations of microorganisms. Regarding the BPR performance, more microbes in the system would theoretically lead to enhanced phosphorus removal. The potential effect of SRT on BPR performance was evaluated at sludge ages of 10 and 20 days.

At the 10-day SRT, the microbial consortia fed the 90:10 substrate exhibited phosphorus cycling patterns consistent with BPR theory (Figure 1) and achieved exceptional effluent phosphorus values on a stable and reliable basis (Table 2); total phosphorus removal ranged from 90 to 98% (mass basis; Table 3). Conversely, the non-augmented SBRs showed highly variable BPR performance at the 10-day SRT (Figures 2 and 3). While reactor NF.10.12.1.3 generally exhibited good overall BPR performance for two of the three sampling cycles (Table 3),

Table 2—Average VSS, food-to-microorganism (F:M) ratio, and effluent phosphorus concentrations for all 12 bioreactor configurations. Results are averages from three sampling events performed on different days within a time period of 1 week after reactors had reached steady-state.

Reactor	Average VSS (mg/L)	Average F:M (mg organic acid as COD·mg VSS^{-1})	Average effluent phosphorus (mg/L)
NF.10.12.1.3	1018	0.020	1.25
NF.10.18.1.3	747	0.018	2.05
F.10.12.1.3	2793	0.023	0.15
F.10.18.1.3	2503	0.018	0.14
F.10.12.1.5	2077	0.053	0.15
F.10.18.1.5	1257	0.059	0.12
NF.20.12.1.3	1753	0.008	0.83
NF.20.18.1.3	1367	0.007	1.43
F.20.12.1.3	2957	0.022	0.18
F.20.18.1.3	2710	0.016	0.18
F.20.12.1.5	3333	0.029	0.15
F.20.18.1.5	3127	0.021	0.11

Table 3—Influent VFA concentrations and phosphorus released anaerobically for each SBR for each sampling event; quantity of phosphorus released anaerobically per organic acid uptake ratio normalized to VSS (P:VFA:VSS ratio); quantity of phosphorus released anaerobically per organic acid uptake (P:C ratio; mmol basis); effluent phosphorus; and percent phosphorus removed for each SRT and bioreactor during each sampling event.

Reactor and sampling date	Influent VFAs (mg/L) Acetic/propionic/valeric/butyric	Phosphorus released (mg)	Phosphorus released per VFA-VSS (mmol P·(mmol C·g VSS)$^{-1}$)	Phosphorus released per VFA (mmol P· mmol C^{-1})	Effluent phosphorus (mg/L)	Phosphorus removed (%)
NF.10.12.1.3						
07/29/08	48.2/7.1/0.0/0.0	0.7	0.0	0.03	3.10	40.3
07/31/08	45.3/3.8/5.1/0.0	4.6	0.20	0.20	0.34	84.6
08/04/08	41.0/3.5/0.0/0.0	3.9	0.17	0.21	0.31	87.1
NF.10.18.1.3						
07/29/08	48.2/7.1/0.0/0.0	0.0	0.0	0.00	3.65	44.5
07/31/08	45.3/3.8/5.1/0.0	2.0	0.10	0.09	1.25	41.2
08/04/08	41.0/3.5/0.0/0.0	1.2	0.08	0.06	1.26	48.7
F.10.12.1.3						
07/29/08	98.7/18.9/20.8/5.2	22.6	0.13	0.35	0.18	92.4
07/31/08	142.9/22.0/9.8/5.3	22.2	0.10	0.28	0.13	94.2
08/04/08	107.3/18.8/10.9/3.9	18.8	0.10	0.30	0.15	93.6
F.10.18.1.3						
07/29/08	98.7/18.9/20.8/5.2	29.2	0.20	0.45	0.13	91.7
07/31/08	142.9/22.0/9.8/5.3	31.1	0.15	0.39	0.12	92.4
08/04/08	107.3/18.8/10.9/3.9	23.4	0.14	0.38	0.17	89.7
F.10.12.1.5						
07/30/08	95.1/23.4/35.2/4.8	16.6	0.11	0.23	0.16	95.2
08/01/08	145.1/26.6/12.9/2.4	16.4	0.10	0.20	0.07	97.9
08/05/08	126.6/40.0/11.4/5.4	16.6	0.09	0.20	0.22	93.4
F.10.18.1.5						
07/30/08	95.1/23.4/35.2/4.8	20.4	0.20	0.28	0.14	93.9
08/01/08	145.1/26.6/12.9/2.4	18.7	0.20	0.23	0.10	95.5
08/05/08	126.6/40.0/11.4/5.4	20.4	0.19	0.25	0.12	94.7
NF.20.12.1.3						
09/05/08	47.0/10.2/0.0/0.0	23.5	0.52	0.96	0.25	85.7
09/08/08	30.3/5.2/0.0/0.0	21.7	0.82	1.44	0.18	89.3
09/10/08	14.9/0.0/0.0/0.0	18.5	1.82*	3.01*	2.05	52.6
NF.20.18.1.3						
09/05/08	47.0/10.2/0.0/0.0	19.0	0.58	0.78	0.25	78.9
09/08/08	30.3/5.2/0.0/0.0	18.4	0.85	1.21	0.52	64.5
09/10/08	14.9/0.0/0.0/0.0	15.8	1.79*	2.60*	3.51	37.7
F.20.12.1.3						
09/05/08	158.6/29.6/17.2/0.0	21.7	0.08	0.24	0.22	90.8
09/08/08	127.2/25.0/6.9/0.0	20.5	0.11	0.30	0.17	94.2
09/10/08	94.9/23.5/6.1/3.5	22.1	0.14	0.39	0.14	93.9
F.20.18.1.3						
09/05/08	158.6/29.6/17.2/0.0	35.2	0.15	0.39	0.23	85.5
09/08/08	127.2/25.0/6.9/0.0	33.7	0.18	0.49	0.15	91.8
09/10/08	94.9/23.5/6.1/3.5	35.9	0.22	0.63	0.16	90.1
F.20.12.1.5						
09/05/08	158.6/29.6/17.2/0.0	37.1	0.12	0.41	0.18	94.8
09/08/08	127.2/25.0/6.9/0.0	35.7	0.16	0.52	0.13	96.2
09/10/08	94.9/23.5/6.1/3.5	31.6	0.18	0.56	0.13	96.2
F.20.18.1.5						
09/05/08	158.6/29.6/17.2/0.0	53.4	0.20	0.59	0.13	94.6
09/08/08	127.2/25.0/6.9/0.0	51.2	0.23	0.74	0.14	94.1
09/10/08	94.9/23.5/6.1/3.5	44.3	0.25	0.78	0.06	97.4

