Laboratory Testing of Process Controls for the Mitigation of Toxic Shock Events at Enhanced Biological Phosphorus Removal Wastewater Treatment Plants

Jeremy S. Guest

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Dr. Nancy G. Love, Chair
Dr. John T. Novak
Dr. John C. Little

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Abstract

Toxic shock events can be detrimental to wastewater treatment systems and can result in long-term losses of system performance. If warned of an impending toxic shock, operators would have the opportunity to implement process controls that could help mitigate the effects of the shock event. The objective of this project was to evaluate the effectiveness of a developed corrective action strategy (involving aerobic endogenous respiration) on an enhanced biological phosphorus removal (EBPR) wastewater treatment plant (WWTP) shocked with chlorine. Three identical, laboratory-scale systems were designed to mimic one train of the Long Creek Water Resources Reclamation Facility (WRRF, Gastonia, NC). The basis of this study is a comparative performance analysis among the three trains; a negative control (unshocked and operated normally), a positive control (shocked with hypochlorite and operated normally), and the corrective action (shocked with hypochlorite and process controls implemented). Comparative performance analysis among the three trains was based on effluent quality, performance stability, and biomass kinetics as indicated by rates of respiration and phosphate release and uptake. The shock event and corrective action strategy both inhibited EBPR. After an initial perturbation, the positive control matched the performance of the negative control. The corrective action, however, exhibited significant instability in EBPR performance. Regardless of whether aerobic or anaerobic sludge storage conditions are selected, endogenous respiration will still result in system instability. It is recommended, therefore, that measures be taken to avoid imposing endogenous conditions on isolated sludge during a short-term toxic shock event.

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Attribution

Dr. Nancy G. Love, *Ph.D.*, Professor, Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Andrew Shaw, Process Specialist, Black and Veatch, Kansas City, MO 64114

Ameet J. Pinto, *M.S.*, Graduate Research Assistant, Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

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Chapter 1

Introduction

Toxic Shock Events and the Threat of "All Hazards"

Toxic shock events can be detrimental to wastewater treatment systems and can result in long-term losses of system performance. In a survey of 101 wastewater treatment plants (WWTPs) conducted by Love and Bott (Love and Bott, 2000), 64% of respondents had experienced an upset event resulting from an upstream chemical spill. The Water Environment Research Foundation (WERF), in partnership with the US Environmental Protection Agency (USEPA), has identified the need to prevent or mitigate upset events at municipal WWTPs. The focus of this project (WERF 04-CTS-11S) is the development of corrective action strategies for WWTP operators to implement during toxic shock events. Projects related to this study include the development of a contaminant prioritization framework (WERF 03-CTS-2S) and the development of a decision support system (DSS) to detect upset conditions (WERF 03-CTS-7S).

The original intent of these WERF projects was to protect WWTPs from direct and indirect terrorist attacks; "direct" meaning the introduction of a toxin directly to the collection system, and "indirect" meaning the use of disinfectants by emergency response personnel near the collection system following a biological attack. Recently, however, the USEPA has shifted its focus to the threat of "all hazards"; natural and manmade disasters, including terrorist attacks. This policy shift means that the likelihood of shock events resulting from hurricanes, earthquakes, and landslides must be considered. After consulting the contaminant prioritization framework currently under development (WERF 03-CTS-2S), calcium hypochlorite ($Ca(OCl)_2$) was identified as a significant threat to municipal WWTPs. Calcium hypochlorite is a commonly used disinfectant that is often stored in 1,000-10,000 gallon volumes. In the event of a natural

disaster, it is possible that tanks or valves could be damaged leading to the unintentional introduction of calcium hypochlorite to the sewer system. Therefore, for the purposes of this study, a 2,500 gallon spill of 6% calcium hypochlorite was simulated.

Corrective Action Strategies

The objective of corrective action strategies is to minimize the short- and long-term impacts of toxic shock events. The intuitive reaction from an operations perspective is to protect the biomass from the toxin to prevent long-term losses in system performance that could result in prolonged environmental pollution. However, if the contaminated influent does not come in contact with activated sludge, it will pass through the plant without biological treatment, resulting in effluent with concentrations of biochemical oxygen demand (BOD), ammonia (NH_3), and phosphorus that could lead to permit violations. Furthermore, the toxin will pass through the treatment plant to the effluent untreated, putting aquatic species in the receiving stream at risk. A certain level of treatment, therefore, must still be achieved to protect the environment from the discharge of untreated sewage and to comply with permit requirements.

Enhanced Biological Phosphorus Removal

Biological wastewater treatment processes can be sensitive to abrupt process changes. Enhanced biological phosphorus removal (EBPR), in particular, can become unstable if system parameters change quickly (Ahn et al., 2006). Polyphosphate accumulating organisms (PAOs), the bacteria responsible for EBPR, are able to produce and degrade numerous biopolymers including polyphosphate (polyP), glycogen, and polyhdroxyalkanoates (PHA) (Keasling et al., 2000, Mino et al., 1998). The production and utilization of these energy pools may provide cells with a means to balance intracellular energy supplies as PAOs are cycled between anaerobic, carbon-rich conditions and aerobic, carbon-limited conditions (Keasling et al., 2000). The ability to quickly uptake and store substrates and achieve this intracellular energy balance provides PAOs with a strong competitive advantage over other microorganisms incapable of this process (van Loosdrecht and Heijnen, 1997). Although the simplest way to protect biomass from an influent toxin may be to stop influent flow to a given train, it has been shown that prolonged endogenous respiration can cause significant shifts in the intracellular biopolymer pools of PAOs, resulting in a loss of EBPR stability (Lopez et al., 2006). It is crucial, therefore, that the set of implemented

process controls, designated the "corrective action strategy", be carefully designed and pre-tested to minimize the immediate and long-term effects on system performance.

Research Overview

With upset early warning devices currently under development (Henriques et al., in press-a; Love and Bott, 2000), WWTPs may be warned of an impending toxic shock by remote sensors in the collection system. With these remote sensors, operators would have the opportunity to implement process controls that could help mitigate the effects of the toxic shock event. The objective of this project was to evaluate the effectiveness of a developed corrective action strategy on a laboratory-scale 5-stage Bardenpho WWTP shocked with calcium hypochlorite. The results of this research have been submitted to *Water Practice* for review and have been accepted to the 2007 Water Environment Federation Technical Exhibition and Conference (WEFTEC). These results could eventually be integrated into a first generation decision support system (DSS) to assist WWTP operators during toxic shock events.

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Chapter 2

Literature Review

Enhanced Biological Phosphorus Removal and PAOs

The development of wastewater treatment plants (WWTPs) designed to achieve enhanced biological phosphorus removal (EBPR) began with the patenting of the Phostrip process by Levin in 1970 (Barnard, 2006). In 1974, Barnard observed significant biological phosphorus removal at a 4-stage nitrogen removal pilot plant in South Africa, and identified the need for a well defined anaerobic zone for optimum biological phosphorus removal performance (Barnard, 2006). These developments were milestones in our understanding of EBPR; a technology that has become widely utilized across the world (Barnard, 2006).

The EBPR process is commonly characterized by the exposure of biomass to influent under anaerobic conditions, followed by aerobic conditions (Barnard, 1975). There are many EBPR WWTP configurations in use today, the most common of which are Phoredox (Anaerobic/Oxic, A/O), Anaerobic/Anoxic/Oxic (A^2O), University of Capetown (UCT), Virginia Initiative Plant (VIP), 5-stage Bardenpho, Johannesburg Process, PhoStrip, and sequencing batch reactors (SBRs) whose cycle includes anaerobic and aerobic stages (Metcalf & Eddy Inc., 2003). Although each of these processes has a different configuration, they are all designed to cultivate poly-phosphate accumulating organisms (PAOs); the bacteria responsible for the "luxury uptake" of phosphorus that enable us to achieve biological phosphorus removal at WWTPs (Mino et al., 1998).

PAO Species of Significance

One defining characteristic of PAOs is their ability to uptake excess phosphorus in the form of inorganic phosphate (P_i) and store it within the cell as the biopolymer polyphosphate (polyP)

(Mino et al., 1998). For over 30 years, researchers have sought to identify and characterize the relevant PAOs in activated sludge (Fuhs and Chen, 1975). In addition to various classes of proteobacteria, gram positive PAOs have also identified (Table 2-1).

Table 2-1. Summary of identified PAO classifications and associated citations.

CLASSIFICATION	COMMONLY IDENTIFIED SPECIES OF BACTERIA	CITATIONS
alphaproteobacteria	-	(Kawaharasaki et al., 1999)
gammaproteobacteria	*Acinetobacteria* sp.	(Auling et al., 1991; Deinema et al., 1985; Fuhs and Chen, 1975; Streichan et al., 1990; Wentzel et al., 1988)
betaproteobacteria	*Rhodocyclus* sp. *Rhodocyclus*-like sp. *Accumulibacter phosphatis*	(Bond et al., 1995; Bond et al., 1999; Crocetti et al., 2000; He et al., 2006; Hesselmann et al., 1999; Kämpfer et al., 1996; Kong et al., 2005; Martin et al., 2006; McMahon et al., 2002a; McMahon et al., 2002b; Okunuki et al., 2002; Okunuki et al., 2004; Wagner et al., 1994; Zilles et al., 2002)
Gram + bacteria with a high G-C content	*Actinobacteria* sp.	(Bond et al., 1999; Christensson et al., 1998; Crocetti et al., 2000; Kämpfer et al., 1996; Kawaharasaki et al., 1999; Kong et al., 2005; Okunuki et al., 2004; Wagner et al., 1994)

As research into identifying PAOs began, studies had to rely on culture-dependent methods to identify the organisms responsible for EBPR (Auling et al., 1991; Deinema et al., 1985; Fuhs and Chen, 1975; Streichan et al., 1990). The use of culture-dependent methods, such as plating, led some researchers to believe that the bacterial genus *Acinetobacter* was the dominant PAO in activated sludge, and was principally responsible for EBPR (Auling et al., 1991; Deinema et al., 1985; Fuhs and Chen, 1975; Streichan et al., 1990; Wentzel et al., 1988). In recent years, some researchers have, in fact, shown certain *Acinetobacter* sp. to be capable of excess phosphate release and uptake (Boswell et al., 1998; Zafiri et al., 1999). When working with mixed or enriched cultures, however, culture dependent methods such as agar-plating with acetate may be biased towards *Acinetobacter* sp. (Christensson et al., 1998; Kämpfer et al., 1996; Wagner et al., 1994). In fact, Wagner and colleagues found that plating EBPR sludges not only leads to

overestimations of *Acinetobacter* content, but also causes inhibition of β-proteobacteria and the gram positive bacteria that may have been principally responsible for the EBPR capabilities of their sludge (Wagner et al., 1994). Recently developed molecular techniques have allowed researchers to identify PAO species without culturing, resulting in the position that the role of *Acinetobacter* sp. in EBPR has been drastically overestimated (Bond et al., 1995; Christensson et al., 1998; Hiraishi et al., 1989; Kämpfer et al., 1996; Van Niel et al., 1998; Wagner et al., 1994).

In particular, some researchers have suggested *Actinobacteria* and the β-2 subclass of the class *Proteobacteria* are particularly important for efficient P-removal (Bond et al., 1999; Crocetti et al., 2000). Species that are commonly identified as PAOs include *Rhodocyclus* (Bond et al., 1995; Crocetti et al., 2000; Kong et al., 2005; Zilles et al., 2002), *Rhodocyclus*-like bacteria (McMahon et al., 2002a; Zilles et al., 2002), *Actinobacteria* (Bond et al., 1999; Crocetti et al., 2000; Kong et al., 2005; Okunuki et al., 2004), and *Accumulibacter* (He et al., 2006; Martin et al., 2006). In acetate-fed systems, recent culture independent studies have provided evidence that *Rhodocyclus*-related species (e.g., *Accumulibacter phosphatis*) may be more significant to EBPR (Bond et al., 1995; Bond et al., 1999; Crocetti et al., 2000; Hesselmann et al., 1999; Martin et al., 2006; Okunuki et al., 2004). It is important to remember, however, that the population structure of each activated sludge community will be unique, and authors should be cautious in discounting organisms simply because they were not observed in a particular EBPR system. Recent developments in the field of microbial ecology have led to discussion over the possibility of random prokaryotic community assembly (Sloan et al., 2006). Evidence from this research suggests that possessing a simple kinetic advantage will not guarantee complete dominance by a particular organism, and will likely not lead to the washout of similar organisms with slightly slower specific growth rates (Sloan et al., 2006).

Although identifying species of bacteria in diverse cultures can be expensive and time-consuming, we must develop our understanding of PAO community structures. It has been previously shown that diverse microbial communities can be far more stable under fluctuating environmental conditions (Miura et al., 2007; Naeem and Li, 1997; von Canstein et al., 2002), but little is known about PAO community structures and how diversity relates to EBPR stability and resistance to perturbations.

Although pure cultures are commonly used to study bacteria (Madigan et al., 2003), researchers have had a difficult time developing axenic cultures of relevant PAO species (Martin et al., 2006; Mino et al., 1998). As a result, PAO studies typically utilize *enriched cultures* of PAOs (Martin et al., 2006; McMahon et al., 2002a; Mino et al., 1998). In the past decade, pure cultures of *Acinetobacter* have been isolated and grown (Boswell et al., 1998; Van Niel et al., 1998) and have become commercially available (ATCC, 2007), but the validity of using this organism as a model for PAOs is still contested (Martin et al., 2006).

PAO Metabolism

PAOs are able to produce and degrade numerous biopolymers including polyP, glycogen, and polyhdroxyalkanoates (PHA) (Keasling et al., 2000; Mino et al., 1998). The production and utilization of these energy pools may provide cells with a means to balance intracellular energy supplies as PAOs are cycled between anaerobic, carbon-rich conditions and aerobic, carbon-limited conditions (Keasling et al., 2000). The ability to quickly uptake and store substrates and achieve this intracellular energy balance provides PAOs with a strong competitive advantage over other microorganisms incapable of this process (van Loosdrecht and Heijnen, 1997). The ability to utilize various biopolymers may also provide PAOs with a unique advantage during shock events with certain toxins (e.g., heavy metals) (Keasling 2000). Although the advantage of these biopolymers is apparent, PAOs rely heavily on a balance among these intracellular energy sources. If an EBPR WWTP were exposed to a toxin that could inhibit the production or consumption of only one of the biopolymers in PAOs, it is likely that phosphorus-removal performance would be lost.

In addition to polyP production, PAOs are often characterized by their unique ability to anaerobically uptake organic substrates without the consumption of an electron acceptor (Mino et al., 1998). Under anaerobic conditions, PAOs solubilize polyP chains and degrade glycogen to produce the energy and reducing power required to uptake organic substrates (Keasling et al., 2000; Mino et al., 1998). Once the organic substrate is inside the cell, the energy from the degradation of polyP and glycogen is also used for the storage of the substrate as polyhydroxyalkanoates (PHAs) (Satoh et al., 1992). Under aerobic conditions, PAOs utilize the PHA produced under anaerobic conditions to produce the energy and reducing power required for polyP synthesis, glycogen synthesis, cell growth and maintenance (Filipe and Daigger, 1998).

Table 2-2. Summary of energy demand and energy sources for PAOs cycling between anaerobic and aerobic conditions (adapted from Mino et al. 1998). DPAOs will have the same energy demands and sources under anoxic conditions as PAOs have under aerobic conditions (Kuba et al., 1996).

PHASE	ENERGY DEMAND	ENERGY SOURCES
Anaerobic	Uptake of organic substrate Conversion of organic substrate to PHA Endogenous respiration/Maintenance	Degradation of polyP Degradation of glycogen
Aerobic	polyP synthesis Glycogen synthesis Cell synthesis Maintenance (Filipe and Daigger, 1998)	Degradation of PHA

As researchers continued to investigate the optimization of EBPR, they discovered that PAOs appear to prefer short chain fatty acids such as acetate and propionate as their carbon source (Satoh et al., 1992; Wentzel et al., 1985). Since that time, enriched PAO cultures have been shown to anaerobically utilize a variety of organic substrates including carboxylic acids, sugars, and amino acids (Mino et al., 1998). The most common organic substrate used in enrichment and pure culture studies, however, is acetate (Bond et al., 1995; Bond et al., 1999; Crocetti et al., 2000; Martin et al., 2006; Okunuki et al., 2004). The uptake of acetate has been shown to lead to the formation of polyhydroxybutyrate (PHB) as the principle PHA (Satoh et al., 1992; Smolders et al., 1994b), and PHB is believed to be the most efficient form of PHA for EBPR (in terms of P·moles taken up per C·moles consumed under aerobic conditions) (Lopez et al., 2006; Pijuan et al., 2004; Randall and Liu, 2002).

Although it has been shown that some species of PAO are capable of utilizing nitrate as their electron acceptor (Chuang et al., 1996; Kerrn-Jespersen and Henze, 1993; Kuba et al., 1993; Kuba et al., 1997; Mino et al., 1998), it has also been proven that not all species of PAO possess the necessary genes (e.g., *nir* genes encoding for nitrite reductase) to achieve denitrification (Martin et al., 2006). Under anoxic conditions, the metabolism of denitrifying PAOs (DPAOs) is the same as aerobic metabolism, except with nitrate as the electron acceptor in place of oxygen (Kuba et al., 1996). The energy production efficiency of DPAO metabolism under anoxic conditions is estimated to be only 60% of aerobic metabolism (Kuba et al., 1994), which may result in a 20% lower yield for anaerobic-anoxic EBPR systems (in comparison with anaerobic-aerobic systems) [Mino et al. (1998) calculated from Murnleitner et al. (1997)].

The anaerobic process of degrading polyP chains is performed by polyphosphatase (Akiyama et al., 1993). The enzyme exopolyphosphatase (PPX), encoded by a gene in the same operon as *ppk* (the gene encoding for polyphosphate kinase, PPK), is capable of cutting off the terminal P_i of a polyP chain (Akiyama et al., 1993). The solubilization of polyP chains may also be achieved by guanosine pentaphosphate phosphohydrolase (GPP), a second polyPase identified in *E. coli* (Keasling et al., 1993).

PolyP degradation leads to the production of ATP (Filipe and Daigger, 1998; Mino et al., 1998). High ATP concentrations have been shown to stimulate *in vitro* synthesis of polyP PPK, while high ADP concentrations have been shown to inhibit polyP synthesis (Ahn and Kornberg, 1990). This correlation further suggests that the production and degradation of biopolymers is a method to balance intracellular energy supplies (Keasling et al., 2000). Poly-P AMP-phosphotransferase has been shown to be significant to EBPR, catalyzing the reaction of $(P_i)_n + AMP \rightarrow (P_i)_{n-1} +$ ADP (van Groenestijn et al., 1989). Adenylate kinase is also believed to be involved in the process of ATP synthesis during polyP degradation (van Groenestijn et al., 1989), but the role this enzyme plays is still not fully understood (Seviour et al., 2003).

Other critical genes in PAOs likely include *phaABC* and *phaZ*, responsible for PHA synthesis and degradation, respectively (Martin et al., 2006). A recent study also identified a potential novel cytochrome in *A. phosphatis* that could allow for the full anaerobic use of the TCA cycle (Martin et al., 2006). It is also important to note that nutrients such as Mg^{2+} (Ahn and Kornberg, 1990; Akiyama et al., 1993) and K^+ (Akiyama et al., 1993) have been shown to be particularly important to the metabolism of PAOs (Martin et al., 2006).

One of the most significant leaps forward in our understanding of PAOs was the sequencing of the genome of *Candidatus* Accumulibacter phosphatis (Martin et al., 2006). By sequencing the genome, Martin and coauthors were able to infer the metabolic pathways of *A. phosphatis* under both aerobic and anaerobic conditions (Martin et al., 2006). It is important to note, however, that the same metabolic pathways may not be common to all PAOs. Glycogen degradation in *A. phosphatis*, for example, is likely achieved via the Embden-Meyerhof-Parnas (EMP) pathway, as all of the EMP pathway genes are present in the sequenced genome (Martin et al., 2006). Other researchers, however, have seen evidence that would suggest that PAOs achieve glycogen

degradation via the Entner-Doudoroff (ED) pathway (Baetens, 2001; Hesselmann et al., 2000; Juni, 1978; Maurer et al., 1997; Wentzel et al., 1991). A compilation of proposed aerobic and anaerobic metabolic pathways in PAOs can be seen in Figures 2-1 and 2-2.

Over the last two decades, several activated sludge models that include EBPR were developed and have become well recognized. These models included the Comeau/Wentzel model (Comeau et al., 1986; Wentzel et al., 1986), ASM No. 2 (Gujer et al., 1995; Henze et al., 1995), Mino 1987 (Mino et al., 1987), adapted-Mino (Wentzel et al., 1991), and Smolders' (Smolders et al., 1994b, 1994c, 1995b; Smolders et al., 1995a). The major differences in these models have been discussed by others (Filipe and Daigger, 1998; Lopez et al., 2006; Mino et al., 1998; Wentzel et al., 1991), and are outlined in Table 2-3. These metabolic models are based on empirical data from various continuous flow (Mino et al., 1987; Wentzel et al., 1989) and SBR systems (Smolders et al., 1995b).

Table 2-3. Summary of major differences among metabolic models for PAOs. "NA" represents items that were not explicitly addressed in the cited works.

MODEL	SOURCE OF REDUCING POWER FOR PHA SYNTHESIS	CHARACTERIZATION OF ENDOGENOUS PROCESSES	METABOLIC PATHWAY FOR GLYCOGEN DEGRADATION
Comeau/Wentzel	Acetate degradation via anaerobic tricarboxylic acid (TCA) cycle (Wentzel et al., 1991)	Decay, endogenous respiration, and maintenance (Wentzel et al., 1989)	NA – Glucose degradation performed by non-PAO heterotrophs (Wentzel et al., 1985)
Mino 1987	Glycogen degradation (Mino et al., 1987)	NA	Embden-Meyerhof-Parnas (Mino et al., 1987)
adapted-Mino	Glycogen degradation (Wentzel et al., 1991)	NA	Entner-Doudoroff (Wentzel et al., 1991)
Smolders	Glycogen degradation (Smolders et al., 1994b)	Maintenance (Smolders et al., 1995b)	Embden-Meyerhof-Parnas (Smolders et al., 1994b, 1994c)
ASM No. 2	NA	Decay (Gujer et al., 1995; Henze et al., 1995)	NA

As previously discussed, researchers have suggested that possessing a simple kinetic advantage will not guarantee complete dominance by a particular organism, and will likely not lead to the washout of similar organisms with slightly slower specific growth rates (Sloan et al., 2006). Therefore, irrespective of feed and operating conditions, the ultimate community structure of a reactor system will be determined by a combination of evolution and chance. This should be an indication to researchers that no one PAO would be optimal for all EBPR WWTPs, and the community structure of a given activated sludge must be taken into consideration when applying metabolic models to predict system performance.

Additionally, it is unclear how specific species of PAOs would be affected by toxic shock events. Heavy metals have been found in polyP granules in certain bacteria (Scott and Palmer, 1990), leading some researchers to suggest that the ability to synthesize polyP granules may provide PAOs with a unique advantage when exposed to heavy metals (Keasling et al., 2000). It has also been proposed that polyP detoxifies heavy metals once they enter the cell (Rachlin et al., 1982) or that PAOs may have more complex defense mechanisms such as the ability to utilize polyP for growth (in place of PHA) in the presence of heavy metals (Aiking et al., 1984; Keasling et

al., 2000). Although many laboratory- and full-scale nutrient limitation studies have been performed, few studies have looked at chemical stressors and their impact on PAOs. To be prepared for toxic shock events at EBPR WWTPs, there exists a need for process- and molecular-level research to better understand the impact of chemical stressors on PAOs. The research discussed in Chapters 3-6 of this document sought to address the need for research on the process-level effects of toxic shock events at EBPR WWTPs.