* These ratios were elevated because of an extremely low amount of carbon in the raw wastewater feedstock during this sampling event.

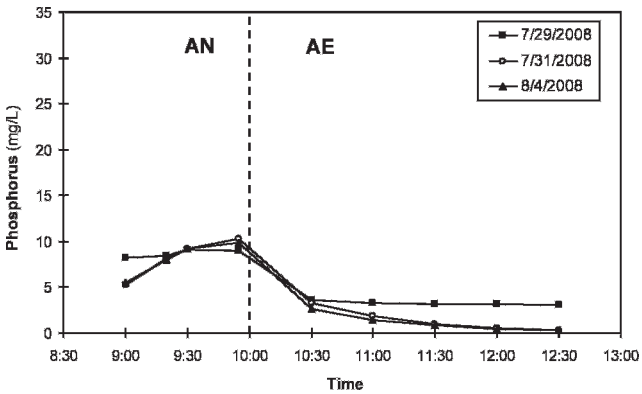

Figure 2—Bulk solution phosphorus profiles for reactor NF.10.12.1.3. Results from each sampling event are shown, with the sampling date noted.

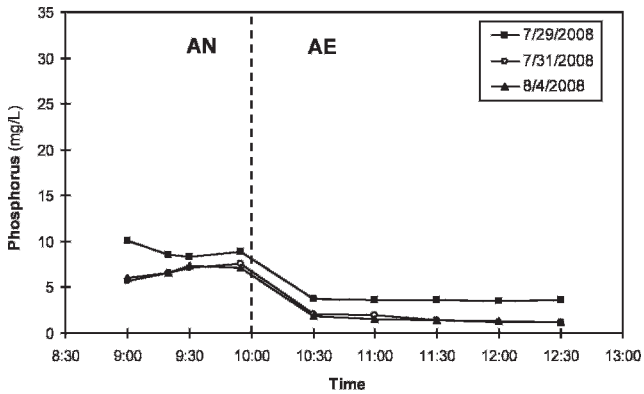

Figure 3—Bulk solution phosphorus profiles for reactor NF.10.18.1.3. Results from each sampling event are shown, with the sampling date noted.

significantly less phosphorus release and uptake was observed during the first assessment cycle (July 29th; Figure 2); bioreactor NF.10.18.1.3 can only marginally be interpreted as performing BPR (Figure 3). For both NF bioreactors, even for the cycles that showed ''typical'' BPR anaerobic–aerobic phosphorus cycling, the relative difference between peak phosphorus release and effluent concentration was markedly less than that observed in the fermenter liquor-augmented reactors. Overall, phosphorus removal in the NF SBRs ranged from approximately 40 to 87% in NF.10.12.1.3 to approximately 41 to 49% in NF.10.18.1.3 (Table 4).

The reduced BPR performance observed in the NF SBRs at the 10-day SRT could have been related to a low PAO concentration, insufficient induction of necessary BPR metabolisms, or some combination thereof. Regarding PAO concentration, compared with the augmented reactors, the total microbial concentration (measured as VSS) in the NF SBRs was significantly lower (Table 2). However, a muted metabolic response cannot be ruled out; the NF SBRs received measurably less VFAs than their augmented counterparts (Table 3), and the BPR metabolisms associated with anaerobic phosphorus release and aerobic phosphorus uptake were linked theoretically and intrinsically to VFA availability. Regarding the seemingly unstable and/or upset condition observed in each first NF assessment cycle, this phenomenon was not a result of a toxic compound in the wastewater, as the augmented reactors did not exhibit a similar response, although they were fed the same raw wastewater and tested on the same days as the NF reactors.

In contrast to the 10-day SRT, all bioreactors exhibited ''typical'' BPR behavior at the 20-day SRT, and three of the six bioreactors exhibited improved effluent quality over their 10-day SRT counterparts (Figures 4 to 6 and Table 3). Further, the microbial consortia in SBRs F.20.12.1.5 and F.20.18.1.5 (Figure 4) both exhibited approximately twice the anaerobic phosphorus release (measured as milligrams phosphorus per liter in bulk solution), as observed in their 10-day SRT counterparts and also as observed in the other 20-day augmented bioreactors. This observed response appears to be associated predominantly with microbial population; the VSS concentration increased substantially in the 1.5 reactors between SRTs, while the relative quantity of VFAs (which theoretically stimulate this phosphorus-release response) remained constant. Thus, a larger concentration of microbes was effectively working harder to survive in the anaerobic environment. Nevertheless, despite this marked increase in phosphorus release, neither consortium exhibited an increase in overall process performance (measured as effluent phosphorus). Phosphorus removal ranged from approximately 86 to 97%, for the F reactors, to approximately 38 to 89%, for the NF reactors (Table 4).

The most significant effect of increasing the SRT was observed in the NF bioreactors. The phosphorus cycling patterns for the 20-day SRT NF SBRs were much more consistent with that predicted by BPR theory (and as compared with their 10-day SRT counterparts); phosphorus release increased 3- to 4-fold from the 10-day SRT, and, commensurately, the respective consortia exhibited significantly better phosphorus removal (Figures 5 and 6). In particular, the consortium in the NF.20.18.1.3 bioreactor, which had performed BPR quite poorly at the 10-day SRT, adapted under the longer SRT to not only exhibit typical BPR behavior (Figures 3 versus 6) but also increase the phosphorus-removal efficiency by approximately 35% (Table 3).

Lee et al. (2007) found that a 20-day SRT was optimum for BPR performance and further suggested that improved process performance was associated with an increased concentration of PAOs. In comparison, for our investigations, the SRT appeared to have a minimal effect on BPR performance for the VFA-augmented SBRs. However, consistent with Lee et al. (2007), the longer SRT did yield improved BPR performance for the NF SBRs. These results indicate that there is a critical PAO population that must be maintained to achieve good BPR, and this critical mass can be realized through modulating the SRT. However, VFA augmentation also appeared to play a potentially critical role in process performance.

Anaerobic Volatile Fatty Acid Augmentation and Biological Phosphorus Removal Performance. Primary solids fermenter liquor is rich in critical macronutrients required for microbial growth, including VFAs. Therefore, similar to the effects of imposing longer SRTs, the augmentation of BPR systems with fermenter liquor can facilitate an increase in VSS (and, presumably, PAOs), as contrasted with bioreactors provided only raw wastewater. The VFAs present in fermenter liquor also theoretically are required to induce necessary anaerobic BPR metabolisms (Seviour et al., 2003). To better understand the effects of VFA augmentation on our BPR reactors, we assessed