Factors Affecting EBPR Performance

There are many factors that can affect the performance of an EBPR system. One parameter that can significantly affect EBPR is the pH in each zone (Filipe et al., 2001a). An aerobic pH of 6.5, for example, has been shown to decrease the phosphate uptake rate of PAOs by 37% and 42% as compared to the rates at 7.0 and 7.5, respectively (Filipe et al., 2001a). While PAOs appear to be less sensitive to pH shifts from 6.5-8.0 under anaerobic conditions (Filipe et al., 2001d; Liu et al., 1996), lower pH values in the anaerobic zone have also been shown to decrease PAO efficiency (defined as P·moles release per C·moles uptake) (Liu et al., 1996; Smolders et al., 1994a; Smolders et al., 1994b). The proposed mechanism for this decrease in PAO performance is the increased energy requirement for acetate transport across the cell membrane at lower pH levels (Liu et al., 1996; Smolders et al., 1994a; Smolders et al., 1994b).

In addition to pH, the dissolved oxygen concentration in the aerobic zone has been shown to significantly affect EBPR performance (Narayanan et al., 2006). Pilot testing by Narayanan and colleagues suggests that the dissolved oxygen concentration at the start of the aerobic phase should be at least 1.5 mg·O_2/L to prevent inhibition of PAOs (Narayanan et al., 2006). Prior to aeration, it is also important that a significant reduction in soluble organics be achieved under anaerobic or anoxic conditions. The presence of organic substrates in the aerobic phase has been shown to create a competitive disadvantage for PAOs by promoting the growth of aerobic bacteria such as *Pseudomonas* sp. (Wentzel et al., 1988).

During the anaerobic phase of EBPR, exposure of PAOs to nitrate has been shown to inhibit the release of phosphate (Chuang et al., 1996; Kuba et al., 1994; Mino et al., 1998; Van Niel et al., 1998). This inhibition may be the result of competition between PAOs and denitrifiers, or the result of less efficient DPAO metabolism.

The EBPR process often relies on consistent organic loading. Smaller WWTPs that experience low organic carbon loadings on the weekends are susceptible to poor EBPR performance on Mondays and Tuesdays (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996). It is believed that this effect is the result of either reduced pools of storage products (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996) or the reduced pools of storage products and the decay of PAOs (Brdjanovic et al., 1998a; Miyake and Morgenroth, 2005). Decreasing aeration during weekends could potentially help mitigate the effect of lower organic carbon loading by helping conserve the internal energy pools of PAOs (Brdjanovic et al., 1998a; Temmink et al., 1996) and reducing their decay rates (Siegrist et al., 1999). Decreased carbon loadings may also result in incomplete denitrification, leaving elevated nitrate concentrations in the anaerobic zone (resulting in anoxic conditions). Increased anoxic volumes (in place of anaerobic) may also increase the decay rates of PAOs (Siegrist et al., 1999).

Like PAOs, glycogen accumulating organisms (GAOs) uptake VFAs under anaerobic conditions. GAOs, however, do not achieve luxury uptake of phosphorus and are, therefore, not beneficial to the EBPR process. GAOs use stored glycogen for energy and reducing power for VFA uptake and conversion to PHA. Because they cannot utilize polyP as an energy source, GAOs consume roughly twice as much glycogen during acetate uptake as PAOs (Filipe et al., 2001b, 2001d). In the aerobic zone, however, PAOs must uptake phosphorus to replenish the polyP supply. This uptake of inorganic phosphate is an energy expenditure that GAOs do not have to make (Filipe et al., 2001a). As a result, despite the utilization of less glycogen by PAOs, the energetic cost of cycling of phosphate into and out of the cell results in similar yield rates (per carbon taken in) between PAOs and GAOs (Filipe et al., 2001a).

GAOs have been shown to have a greater rate of PHA degradation than PAOs (Filipe et al., 2001a). Brdjanovic and colleagues showed that the minimum solids retention time (SRT_{min}) of a EBPR system was dependent on the rate of PHA degradation, where the minimum aerobic SRT ($SRT_{min,aer}$) was defined as the minimum time required to aerobically consume the anaerobically stored PHA (Brdjanovic et al., 1998b). Subsequently, Filipe and Daigger proposed that GAOs will always have a smaller SRT_{min} than that of PAOs, and any SRT that allows for PAO cultivation will allow for GAO cultivation (Filipe et al., 2001a).

Elevated pH levels (7.0 to 8.0) under anaerobic conditions inhibit the uptake of acetate by GAOs (Filipe and Daigger, 1999; Filipe et al., 2001b, 2001d), while PAOs have been shown to have a relatively constant rate of acetate uptake if pH values are between 6.5 and 8.0 (Liu et al., 1996; Filipe et al., 2001d). An aerobic pH of 6.5, however, has been shown to inhibit PAO activity without negatively affecting GAO activity (Filipe et al., 2001a). Filipe and colleagues come to the conclusion that anaerobic pH values greater than 7.25 will give a competitive advantage to PAOs, and aerobic pH values greater than 7.0 will prevent inhibition of both PAOs and GAOs (Filipe et al., 2001a, 2001c). In fact, elevated pH values (7.5 – 8.0) in both zones may enhance the performance of PAOs (Filipe et al., 2001a, 2001c).

In addition to these factors, a number of PAO stressors have been identified and are discussed in the following section.

Table 2-4. Summary of terms from Figures 2-1 and 2-2.

PHV	polyhydroxyvalerate
PH2MB	polyhydroxy-2-methylbutyrate
PHB	polyhydroxybutyrate
PH2MV	polyhydroxy-2-methylvalerate
NAD^+/NADH	nicotinamide adenine dinucleotide (oxidized/reduced)
$NADP^+$/NADPH	nicotinamide adenine dinucleotide phosphate (oxidized/reduced)
ATP	adenosine triphosphate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
PP_i	poly-phosphate
P_i	orthophosphate
Fd_{red}	ferredoxin – reduced
Fd_{ox}	ferredoxin – oxidized
Q	oxidized quinone
QH_2	reduced quinone

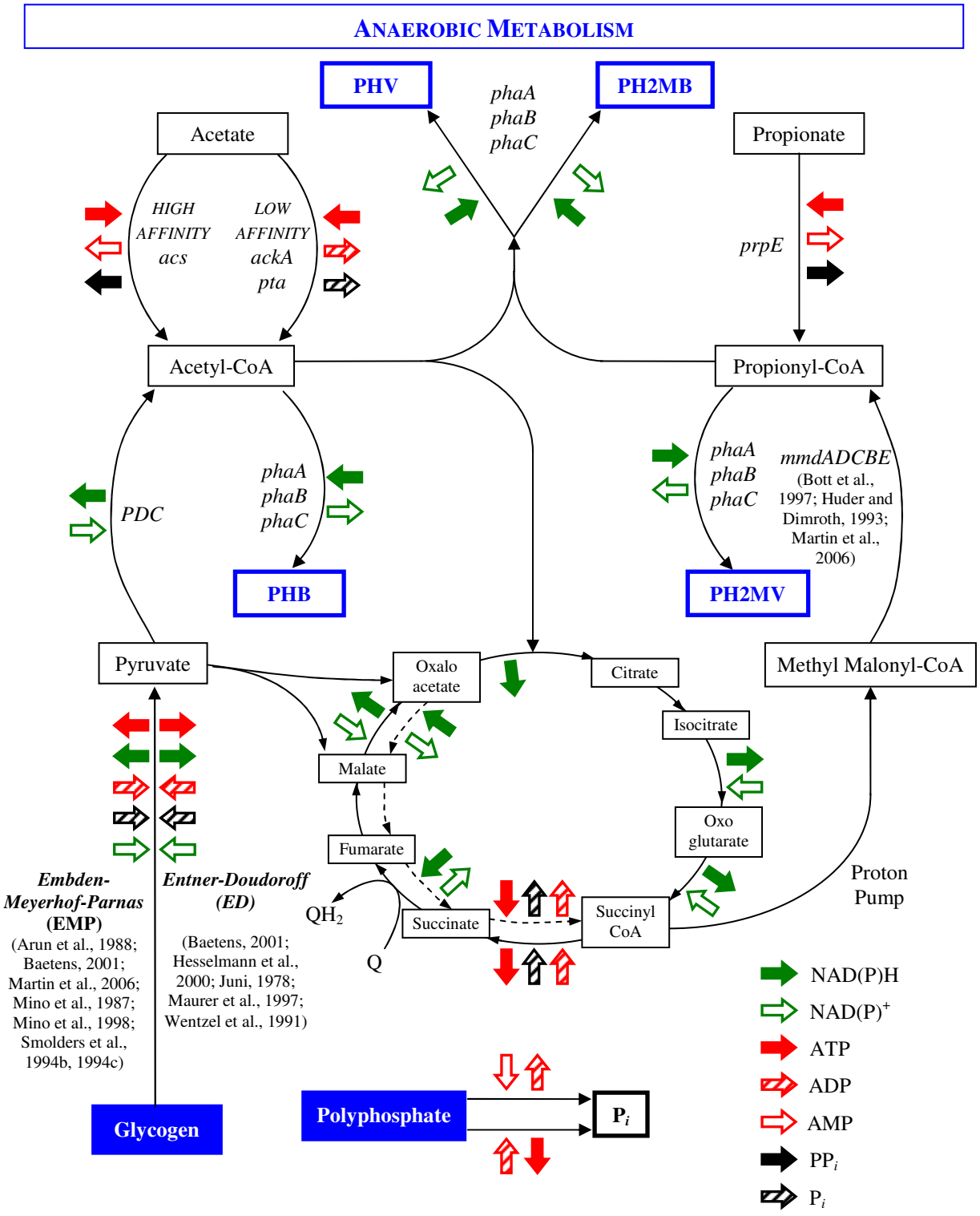

Figure 2-1. Qualitative model for the anaerobic metabolism of PAOs. Green, red, and black arrows represent reducing power, energy, and the biopolymer polyP, respectively. Closed arrows represent molecules that are reduced or energy-rich, while open arrows represent oxidized or energy-depleted molecules. Arrows pointed towards a given pathway are reactants, while arrows pointed away from the pathway are products. This figure was adapted from Martin et al. (2006) unless otherwise noted.

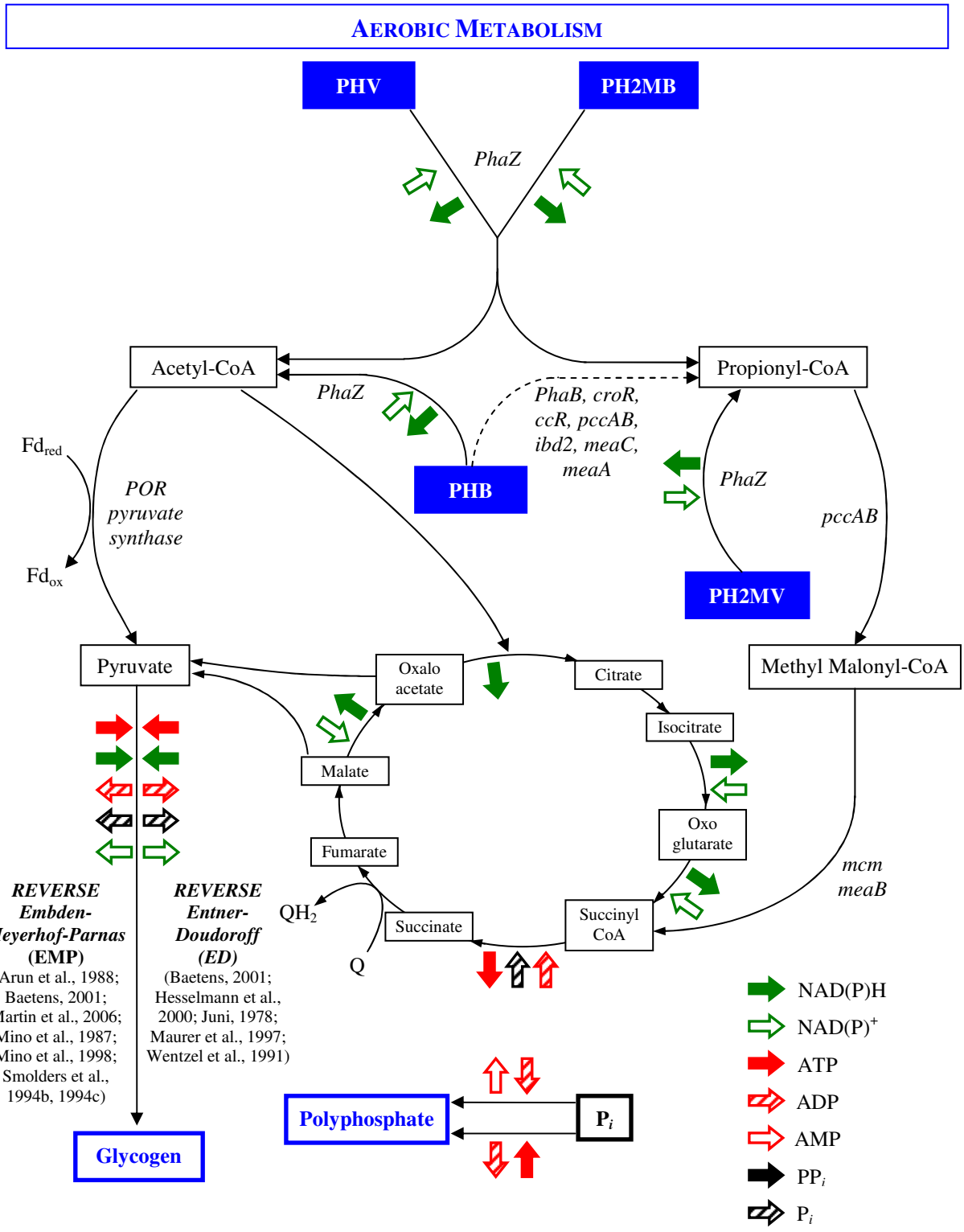

Figure 2-2. Qualitative model for the aerobic metabolism of PAOs. Green, red, and black arrows represent reducing power, energy, and the biopolymer polyP, respectively. Closed arrows represent molecules that are reduced or energy-rich, while open arrows represent oxidized or energy-depleted molecules. Arrows pointed towards a given pathway are reactants, while arrows pointed away from the pathway are products. This figure was adapted from Martin et al. (2006) unless otherwise noted.

PAO Stress and Stress Response

As with most relevant bacteria in wastewater treatment processes, a number of stressors have been identified for PAOs. PAO stressors may result in a mechanistic inhibition, a competitive disadvantage, or cell deterioration. Stressors that have been identified include NO_x species (Van Niel et al., 1998), heavy metals (Hao and Chang, 1988), and endogenous respiration (Lopez et al., 2006).

NO_x Species

Studies have shown that the presence of NO_x species in EBPR sludge can be detrimental to PAO performance (Meinhold et al., 1999; Van Niel et al., 1998; Weon et al., 2002). NO_x species include: nitrate, NO_3^- (the end point of nitrification); nitrite, NO_2^- (an intermediate product of nitrification and denitrification); nitric oxide, NO (an intermediate gas formed during denitrification); and nitrous oxide, N_2O (an intermediate gas formed during denitrification).

Under anaerobic conditions, nitrate is commonly thought to result in a competitive disadvantage to PAOs for one of two reasons; (i) competition between PAOs and denitrifiers for the same carbon source and/or (ii) the presence of denitrifying PAOs capable of anoxic phosphate uptake (Egli and Zehnder, 1994; Kerrn-Jespersen and Henze, 1993; Kuba et al., 1994). To further investigate the deterioration of the anaerobic performance of PAOs, Van Niel and colleagues performed enzyme assays to determine if NO_x species would be inhibitory to specific enzymes of interest (e.g., enzymes involved in polyphosphate degradation) (Van Niel et al., 1998). This study showed that aqueous NO was detrimental to adenylate kinase activity, while nitrate and nitrite had no apparent effect (Van Niel et al., 1998). This supports the findings of Appeldoorn (Appeldoorn, 1993) who observed that nitrate interference with anaerobic phosphate release was removed when denitrification was inhibited by cyanide and azide. NO has also been shown to inhibit the uptake of acetate, the formation of PHB, and the consumption of glycogen (Van Niel et al., 1998).

Under aerobic or anoxic conditions, nitrite is believed to be particularly inhibitory to the EBPR process (Meinhold et al., 1999; Weon et al., 2002). The inhibitory effect of nitrite on many species of bacteria has been shown to be strongly related to the solution pH, suggesting that

inhibition results from the protonated species, HNO_2, which can cross the cell membrane (Almeida et al., 1995; Parsonage, 1985). It has been proposed that the inhibition of PAOs results from the decrease in intracellular pH and proton motive force once nitrous acid enters the cell membrane (Weon et al., 2002). This decrease in the proton motive force would lessen the cell's ability to produce ATP, and would likely lead to a decrease in the energy state of the cell (Weon et al., 2002). As it has been previously shown that the synthesis of poly-P is dependent upon the energy state of the cell (Deinema et al., 1985; Fuhs and Chen, 1975), this shift in energy state could reduce the synthesis of poly-P and uptake of phosphate (Weon et al., 2002). This mechanism for inhibition is speculated, however, as the impact of nitrite on specific metabolic pathways was not investigated (Meinhold et al., 1999; Weon et al., 2002).

Metal Toxicity

Studies have shown that PAOs may have an advantage over other bacteria during exposure to heavy metals. PolyP is typically associated with positively charged, low-molecular weight compounds (e.g., amino acids, K^+, Mg^{2+}, Ca^{2+}) (Keasling et al., 2000). Despite this fact, heavier metals have been found in polyP granules in certain organisms (Crang and Jenson, 1975; Pettersson et al., 1985; Scott and Palmer, 1990). Surface-associated polyP may also be important to chelation of cations on the cell surface, which could result in the precipitation of heavy metals before entering the cell because of the highly insoluble nature of metal-phosphates (Keasling et al., 2000). It has also been proposed that polyP detoxifies heavy metals once they enter the cell, or that PAOs may have the ability to utilize polyP (in place of PHA) for growth in the presence of heavy metals (Keasling et al., 2000). Keasling and Hupf (1996) showed that increasing doses of cadmium had less of an effect on the growth rate of *E. coli* cells if they had the ability to synthesize and degrade polyP, further suggesting that polyP plays a significant role in heavy metal resistance among PAOs.

Additional studies have been performed to investigate the potential use of PAOs for remediation of wastewaters contaminated with copper (Cu^{2+}), cadmium (Cd^{2+}), nickel (Ni^{2+}), and uranyl (UO_2^{2+}) (Boswell et al., 1998). Exposure of a pure culture of *Acinetobacter johnsonii* to 0.1 mM of Cd^{2+}, Cu^{2+}, or Ni^{2+} resulted in the apparent abolition of protein synthesis (Boswell et al., 1998). Boswell et al. (1998) investigated the mechanisms for inhibition resulting from these three metals using electron microscopy and flow cytometry after incubation with propidium

iodide and bis-oxonol. Propidium iodide, used as the "dead" stain in live/dead staining, enters cells with compromised membranes and binds to nucleic acids. Bis-oxonol is lipophilic and anionic, and accumulates intracellularly when the cytoplasmic membrane is depolarized (Nebe-Von Caron and Badley, 1995). Exposure of *Acinetobacter johnsonii* to 0.1 mM of Cu^{2+} or Ni^{2+} resulted in significant loss of membrane integrity, indicated by the presence of both dyes in the majority of cells (Boswell et al., 1998). Ultimately, this concentration of copper and nickel left few cells intact. Exposure to 0.1 mM of Cd^{2+} resulted in only a small percentage of cells with depolarized cytoplasmic membranes, and 0.1 mM of UO_2^{2+} had little effect on membrane integrity. The apparent toxicity of these metals is as follows: $Ni^{2+} > Cu^{2+} > Cd^{2+} > UO_2^{2+}$ (Boswell et al., 1998).

Inorganic forms of other heavy metals (e.g., zinc, lead) have also been shown to inhibit EBPR at concentrations that do not appear to impact nitrification or organic carbon removal (Hao and Chang, 1988). Additionally, tin concentrations as low as 4 μg/L at a municipal WWTP have been correlated with elevated effluent phosphate concentrations without noticeable effects on other processes (Rayne et al., 2005).

Phosphate Limitation

The genes encoding for PPX and PPK are in the same operon. Studies suggest that the production of these enzymes is linked (Akiyama et al., 1993), and that P_i starvation would induce both PPX and PPK production (Keasling et al., 2000). This upregulation results in additional degradation of polyP by PPX, which increases the concentration of P_i. This increase in concentration is then sensed by the external P_i sensor, which represses the phosphate-starvation response (Van Dien et al., 1997). When P_i becomes available later, the cell is ready to produce and degrade polyP (Keasling et al., 2000). It has been speculated that this regulatory feature may be similar for other nutritional stresses in PAOs (e.g., carbon starvation to surplus, oxygen starvation to surplus) (Keasling et al., 2000).

Endogenous Respiration

Cellular metabolism under starvation conditions may differ significantly from metabolism under nutrient-rich conditions (Lopez et al., 2006): differences in metabolism may result from

decreased energy requirements under starvation conditions (Harder, 1997) or a decrease in the cell lysis rate (Lavallee et al., 2002).

EBPR performance relies not only on the number of active PAOs, but also the relative concentrations of storage compounds (PHA, glycogen, poly-P) (Lopez et al., 2006). One cause of EBPR instability is low organic carbon loading, which can result in a loss of EBPR performance even days after normal carbon loading resumes (Brdjanovic et al., 1998a; Carucci et al., 1999; Rieger et al., 2001; Temmink et al., 1996). It has been suggested that this effect is the result of either reduced pools of storage products (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996) or the reduced pools of storage products and the decay of PAOs (Brdjanovic et al., 1998a; Miyake and Morgenroth, 2005).

It has been well documented that under endogenous conditions, the decay of bacteria is faster in the presence of oxygen (Brdjanovic et al., 1998a; Lopez et al., 2006; Siegrist et al., 1999; Temmink et al., 1996). Under anaerobic conditions, decay processes do not appear to be nearly as significant; instead, the utilization of specific pools of cellular storage compounds may be accelerated (Lopez et al., 2006). These energy pools are designed to be dynamic (van Loosdrecht and Heijnen, 1997), but even short-term (< 1 day) starvation can lead to imbalances that are detrimental to PAO performance (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996).

The remainder of this section highlights the results of a study of PAO starvation performed by Lopez and colleagues, in which they monitored relevant biopolymers over the course of 3-4 weeks of sludge storage (Lopez et al., 2006). The authors were able to identify potential causes of reduction in phosphorus removal performance resulting from short- (Table 2-5) and long-term (Table 2-7) periods of starvation under anaerobic and aerobic conditions.

Within the first day of anaerobic storage Lopez and colleagues observed considerable reduction of P-release and uptake rates, significant glycogen consumption, and complete polyP degradation (Table 2-5). Additionally, a shift in the ratio of PHB to polyhydroxyvalerate (PHV) was observed (switching from PHB-rich to PHV-rich): this shift is significant because PHB is believed to be a more efficient source of energy and reducing power during substrate uptake (Lopez et al., 2006; Pijuan et al., 2004; Randall and Liu, 2002).

After one day of aerobic storage, the rates of P-release and uptake were impacted much less severely and the only biopolymer consumed to a greater extent than under anaerobic storage was PHA (which was depleted within the first 4-5 hours). After PHA pools had been depleted, PAOs began to utilize glycogen and polyP. The degradation of glycogen appeared to stop some time between 1 and 7 days of starvation, leaving cells with a minimum glycogen content roughly 4-times larger than the long-term anaerobic minimum. Also within the first day of aerobic starvation, the utilization of polyP began; this process was slow and appeared to only begin after PHA depletion.

Table 2-5. Summary of the effects of 1 day of endogenous respiration on an enriched culture of PAOs (adapted from Lopez et al. 2006). Within each column the more detrimental storage method is shaded.