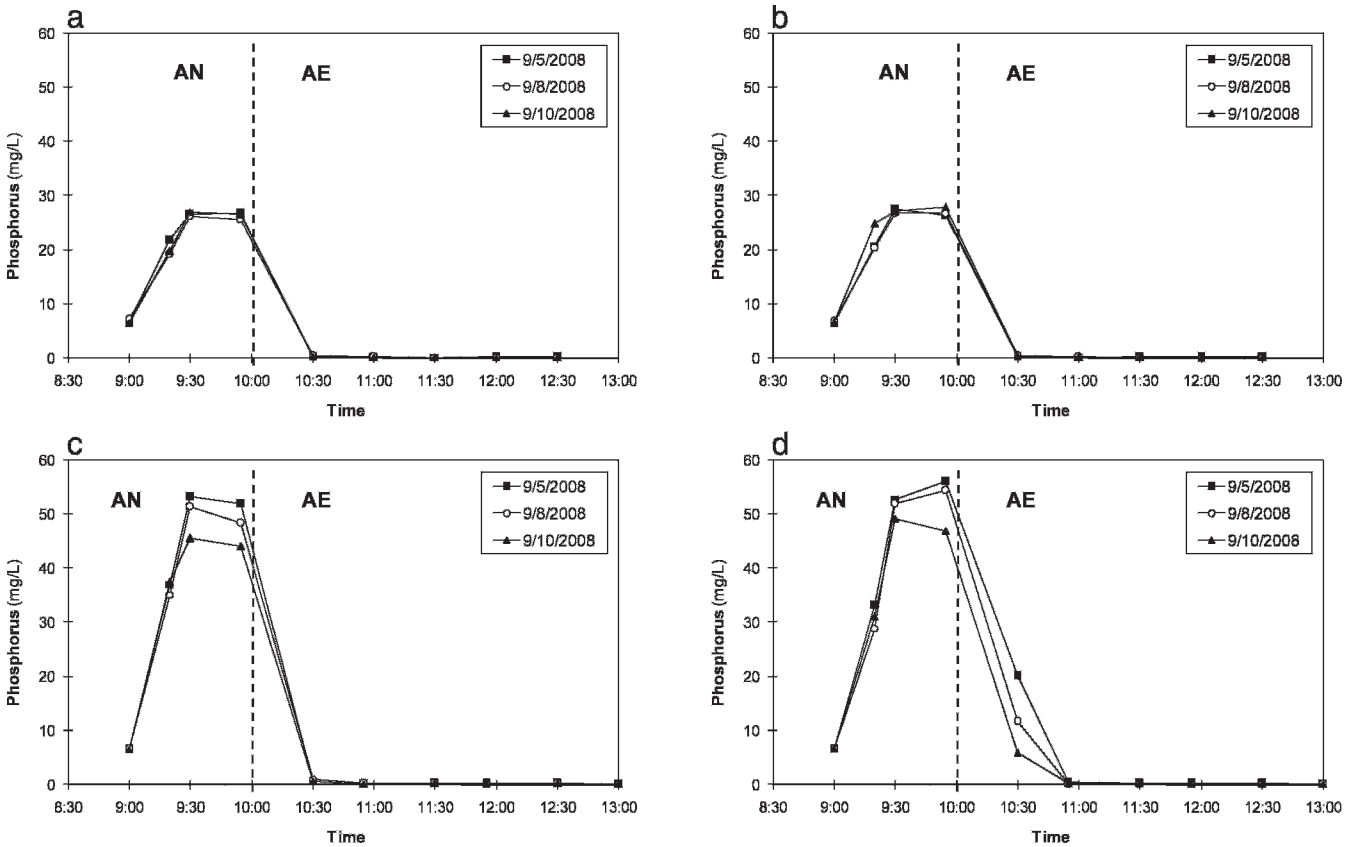

Figure 4—Bulk solution phosphorus profiles for reactor (a) F.20.12.1.3, (b) F.20.18.1.3, (c) F.20.18.1.5, and (d) F.20.12.1.5. Results from each sampling event are shown, with the sampling date noted.

the performance data from the following perspectives: (1) broadly and generally comparing VFA augmentation versus fed-raw-wastewater-only, (2) from a food-to-microorganism ratio (F/M) perspective, and (3) considering phosphorus released anaerobically relative to the VFAs used.