STORAGE CONDITIONS	P-RELEASE AND UPTAKE RATES	PHA	PHB/PHV	GLYCOGEN	POLYP
Anaerobic	~70% reduction	increased content	from 91% PHB to >75% PHV	reached 2 week aerobic minimum	complete degradation
Aerobic	5-30% reduction	depletion after ~ 4 hours	no change	slight decrease	degradation began after ~ 0.7 days

Cellular decay and changes in membrane integrity were not observed during the first day of storage. However, during long-term (3-4 weeks) storage anaerobic starvation maintained a higher percentage of cells with membrane integrity than aerobic starvation (based on fluorescence microscopy with propidium iodide and SYTO 9) (Table 2-6). Additionally, anaerobic storage resulted in less decay (based on MLVSS measurements) (Table 2-6) and an increase in cellular PHA content.

Table 2-6. Effects of 21 days of endogenous respiration on the MLVSS and membrane integrity of an enriched culture of PAOs (adapted from Lopez et al. 2006). Within each column the more detrimental storage method is shaded.

STORAGE CONDITIONS	MLVSS	MEMBRANE INTEGRITY (QUALITATIVE
Anaerobic	No change	Slight decrease in % of cells with membrane integrity
Aerobic	Gradual decrease to 29% of original concentration	Significant decrease in % of cells with membrane integrity

During anaerobic storage there was little change in the biopolymer content of cells or the rates of P-release and uptake after the first day. One proposed explanation for the relatively constant level of PAO activity during anaerobic starvation is that the maintenance energy demand significantly decreased in the absence of energy sources (Harder, 1997). Under aerobic storage conditions, however, biopolymers were utilized for the duration of the study and P-release and uptake rates continued to deteriorate. After the depletion of PHA pools, glycogen and polyP were degraded for the duration of the study to achieve aerobic endogenous respiration.

Table 2-7. Summary of the effects of 21 days of endogenous respiration on an enriched culture of PAOs (adapted from Lopez et al. 2006). Within each column the more detrimental storage method is shaded.

STORAGE CONDITIONS	P-RELEASE AND UPTAKE RATES	PHA	PHB/PHV	GLYCOGEN	POLYP
Anaerobic	~70% reduction	Increased content	From 91% PHB to >70% PHV	Depleted	Complete degradation
Aerobic	First order decay to ~100% reduction after ~21 days	Depleted	No change	~35% remaining after 14 days	Linear degradation complete after ~21 days

During anaerobic starvation, most dynamics among the cellular storage products occurred in the first day. These biopolymer dynamics appear to be the most influential parameter on PAO performance. During aerobic storage, decay processes appear to play a significant role in the deterioration of PAO performance, but only if storage lasts multiple weeks. However, little is known about the short-term effects of endogenous respiration. Although decay is commonly thought to be the major drawback of aerobic starvation, it may not be as detrimental as biopolymer utilization over the course of 8-16 hours of storage. Additional research needs to be conducted to better understand the dynamics of short-term storage on EBPR performance. The research presented here will investigate the impact of short-term aerobic storage on PAOs by monitoring the process performance of a laboratory-scale 5-stage Bardenpho system.

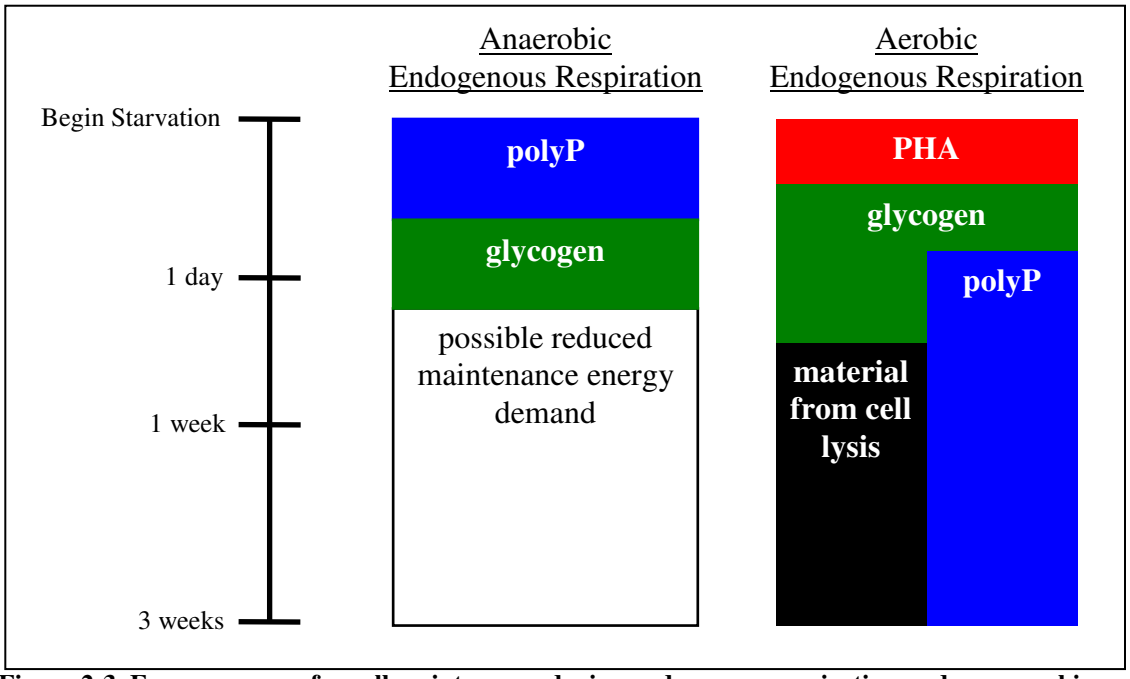

Figure 2-3. Energy sources for cell maintenance during endogenous respiration under anaerobic and aerobic conditions. This figure is qualitative and is based on the findings of Lopez et al. (2006).

Chlorine Toxicity and Mitigation of Toxic Shock Events

Mechanisms of Chlorine Toxicity

For over half a century scientists have been investigating the mechanism for bacterial toxicity of chlorine (Fair et al., 1948; Green and Stumpf, 1946; Knox et al., 1948; Venkobachar et al., 1977). The most common species of reactive chlorine are hypochlorous acid (HOCl), hypochlorite (OCl^-), and monochloramine (NH_2Cl). In these reactive forms, the chlorine atom has an oxidation state of +1, yet concentrations of these species are typically represented as elemental chlorine (Cl_2) which has an oxidation state of zero. The chlorine atom is in its most stable form when it exists as chloride (Cl^-). Chlorine atoms with a +1 oxidation state are strong oxidants. Although the relative toxicities of +1 oxidation state chlorine species may vary depending on the species of bacteria, all of these reactive chlorine species have been shown to be bacteriocides (Chesney et al., 1996; Morris, 1966b; Winterbourn et al., 1992). Chlorine gas (Cl_2) is often used to make hypochlorite by dissolving the gas into a caustic solution with either sodium or calcium, forming sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl)_2$), respectively. Hypochlorous acid (HOCl) is a weak acid with a pKa of approximately 7.5 (Morris, 1966a), and is believed to be toxic to virtually all cell types (Barrette et al., 1989). Additionally, lethal levels of HOCl may affect function and viability within 100 milliseconds of exposure (Albrich and Hurst, 1982).

Some of the earliest chlorine toxicity studies showed an inhibition of glucose oxidation by trace amounts of chlorine (Green and Stumpf, 1946), and an inhibition of thiol-containing enzymes at higher chlorine doses (Knox et al., 1948). Since these preliminary studies, numerous mechanisms of inhibition have been reported.

Thiol groups are among the most nucleophilic of all substituent groups (Albrich et al., 1981; Solomons, 1996). When bacteria are exposed to chlorine, however, many biomolecules can be oxidized and result in cellular inhibition. Components commonly affected by oxidants include thioethers (Chesney et al., 1996), conjugated double bonds (Chesney et al., 1996), and iron sulfur centers (Rosen and Klebanoff, 1982). Additionally, reactive chlorine species have been shown to inhibit cellular respiration (Albrich and Hurst, 1982; Albrich et al., 1981; Barrette et al., 1989) and metabolite/substrate transport (Barrette et al., 1989; Albrich et al., 1986).

In addition to various inhibitory mechanisms, chlorine exposure can damage the structure of the cell membrane. Friberg (1957) used bacteria with radioactive phosphorus (^{32}P) to determine the effect of chlorine on membrane integrity. Cells were exposed to chlorine and leakage of cellular components was observed (Friberg, 1957). This deterioration of membrane integrity and loss of cellular components has also been seen by other researchers (Venkobachar et al., 1977). Venkobachar and colleagues developed a dose-response curve that confirmed increasing leakage of cellular macromolecules with increasing chlorine doses (Venkobachar et al., 1977). Proteins and RNA were seen in the supernatant at low doses of chlorine (1.5 mg·Cl_2/L and greater) and DNA was seen at higher doses (approximately 11 mg·Cl_2/L and greater) (Venkobachar et al., 1977). However, others studies have suggested that the integrity of the plasma membrane is not compromised, because small molecules and ions were retained inside the cell after chlorine exposure (Albrich et al., 1986; Barrette et al., 1987).

Chlorine has also been shown to decrease the zeta potential and the oxidative phosphorylation capabilities of *E. coli* cells, potentially resulting from the inactivation of trans-phosphorylases (Venkobachar et al., 1977). Additionally, a chlorine dose of 0.4 mg·Cl_2/L resulted in the complete cessation of phosphate uptake by *E. coli*, but oxygen uptake by the cells continued (Venkobachar et al., 1977).

It has also been proposed that chlorine disrupts ATP production by oxidative and fermentative pathways by inhibiting inner membrane bound systems that achieve these processes (Barrette et al., 1989). This inhibition may result from the impedance of amino acid (and various forms of organic carbon) transport into the cell (Albrich et al., 1986; Barrette et al., 1989).

Normal bacteria maintain energy charge (EC) levels of 0.8-0.9 when sufficient nutrients are available, and protein synthesis and cellular replication stop when the EC drops below this range (Barrette et al., 1989). *E. coli* cells exposed to lethal doses of HOCl, however, are unable to attain the EC levels required for growth (Barrette et al., 1989). This may be the result of the oxidative modification of the F1 complex of the proton-translocating ATP synthase (Barrette et al., 1989). HOCl may also degrade (or change the formation rate of) mRNA (Barrette et al., 1989). Ultimately, there appears to be no recovery from HOCl exposure (Barrette et al., 1989).

Chlorine Usage at WWTPs

Disinfection is a critical process at municipal wastewater treatment plants because WWTP effluent must be disinfected before being discharged to a receiving body of water. The most common disinfection process is chlorination, where reactive chlorine species kill or inactivate bacteria and viruses via chemical oxidation. When using chlorination for disinfection, WWTPs must achieve a given *Ct*, where "*C*" is the concentration of reactive chlorine and "*t*" is the duration of chlorine contact with the plant effluent. However, the use of chlorination for effluent disinfection does not affect plant performance because the mixed liquor never comes in contact with this chlorine. A practice that does expose WWTP biomass to reactive chlorine species is the use of chlorination to reduce the prevalence of filamentous bacteria (Blackbeard et al., 1988; Caravelli et al., 2003; Casey et al., 1995; Lakay et al., 1988; Mascarenhas et al., 2004; Ramirez et al., 2000).

The prevalence of filamentous organisms can result in bulking, deteriorating the settleability of a mixed liquor and making it difficult to control the residence time of biomass in the system. Additionally, the growth of certain species of filamentous organisms can result in foaming, which is also problematic for WWTPs. There are two main methods of control for reducing the prevalence of filamentous organisms in a given sludge: the specific method and the nonspecific method. The specific method to inhibit the growth of filamentous organisms is to use process controls to select for floc-forming bacteria. Increasing dissolved oxygen levels (or minimizing areas of low DO), decreasing SRT, or adding an anaerobic selector are all methods to select for desirable bacteria (Campbell et al., 1985; Jenkins et al., 1984a). Although these methods may be effective, process configurations and controls are typically dictated by economics and effluent characteristics. Additionally, adjusting process controls can be particularly dangerous at biological nutrient removal WWTPs with stringent effluent N and P limits because these plants may have to meet effluent concentrations less than 1 mg/L. In order to avoid changing process parameters, nonspecific controls can be used. A common nonspecific control for filamentous growth is the use of low chlorine doses in the mixed liquor. Chlorination for control of filamentous organisms has been implemented for over 70 years (Smith and Purdy, 1936). The method became more refined in the early 1980's, when Jenkins and colleagues published

extensive material on the use of chlorination to control mixed liquor bulking (Jenkins et al., 1984a; Jenkins et al., 1984b).

Floc-forming bacteria at WWTPs often have the ability to excrete extracellular polymeric substances (EPS) that can enhance cellular agglomeration. Production of EPS has been linked with exposure to toxic substances, suggesting that EPS could be a cellular defense to toxins (Henriques and Love, 2007). Unlike floc-forming bacteria, the majority of filamentous bacteria in mixed liquor are not protected by EPS, and tend to be far more susceptible to oxidative stressors (Kim et al., 1994). For this reason, chlorination is often the quickest and most cost-effective way to decrease the abundance of filamentous bacteria. However, overdosing with chlorine (via high concentrations or prolonged dosing) can result in deterioration of floc structure and increased turbidity in secondary clarifier effluent (Campbell et al., 1985; Lakay et al., 1988; Mascarenhas et al., 2004; Ramirez et al., 2000). Although the reason for this increase in turbidity is not entirely understood, Wimmer and Love (2004) showed that exposure to hypochlorite and the resulting increased turbidity can be correlated with increased soluble phase potassium. These results suggest that glutathione-gated potassium efflux and the resulting increase in monovalent:divalent cation ratio is responsible for the poor flocculation (Wimmer and Love, 2004; Bott and Love, 2004). The significance of the ratio of monovalent to divalent cations has been previously discussed by Higgins and colleagues (Higgins and Novak, 1997a, 1997b).

Mitigation of Chlorine Toxic Shock Events

There are a number of factors that can affect a bacterium's resistance to chlorine. One of the most influential factors is believed to be the glutathione (L-γ-glutamyl-L-cysteinylglycine, GSH) content of a cell (Saby et al., 1999). Reactive chlorine and other oxidants have been shown to preferentially oxidize thiol groups (Albrich et al., 1986; Albrich et al., 1981), and GSH is the predominant thiol compound in *E. coli* and many other bacteria (Fahey et al., 1978). Chesney et al. (1996) conducted studies in which *E. coli* mutants incapable of producing GSH were supplemented with equivalent levels of extracellular GSH. This extracellular addition of GSH provided GSH-deficient mutants with the same resistance to chlorine as wild-type *E. coli* cells capable of producing GSH. Interestingly, however, mutant cells supplemented with extracellular GSH were less resistant to H_2O_2 than wild-type cells. Since GSH cannot pass the inner

membrane of *E. coli* cells, it is likely that intracellular GHS is part of a more complex mechanism by which cells protect themselves from H_2O_2 (Chesney et al., 1996). GHS, therefore, can act as a sacrificial defense against oxidative stressors and may also be involved in more complex cellular defense mechanisms against oxidative stress (Chesney et al., 1996; Saby et al., 1999).

Saby and coauthors have shown that *E. coli* has increased resistance to chlorine when operated under starvation conditions for 24 hours prior to exposure (Saby et al., 1999). In fact, many researchers have shown that the sensitivity of bacteria to disinfectants is reduced when subjected to starvation conditions (Berg et al., 1982; Lisle et al., 1998; Saby et al., 1999). Several mechanisms for increased resistance have been suggested, including: the development of physical barriers from floc formation and EPS accumulation (Henriques and Love, 2007), and physiological developments such as changes in membrane permeability (Lisle et al., 1998) and GSH metabolism (Saby et al., 1999).

It has previously been suggested that cellular GSH levels may be significantly higher in stationary-phase *E. coli* cells (Fahey et al., 1978). Saby et al. (1999) observed an increased chlorine resistance in wild-type *E. coli* cells after they had been starved for 24 hours; the resistance of a GSH-deficient *E. coli* mutant remained unchanged. These results further suggest that GSH production may be induced under nutrient-limiting conditions (Saby et al., 1999).

Under aerobic conditions, cells are constantly exposed to low levels of reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals (Fridovich, 1978). Using the same GSH-deficient *E. coli* mutant as in their starvation experiment (discussed above), Saby and coauthors investigated the impact of these reactive oxygen species on the chlorine resistance of *E. coli* (Saby et al., 1999). The study showed that increasing the concentration of dissolved oxygen in the medium from 10% to 100% of saturation (and thereby increasing the concentration of reactive oxygen species) increased the GSH content of wild-type cells threefold. This increase in GSH provided the wild-type cells with an increased resistance to chlorine while the GSH-deficient mutant exhibited no change. Therefore, non-lethal levels of oxidative stress may increase cell resistance to chlorine by increasing cellular GSH content (Saby et al., 1999). The work of Dukan and Touati (1996) further supports the belief that exposure to sub-lethal levels of

reactive oxygen species increases cell resistance to chlorine: they found that exposure to sub-lethal doses of H_2O_2 increased *E. coli* resistance to chlorine.

Although aerobic conditions prior to exposure have been shown to increase cellular resistance to chlorine (Saby et al., 1999), the presence of reactive oxygen species after chlorine exposure may be detrimental to cells (Dukan et al., 1999). Hypochlorous acid has been shown to damage DNA in *E. coli*, as a *recA* and *recB* mutants (unable to achieve DNA recombinational repair) were slightly more sensitive to HOCl exposure than wild type cells (Dukan and Touati, 1996). However, no additional sensitivity (when compared to the wild-type) was seen in a *recA* mutant when cells were not exposed to oxygen after HOCl exposure (Dukan et al., 1999). These results suggest that DNA damage may result from reactive oxygen species after HOCl exposure (Dukan et al., 1999). Dukan et al. (1999) also observed that at lower HOCl concentrations ($\leq$ 38 μM), in addition to the loss of reduced GSH, other antioxidant defenses may be significantly damaged (decrease in activities of glucose-6-phosphate dehydrogenase and hydroperoxidase I catalase). At higher HOCl concentrations (57 and 76 μM), other enzymes such as superoxide dismutase (SOD) and hydroperoxydase II are inactivated. The loss of these cellular defenses against oxidative stress increase *E. coli* sensitivity to hydroxyl radicals, superoxide, and hydrogen peroxide (Dukan et al., 1999). It should also be noted that exposure to oxidants has been shown to mobilize intracellular transition metals which could increase the formation of reactive oxygen species via the Fenton reaction (Dukan et al., 1999; Rosen and Klebanoff, 1982). Therefore, exposure to HOCl may not only inhibit cellular defense to reactive oxygen species, but may also increase their formation (Dukan et al., 1999). In summary, during the recovery period following exposure to chlorine species, cells are more vulnerable to oxidative stressors, and exposure to oxygen during this time may significantly increase the toxicity of the chlorine exposure (Dukan et al., 1999).

Although these fundamental mechanisms of chlorine toxicity have been investigated using *Escherichia coli* and other model organisms, little is known about chlorine toxicity and PAOs. Chlorine is commonly used at WWTPs in small doses for filamentous control without an observable impact on PAOs. With the complex metabolic pathways of PAOs, it is unclear how higher doses of chlorine would impact the ability of PAOs to release and uptake phosphate. PAOs rely heavily on a balance of intracellular biopolymers. Although PAOs have multiple

pathways for energy production (glycogen degradation, polyP degradation, PHA degradation), an inhibition of any one pathway would likely result in unstable EBPR performance. Additional studies, therefore, are needed to understand the toxic mechanisms of chlorine in PAOs.

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Chapter 3

Materials and Methods

Ameet J. Pinto, Andrew Shaw, and Nancy G. Love contributed to the content in this chapter.

Standard methods. Methods identified in this section that are accompanied by a four-digit number (e.g., 5220) refer to the procedures and guidelines found in Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

Corrective Action Strategies

Identification of potential corrective actions. At the start of this study, an expert workshop was held to identify potential corrective action strategies for Long Creek Water Resources Reclamation Facility (WRRF) in Gastonia, North Carolina. The workshop included WWTP operations personnel, wastewater process specialists, and academic researchers. One basic assumption underlying the corrective action strategies is that with technologies currently under development, operators will be warned by remote sensors in the collection system that the toxin is coming. The operators will then have the opportunity to implement the optimal corrective actions, as defined by the DSS, to mitigate the toxic shock event.

It was also assumed for the purposes of this study that the toxin would arrive at the plant as a shock load lasting between 15 minutes and 8 hours. This range was selected based on the assumption that the toxin was discharged to the sewer in a matter of minutes, either immediately upstream of the WRRF (resulting in a high concentration, short duration shock) or near the perimeter of the service area (resulting in a lower concentration, longer duration shock). The corrective action strategy would be implemented immediately preceding the arrival of the toxin, and would continue until no toxin could be detected in the mixed liquor or effluent of the plant. Once the system was free of the toxin, normal operation would resume.

It was unanimously agreed upon that the most preferable corrective action during a toxic shock event would be to route all contaminated influent to a storage basin, at which time the toxin could be slowly introduced to the system, treated by an alternative method, or removed from the site for treatment at an alternate facility. For the purposes of this study, however, it was assumed that the Long Creek WRRF had no influent storage capacity.

The intuitive reaction from an operations perspective is to protect the biomass from the toxin to prevent long-term losses in system performance that could result in prolonged environmental pollution. However, if the contaminated influent does not come in contact with activated sludge, it will pass through the plant without biological treatment, resulting in effluent with concentrations of biochemical oxygen demand (BOD), ammonia (NH_3), and phosphorus that could lead to permit violations. Furthermore, the toxin will pass through the treatment plant to the effluent untreated, putting aquatic species in the receiving stream at risk. A certain level of treatment, therefore, must still be achieved to protect the environment from the discharge of untreated sewage and to comply with permit requirements.

Plant-specific corrective action strategies. The Long Creek WRRF is a 5-stage Bardenpho system with a design capacity of 16 MGD. The plant has four parallel trains for the first three biological treatment zones (anaerobic – ANA, anoxic 1 – ANX 1, aerobic 1 – AER 1), one train for the final two biological treatment zones (anoxic 2 – ANX 2 and aerobic 2 – AER 2, together designated the "denitrification basin"), and four secondary clarifiers. Taking into consideration the process controls available to Long Creek operators, a number of corrective actions were considered for laboratory-scale testing.

One potential corrective action would be to stop all aeration and mixing in the reactors (Figure 3-1B). This would allow biomass to settle and could potentially help short-circuit flow through the reactors, pushing the contaminant through the system quickly and minimizing biomass exposure. This corrective action, however, does not offer much control over the balance between effluent quality and biomass exposure to the toxin. If the contaminant were volatile, another option would be to turn off the mixing in the anaerobic and anoxic zones, but increase aeration to volatilize the chemical (Figure 3-1C). The increase in aeration may provide some level of treatment, but it could also expose operators and neighbors to unacceptable levels of gaseous

contaminant or potentially represent an air permit violation. Efforts to model the fate of volatile organic chemicals at WWTPs have previously been made (Monteith et al., 2006).

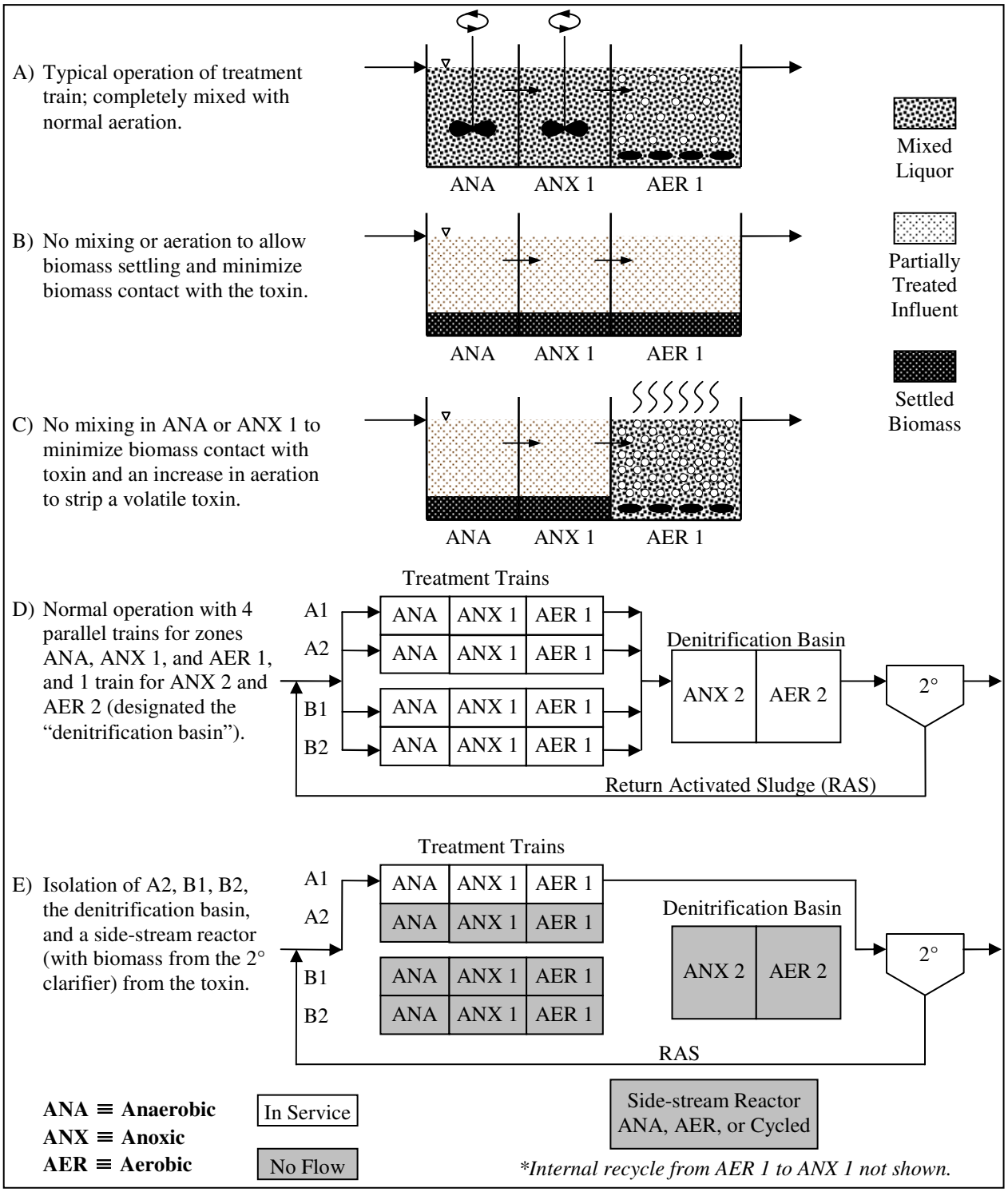

Figure 3-1. Potential corrective action strategies for the mitigation of toxic shock events. Normal operation is illustrated in figures A and D. Operators may stop mixing or adjust aeration (figures B and C), or isolate treatment trains from influent flow (figure E).

The Long Creek WRRF also has the ability to stop flow to any treatment train and bypass the denitrification basin. In the event of a shock event, an additional corrective action could be to bypass 1-3 of the treatment trains and/or the denitrification basin. This alternative offers a great deal of operational flexibility since the exposure of the contaminated influent to activated sludge for treatment can be adjusted by changing the number of trains in service. Any trains that were isolated from the shock would be put back into service after the contaminant had left the WRRF. The effect of endogenous respiration on the performance of the biomass may be relatively significant, but the system would likely be achieving some level of biological phosphorus removal (BPR) in a few days time (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996). Depending on the bacteriocidal effect of the contaminant, however, exposure of biomass to the toxic shock could result in long-term losses of system performance due to death and washout of crucial organisms such as PAOs or ammonia oxidizing bacteria (AOBs). If EBPR performance is lost it could potentially take several weeks to recover, even with the use of bioaugmentation. Such a long recovery period could significantly compromise a facility's permit compliance.

For the purposes of this study, we investigated the effect of a *worst case scenario* shock event and a *maximum response* corrective action strategy. The *worst case scenario* was defined as the discharging of a contaminant directly upstream of the WRRF over 10-15 minutes. The *maximum response* was defined as minimizing the biomass exposure to the contaminant.

In order to effectively minimize the exposure of biomass to the toxin, the simplest solution would be to route all influent flow to one treatment train, bypass the denitrification basin, and route all flow to one secondary clarifier (Figure 3-1E). In this case, only one train would be exposed to the toxin. By routing all flow to one train, however, that train would be hydraulically overloaded and poor solids removal would likely be achieved in the secondary clarifier. Long Creek WRRF utilizes media filtration after secondary clarification, a solids-separation process that could help prevent the discharging of significant solids. The WRRF also has the ability to add alum prior to the secondary clarifier which could help solids settling and the precipitation of excess effluent phosphate.

Additional biomass could potentially be isolated if, immediately preceding the shock, the sludge blanket of the exposed secondary clarifier were routed to an out-of-service reactor for storage. To make sure some level of treatment would still be achieved, however, return activated sludge (RAS) flow would resume after the sludge blanket was depleted. The side-stream reactor could potentially be aerated, unaerated, or could cycled between the two.

Selection of corrective action strategy. In order to test the *maximum response* and isolate as much biomass as possible, the denitrification basin and three (of four) treatment trains would be bypassed. Additionally, the sludge blanket from the secondary clarifier would be pumped to a side-stream reactor for storage (Figure 3-1E). Once the sludge blanket was depleted, the RAS flow would be restored. The internal recycle would also be discontinued to minimize mixing of the contaminant in the system. Once the contaminant was no longer detected in the mixed liquor or effluent, biomass from the side-stream reactor would be reintroduced to the system and normal operation would resume.

Storage of activated sludge. Storage conditions in the side-stream reactor offer another level of operator control. It has been well documented that under endogenous conditions, the decay of bacteria is faster in the presence of oxygen (Brdjanovic et al., 1998a; Lopez et al., 2006; Siegrist et al., 1999; Temmink et al., 1996). Under anaerobic conditions, decay processes do not appear to be nearly as significant; instead, the utilization of specific pools of cellular storage compounds may be accelerated (Lopez et al., 2006). These energy pools are designed to be dynamic (van Loosdrecht and Heijnen, 1997), but even short-term (< 1 day) starvation can lead to imbalances that are detrimental to PAO performance (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996).

Lopez and colleagues conducted a thorough study of PAO starvation, monitoring relevant biopolymers over the course of 4 weeks (Lopez et al., 2006). Although the study was designed to elucidate the effect of long-term starvation on PAOs, the authors were able to identify potential causes of PAO inhibition after only one day of starvation (Table 2-5).

Within the first day of storage, Lopez et al. (2006) observed considerable inhibition of P-release and uptake rates, significant glycogen consumption, and complete polyP degradation. Additionally, a shift in the ratio of polyhydroxybutyrate (PHB) to polyhydroxyvalerate (PHV)

was observed; this shift is significant because PHB is believed to be more a more efficient source of energy and reducing power during substrate uptake (Randall and Liu, 2002).

Under aerobic storage, the rates of P-release and uptake were impacted much less severely, and the only biopolymer consumed to a greater extent than under anaerobic storage was PHA. Decay, which is commonly thought to be the major drawback to aerobic activated sludge storage, may not be as significant as biopolymer utilization over the course of 8-16 hours. For the purposes of this study, therefore, the side-stream reactor was maintained under aerobic conditions.

Laboratory-Scale Reactor Testing

Three identical, laboratory-scale, 5-stage Bardenpho systems were constructed and housed in a remote storage shed adjacent to a municipal sanitary sewer system manhole. Operational parameters such as recycle rates and hydraulic retention times (HRTs) were designed to mimic train A1 of the Long Creek Water Resources Reclamation Facility (WRRF) in Gastonia, North Carolina; the effect of the corrective action strategy on trains A2, B1, and B2 was not investigated. Each train included a primary clarifier (6.0 liters), an anaerobic zone (4.5 liters), a preliminary anoxic zone (3.0 liters), a preliminary aerobic zone (14.5 liters), a secondary anoxic zone (5.3 liters), a secondary aerobic zone (2.2 liters), and a secondary clarifier (5.9 liters) (Figure 3-1D). The systems were operated at 22°C ± 3°C and the influent flow rate was maintained at 50 mL/min to simulate the design capacity of Long Creek's A1 train, 3.5 million gallons per day (MGD). Wastewater was pumped daily from the manhole into an unmixed 200 liter storage tank from which the influent for each train was pumped. Internal recycle and return activated sludge (RAS) flow rates were maintained at 150% and 100% forward flow, respectively. Solids retention time (SRT) was calculated based on a four-day average and maintained between 12 and 16 days.

Selection of contaminant. The contaminant was selected based on a contaminant prioritization list developed as part of WERF project 03-CTS-2S. The prioritization framework takes into consideration a number of parameters ranging from availability of the toxin to its ease of handling and introduction to the sewer system (Leonard Casson, personal communication).

Simulated toxic shock events. Prior to each shock event, the laboratory reactors were seeded with RAS, shipped unchilled and overnight from Long Creek Water Resources Reclamation Facility (WRRF). The trains were operated identically for 2.5 HRTs, after which the biomass from all three trains was removed, remixed, and redistributed to the biological reactors. All three trains were then operated an additional 2.5 HRTs prior to the shock event. These reactors were not necessarily given sufficient time to reach steady-state: the basis of this study would be a comparative performance analysis between the three trains.

Immediately preceding the toxic shock event, the following corrective actions were implemented in the corrective action train: the internal recycle was stopped, the sludge blanket in the secondary clarifier was pumped to an aerated sidestream reactor, influent flow was increased to 229 mL/min to simulate an increase in flow to 16 MGD, and the second anoxic and aerobic zones were bypassed. A stock solution of 1% calcium hypochlorite was prepared in glassware that had been nitric acid washed and chlorine bathed to eliminate the chlorine demand of the glassware. The 1% calcium hypochlorite solution was added directly to the primary clarifier of the corrective action and positive control trains in volumes of 300 mL and 66 mL, respectively. Additionally, 618 mg of alum was added to the primary clarifier of the corrective action train (this alum dose was designed to simulate the addition of 90 mg aluminum sulfate per liter of influent for one hour). The chlorine and alum were introduced near the primary clarifier inlets over the course of 15 minutes.

The positive control and corrective action trains were monitored for chlorine until none was detected in the mixed liquor or effluent. Once chlorine was no longer detected in the corrective action train, the corrective actions were terminated, biomass from the sidestream reactor was reintroduced to the first aerobic zone of the corrective action train, and normal operation resumed. Trains were operated and monitored until their process performance converged. This experiment was run twice, independently. During each shock event, one laboratory train remained unshocked (NC, negative control) while hypochlorite was introduced to the remaining two trains (PC, positive control and CA, corrective action). The corrective action strategy was only implemented on the CA train, and the PC train represented the “do nothing” approach to mitigating the shock event. This study simulated a shock of 2,500 gallons of 6% calcium hypochlorite ($Ca(OCl)_2$) arriving at the WRRF over the course of 15 minutes, with the total

influent flow at plant capacity (16 MGD). A summary of the implemented corrective actions can be seen in Table 4.2. It is important to note that these corrective actions were only implemented while chlorine (free or total) was detected in the mixed liquor or effluent of the system. Once chlorine was no longer detected, biomass from the side-stream reactor was reintroduced to the CA train and normal operation resumed. Trains were operated and monitored until their process performance converged. Comparative performance analysis among the three trains was based on effluent quality, performance stability, and biomass kinetics as indicated by rates of respiration and phosphate release and uptake.

Table 3-1. Summary of laboratory-scale reactor setup and the tested corrective action strategy. Each train was designed to simulate train A1 of Long Creek WRRF which treats 3.5 MGD of the plant's 16 MGD capacity. The flow to the PC train was not changed, resulting in a shock of 550 gallons of 6% calcium hypochlorite.

TRAIN	SHOCK	CORRECTIVE ACTIONS	OBJECTIVE OF CORRECTIVE ACTION
Negative Control, NC	None	None	-
Positive Control, PC	550 gallons of 6% Calcium Hypochlorite	None	-
Corrective Action, CA	2,500 gallons of 6% Calcium Hypochlorite	Increase influent flow 3.5 → 16 MGD	Isolate trains A2, B1, and B2 from toxin
		Bypass denitrification basin (ANX 2 and AER 2)	Isolate denitrification basin from toxin
		Stop internal recycle	Prevent additional mixing of toxin and minimize duration of shock event
		Transfer secondary (2°) sludge blanket to aerated side-stream reactor	Isolate additional biomass from the toxin
		Add alum prior to primary (1°) clarifier during shock event	Enhance 1° treatment to lessen nutrient load on system

On-Site Analyses

Dissolved oxygen (DO). DO measurements were taken in the first aerobic zone and second anoxic zone using a YSI model 58 DO meter with a YSI 5905 probe. The meter was calibrated immediately preceding each set of measurements using a BOD bottle with water-saturated head space, taking into consideration the elevation and temperature at the time of sampling.

pH. pH measurements were taken in the first aerobic zone during both experimental runs using a Oakton pH 6 Acorn series model 9708 pH/mV/°C meter with an Accumet pH probe. Additionally, pH was monitored in the anaerobic zone during the first experimental run and in the secondary clarifier during the second. The pH meter was calibrated at 7.0 and 4.0 approximately 20 minutes before each set of pH readings.

Sludge volume index (SVI). A mixed liquor sample from the first aerobic zone of each train was placed into a 250 mL graduated cylinder. Samples were given 30 minutes to settle, at which time the volume of the sludge blanket was recorded. SVI readings were estimated by multiplying the sludge blanket reading by 4 (to normalize to 1 L) and dividing by the average MLSS at that time point. One SVI measurement was made at each sampling point.

Chlorine. Chlorine samples were collected from reactors and secondary clarifiers to monitor the progress of chlorine through the system. Samples were filtered through 0.45 μm nitrocellulose filters and placed in acid-washed, chlorine-bathed glassware. Chlorine concentration was determined immediately after sampling using the DPD ferrous titrimetric method (4500-Cl-F) adjusted for 10 mL sample volumes.

Inorganics

Orthophosphate. Samples were collected using plastic syringes and filtered through 0.45 μm nitrocellulose filters. For the first experimental run, samples were stored in 50 mL centrifuge tubes at -20 °C. Frozen samples were later thawed and analyzed using a Dionex model DX-120 ion chromatograph with an IonPac AG9-HC guard column, an IonPac AS9-HC analytical column, and 10.0 mM Na_2CO_3 eluent. For the second experimental run, orthophosphate samples were stored in hydrochloric acid-washed glassware, acidified with hydrochloric acid, and stored at 4 °C prior to analysis. Acidified samples were analyzed using the ascorbic acid method (4500-P-E) adjusted for 175 μL sample volumes. Samples were added to hydrochloric acid-washed microplates and diluted in-well. Reagents were mixed and 28 μL of the mixture was added to each well and mixed using automatic pipettors. Microplates were analyzed for absorbance at 880 nm using a Biotek μQuant model MQX200. The linear range for this analysis was 0.14 mg·P/L to 0.93 mg·P/L. Samples were analyzed in triplicate, and any samples with a standard deviation greater than 5% were re-run.

Nitrite and nitrate. Samples were collected using plastic syringes, filtered through 0.45 μm nitrocellulose filters, and stored in 50 mL centrifuge tubes at -20 °C. Frozen samples were later thawed and analyzed using a Dionex model DX-120 ion chromatograph with an IonPac AG9-HC guard column, an IonPac AS9-HC analytical column, and 10.0 mM Na_2CO_3 eluent.

Alkalinity. Samples were collected from the effluent of each secondary clarifier and analyzed in duplicate using the titration method for alkalinity (2320-B) with 0.02 N sulfuric acid.

Organics

Chemical oxygen demand (COD). Samples were acidified with concentrated sulfuric acid and stored in 15 mL centrifuge tubes at 4 °C. Soluble COD (sCOD) samples were filtered through 0.45 μm nitrocellulose filters prior to acidification and storage. Samples were analyzed using the closed reflux titrimetric method (5220-C).

Acetate. Samples were filtered through 0.45 μm nitrocellulose filters stored in 15 mL centrifuge tubes at -20 °C. Frozen samples were later thawed and analyzed using a Hewlett Packard model 5890 gas chromatograph with a Supelco Nukol™ fused silica capillary column (15 m x 0.53 mm with 0.5 μm film thickness) and flame ionization detector (FID). The injection port and FID were held at 200 °C and 250 °C, respectively. The column is held at 80 °C for 3 minutes after injection and then increased at 6 °C per minute for 10 minutes, reaching a final temperature of 140 °C.

Ammonia. Samples were filtered through 0.45 μm nitrocellulose filters, acidified with concentrated sulfuric acid, and stored in 15 mL centrifuge tubes at 4 °C. Samples were analyzed using the manual phenate method (4500-NH_3-F) adjusted for 240 μL samples volumes. Samples were diluted and reagents were added to 1.5 mL centrifuge tubes. Sample/reagent mixes were added to nitric acid-washed microplates to determine absorbance at 640 nm using a Biotek μQuant model MQX200.

Total Kjeldahl nitrogen (TKN). Samples were acidified with concentrated sulfuric acid and stored in 15 mL centrifuge tubes at 4 °C. Samples were analyzed using the semi-micro-Kjeldahl method (4500-N_{org}-C).

Solids

Replication. During the first shock event, all solids analyses were performed in triplicate. Relatively low standard deviations were achieved, and only duplicate analyses were run during the second shock event.

Total suspended solids (TSS) and volatile suspended solids (VSS). Effluent TSS/VSS was initially intended to be a performance indicator for the systems. For the first shock event, a 1 liter container was placed at the effluent of each secondary clarifier to collect an effluent TSS/VSS sample. However, effluent TSS/VSS appeared to be more dependent upon the researchers' movement in the shed than on process performance (vibrations led to increased effluent turbidity). As a result, effluent TSS/VSS samples during the second shock event were collected from each effluent basin to estimate biomass lost in the effluent. Samples were transported to the laboratory and filtered (100 mL of sample per filter) within 1 hour.

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Samples were collected from the first aerobic zone of each train. Samples were transported to the laboratory and filtered (10 mL of sample per filter) within 1 hour.

For all solids analysis, glass fiber filters with 1.5 μm pore size were rinsed with distilled (Type II) water, placed in numbered aluminum tins, baked at 550 °C for 20 minutes, placed in a dessicator for 3 minutes, and massed (tare mass, M_o). Samples were then filtered through 1.5 μm glass fiber filters. Glass fiber filters were replaced in their respective aluminum tins and placed at 105 °C for 1 hour. After baking at 105 °C, samples were placed in a dessicator for 3 minutes and massed (dry mass, M_{105}). After this "dry" measurement was recorded, samples were baked at 550 °C for 20 minutes, placed in a dessicator for 3 minutes, and massed (inert mass, M_{550}). Total and volatile solids were determined as follows:

$$\text{TSS or MLSS} = (M_{105} - M_o)/V_{\text{sample filtered}}$$

$$\text{VSS or MLVSS} = (M_{105} - M_{550})/V_{\text{sample filtered}}$$

All mass measurements were made using a Mettler H10 balance with sensitivity to 0.0001 grams.

Kinetics

Mixed liquor samples were collected from the first aerobic zone of each train and transported back to the laboratory for analysis.

Specific oxygen uptake rate (sOUR). Samples were aerated and 80 mL was added to each of two OUR bottles with stir bars. Each OUR bottle was placed on a magnetic stir plate and mixed such that a vortex was visible. A nutrient mix was previously prepared in distilled water (Type II) and contained 10.5 g/L beef extract, 10.5 g/L bacto-casilone, 10.5 g/L yeast extract, 2.8 g/L fructose, 2.8 g/L galactose, 2.8 g/L glucose, 29.5 g/L acetic acid, and 26.3 g/L glycerol. While mixing, 76.8 µL of nutrient mix was added to each OUR bottle. The OUR bottles were sealed by submerging Thermo Orion model 9708 O_2 electrodes in each bottle through a rubber stopper. DO readings were recorded using a Accumet AR25 dual channel pH/ion meter connected and LabVIEW 7 Express. A decrease of at least 2 mg·O_2/L was observed for all OUR measurements. Linear regression was used to determine the rate of oxygen utilization (OUR). This OUR value was then normalized to the concentration of biomass as estimated by MLVSS.

$$sOUR = OUR/MLVSS$$

All mixed liquor samples were analyzed in duplicate.

Maximum phosphate release/uptake rates and inhibition of maximum rates. 100 mL of each train's mixed liquor was added to each of four 150 mL beakers, totaling twelve beakers per time point. Samples were well-mixed with magnetic stirrers and sparged with N_2 gas until the DO of all beakers was below 0.3 mg·O_2/L. A concentrated calcium hypochlorite solution was added to two of the four beakers for each mixed liquor to reach a final concentration of 5 mg·Cl_2/L. After chlorine addition, beakers were given 2 minutes to mix. After 2 minutes, a sodium acetate and phosphate solution was added to each beaker to achieve a final concentration of 50 mg·CH_3COO^-/L and 5 mg·P/L. Orthophosphate samples were collected, filtered through 0.45 µm nitrocellulose filters, acidified with hydrochloric acid, and stored in hydrochloric acid-washed glassware at 4 °C. Samples were collected 2, 30, and 60 minutes after phosphate and acetate addition to monitor anaerobic phosphate release while sparging with N_2. After the 60 minute anaerobic time point, all beakers were aerated and samplers were collected 20, 40, and 60

minutes after aeration began. Maximum phosphate release/uptake rates were determined by normalizing the change in orthophosphate concentration to time. Inhibition of orthophosphate release/uptake was estimated by comparing the maximum rates from control beakers to those exposed to 5 mg·Cl_2/L. Orthophosphate concentrations were analyzed using the ascorbic acid method (4500-P-E) adjusted for 175 µL of sample volume, as previously discussed.

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Chapter 4

Results and Discussion

Ameet J. Pinto, Andrew Shaw, and Nancy G. Love contributed to the content in this chapter.

Experimental Results

As previously documented (Neethling et al., 1987), free chlorine immediately dissipated once in contact with the influent wastewater. Total chlorine (defined as monochloramines plus free chlorine), however, was still measurable. The corrective actions were maintained until total chlorine was no longer detected; 18 hours and 10 hours in the first and second shock events, respectively. Once the toxin had left the system, CA train flow rates were returned to normal and the biomass from the aerobic side-stream reactor was reintroduced to the system. All three systems were then monitored until their performance converged.

Both shock events had similar results, but, as could be expected, the 18 hour starvation during the first shock event resulted in more significant process upset. For this paper we will focus on the impact the second shock event had on the EBPR process: the results of the first shock event on nutrient removal have been discussed in detail elsewhere (Pinto et al., submitted).

Influent parameters were estimated based on daily composite samples. The system influent was raw municipal sewage pumped from a manhole, resulting in fluctuations in nutrient loading each day. Typical influent parameters can be seen in Table 4-1.

Table 4-1. Typical parameters of raw sewage influent. The ± represents the 95% confidence interval.

PARAMETER	INFLUENT CONCENTRATION
sCOD	226 ± 20 mg·COD/L
PO_4^{3-}	4.4 ± 0.7 mg·P/L
NH_3	38 ± 5 mg·N/L
Alkalinity	255 ± 28 mg·$CaCO_3$/L
pH	6.8 ± 0.1

The most challenging aspect of operating a laboratory-scale, 5-stage Bardenpho system was DO control. Low DO levels in AER 1 could potentially inhibit P-uptake (Narayanan et al., 2006), as well as nitrification and organic carbon removal. If DO levels were too high, the internal recycle and forward flow would transport that oxygen to ANX 1 and 2, potentially abolishing the anoxic zones altogether. The DO in both AER 1 and ANX 2 were measured at each sampling time, and air flow rates were adjusted periodically as needed.