For those reactors augmented with VFA-rich fermenter liquor, effluent phosphorus concentrations were consistently low (Table 2), excellent overall phosphorus removal was achieved (Table 3), and the consortia all exhibited the typical BPR phosphorus cycling patterns. In contrast, the non-augmented reactors exhibited much higher average effluent phosphorus values and generally variable (poor to excellent) overall phosphorus removal. These results might suggest that VFA augmentation was critical to induce prerequisite BPR metabolisms. However, in the NF SBRs, overall BPR performance improved substantially with increasing VSS concentrations, despite the fact that the microbes, on a unit basis, received the lowest quantity of VFAs both compared with their 10-day SRT counterparts and to all the augmented reactors (Tables 2 and 3). Moreover, the consortia in the 20-day SRT NF reactors also

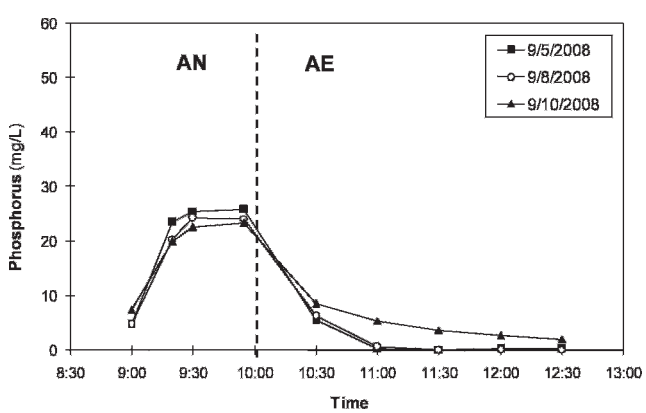

Figure 5—Bulk solution phosphorus profiles for reactor NF.20.12.1.3. Results from each sampling event are shown, with the sampling date noted.

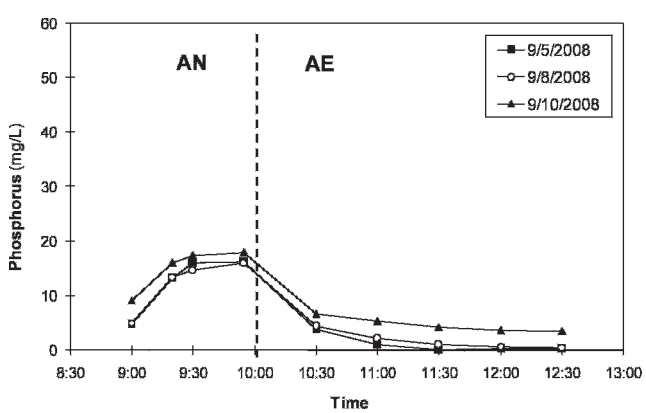

Figure 6—Bulk solution phosphorus profiles for reactor NF.20.18.1.3. Results from each sampling event are shown, with the sampling date noted.

exhibited ''typical'' phosphorus cycling patterns. Thus, while from a macro-perspective, VFA augmentation did appear to stabilize BPR, the results also suggest that BPR success can be realized when a critical mass of microbes simply is exposed to cyclical anaerobic–aerobic conditions.

A more detailed perspective in which to assess the effects of VFA augmentation lies in the F/M ratio, which was calculated as the mass of organic acids (on a COD basis) to the mass of VSS (standard stoichiometric ratios were used to convert the respective VFAs to a COD basis [Grady Jr. et al., 1999]). This parameter expresses the amount of readily biodegradable carbon provided per microbe, and, in contrast to the F versus NF discussion above, allows for a direct comparative assessment between and across all tested bioreactors. Key observations from this comparison are as follows:

(1) While the F/M ratio did vary across the fermenter liquor-augmented bioreactors and decreased from the 10- to 20-day SRT, there was no apparent correlation with effluent phosphorus concentrations (Table 2); however, the F.20.12/18.1.5 consortia received significantly less food (on a unit basis) than at the 10-day SRT, yet performance remained stable.
(2) In contrast to that observed in the augmented reactors, effluent phosphorus concentrations decreased substantially in the NF reactors with a reduced F/M ratio; in other words, a higher concentration of microbes received less food, yet achieved better BPR. However, at each SRT, the NF reactor receiving the most VFAs per cycle (i.e., the 12-hour HRT reactors) performed the best BPR, and the NF consortium receiving the most VFAs and exhibiting the highest VSS (NF.20.12.1.3) achieved the lowest effluent phosphorus concentration and best overall phosphorus removal.
(3) Contrasting NF.10.18.1.3 and F.10.18.1.3, while both reactors were operated fundamentally the same and both consortia realized the same F/M ratio, the augmented reactor VSS was approximately 3.5 times higher, and the effluent phosphorus was more than 1 order of magnitude lower than the NF reactor. In this scenario, the augmented SBR exhibited a significantly higher concentration of microbes, principally as a result of receiving more VFAs.

In summary, the F/M comparative analysis again suggested that VFA augmentation was not necessarily a critical BPR process driver.