Effect on EBPR

The shock event and corrective action strategy both inhibited EBPR. One and a half days after the shock event, the effluent orthophosphate in the positive control reached a peak of 2.3 mg·P/L, as compared to the negative control effluent of 0.2 mg·P/L. After this initial perturbation, the positive control nearly matched the performance of the negative control through day 9, save one elevated point on day 5. The corrective action train, however, exhibited unstable performance through day 7 of the study, after which it began a steady downward trend of effluent orthophosphate (Figure 4-1). These results are similar to those from the first shock event, with significantly unstable EBPR in the corrective action train for several days following the implementation of the corrective action strategy (Pinto et al., submitted).

The fluctuations in influent parameters made estimates of percent removal less definitive. To estimate the percentage of influent phosphate removed, effluent grab samples were compared with composite influent samples from the preceding 24 hours (Figure 4-2). This method was acceptable for comparative analysis but only allowed for daily estimates of EBPR performance.

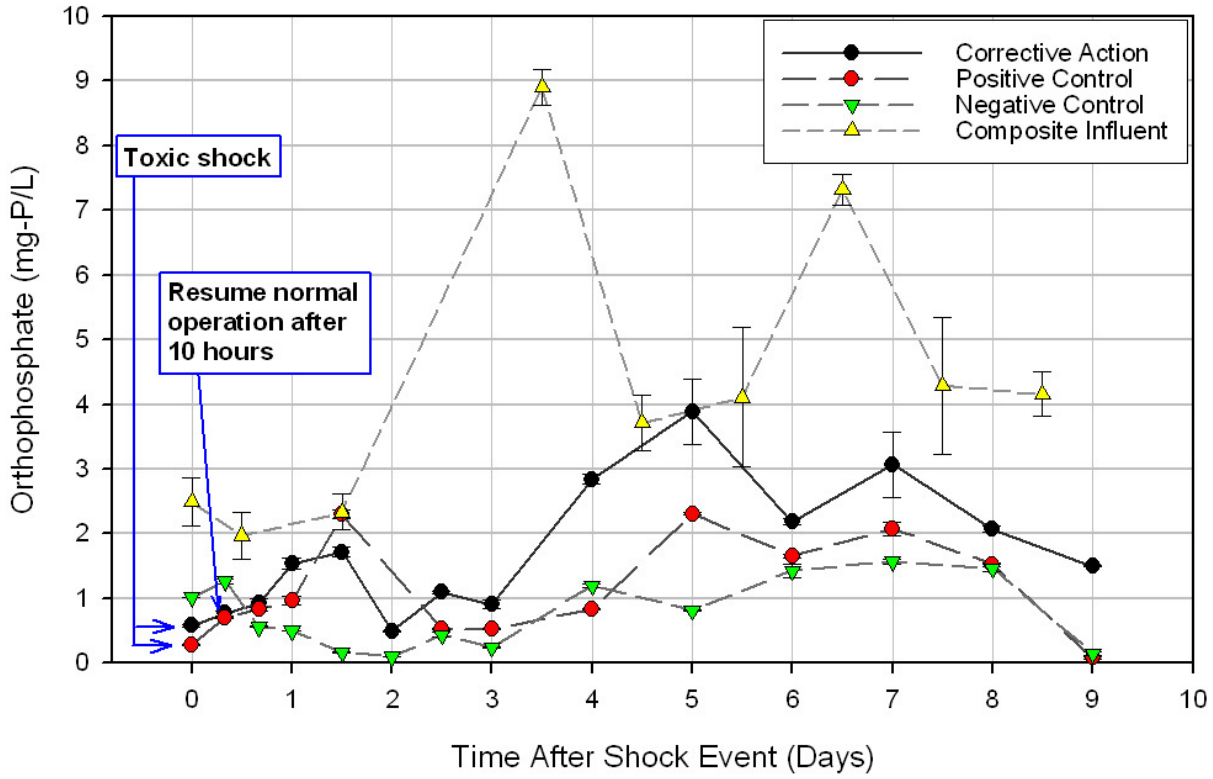

Figure 4-1. Influent and effluent orthophosphate concentrations during shock event 2. Error bars represent ± standard deviation of triplicate analysis. Raw data can be seen in Tables A-2, A-3 and A-4.

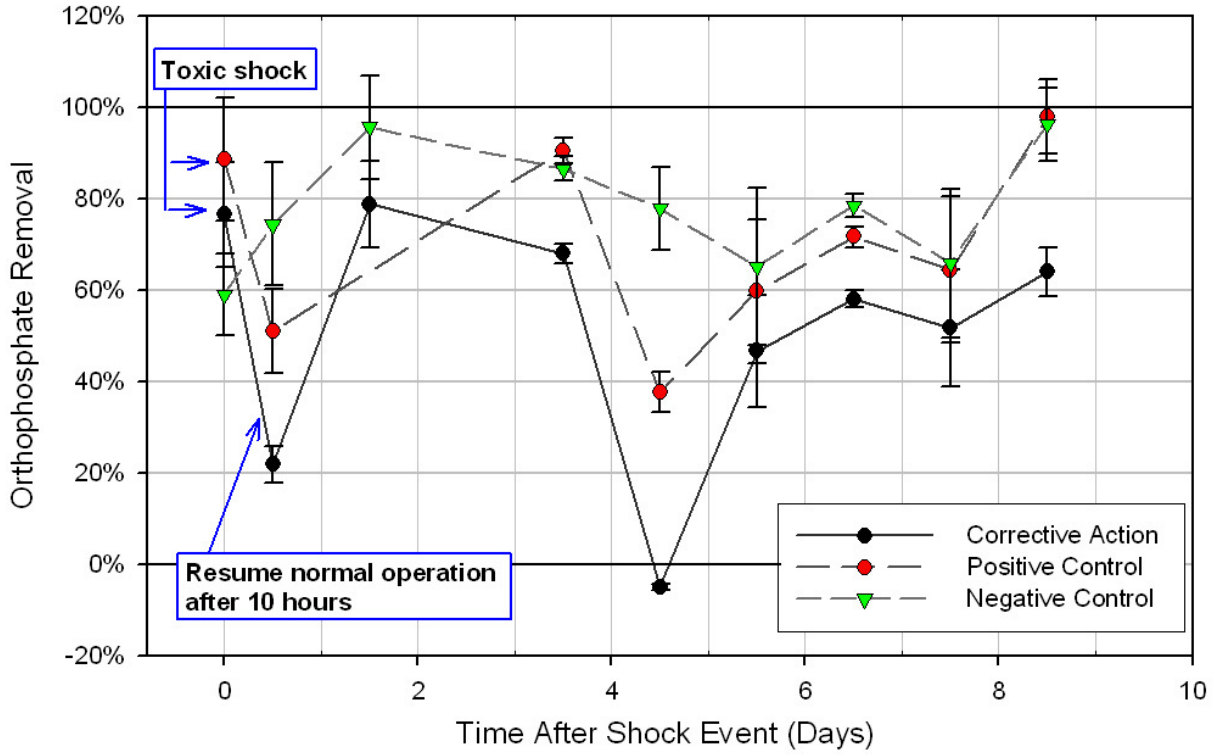

Figure 4-2. Estimated percent removal of orthophosphate during shock event 2. Values are based on effluent concentrations relative to the composite influent concentration from the preceding 24 hours. Error bars represent ± propagated error from triplicate influent and effluent orthophosphate analyses (see Table A-13).

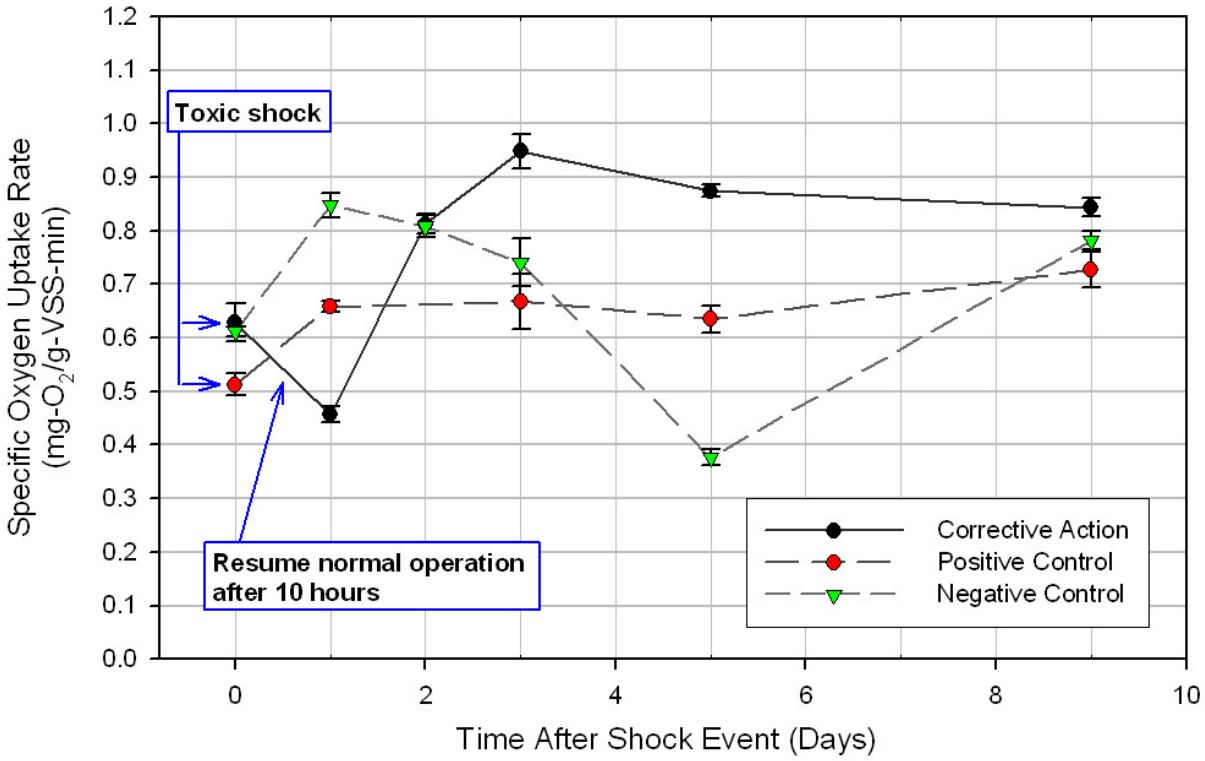

Figure 4-3. Mixed liquor specific oxygen uptake rates (sOURs) during shock event 2. Error bars represent ± propagated error from duplicate OUR and solids analyses (see Appendix A).

Phosphate Release/Uptake Rates and Resilience

Effluent phosphate concentrations did not correlate with the maximum mixed liquor or maximum specific phosphate release (Table 4-2) and uptake (Table 4-3) rates. Additionally, due to the variation between biological duplicates, there were no discernable trends in the resilience of the sludges (defined by the inhibition resulting from exposure to 5 mg·Cl_2/L of calcium hypochlorite).

Table 4-2. Maximum specific phosphate release rates (mg·P/g·VSS·hr). The ± represents original readings from biological duplicates.

Days After Shock Event	CA Train	PC Train	NC Train
0	3.6 ± 1.1	4.7 ± 1.0	3.0 ± 1.3
1	2.7 ± 0.5	6.1 ± 0.5	5.6 ± 2.1
3	5.0 ± 3.2	4.0 ± 0.1	6.2 ± 0.7
7	6.2 ± 1.8	5.9 ± 2.4	17.8 ± 0.2

Table 4-3. Maximum specific phosphate uptake rates (mg·P/g·VSS·hr). The ± represents original readings from biological duplicates.

Days After Shock Event	CA Train	PC Train	NC Train
0	6.8 ± 1.0	3.1 ± 0.1	4.2 ± 0.3
1	1.5 ± 1.5	6.6 ± 0.9	6.4 ± 4.0
3	2.6 ± 0.2	2.7 ± 1.2	4.4 ± 0.1
7	12.8 ± 1.9	4.5 ± 0.1	26.2 ± 0.3

It should be noted that the maximum rates of phosphate release and uptake were estimated based on a kinetic study with unadjusted pH. The objective of this kinetic measurement was to characterize the potential EBPR performance of the mixed liquor in its existing state, even if the PAOs would benefit from an increase in pH. The pH of the AER 1 zone was monitored regularly (Figure 4-4).

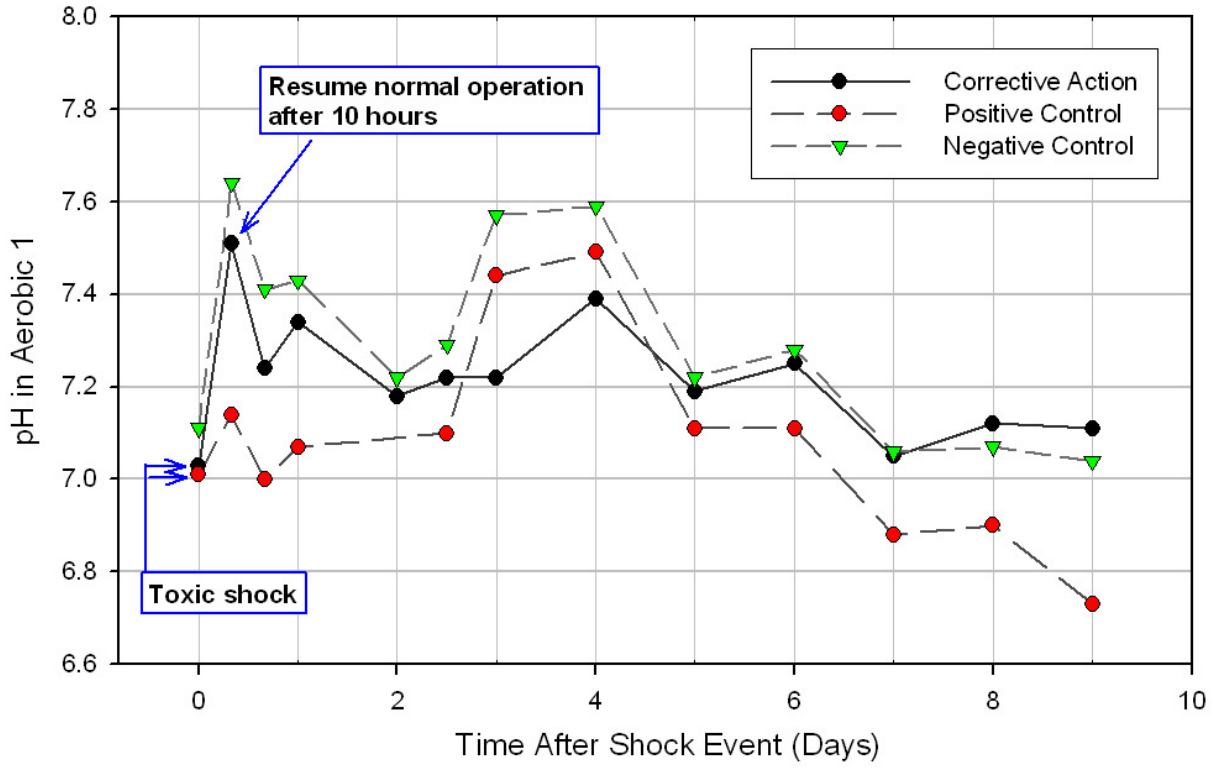

Figure 4-4. pH of first aerobic zone during shock event 2 (see Table A-12).

It is likely the pH in the aerobic and anaerobic zones significantly impacted the rates of phosphate release and uptake. An aerobic pH of 6.5, for example, has been shown to decrease the phosphate uptake rate of PAOs by 37% and 42% as compared to the rates at 7.0 and 7.5,

respectively (Filipe et al., 2001a). While PAOs appear to be less sensitive to pH shifts from 6.5-8.0 under anaerobic conditions (Filipe et al., 2001d; Liu et al., 1996), lower pH values in the anaerobic zone have still been shown to decrease PAO efficiency (defined as P·moles released per C·moles taken in) (Liu et al., 1996; Smolders et al., 1994a; Smolders et al., 1994b). It is clear, therefore, that a shift in pH as a result of the shock event or corrective action strategy can inhibit EBPR. Although all three trains began with a pH between 7.01-7.11, only the corrective action and negative control trains were similar for the duration of the study (Figure 4-4).

Discussion

In both experimental runs it was clear that the positive control and corrective action trains were negatively affected by the toxic shock event. The positive control experienced a brief perturbation event, in which effluent phosphate peaked roughly 16-36 hours after the shock arrived in the primary clarifier (the impact of the primary clarifier on contaminant dissipation has been discussed elsewhere (Pinto et al., submitted). However, there was no noticeable impact on the system's carbon removal or nitrification ability. The denitrification capacity of the positive control was unstable, but this was likely the result of fluctuating dissolved oxygen levels in the aerobic zones which led to DO transfer into the anoxic zones. If a larger contaminant dose were used, it is likely that the positive control would experience a more significant loss in system performance, while the impact on the corrective action train would be similar to the results shown here.

It is worth noting that the pH of the corrective action train closely followed that of the negative control. The positive control, however, had a pH consistently below the negative control. This trend was only observed in the second shock event, making it unclear as to whether or not it was actually caused by the toxic shock.

In the corrective action train, both the side-stream reactor and AER 2 zones were aerated during storage. Dissolved oxygen from the AER 2 zone also bled into ANX 2, resulting in aerobic conditions throughout all stored sludge. Since both shock events lasted less than 16 hours, it is unlikely that significant polyP degradation took place during aerobic storage. This argument is

supported by the fact that the corrective action train's effluent phosphate did not approach or surpass the influent concentration after flow resumed.

Although the EBPR performance of the corrective action train was unstable in the days following the toxic shock event, the direct cause of this performance upset was unclear. It is well known that smaller WWTPs that experience low organic carbon loadings on weekends are susceptible to poor EBPR performance on Mondays and Tuesdays (Brdjanovic et al., 1998a; Carucci et al., 1999; Temmink et al., 1996), and it has been suggested that decreasing the aeration during periods of low carbon loading could potentially help conserve the internal energy pools of PAOs (Brdjanovic et al., 1998a; Temmink et al., 1996). In this study, dissolved oxygen levels were not controlled during storage. In fact, the ANX 2 DO rose as high as 6.0 $mg \cdot O_2/L$ before flow was restored. This elevated level of DO may have expedited the utilization of biopolymers, resulting in long-term loss of system performance. By maintaining a lower DO, the impact of aerobic endogenous respiration on the stability of the system could potentially be reduced.

If unaerated storage had been used, the available nitrate and organic carbon would have first been utilized by denitrifiers, including denitrifying PAOs (if present). After anaerobic conditions had been achieved, the PAOs would have begun to utilize their available biopolymers for cell maintenance (as seen in Lopez et al. (2006)). As the toxin passed through the train, isolated PAOs would likely have consumed the majority of their glycogen and polyP, resulting in elevated soluble phosphate concentrations. Additionally, the anaerobic storage would likely have resulted in a significant and rapid reduction in phosphate release and uptake rates. Finally, a reduction in pH resulting from fermentation reactions could also be expected.

It is unclear how anaerobic storage would affect the EBPR capacity of the system in the days following the corrective action. The stored sludge would have significant levels of soluble phosphate and would likely need to be returned to the front of the plant to achieve some level of EBPR. Otherwise, significant chemical addition would be required to precipitate out the excess phosphate. The initial performance (release/uptake rates) of the isolated PAOs would also be significantly inhibited, but it is unclear how quickly they would recover in the following days.

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Chapter 5

Conclusion

A number of process controls could potentially be implemented to mitigate the effects of a toxic shock. Ideally, contaminated influent would be stored in an on-site basin and the appropriate management method could be identified with the help of the regulatory agencies. Unfortunately, however, very few WWTPs have the capacity to store significant volumes of influent. Without this influent storage capability, the WWTP's next line of defense is operational flexibility. By isolating trains or reactors from contaminated influent, plants have the ability to protect biomass from toxins and prevent the washout of microorganisms that are crucial to the biological treatment of wastewater. However, potential process controls must be experimentally tested to better understand their short- and long-term effects on system performance. In order to test one set of identified process controls (designated the "corrective action strategy") on perturbation by a soluble toxic agent, a laboratory-scale 5-stage Bardenpho system was designed and operated. The effect of the corrective action strategy was evaluated during a simulated calcium hypochlorite shock event. The effect of the corrective action strategy was evaluated by comparing the performance of three identical trains; a negative control train (no process change, no shock), a positive control train (no process change, hypochlorite shock), and a corrective action train (process controls implemented, hypochlorite shock). The process controls implemented include isolating biomass from the toxin while storing it aerobically, stopping the internal recycle, and adding alum to the primary clarifier. This experiment was run twice, independently. Aerobic storage of biomass was selected based on a previous study by Lopez et

al. (2006) that showed less inhibition of phosphate release/uptake rates after one day of aerobic starvation versus anaerobic starvation.

The results of this study indicate that WWTPs may not be as susceptible to calcium hypochlorite as the contaminant prioritization framework suggests. Although a higher toxin load may have had more detrimental effects on system performance, a simulated 2,500 gallon spill of 6% calcium hypochlorite over 15 minutes did not significantly impact the system performance. The corrective action implemented, however, did severely inhibit EBPR stability for several days even after normal operation had resumed.

Regardless of whether aerobic or anaerobic storage conditions are selected, endogenous respiration will still result in the loss of important intracellular pools of energy among PAOs. It is recommended, therefore, that measures be taken to avoid imposing endogenous conditions on isolated sludge during a short-term toxic shock event. Many nutrient removal WWTPs have carbon sources on-site to aid denitrification processes. If the WWTP has the capability, the addition of organic carbon to isolated EBPR sludge under cycling conditions (ANA-AER-ANA-AER) could be the optimal storage alternative. The isolation of biomass, however, would only be recommended if the influent toxin were sufficient to significantly impact the performance of the WWTP or the community structure of the activated sludge. If biomass isolation is deemed necessary, recently developed techniques for mitigating the negative effects of sludge storage may be useful. One useful technique may be the cycling of stored biomass between aerobic and anaerobic conditions (Yilmaz et al., 2007). By aerating 15 minutes every 6 hours, it may be possible to store sludge for up to 2 weeks without a significant inhibition of P-release and uptake rates (Yilmaz et al., 2007).

It is clear from this study, however, that EBPR systems may have sufficient resilience to absorb toxic shocks of this scale without the need for corrective actions.

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Chapter 6

Engineering Significance

Toxic shock events can be detrimental to wastewater treatment systems and can result in long-term losses of system performance. If a plant has sufficient operational flexibility, it may have the ability to minimize biomass exposure to the influent toxin to prevent inhibition of biological processes. However, bacteria are sensitive to changes in environmental conditions, and process controls designed to mitigate a toxic shock may also be detrimental to system performance. By implementing carefully designed and pre-tested process controls during toxic shock events, WWTPs may be able to minimize the short- and long-term effects of a toxic shock. EBPR processes, in particular, are known to be particularly sensitive to changes in process parameters. From changes in DO and nitrate concentrations to variable pH and organic loading, many factors can negatively impact PAOs, the bacteria responsible for the EBPR process. It is crucial, therefore, that corrective action strategies be designed to minimize the impact on PAOs. Additionally, these corrective action strategies must be tested at the laboratory-scale to understand the potential effects of their implementation.

This study showed that calcium hypochlorite spills on the order of 2,500 gallons (at 6% $Ca(OCl)_2$) may not detrimental to WWTPs of this capacity (16 MGD). In the positive control train (simulating a WWTP that did not implement the corrective action strategy during the toxic shock), biological carbon, nitrogen, and phosphorus removal processes were not significantly affected. These results notwithstanding, the threat of performance losses resulting from toxic shocks is very real. If a more inhibitory toxin were used or if a greater dose of calcium hypochlorite had been simulated, the toxic shock may have caused the washout of crucial microorganisms. Even with bioaugmentation, washout of PAOs or nitrifiers could result in

weeks of permit violations. For this reason, we must continue to develop corrective action strategies that will minimize the impact on system performance.

Additionally, studies should be conducted to determine the impact of toxic shock events with insoluble contaminants. This study investigated the effect of a single shock with a soluble toxin. Had an insoluble toxin been used, the toxin may have become associated with biomass and remained in the system for an extended period. Had this prolonged exposure taken place, it may have had a drastically different effect on system performance. As a result, alternative corrective actions would need to be developed and tested to facilitate the removal of the biomass-associated toxin from the system.