The BPR success is predicated theoretically and centrally on significant anaerobic phosphorus release associated with VFA uptake and storage; in fact, the phosphorus released-to-VFA uptake ratio has been proposed as a measure of process performance (Filipe et al., 2001; Smolders et al., 1994). Thus, as a final comparative assessment related to VFA availability on BPR, we assessed the ratios of phosphorus released anaerobically (on a mmol phosphorus basis) to the VFA uptake (on a mmol carbon basis) (commonly referred to as the P:C ratio) and the P:C:VSS ratio (Table 3).

First considering the augmented reactors, for the F.12.1.3 SBRs, little average difference was observed for both the P:C and P:C:VSS ratios across SRTs; comparable total anaerobic phosphorus release also was observed. However, between the 12- and 18-hour HRT (for both SRTs), the two ratios increased markedly, as did gross phosphorus release. In other words, the consortia exposed to longer HRTs, which received less VFAs and were characterized by lower VSS, hydrolyzed more polyphosphate for energy; however, effluent phosphorus remained consistently low. For the F.1.5 SBRs, anaerobic phosphorus release increased substantially as both HRT and SRT increased (on a P:C and P:C:VSS basis and also on a mass basis; Table 3), as with the F.1.3 SBRs. Second, considering the NF reactors, the 20-day SRT consortia, which increased in concentration substantially over their 10-day counterparts, received significantly less VFAs (much lower F/M; Table 2); yet, the P:C and P:C:VSS ratios and the mass of phosphorus released anaerobically increased significantly over that observed at the 10-day SRT; this response appeared to correlate with improved effluent quality. There were also some interesting anomalies observed for the NF reactors. For each 20-day SRT NF scenario, on the September 10th sampling event, the respective consortia received significantly less VFAs than the previous two sampling events, and effluent quality was degraded (Table 3). However, the microbes released a large quantity of phosphorus; the P:C and P:C:VSS ratios were artificially high, as a result of the low VFAs. In direct contrast, considering the NF 10-day SRT data, VFA concentrations for the July 29th event were comparable with that measured for the other two events; however, little to no phosphorus was released anaerobically (Table 3); similar to the NF 20-day SRT anomaly, effluent levels were degraded. Combined, these P:C and P:C:VSS results suggest that, while VFAs may contribute toward inducing anaerobic phosphorus release, the true value of VFAs to BPR success ultimately may be in driving growth aerobically (albeit associated with the use of PHA that was synthesized anaerobically with VFA uptake); with more microbes present aerobically (induced by providing more VFAs, by increasing SRT, or both), more phosphorus removal can occur.

It could be suggested that the observed increases in anaerobic phosphorus release were associated with a phenomenon known as *secondary phosphorus release* (i.e., when VFAs are depleted and microbes are deprived of external electron acceptors, it is postulated that microbes will anaerobically hydrolyze polyphosphate for energy and release excess phosphorus to bulk solution [Wouters-Wasiak et al., 1996]). However, it is unlikely that the short anaerobic period applied in our reactors (1 hour) would have been sufficient to fully deplete VFAs and induce such a response. Also, in related research, we observed that anaerobic phosphorus release can end with the depletion of VFAs (unpublished data).

Influent Volatile Fatty Acids and Readily Biodegradable Chemical Oxygen Demand. Applying typical stoichiometric ratios to convert VFAs to a COD basis (Grady Jr. et al., 1999), the raw wastewater VFA fraction was consistently $>80\%$ of the readily biodegradable COD (rbCOD); for the 90:10 substrate, the VFA concentration was consistently $>90\%$ of the rbCOD. The rbCOD was defined as the difference between soluble COD following reactor feed and the end-of-cycle residual soluble COD. Consistent with BPR theory, the VFAs were consumed effectively by the respective consortia during the anaerobic period. However, limited COD testing showed that some of the non-VFA rbCOD was used aerobically.

Influent Volatile-Fatty-Acid-to-Phosphorus Ratio. The ratio of influent VFA-to-influent phosphorus (VFAinfl:Pinfl) has been suggested as a potential indicator for achieving successful BPR (Grady Jr. et al., 1999). Specifically, as the ratio increases, more organic carbon (i.e., VFAs) would be available to drive BPR