Previous research suggested that short-term (less than one day) aerobic endogenous respiration would not significantly impact the rates of phosphate release and uptake by PAOs (Lopez et al., 2006). This research has shown, however, that this sludge storage technique can be detrimental to the stability of the EBPR system. More research needs to be performed to gain a better understanding of the impact of endogenous respiration (anaerobic, aerobic, and cycled anaerobic-aerobic) on PAO biopolymers, and how these intracellular energy pools translate to system stability. Additionally, the impact of endogenous respiration on PAO community structure should be investigated to determine how different storage strategies affect the diversity of PAO species in a EBPR sludge: it has been previously shown that diverse microbial communities can be far more stable under fluctuating environmental conditions (Miura et al., 2007; Naeem and Li, 1997; von Canstein et al., 2002).

Ultimately, the results of this research will be incorporated into a decision support system (DSS) for operators during toxic shock events. As a preliminary step towards the development of a DSS, a decision tree was developed based on the corrective actions discussed here (Pinto et al., submitted). This decision-making tool took into consideration the location in the system where the toxin was identified, the physical properties of the toxin (a tendency to remain in solution or sorb to biomass), and the operational flexibility of Long Creek WRRF. As laboratory testing of process controls continues, this preliminary DSS will be modified to incorporate superior corrective action strategies that will help operators mitigate the effects of toxic shock events. Ultimately, the likely process effects of contaminants will be incorporated into the DSS using

previously completed SBR studies that investigated the effects of various classes of toxins (e.g., electrophiles) on activated sludge (Henriques, in press-b).

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Appendix A

Supporting Data

The following tables list the raw values of the data represented in Chapter 4. These data were obtained from analytical techniques outlined in the Materials and Methods section (Chapter 3) of this thesis.

+/- : For analyses performed with three or more replicates, the +/- value represents the standard deviation of the values obtained. For analyses performed in duplicate, the +/- value is the larger replicate minus the average value of duplicate samples.

Table A-1. Effluent orthophosphate concentrations from shock event 1 – replicate data. Analysis performed in duplicate on an ion chromatograph.

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/L$)				Positive Control ($mg \cdot PO_4^{3-} \cdot P/L$)				Negative Control ($mg \cdot PO_4^{3-} \cdot P/L$)			
	Rep 1	Rep 2	Avg	+/-	Rep 1	Rep 2	Avg	+/-	Rep 1	Rep 2	Avg	+/-
0.0	2.37	2.75	2.56	0.19	0.44	0.49	0.46	0.03	1.39	1.37	1.38	0.01
0.7	6.15	6.23	6.19	0.04	1.73	1.77	1.75	0.02	2.02	2.20	2.11	0.09
1.2	1.32	1.83	1.57	0.26	0.55	0.36	0.45	0.09	1.56	1.49	1.53	0.04
1.8	0.82	0.91	0.87	0.04	0.92	0.97	0.95	0.02	1.15	1.57	1.36	0.21
2.2	1.72	1.90	1.81	0.09	1.66	1.72	1.69	0.03	2.40	2.35	2.37	0.03
2.7	4.52	4.59	4.56	0.04	1.02	1.00	1.01	0.01	0.23	n.a.	0.23	0.00
3.2	1.89	1.89	1.89	0.00	0.55	0.64	0.59	0.05	0.50	0.50	0.50	0.00
3.9	2.51	2.45	2.48	0.03	0.74	0.78	0.76	0.02	0.00	0.25	0.13	0.13
4.8	0.00	0.26	0.13	0.13	0.27	0.00	0.13	0.13	0.00	0.00	0.00	0.00
5.8	0.13	0.08	0.11	0.03	0.23	0.22	0.23	0.01	0.00	0.00	0.00	0.00
6.8	4.96	5.13	5.05	0.09	3.99	3.95	3.97	0.02	3.92	4.19	4.06	0.14
7.7	2.23	2.50	2.36	0.14	2.43	2.76	2.60	0.17	2.80	3.35	3.08	0.27
9.7	3.49	3.59	3.54	0.05	2.50	2.59	2.55	0.04	2.54	2.48	2.51	0.03

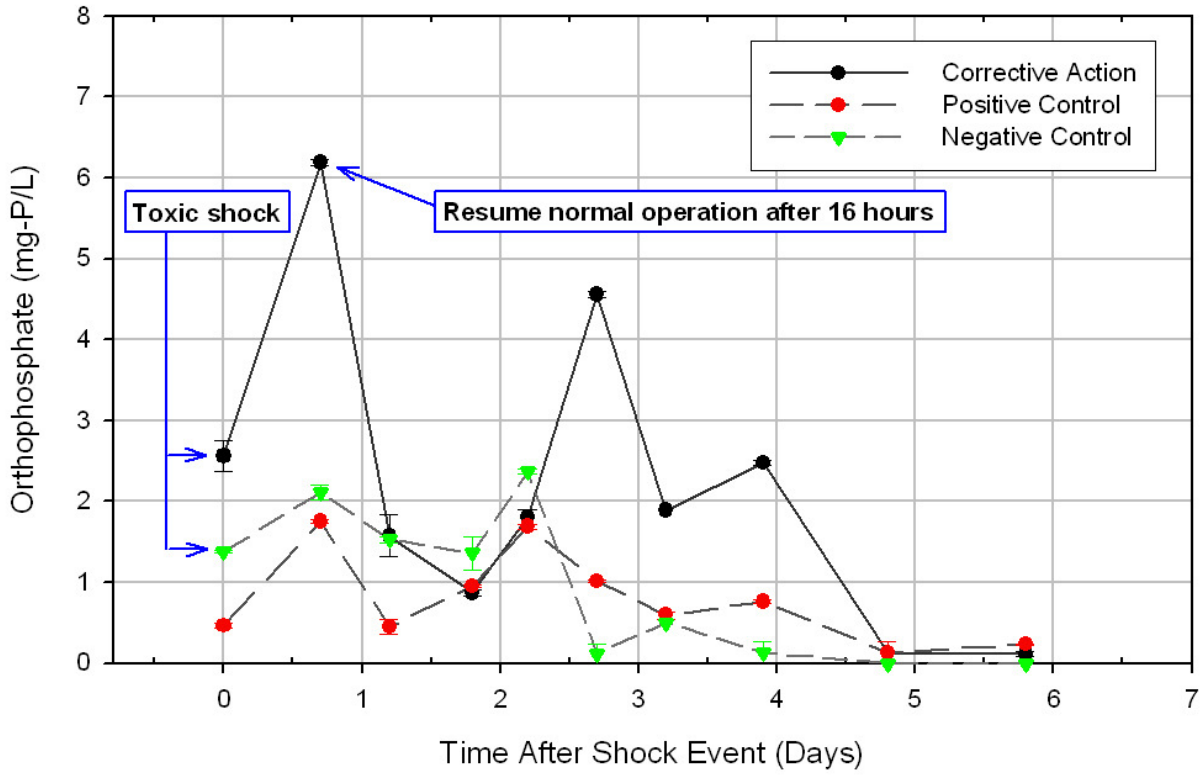


Figure A-1. Effluent orthophosphate concentrations during shock event 1.

Table A-2. Effluent orthophosphate concentrations from shock event 2 – replicate data (corrective action and positive control). Analysis performed in triplicate using the ascorbic acid method. The averages in this table are graphed in Figure 4-1 with error bars extending to ± the standard deviation.

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/L$)					Positive Control ($mg \cdot PO_4^{3-} \cdot P/L$)				
	Rep 1	Rep 2	Rep 3	Avg	Stdev	Rep 1	Rep 2	Rep 3	Avg	Stdev
0.0	0.58	0.60	0.56	0.58	0.02	0.30	0.28	0.27	0.28	0.01
0.3	0.82	0.77	0.74	0.78	0.04	0.71	0.69	0.72	0.71	0.01
0.7	1.00	0.94	0.86	0.93	0.07	0.84	0.86	0.80	0.84	0.03
1.0	1.64	1.49	1.49	1.54	0.09	1.03	0.89	0.97	0.96	0.07
1.5	1.81	1.69	1.65	1.72	0.08	2.38	2.27	2.26	2.30	0.07
2.0	0.48	0.50	0.50	0.49	0.01	-	-	-	-	-
2.5	1.10	1.07	1.11	1.10	0.02	0.54	0.53	0.53	0.53	0.01
3.0	0.97	0.90	0.84	0.90	0.07	0.54	0.53	0.51	0.52	0.01
4.0	2.92	2.84	2.77	2.85	0.07	0.85	0.84	0.83	0.84	0.01
5.0	4.46	3.71	3.51	3.89	0.50	2.29	2.31	2.33	2.31	0.02
6.0	2.22	2.22	2.13	2.19	0.05	1.68	1.66	1.62	1.65	0.03
7.0	3.66	2.75	2.80	3.07	0.51	1.97	2.17	2.08	2.07	0.10
8.0	2.13	2.07	2.02	2.07	0.05	1.50	1.56	1.53	1.53	0.03
9.0	1.50	1.50	1.48	1.50	0.01	0.09	0.10	0.06	0.08	0.02
10.0	1.53	2.18	1.53	1.75	0.37	2.70	2.63	2.70	2.68	0.04
11.0	1.21	1.15	1.18	1.18	0.03	2.09	2.11	2.11	2.10	0.01
12.0	1.42	1.39	1.35	1.39	0.04	3.72	3.93	3.77	3.80	0.11
13.0	0.91	0.96	0.88	0.92	0.04	1.99	1.86	2.05	1.97	0.10
14.0	1.55	1.54	1.36	1.48	0.11	0.56	0.55	0.57	0.56	0.01
16.0	0.66	0.67	0.64	0.66	0.02	0.06	0.06	0.06	0.06	0.00
18.0	0.58	0.56	0.57	0.57	0.01	0.03	0.03	0.03	0.03	0.00

Table A-3. Effluent orthophosphate concentrations from shock event 2 – replicate data (negative control). Analysis performed in triplicate using the ascorbic acid method. The averages in this table are graphed in Figure 4-1 with error bars extending to ± the standard deviation.

Days After Shock Event	Negative Control ($mg \cdot PO_4^{3-} \cdot P/L$)				
	Rep 1	Rep 2	Rep 3	Avg	Stdev
0.0	1.01	1.02	1.01	1.02	0.01
0.3	1.30	1.21	1.27	1.26	0.05
0.7	0.55	0.57	0.55	0.56	0.01
1.0	0.51	0.50	0.50	0.50	0.00
1.5	0.17	0.16	0.16	0.16	0.00
2.0	0.10	0.10	0.10	0.10	0.00
2.5	0.47	0.44	0.40	0.44	0.03
3.0	0.25	0.25	0.23	0.24	0.01
4.0	1.23	1.20	1.17	1.20	0.03
5.0	0.82	0.83	0.81	0.82	0.01
6.0	1.54	1.36	1.38	1.43	0.10
7.0	1.62	1.56	1.53	1.57	0.05
8.0	1.40	1.47	1.52	1.46	0.06
9.0	0.16	0.18	0.12	0.15	0.03
10.0	0.86	0.93	0.92	0.91	0.04
11.0	3.71	3.51	3.21	3.48	0.25
12.0	4.36	4.26	4.46	4.36	0.10
14.0	1.17	1.09	1.15	1.14	0.05
16.0	4.29	3.88	3.95	4.04	0.22
18.0	9.26	8.56	8.32	8.71	0.48

Table A-4. Composite influent orthophosphate concentrations from shock event 2 – replicate data. Analysis performed in triplicate using the ascorbic acid method. The averages in this table are graphed in Figure 4-1 with error bars extending to ± the standard deviation.

Days After Shock Event	Composite Influent ($mg \cdot PO_4^{3-} \cdot P/L$)				
	Rep 1	Rep 2	Rep 3	Avg	Stdev
-1 → 0	2.50	2.85	2.10	2.49	0.38
0 → 1	2.37	1.83	1.70	1.97	0.36
1 → 2	2.52	2.01	2.46	2.33	0.28
3 → 4	8.58	9.03	9.08	8.90	0.28
4 → 5	4.11	3.76	3.26	3.71	0.43
5 → 6	5.16	4.16	3.01	4.11	1.08
6 → 7	7.18	7.58	7.18	7.32	0.23
7 → 8	5.26	4.46	3.16	4.29	1.06
8 → 9	3.76	4.36	4.36	4.16	0.35
9 → 10	4.26	4.16	4.11	4.17	0.08
10 → 11	5.81	5.81	5.66	5.76	0.09
11 → 12	4.36	4.26	4.41	4.34	0.08
12 → 13	4.96	5.11	4.91	4.99	0.10
13 → 14	5.56	6.01	5.71	5.76	0.23
14 → 15	5.46	5.16	4.06	4.89	0.74
16 → 17	6.66	6.56	6.86	6.69	0.15

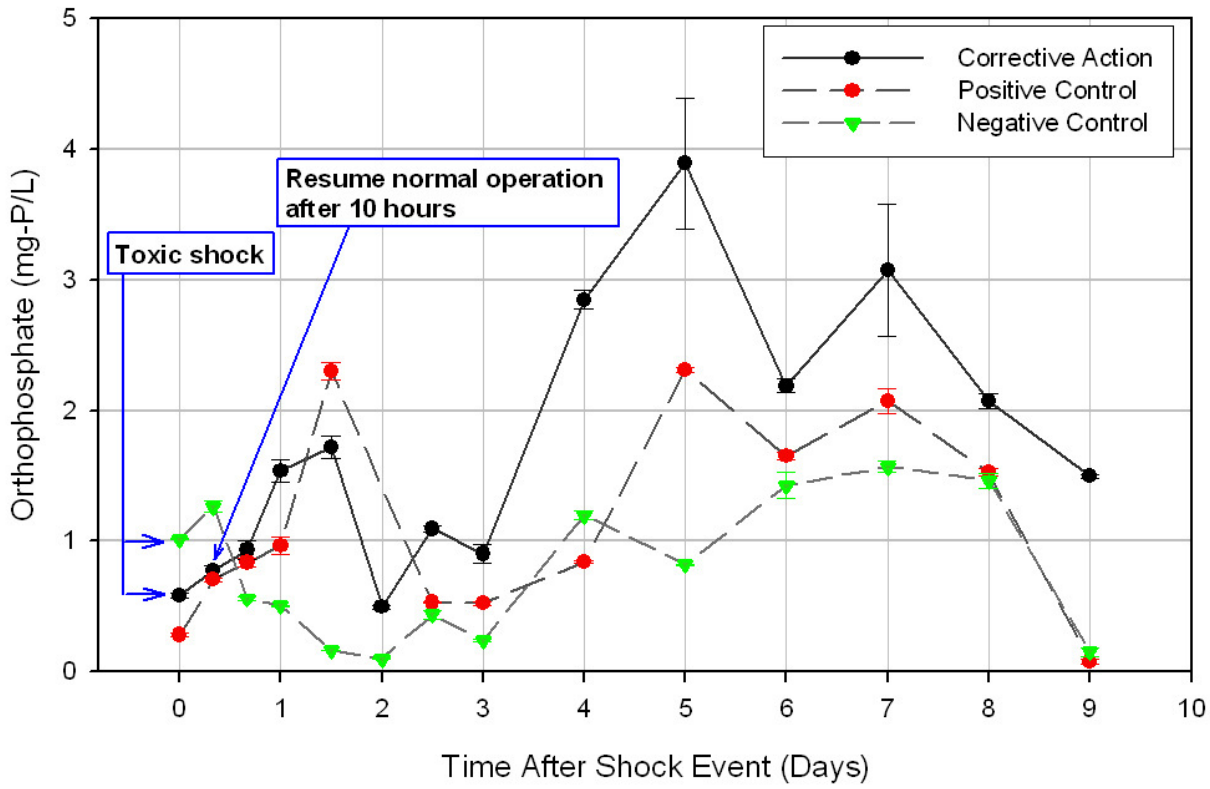


Figure A-2. Effluent orthophosphate concentrations during shock event 2.

During the second shock event, the EBPR capability of the negative and positive controls appeared to be significantly inhibited after day 9 of the study. This, however, did not appear to be related to the shock event. From days 9 to 13, it is likely that the readily biodegradable organic carbon in the influent was diminished. This argument is supported by an increase in effluent nitrate in all three trains and an increase in AER 1 DO levels in both the positive control and corrective action trains (data not shown). Following day 14, the effluent phosphate from the negative control increased drastically, surpassing the estimated influent concentration. It is likely that this perturbation was the result of insufficient DO in the AER 1 zone of the negative control: between days 15 and 16, the negative control DO in AER 1 had dropped from 1.5 mg·O_2/L to 0.5 mg·O_2/L. In addition to causing PAO phosphate release into the effluent, the drop in DO also resulted in increased effluent ammonia and complete denitrification that may have resulted from increased anoxic volumes (data not shown).

The argument that the performance on days 10-18 is unrelated to the corrective action strategy is further supported by the specific oxygen uptake rates (sOURs) of the mixed liquors (Figure 4.4).

Although there were large variations in the sOURs over the first 5 days of the study, it appears that they had begun to converge by day 9.

Table A-5. Oxygen uptake rates (OURs) during shock event 1 – replicate data. Analyses performed in duplicate with mixed liquor from aerobic 1.

Days After Shock Event	Corrective Action ($mg \cdot O_2/min$)				Positive Control ($mg \cdot O_2/min$)				Negative Control ($mg \cdot O_2/min$)			
	Rep 1	Rep 2	Avg	+/-	Rep 1	Rep 2	Avg	+/-	Rep 1	Rep 2	Avg	+/-
0.0	0.89	0.82	0.85	0.04	1.35	1.34	1.34	0.01	1.15	0.97	1.06	0.09
0.7	0.39	0.43	0.41	0.02	1.07	1.13	1.10	0.03	0.70	0.76	0.73	0.03
1.8	1.04	0.81	0.92	0.12	1.27	0.99	1.13	0.14	0.89	0.69	0.79	0.10
2.7	0.56	0.67	0.61	0.05	1.74	2.11	1.93	0.18	1.43	1.70	1.57	0.13
4.8	1.82	1.63	1.72	0.10	1.98	1.71	1.85	0.14	1.62	1.37	1.49	0.13
6.8	2.90	2.50	2.70	0.20	2.77	2.38	2.58	0.20	2.73	2.25	2.49	0.24

Table A-6. Mixed liquor volatile suspended solids (MLVSS) in Aerobic 1 during shock event 1 – replicate data (corrective action and positive control). Analysis performed in triplicate as described in Chapter 3. If data from only 2 replicates are available, the value in the standard deviation column is the larger replicate minus the average value of duplicate samples.

Days After Shock Event	Corrective Action (mg·MLVSS/L)					Positive Control (mg·MLVSS/L)				
	Rep 1	Rep 2	Rep 3	Avg	Stdev	Rep 1	Rep 2	Rep 3	Avg	Stdev
0.0	-	1,550	1,620	1,585	35*	1,680	1,490	1,610	1,593	96
0.7	400	570	640	537	123	1,810	1,740	1,900	1,817	80
1.8	1,250	1,200	1,180	1,210	36	1,640	1,590	1,510	1,580	66
2.7	600	610	500	570	61	1,740	1,800	1,770	1,770	30
4.8	1,660	1,700	1,630	1,663	35	1,700	1,730	1,730	1,720	17
6.8	1,560	1,520	1,600	1,560	40	2,330	2,400	2,400	2,377	40

Table A-7. Mixed liquor volatile suspended solids (MLVSS) in Aerobic 1 during shock event 1 – replicate data (negative control). Analysis performed in triplicate as described in Chapter 3.

Days After Shock Event	Negative Control (mg·MLVSS/L)				
	Rep 1	Rep 2	Rep 3	Avg	Stdev
0.0	1,370	1,330	1,310	1,337	31
0.7	1,080	960	1,070	1,037	67
1.8	980	980	990	983	6
2.7	1,430	1,530	1,510	1,490	53
4.8	1,430	1,350	1,390	1,390	40
6.8	2,200	2,130	2,140	2,157	38

Specific oxygen uptake rates (sOURs) were calculated as follows:

$$C \pm c = \frac{A}{B} \pm \left[\left(\frac{A}{B} \right) * \sqrt{\left(\frac{a}{A} \right)^2 + \left(\frac{b}{B} \right)^2} \right]$$

Where:

C	≡	average sOUR (mg·O_2/g·MLVSS·min)
c	≡	+/- sOUR (mg·O_2/g·MLVSS·min)
A	≡	average OUR (mg·O_2/min)
a	≡	+/- OUR (mg·O_2/min)
B	s≡	average MLVSS (g·MLVSS/L)
b	≡	+/- MLVSS (g·MLVSS/L)

Table A-8. Specific oxygen uptake rates (sOURs) during shock event 1.

Days After Shock Event	Corrective Action (mg·O_2/g·MLVSS·min)	Positive Control (mg·O_2/g·MLVSS·min)	Negative Control (mg·O_2/g·MLVSS·min)
0.0	0.54 ± 0.03	0.84 ± 0.05	0.79 ± 0.07
0.7	0.77 ± 0.18	0.60 ± 0.03	0.70 ± 0.05
1.8	0.76 ± 0.10	0.72 ± 0.09	0.80 ± 0.11
2.7	1.08 ± 0.15	1.09 ± 0.11	1.05 ± 0.10
4.8	1.04 ± 0.06	1.07 ± 0.08	1.07 ± 0.10
6.8	1.73 ± 0.14	1.08 ± 0.08	1.16 ± 0.11

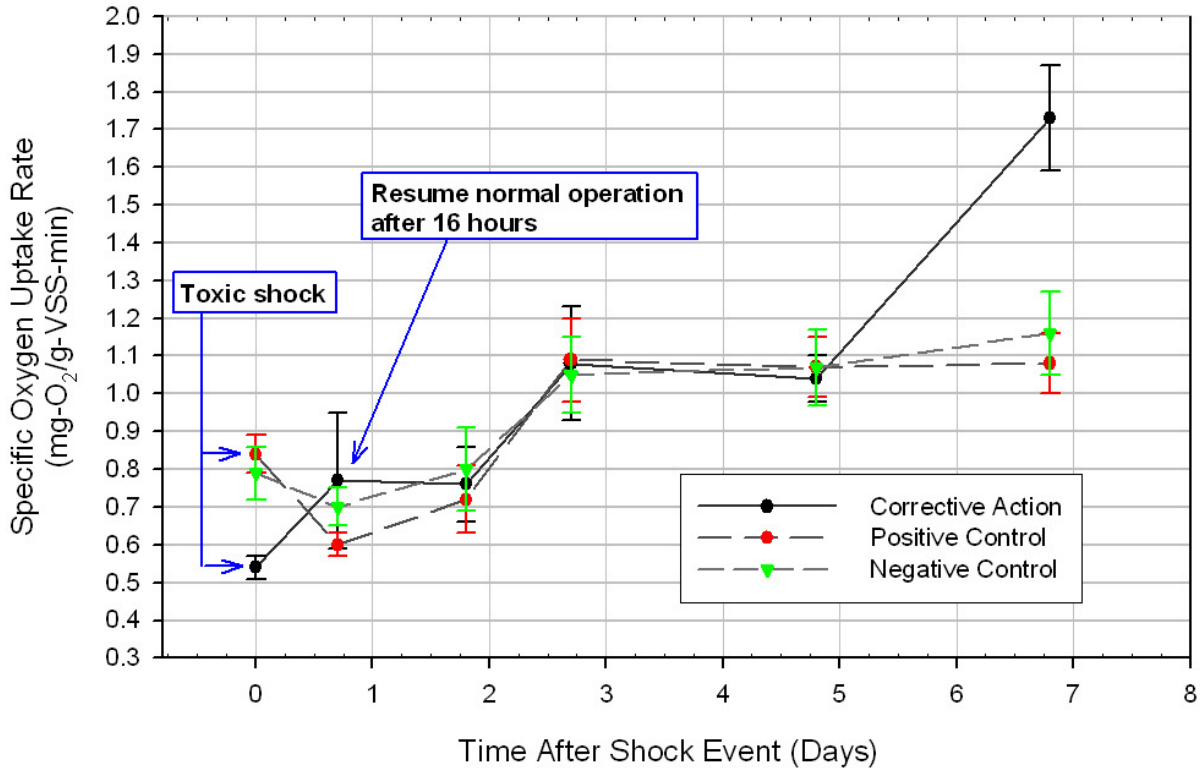


Figure A-3. sOURs during shock event 1.