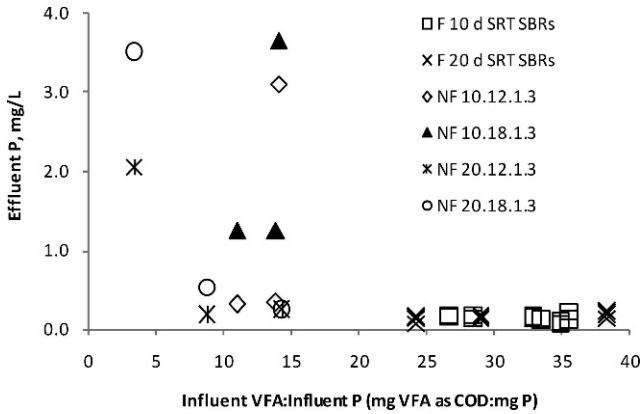

Figure 7—Influent VFA (recorded as COD) to influent phosphorus ratio versus effluent phosphorus for the tested bioreactors. Results for the augmented reactors (F) were broadly combined, given their similarity. Results for each NF reactor are shown independently.

metabolisms, and the consortia would theoretically become more enriched for PAOs. To contrast wastewater feedstocks versus BPR performance and to contribute to the broader body of knowledge on this subject, we calculated the VFAinfl:Pinfl for each reactor tested. As shown in Figure 7, there was a clear distinction between those reactors augmented with fermenter liquor and those receiving only raw wastewater. For the F reactors, the VFAinfl:Pinfl ratio exceeded 24, with consistently low effluent phosphorus. Conversely, the results were mixed for the NF reactors; while ratios of 8 to 15 correlated with low effluent phosphorus (6 data points), there were nearly as many (4 data points) within the same VFAinfl:Pinfl range, which correlated with high effluent phosphorus. These 4 data points, corresponding to ''poor quality'' effluent phosphorus, also correlated with the lowest observed VSS concentrations, which might indicate that the VSS either was enriched insufficiently for PAOs or that, more broadly, an insufficient concentration of microbes was present.

Qualitative Analysis of the Microbial Population. By operating a WWTF in the prescriptive BPR manner, it is assumed broadly that the mixed microbial consortium will be enriched for the requisite PAO population (Metcalf & Eddy, 2003; Oehmen et al., 2007). However, some debate remains as to both the uniqueness (Zhou et al., 2008) and the ubiquity (He et al., 2007, 2008) of PAOs. To investigate the relative microbial diversity in our BPR reactors, DNA was extracted from each bioreactor during steady-state operations, and PCR was amplified with broadly conserved 16S rDNA primers, then separated through DGGE (Figure 8). Each DGGE band (labeled alphabetically) within a lane (labeled numerically) represents a unique amplified DNA sequence present in the sample. Although the individual bands alone do not provide any phylogenetic information about the populations in the reactors, comparatively, this molecular technique can be used to broadly assess the potential similarity between treatment reactors. While DGGE is limited, in that it