Table A-9. OURs during shock event 2 – replicate data. Analyses performed in duplicate with mixed liquor from aerobic 1.

Days After Shock Event	Corrective Action ($mg \cdot O_2/min$)				Positive Control ($mg \cdot O_2/min$)				Negative Control ($mg \cdot O_2/min$)			
	Rep 1	Rep 2	Avg	+/-	Rep 1	Rep 2	Avg	+/-	Rep 1	Rep 2	Avg	+/-
0.0	0.50	0.51	0.51	0.01	1.03	0.95	0.99	0.04	0.89	0.88	0.89	0.00
1.0	0.35	0.33	0.34	0.01	1.12	1.11	1.12	0.01	0.55	0.53	0.54	0.01
2.0	0.87	0.84	0.85	0.02	-	-	-	-	0.86	0.82	0.84	0.02
3.0	0.82	0.82	0.82	0.00	0.73	0.85	0.79	0.06	0.56	0.63	0.60	0.04
5.0	0.76	0.74	0.75	0.01	0.80	0.74	0.77	0.03	0.47	0.43	0.45	0.02
9.0	1.29	1.34	1.32	0.03	1.21	1.11	1.16	0.05	0.96	0.92	0.94	0.02
12.0	1.24	1.12	1.18	0.06	0.95	1.00	0.97	0.03	0.64	0.70	0.67	0.03
14.0	1.08	1.00	1.04	0.04	0.92	0.87	0.90	0.02	1.12	1.22	1.17	0.05
16.0	2.08	2.34	2.21	0.13	2.11	-	2.11	-	1.85	1.83	1.84	0.01

Table A-10. MLVSS in Aerobic 1 during shock event 2 – replicate data. Analysis performed in duplicate as described in Chapter 3.

Days After Shock Event	Corrective Action (mg·MLVSS/L)	Positive Control (mg·MLVSS/L)	Negative Control (mg·MLVSS/L)
0.0	805 ± 45	1925 ± 15	1450 ± 20
1.0	740 ± 0	1695 ± 25	635 ± 15
2.0	1053 ± 12	1715 ± 25	1035 ± 5
3.0	*863 ± 30	*1177 ± 21	*808 ± 8
5.0	855 ± 5	1210 20	1195 ± 15
9.0	1560 ± 10	1600 ± 20	1205 ± 5
12.0	2205 ± 15	1865 ± 35	1390 ± 30
14.0	2305 ± 35	2095 ± 15	2025 ± 35
16.0	2895 ± 5	2445 ± 25	2435 ± 5

Table A-11. sOURs during shock event 2. The averages in this table are graphed in Figure 4.3 with error bars extending to ± the difference between the larger replicate and the average replicate (of two samples).

Days After Shock Event	Corrective Action ($mg \cdot O_2/g \cdot MLVSS \cdot min$)	Positive Control ($mg \cdot O_2/g \cdot MLVSS \cdot min$)	Negative Control ($mg \cdot O_2/g \cdot MLVSS \cdot min$)
0.0	0.63 ± 0.04	0.51 ± 0.02	0.61 ± 0.01
1.0	0.46 ± 0.01	0.66 ± 0.01	0.85 ± 0.02
2.0	0.81 ± 0.02	- ± -	0.81 ± 0.02
3.0	0.95 ± 0.03	0.67 ± 0.05	0.74 ± 0.04
5.0	0.87 ± 0.01	0.64 ± 0.03	0.38 ± 0.02
9.0	0.84 ± 0.02	0.73 ± 0.03	0.78 ± 0.02
12.0	0.54 ± 0.03	0.52 ± 0.02	0.48 ± 0.02
14.0	0.45 ± 0.02	0.43 ± 0.01	0.58 ± 0.03
16.0	0.76 ± 0.04	0.86 ± 0.01	0.76 ± 0.01

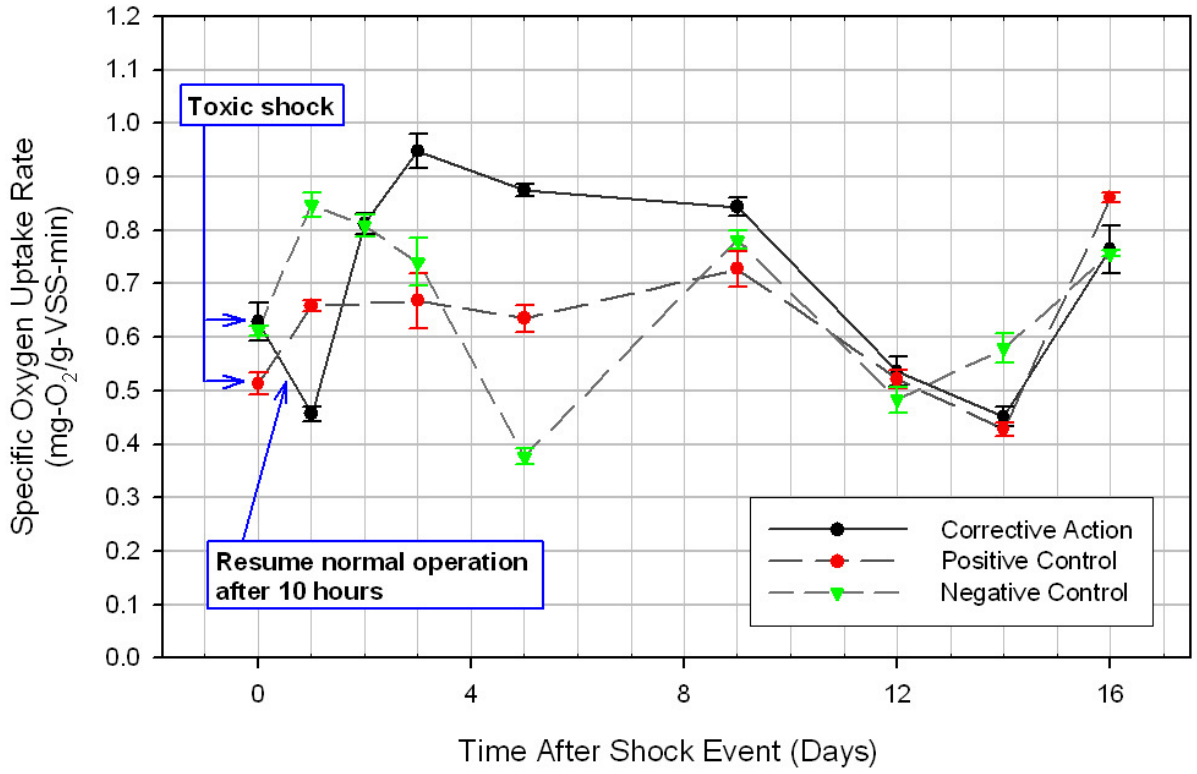


Figure A-4. sOURs during shock event 2.

Table A-12. System pH readings during shock event 2. These values are graphed in Figure 4-4.

Days After Shock Event	Corrective Action pH	Positive Control pH	Negative Control pH
	Aerobic 1	Aerobic 1	Aerobic 1
0.0	7.03	7.01	7.11
0.3	7.51	7.14	7.64
0.7	7.24	7.00	7.41
1.0	7.34	7.07	7.43
2.0	7.18	-	7.22
2.5	7.22	7.10	7.29
3.0	7.22	7.44	7.57
4.0	7.39	7.49	7.59
5.0	7.19	7.11	7.22
6.0	7.25	7.11	7.28
7.0	7.05	6.88	7.06
8.0	7.12	6.90	7.07
9.0	7.11	6.73	7.04
10.0	7.17	6.85	7.03
11.0	7.19	6.77	7.05
13.0	7.08	6.86	7.12
14.0	7.08	6.75	7.10
15.0	7.21	6.85	7.22
16.0	7.29	7.01	7.30
17.0	7.10	6.93	7.26

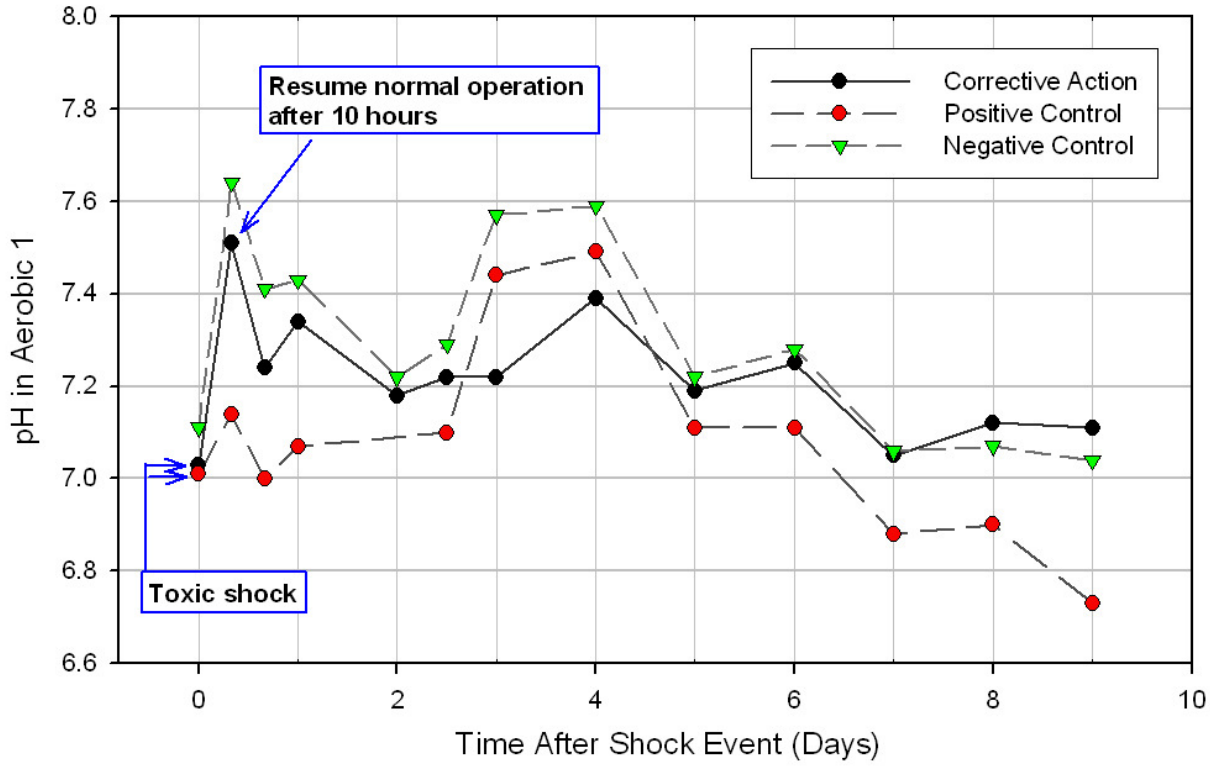


Figure A-5. pH of first aerobic zone during shock event 2.

Table A-13. Estimated percent removal of orthophosphate during shock event 2. Values are based on effluent concentrations relative to the composite influent concentration from the preceding 24 hours. The ± represents propagated error from triplicate influent and effluent orthophosphate analyses. The averages in this table are graphed in Figure 4.2 with error bars extending to ± the propagated error.

Days After Shock Event	Corrective Action (% of influent removed)	Positive Control (% of influent removed)	Negative Control (% of influent removed)
0.0	76.5 ± 11.6	88.6 ± 13.4	59.1 ± 8.9
0.5	21.9 ± 4.0	51.0 ± 9.2	74.4 ± 13.4
1.5	78.8 ± 9.4	±	95.6 ± 11.4
3.5	68.0 ± 2.1	90.6 ± 2.8	86.5 ± 2.7
4.5	-5.0 ± 0.6	37.7 ± 4.3	77.9 ± 9.0
5.5	46.7 ± 12.3	59.7 ± 15.7	65.2 ± 17.1
6.5	58.0 ± 1.8	71.7 ± 2.3	78.5 ± 2.5
7.5	51.7 ± 12.8	64.4 ± 15.9	65.9 ± 16.3
8.5	64.0 ± 5.3	98.0 ± 8.2	96.3 ± 8.0

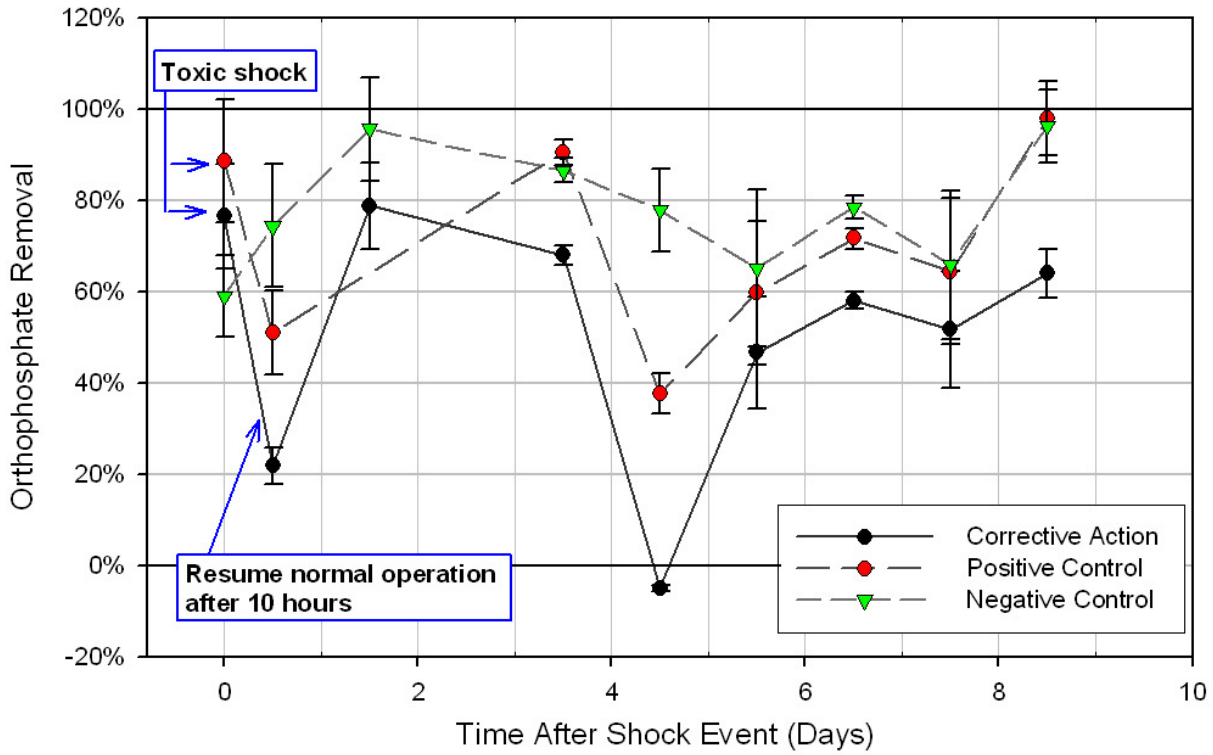


Figure A-6. Estimated percent removal of orthophosphate during shock event 2. Values are based on effluent concentrations relative to the composite influent concentration from the preceding 24 hours. Error bars represent ± propagated error from triplicate influent and effluent orthophosphate analyses (see Table A-13).

Appendix B

Supplementary Data

Composite sampling: The 200 liter influent tank was filled daily with raw sewage. Composite samples were collected hourly from the influent tank and stored below 4 °C until collection, filtration, and storage.

Table B-1. Effluent orthophosphate concentrations during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/L$)	Positive Control ($mg \cdot PO_4^{3-} \cdot P/L$)	Negative Control ($mg \cdot PO_4^{3-} \cdot P/L$)
0.0	2.56 ± 0.19	0.46 ± 0.03	1.38 ± 0.01
0.7	6.19 ± 0.04	1.75 ± 0.02	2.11 ± 0.09
1.2	1.57 ± 0.26	0.45 ± 0.09	1.53 ± 0.04
1.8	0.87 ± 0.04	0.95 ± 0.02	1.36 ± 0.21
2.2	1.81 ± 0.09	1.69 ± 0.03	2.37 ± 0.03
2.7	4.56 ± 0.04	1.01 ± 0.01	0.12 ± 0.12
3.2	1.89 ± 0.00	0.59 ± 0.05	0.50 ± 0.00
3.9	2.48 ± 0.03	0.76 ± 0.02	0.13 ± 0.13
4.8	0.13 ± 0.13	0.13 ± 0.13	0.00 ± 0.00
5.8	0.11 ± 0.03	0.23 ± 0.01	0.00 ± 0.00
6.8	5.05 ± 0.09	3.97 ± 0.02	4.06 ± 0.14
7.7	2.36 ± 0.14	2.60 ± 0.17	3.08 ± 0.27
9.7	3.54 ± 0.05	2.55 ± 0.04	2.51 ± 0.03

Table B-2. Effluent orthophosphate concentrations during shock event 2 (analyses performed in triplicate).

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/L$)	Positive Control ($mg \cdot PO_4^{3-} \cdot P/L$)	Negative Control ($mg \cdot PO_4^{3-} \cdot P/L$)
0.0	0.58 ± 0.02	0.28 ± 0.01	1.02 ± 0.01
0.3	0.78 ± 0.04	0.71 ± 0.01	1.26 ± 0.05
0.7	0.93 ± 0.07	0.84 ± 0.03	0.56 ± 0.01
1.0	1.54 ± 0.09	0.96 ± 0.07	0.50 ± 0.00
1.5	1.72 ± 0.08	2.30 ± 0.07	0.16 ± 0.00
2.0	0.49 ± 0.01	- ± -	0.10 ± 0.00
2.5	1.10 ± 0.02	0.53 ± 0.01	0.44 ± 0.03
3.0	0.90 ± 0.07	0.52 ± 0.01	0.24 ± 0.01
4.0	2.85 ± 0.07	0.84 ± 0.01	1.20 ± 0.03
5.0	3.89 ± 0.50	2.31 ± 0.02	0.82 ± 0.01
6.0	2.19 ± 0.05	1.65 ± 0.03	1.43 ± 0.10
7.0	3.07 ± 0.51	2.07 ± 0.10	1.57 ± 0.05
8.0	2.07 ± 0.05	1.53 ± 0.03	1.46 ± 0.06
9.0	1.50 ± 0.01	0.08 ± 0.02	0.15 ± 0.03
10.0	1.75 ± 0.37	2.68 ± 0.04	0.91 ± 0.04
11.0	1.18 ± 0.03	2.10 ± 0.01	3.48 ± 0.25
12.0	1.39 ± 0.04	3.80 ± 0.11	4.36 ± 0.10
13.0	0.92 ± 0.04	1.97 ± 0.10	4.00 ± 0.20
14.0	1.48 ± 0.11	0.56 ± 0.01	1.14 ± 0.05
16.0	0.66 ± 0.02	0.06 ± 0.00	4.04 ± 0.22
18.0	0.57 ± 0.01	0.03 ± 0.00	8.71 ± 0.48

Table B-3. Composite influent orthophosphate concentrations during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Composite Influent ($mg \cdot PO_4^{3-} \cdot P/L$)
1.8 → 2.7	4.18 ± 0.06
2.7 → 3.9	3.23 ± 0.32
3.9 → 4.8	3.56 ± 0.02
4.8 → 5.8	4.52 ± 0.01
5.8 → 6.8	5.01 ± 0.01
6.8 → 7.7	1.77 ± 0.00
8.7 → 9.7	2.66 ± 0.00

Table B-4. Composite influent orthophosphate concentrations during shock event 2 (analyses performed in triplicate).

Days After Shock Event	Composite Influent ($mg \cdot PO_4^{3-} \cdot P/L$)
-1 → 0	2.49 ± 0.38
0 → 1	1.97 ± 0.36
1 → 2	2.33 ± 0.28
3 → 4	8.90 ± 0.28
4 → 5	3.71 ± 0.43
5 → 6	4.11 ± 1.08
6 → 7	7.32 ± 0.23
7 → 8	4.29 ± 1.06
8 → 9	4.16 ± 0.35
9 → 10	4.17 ± 0.08
10 → 11	5.76 ± 0.09
11 → 12	4.34 ± 0.08
12 → 13	4.99 ± 0.10
13 → 14	5.76 ± 0.23
14 → 15	4.89 ± 0.74
16 → 17	6.69 ± 0.15

Table B-5. Effluent ammonia concentrations during shock event 1 (duplicate dilutions analyzed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot NH_3 \cdot N/L$)	Positive Control ($mg \cdot NH_3 \cdot N/L$)	Negative Control ($mg \cdot NH_3 \cdot N/L$)
0.0	1.1 ± 0.0	1.7 ± 0.2	0.9 ± 0.1
0.7	4.2 ± 0.1	0.3 ± 0.0	0.7 ± 0.1
1.2	2.2 ± 0.0	0.7 ± 0.1	0.9 ± 0.0
1.8	1.0 ± 0.1	0.6 ± 0.0	1.0 ± 0.1
2.2	0.7 ± 0.2	1.2 ± 0.1	1.6 ± 0.3
2.7	1.5 ± 0.0	1.3 ± 0.5	0.0 ± 0.0
3.2	1.0 ± 0.0	1.3 ± 0.1	0.8 ± 0.0
3.9	6.1 ± 1.2	6.6 ± 0.3	1.4 ± 0.5
4.8	0.0 ± 0.0	9.6 ± 0.8	0.7 ± 0.9
5.8	0.0 ± 0.0	3.3 ± 0.6	0.0 ± 0.0
6.8	0.0 ± 0.0	11.0 ± 0.1	5.6 ± 1.6
7.7	0.0 ± 0.0	18.1 ± 1.0	11.2 ± 0.6
9.7	0.0 ± 0.0	12.3 ± 1.0	1.9 ± 1.2

Table B-6. Effluent ammonia concentrations during shock event 2 (duplicate dilutions analyzed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot NH_3 \cdot N/L$)	Positive Control ($mg \cdot NH_3 \cdot N/L$)	Negative Control ($mg \cdot NH_3 \cdot N/L$)
0.0	1.1 ± 0.1	0.0 ± 0.0	7.1 ± 0.2
0.3	0.0 ± 0.0	0.0 ± 0.0	17.4 ± 0.2
0.7	17.2 ± 0.3	0.0 ± 0.0	10.2 ± 0.1
1.0	14.7 ± 0.1	0.0 ± 0.0	6.3 ± 0.1
1.5	16.3 ± 0.3	0.0 ± 0.0	0.9 ± 0.0
2.0	11.6 ± 0.0	- ± -	1.1 ± 0.3
2.5	12.2 ± 0.2	2.2 ± 0.0	0.0 ± 0.0
3.0	12.4 ± 0.3	0.1 ± 0.0	0.1 ± 0.2
4.0	9.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
5.0	20.3 ± 0.2	8.1 ± 0.1	5.6 ± 0.2
6.0	25.9 ± 0.4	11.3 ± 0.0	10.8 ± 0.2
7.0	23.5 ± 0.3	11.3 ± 0.1	12.0 ± 0.3
8.0	18.3 ± 0.3	4.9 ± 0.0	9.4 ± 0.8
9.0	21.7 ± 0.5	3.2 ± 0.0	10.6 ± 0.1
10.0	23.7 ± 0.2	6.3 ± 0.1	9.9 ± 0.0
11.0	24.1 ± 0.4	1.5 ± 0.0	9.9 ± 0.2
12.0	11.4 ± 0.1	1.0 ± 0.0	8.7 ± 0.1
13.0	10.4 ± 0.1	0.0 ± 0.0	9.2 ± 0.2
14.0	15.3 ± 0.2	0.3 ± 0.0	15.0 ± 0.1
16.0	24.9 ± 0.3	2.1 ± 0.0	24.1 ± 0.1

Table B-7. Composite influent ammonia concentrations during shock event 1 (duplicate dilutions analyzed in duplicate).