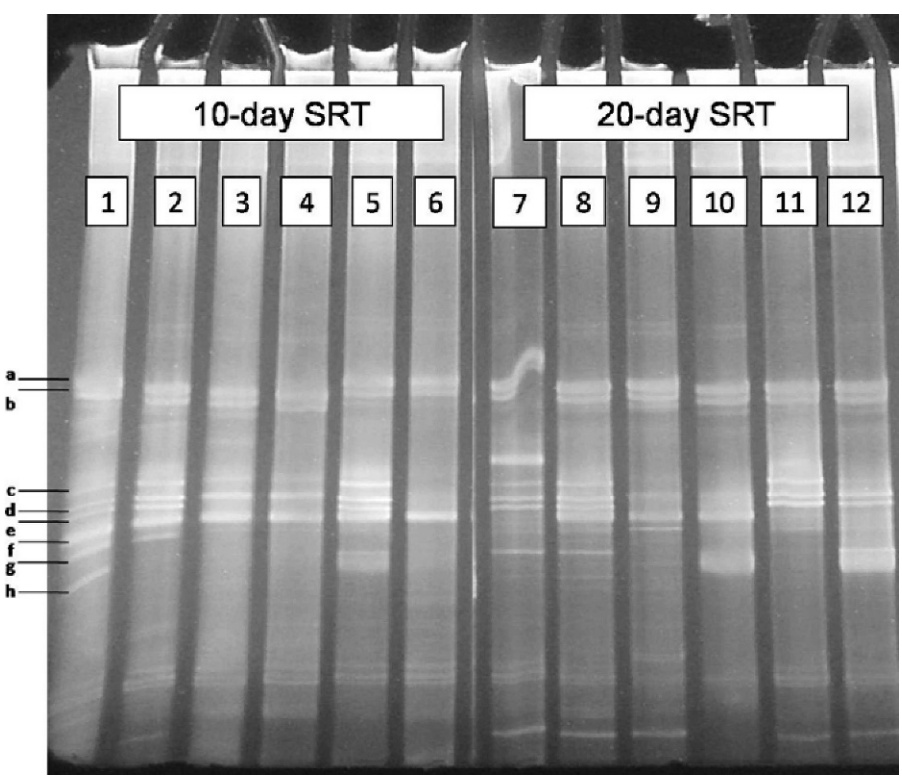

Figure 8—DGGE gels with DNA from each reactor at both SRTs. Lane designations are as follows: (1) NF.10.12.1.3, (2) NF.10.18.1.3, (3) F.10.12.1.3, (4) F.10.18.1.3, (5) F.10.18.1.5, (6) F.10.12.1.5, (7) NF.20.12.1.3, (8) NF.20.18.1.3, (9) F.20.12.1.3, (10) F.20.18.1.3, (11) F.20.18.1.5, and (12) F.20.12.1.5. Naming scheme: ''F'' or ''NF'' designation represents ''fermenter'' or ''non-fermenter'' (i.e., 90:10 feedstock versus 100% municipal raw); the first number is overall HRT (hours); and the last two numbers are anaerobic and aerobic cycle time, respectively (hours).

cannot directly be applied to identify dominant organisms in a given community, the procedure has been shown to be suitable for identification of important mixed-microbial consortia community members (de Araujo and Schneider, 2008).

Given the performance variability in the tested BPR reactors, the central goal of these molecular investigations was to determine if the respective populations exhibited certain similarities that would indicate if the process was more species specific or non-specific; to that end, several relevant observations can be drawn.

(1) A comparison of the 10-day SRT bioreactor populations shows significant similarity across all six systems. While bioreactor NF.10.12.1.3 exhibited the most diversity (lane 1), with the exception of bioreactor F.10.12.1.5 (lane 6), the six most dominant populations (bands a-f) also were observed in the other bioreactors.
(2) Also at the 10-day SRT, the microbial population in the bioreactor exhibiting the best BPR performance (F.10.18.1.5 [lane 5]) is highly similar to those consortia performing the worst BPR (NF bioreactors [lanes 1 and 2]). Similar observations can be made between the same three bioreactors at the 20-day SRT (lane 11 versus lanes 7 and 8).
(3) All dominant bands observed in the fermenter liquor-augmented bioreactors also were observed in the non-augmented bioreactors (for both the 10- and 20-day SRTs), indicating that the NF bioreactors indeed contained PAOs; however, given that the NF SBRs did not perform as efficient BPR, it is unclear if the microbes were either not present in sufficient quantity and/or the critical metabolisms were not sufficiently induced to achieve low effluent phosphorus values. This conclusion is supported, in part, by both Onuki et al. (2002) and Ren et al. (2007), who, after applying PCR/DGGE on BPR reactors, concluded that simply exposing microbes to repeated anaerobic/aerobic cycling ultimately eliminated microbes that were not capable of BPR.

These results further correlate well with those of Lopez-Vazquez et al. (2008), who proposed optimal ranges of VFA species (e.g., acetate-to-propionate) to select for PAOs; consistent with their findings, the operating conditions in all our bioreactors were such that PAOs should broadly predominate. Lee et al. (2007) additionally proposed that microorganisms capable of performing phosphorus removal are more predominant at longer SRTs. As a final comparison, although the BPR performance improved markedly for the NF bioreactors between the 10- and 20-day SRTs, the populations seemingly did not change drastically, and no new bands were observed that were otherwise unique only to the augmented bioreactors (which performed better BPR). Although PCR does bias toward more dominant populations in a sample, given that (1) PAOs have been found to be a significant fraction in activated sludge (up to 15% of the population [Gu et al., 2008]) and (2) PCR-DGGE does identify the relative breadth of all important community members, we believe the molecular results presented herein accurately define and capture the critical species performing BPR.

Conclusions

The main goal of the research presented and discussed herein was to develop a better understanding of the effects of WWTF operational factors on BPR within real wastewater environments. The key conclusions from this study are as follows:

- Longer SRTs can improve BPR performance in WWTFs that cannot augment with VFA-rich fermenter liquor;
- VFA augmentation can stabilize BPR at shorter SRTs;
- Higher VSS concentrations correlate well with improved phosphorus removal;
- VFAs may be most critical in driving process success associated with anaerobic PHA synthesis, which then leads to aerobic growth; and
- Given the population similarity across all bioreactors, BPR variability appears to be less influenced by the presence of specific microbes and more affected by the induction of critical metabolisms.

Credits

This material is based on work supported by the National Science Foundation (Arlington, Virginia) under grant number 0607329. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding agency.

Submitted for publication June 12, 2009; revised manuscript submitted July 27, 2009; accepted for publication August 3, 2009.

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