Days After Shock Event	Composite Influent ($mg \cdot NH_3 \cdot N/L$)		
0.7 → 1.8	6.1	±	0.2
1.8 → 2.7	4.3	±	0.1
2.7 → 3.9	18.2	±	0.9
3.9 → 4.8	24.0	±	1.0
4.8 → 5.8	16.1	±	5.0
5.8 → 6.8	13.5	±	3.8
6.8 → 7.7	24.3	±	4.2
7.7 → 8.7	21.8	±	1.6
8.7 → 9.7	13.1	±	2.9

Table B-8. Composite influent ammonia concentrations during shock event 2 (duplicate dilutions analyzed in duplicate).

Days After Shock Event	Composite Influent ($mg \cdot NH_3 \cdot N/L$)		
-1 → 0	28.4	±	0.4
0 → 1	24.5	±	0.4
1 → 2	25.8	±	0.3
2 → 3	32.0	±	0.7
3 → 4	30.4	±	1.4
4 → 5	40.3	±	0.4
5 → 6	42.4	±	0.5
6 → 7	42.7	±	1.0
8 → 9	41.9	±	1.1
9 → 10	32.5	±	0.8
10 → 11	50.6	±	1.2
11 → 12	44.3	±	0.7
12 → 13	35.1	±	1.3
13 → 14	65.5	±	1.4
15 → 16	39.4	±	0.8

Table B-9. Effluent nitrate concentrations during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot NO_3^- \cdot N/L$)			Positive Control ($mg \cdot NO_3^- \cdot N/L$)			Negative Control ($mg \cdot NO_3^- \cdot N/L$)		
0.0	9.5	±	0.3	10.0	±	1.0	6.1	±	0.0
0.7	1.9	±	0.0	9.5	±	0.0	7.4	±	1.2
1.2	6.0	±	0.9	7.3	±	0.9	7.9	±	0.0
1.8	7.3	±	0.0	7.6	±	0.0	6.7	±	1.4
2.2	7.7	±	0.0	7.1	±	0.0	8.3	±	0.0
2.7	3.7	±	0.0	6.8	±	0.1	10.0	±	0.0
3.2	5.8	±	0.0	4.9	±	0.0	7.8	±	0.0
3.9	5.0	±	0.0	4.5	±	0.0	7.3	±	0.0
4.8	7.4	±	0.1	2.7	±	0.1	5.2	±	0.0
5.8	8.2	±	0.0	3.9	±	0.0	10.0	±	0.0
6.8	33.3	±	0.1	29.7	±	0.0	0.5	±	0.0
7.7	21.9	±	0.4	0.5	±	0.0	0.4	±	0.0
9.7	30.1	±	0.3	0.4	±	0.1	20.1	±	0.1

Table B-10. Effluent nitrate concentrations during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot NO_3^- \cdot N/L$)	Positive Control ($mg \cdot NO_3^- \cdot N/L$)	Negative Control ($mg \cdot NO_3^- \cdot N/L$)
0.0	2.2 ± 0.0	1.9 ± 0.0	0.9 ± 0.0
0.3	3.3 ± 0.1	5.6 ± 0.3	0.5 ± 0.0
0.7	0.7 ± 0.0	4.7 ± 0.2	1.7 ± 0.0
1.0	1.3 ± 0.0	2.1 ± 0.1	2.2 ± 0.0
1.5	0.7 ± 0.0	7.1 ± 0.3	2.5 ± 0.1
2.0	2.3 ± 0.0	4.9 ± 0.2	3.0 ± 0.0
2.5	1.6 ± 0.0	3.2 ± 0.1	7.6 ± 0.3
3.0	1.7 ± 0.0	3.6 ± 0.1	2.4 ± 0.0
4.0	0.5 ± 0.1	2.0 ± 0.0	7.7 ± 0.1
5.0	0.0 ± 0.0	2.9 ± 0.0	1.5 ± 0.1
6.0	0.3 ± 0.0	0.0 ± 0.0	5.3 ± 0.4
7.0	0.4 ± 0.0	2.0 ± 0.0	2.4 ± 0.1
8.0	0.2 ± 0.0	1.5 ± 0.0	2.4 ± 0.1
9.0	0.5 ± 0.1	3.8 ± 0.0	2.6 ± 0.0
10.0	0.1 ± 0.0	2.9 ± 0.1	1.6 ± 0.0
11.0	0.1 ± 0.0	6.1 ± 0.2	2.1 ± 0.0
12.0	2.0 ± 0.6	5.7 ± 0.1	3.4 ± 0.1
13.0	1.2 ± 0.4	3.5 ± 0.0	0.1 ± 0.0
14.0	2.9 ± 0.1	8.2 ± 0.8	1.9 ± 0.2
16.0	0.1 ± 0.0	1.5 ± 0.0	0.1 ± 0.0

Table B-11. Mixed liquor suspended solids (MLSS) concentrations in Aerobic 1 during shock event 1 (analyses performed in triplicate).

Days After Shock Event	Corrective Action (mg·MLSS/L)	Positive Control (mg·MLSS/L)	Negative Control (mg·MLSS/L)
0.0	2283 ± 150	2353 ± 55	2007 ± 49
0.7	940 ± 14	2543 ± 80	1503 ± 86
1.8	1807 ± 81	2243 ± 86	1380 ± 36
2.7	750 ± 62	2430 ± 66	2033 ± 38
3.9	1777 ± 272	2463 ± 482	2390 ± 96
4.8	2180 ± 44	2403 ± 23	1467 ± 15
5.8	1607 ± 38	2663 ± 32	2113 ± 91
6.8	2023 ± 49	2977 ± 35	2763 ± 81
7.7	2153 ± 51	2963 ± 50	2307 ± 38
9.7	3423 ± 112	3350 ± 225	2510 ± 26

Table B-12. MLSS concentrations in Aerobic 1 during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (mg·MLSS/L)	Positive Control (mg·MLSS/L)	Negative Control (mg·MLSS/L)
0.0	825 ± 5	2140 ± 120	1655 ± 15
1.0	835 ± 45	2210 ± 10	770 ± 60
2.0	1335 ± 5	2215 ± 5	1340 ± 55
3.0	1015 ± 35	1385 ± 25	950 ± 10
4.0	735 ± 5	1565 ± 15	1175 ± 5
5.0	1075 ± 5	1520 30	1540 ± 10
6.0	1670 ± 0	2450 ± 10	1535 ± 45
7.0	1895 ± 95	2160 ± 10	1640 ± 10
8.0	2175 ± 55	2445 ± 35	1820 ± 20
9.0	1800 ± 30	1860 ± 50	1400 ± 0
10.0	2340 ± 60	2350 ± 10	1730 ± 20
12.0	2670 ± 20	2290 ± 50	1750 ± 30
14.0	2760 ± 20	2470 ± 10	2435 ± 35
16.0	3510 ± 0	2965 ± 25	2940 ± 50

Table B-13. Mixed liquor volatile suspended solids (MLVSS) concentrations in Aerobic 1 during shock event 1 (analyses performed in triplicate).

Days After Shock Event	Corrective Action (mg·MLVSS/L)	Positive Control (mg·MLVSS/L)	Negative Control (mg·MLVSS/L)
0.0	1585 ± 49	1593 ± 96	1337 ± 31
0.7	537 ± 123	1817 ± 80	1037 ± 67
1.8	1210 ± 36	1580 ± 66	983 ± 6
2.7	570 ± 61	1770 ± 30	1490 ± 53
3.9	1290 ± 36	1980 ± 79	1790 ± 106
4.8	1663 ± 35	1720 ± 17	1390 ± 40
5.8	1193 ± 40	1973 ± 81	1670 ± 92
6.8	1560 ± 40	2377 ± 40	2157 ± 38
7.7	1633 ± 49	2287 ± 21	1707 ± 47
9.7	2653 ± 93	2620 ± 149	1913 ± 21

Table B-14. MLVSS concentrations in Aerobic 1 during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (mg·MLVSS/L)	Positive Control (mg·MLVSS/L)	Negative Control (mg·MLVSS/L)
0.0	805 ± 45	1925 ± 15	1450 ± 20
1.0	740 ± 0	1695 ± 25	635 ± 15
2.0	1053 ± 12	1715 ± 25	1035 ± 5
3.0	*863 ± 30	*1177 ± 21	*808 ± 8
4.0	645 ± 15	1200 ± 0	925 ± 5
5.0	855 ± 5	1210 20	1195 ± 15
6.0	1390 ± 30	1980 ± 20	1275 ± 5
7.0	1585 ± 85	1765 ± 5	1355 ± 15
8.0	1840 ± 0	2025 ± 5	1495 ± 15
9.0	1560 ± 10	1600 ± 20	1205 ± 5
10.0	2025 ± 35	1970 ± 10	1450 ± 20
12.0	2205 ± 15	1865 ± 35	1390 ± 30
14.0	2305 ± 35	2095 ± 15	2025 ± 35
16.0	2895 ± 5	2445 ± 25	2435 ± 5

*Note – Day 3.0 MLVSS data was not obtained, values were estimated at 85% of MLSS.

Table B-15. Mixed liquor wasting during shock event 1.

Days After Shock Event	Corrective Action (L)	Positive Control (L)	Negative Control (L)
-2.0	0.0	0.0	0.0
-1.0	3.0	3.0	3.0
0.0	1.5	1.5	1.5
0.7	1.5	1.5	1.5
1.8	2.3	2.3	2.3
2.7	1.5	2.1	2.3
3.9	2.3	2.3	2.3
4.8	2.2	2.2	2.2
5.8	2.2	2.2	2.2
6.8	2.2	2.2	2.2
7.7	2.2	2.2	2.2
8.7	2.2	2.2	2.2
9.7	2.2	2.2	2.2

Table B-16. Mixed liquor wasting during shock event 2.

Days After Shock Event	Corrective Action (L)	Positive Control (L)	Negative Control (L)
-1.0	2.0	2.0	2.0
0.0	1.0	1.0	1.0
0.3	0.3	0.2	0.2
0.7	0.1	0.1	0.1
1.0	1.1	1.1	1.1
2.0	1.5	1.5	1.5
2.5	0.1	4.0	0.1
3.0	1.5	0.1	1.5
4.0	2.0	1.0	2.0
5.0	2.2	2.2	2.2
6.0	2.2	2.2	2.2
7.0	2.2	2.2	2.2
8.0	2.2	2.2	2.2
9.0	2.3	2.3	2.3
10.0	2.4	2.4	2.4
11.0	2.6	2.6	2.6
12.0	2.6	2.1	2.1
13.0	2.4	2.1	2.1
14.0	2.5	2.2	2.2
15.0	2.0	2.5	2.5
16.0	2.4	2.4	2.4
17.0	2.0	2.0	2.0
18.0	2.4	2.4	2.4

Table B-17. Dissolved oxygen readings during shock event 1.

Days After Shock Event	Corrective Action ($mg \cdot O_2/L$)		Positive Control ($mg \cdot O_2/L$)		Negative Control ($mg \cdot O_2/L$)	
	Aerobic 1	Anoxic 2	Aerobic 1	Anoxic 2	Aerobic 1	Anoxic 2
0.0	4.2	3.7	5.0	0.7	1.4	3.2
0.7	5.1	3.4	2.2	3	3.9	1.7
1.2	5.2	5.9	2.4	2.5	3.7	2.6
1.8	4.8	3.5	2.5	0.2	4.6	0.6
2.7	4.4	2.4	0.9	0.2	1.8	0.3
3.9	2.1	1.2	1.0	0.4	0.8	0.3
4.8	1.2	2.8	0.4	0.2	0.7	0.4
5.8	2.9	0.5	0.7	0.2	0.9	0.2
6.8	1.1	4.1	0.3	0.2	0.3	0.2
8.7	5.2	4.1	0.3	0.2	0.4	0.5
9.7	1.3	3.7	0.8	0.7	0.7	3.2

Table B-18. Dissolved oxygen readings during shock event 2.

Days After Shock Event	Corrective Action ($mg \cdot O_2/L$)		Positive Control ($mg \cdot O_2/L$)		Negative Control ($mg \cdot O_2/L$)	
	Aerobic 1	Anoxic 2	Aerobic 1	Anoxic 2	Aerobic 1	Anoxic 2
0.0	3.5	4.5	4.5	4.6	0.8	1.9
0.3	6.6	6.0	5.1	1.6	4.2	0.2
0.7	2.4	1.4	3.8	1.0	5.3	1.4
1.5	0.7	0.3	2.6	0.3	4.7	1.0
2.0	3.1	0.6	-	-	5.4	1.4
2.5	3.1	0.9	3.3	0.3	5.2	1.4
3.0	3.5	1.3	5.9	4.5	7.0	6.0
4.0	5.4	1.7	6.4	3.6	7.1	5.5
5.0	1.5	0.3	3.6	0.3	5.0	0.6
6.0	0.7	0.2	2.7	0.2	4.9	0.4
7.0	0.6	0.2	1.4	0.2	4.0	0.3
8.0	1.4	0.2	1.5	0.2	3.3	0.2
9.0	2.0	0.2	2.6	0.3	4.1	0.4
10.0	0.6	0.2	1.2	0.2	3.1	0.2
11.0	0.9	0.2	2.5	0.2	3.2	0.2
13.0	2.2	0.2	2.8	0.2	3.1	0.2
14.0	2.5	0.3	2.5	0.2	2.5	0.2
15.0	1.0	0.1	1.1	0.1	1.5	0.1
16.0	0.7	0.3	0.7	0.2	0.5	0.1
17.0	1.8	0.1	2.2	0.1	0.8	0.1
18.0	2.5	0.3	3.9	0.2	5.4	0.2

Table B-19. System pH readings during shock event 1.

Days After Shock Event	Corrective Action pH		Positive Control pH		Negative Control pH	
	Anaerobic	Aerobic 1	Anaerobic	Aerobic 1	Anaerobic	Aerobic 1
0.3	6.59	6.89	6.92	6.74	7.06	7.01
0.7	6.84	7.11	7.00	6.80	7.10	7.16
1.2	7.03	7.07	6.95	6.74	7.05	7.01
1.8	7.00	7.02	6.96	6.77	6.98	7.01
2.7	6.84	7.34	6.89	6.90	6.93	6.88
3.9	6.95	7.06	6.94	7.01	6.92	6.99
4.8	6.75	6.85	6.82	7.01	6.81	6.96

Table B-20. System pH readings during shock event 2.

Days After Shock Event	Corrective Action pH		Positive Control pH		Negative Control pH	
	Aerobic 1	Effluent	Aerobic 1	Effluent	Aerobic 1	Effluent
0.0	7.03	7.29	7.01	7.35	7.11	7.29
0.3	7.51	7.45	7.14	7.07	7.64	7.33
0.7	7.24	7.21	7.00	6.94	7.41	7.11
1.0	7.34	7.28	7.07	7.02	7.43	7.09
2.0	7.18	7.11	-	-	7.22	6.95
2.5	7.22	7.16	7.10	7.00	7.29	6.95
3.0	7.22	7.11	7.44	7.09	7.57	7.14
4.0	7.39	7.20	7.49	7.07	7.59	7.05
5.0	7.19	7.24	7.11	7.03	7.22	6.99
6.0	7.25	7.29	7.11	7.08	7.28	7.08
7.0	7.05	7.14	6.88	6.98	7.06	7.03
8.0	7.12	7.15	6.90	6.96	7.07	7.06
9.0	7.11	7.11	6.73	6.80	7.04	6.98
10.0	7.17	7.18	6.85	6.97	7.03	7.08
11.0	7.19	7.18	6.77	6.86	7.05	7.06
13.0	7.08	7.09	6.86	6.91	7.12	7.13
14.0	7.08	7.03	6.75	6.80	7.10	7.12
15.0	7.21	7.19	6.85	6.96	7.22	7.23
16.0	7.29	7.25	7.01	7.12	7.30	7.27
17.0	7.10	7.15	6.93	7.02	7.26	7.24

Table B-21. Influent pH readings during shock event 2.

Days After Shock Event	Influent pH
0.0	7.00
0.3	7.15
0.7	6.96
1.0	7.07
2.0	7.05
2.5	7.07
3.0	6.92
4.0	7.05
5.0	6.95
6.0	7.08
7.0	6.80
8	6.50
9	6.74
10	6.50
11	6.63
13	6.51
14	6.61
15	6.76
16	6.91
17	6.58

Table B-22. Sludge volume index (SVI) measurements during shock event 1.

Days After Shock Event	Corrective Action (mL/g·MLSS)	Positive Control (mL/g·MLSS)	Negative Control (mL/g·MLSS)
0.0	39	63	56
0.7	15	58	35
1.8	62	66	70
2.7	53	63	59
3.9	68	62	54
4.8	64	65	95
5.8	75	66	53
6.8	81	63	61
7.7	85	72	87
9.7	61	81	80

Table B-23. SVI measurements during shock event 2.

Days After Shock Event	Corrective Action (mL/g·MLSS)	Positive Control (mL/g·MLSS)	Negative Control (mL/g·MLSS)
0.0	68	56	34
1.0	48	51	73
2.0	60	-	60
3.0	71	72	80
5.0	67	66	57
6.0	69	42	60
7.0	74	61	63
8.0	81	62	62
9.0	116	88	80
10.0	126	80	79
12.0	198	73	69
14.0	183	78	61
16.0	187	76	68

Table B-24. Effluent soluble chemical oxygen demand (sCOD) concentrations during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (mg·COD/L)	Positive Control (mg·COD/L)	Negative Control (mg·COD/L)
0.0	84 ± 4	55 ± 6	54 ± 1
0.7	46 ± 4	15 ± 11	137 ± 4
1.2	13 ± 2	25 ± 2	15 ± 8
1.8	22 ± 1	16 ± 1	29 ± 9
2.2	136 ± 24	52 ± 5	104 ± 9
2.7	64 ± 4	36 ± 2	38 ± 0
3.2	56 ± 4	46 ± 1	40 ± 3
3.9	61 ± 8	45 ± 4	43 ± 0
4.8	52 ± 3	45 ± 15	50 ± 3
5.8	49 ± 4	34 ± 4	64 ± 11
6.8	31 ± 7	48 ± 12	30 ± 0
7.7	51 ± 21	49 ± 7	26 ± 22
9.7	19 ± 0	24 ± 0	30 ± 4

Table B-25. Effluent sCOD concentrations during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (mg·COD/L)	Positive Control (mg·COD/L)	Negative Control (mg·COD/L)
0.0	37 ± 4	25 ± 3	28 ± 3
0.3	105 ± 2	25 ± 3	49 ± 2
0.7	43 ± 6	26 ± 4	49 ± 4
1.0	52 ± 3	44 ± 3	30 ± 4
1.5	80 ± 4	65 ± 1	60 ± 2
2.0	57 ± 1	- ± -	26 ± 18
2.5	51 ± 13	41 ± 4	54 ± 3
3.0	64 ± 1	54 ± 7	63 ± 3
4.0	68 ± 2	57 ± 4	73 ± 11
5.0	26 ± 11	5 ± 1	19 ± 1
6.0	4 ± 2	14 ± 5	7 ± 5
7.0	2 ± 2	0 ± 4	0 ± 2
8.0	19 ± 6	6 ± 3	150 ± 4
9.0	144 ± 18	101 ± 22	58 ± 18
10.0	29 ± 1	11 ± 4	36 ± 8
11.0	21 ± 2	0 ± 2	0 ± 6
12.0	10 ± 14	5 ± 1	58 ± 2
14.0	85 ± 2	96 ± 20	29 ± 9
16.0	13 ± 2	30 ± 2	7 ± 8
18.0	9 ± 2	22 ± 10	63 ± 3

Table B-26. Composite influent sCOD concentrations during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Influent (mg·COD/L)
0.7 → 1.8	188 ± 2
1.8 → 2.7	236 ± 3
2.7 → 3.9	245 ± 26
3.9 → 4.8	249 ± 8
4.8 → 5.8	203 ± 15
5.8 → 6.8	237 ± 4

Table B-27. Composite influent sCOD concentrations during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Influent (mg·COD/L)
0 → 1	168 ± 4
1 → 2	157 ± 7
2 → 3	319 ± 6
3 → 4	295 ± 1
4 → 5	237 ± 7
5 → 6	229 ± 15
6 → 7	219 ± 9
8 → 9	244 ± 5
9 → 10	271 ± 32
10 → 11	423 ± 5
11 → 12	528 ± 9
13 → 14	511 ± 3
15 → 16	240 ± 15
17 → 18	464 ± 23

Table B-28. Anoxic 2 dissolved organic carbon (DOC) during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (mg·DOC/L)	Positive Control (mg· DOC/L)	Negative Control (mg· DOC/L)
0.0	5.61 ± 0.16	6.05 ± 0.06	7.28 ± 0.06
1.0	8.46 ± 0.06	5.05 ± 0.03	5.83 ± 0.03
2.0	6.13 ± 0.06	- ± -	5.62 ± 0.05
3.0	6.69 ± 0.14	4.29 ± 0.02	5.06 ± 0.04
4.0	5.69 ± 0.01	5.02 ± 0.00	5.19 ± 0.07
5.0	10.10 ± 0.13	6.49 ± 0.03	6.52 ± 0.08
6.0	9.13 ± 0.04	6.03 ± 0.03	5.61 ± 0.03
7.0	8.83 ± 0.02	6.32 ± 0.01	5.92 ± 0.03
8.0	8.50 ± 0.03	5.49 ± 0.04	8.32 ± 0.05
9.0	7.39 ± 0.01	6.52 ± 0.00	5.54 ± 0.03
10.0	8.45 ± 0.09	6.20 ± 0.00	6.43 ± 0.07

Table B-29. Specific oxygen uptake rates (sOURs) during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot O_2/g \cdot MLVSS \cdot min$)	Positive Control ($mg \cdot O_2/g \cdot MLVSS \cdot min$)	Negative Control ($mg \cdot O_2/g \cdot MLVSS \cdot min$)
0.0	0.54 ± 0.03	0.84 ± 0.05	0.79 ± 0.07
0.7	0.77 ± 0.18	0.60 ± 0.03	0.70 ± 0.05
1.8	0.76 ± 0.10	0.72 ± 0.09	0.80 ± 0.11
2.7	1.08 ± 0.15	1.09 ± 0.11	1.05 ± 0.10
4.8	1.04 ± 0.06	1.07 ± 0.08	1.07 ± 0.10
6.8	1.73 ± 0.14	1.08 ± 0.08	1.16 ± 0.11

Table B-30. sOURs during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot O_2/g \cdot MLVSS \cdot min$)	Positive Control ($mg \cdot O_2/g \cdot MLVSS \cdot min$)	Negative Control ($mg \cdot O_2/g \cdot MLVSS \cdot min$)
0.0	0.63 ± 0.04	0.51 ± 0.02	0.61 ± 0.01
1.0	0.46 ± 0.01	0.66 ± 0.01	0.85 ± 0.02
2.0	0.81 ± 0.02	- ± -	0.81 ± 0.02
3.0	0.95 ± 0.03	0.67 ± 0.05	0.74 ± 0.04
5.0	0.87 ± 0.01	0.64 ± 0.03	0.38 ± 0.02
9.0	0.84 ± 0.02	0.73 ± 0.03	0.78 ± 0.02
12.0	0.54 ± 0.03	0.52 ± 0.02	0.48 ± 0.02
14.0	0.45 ± 0.02	0.43 ± 0.01	0.58 ± 0.03
16.0	0.76 ± 0.04	0.86 ± 0.01	0.76 ± 0.01

Table B-31. Maximum phosphate release rate during shock 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Positive Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Negative Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)
0.7	3.1 ± 0.7	7.0 ± 0.3	8.0 ± 1.7
3.0	5.6 ± 2.5	6.5 ± 0.3	10.8 ± 1.7

Table B-32. Maximum phosphate release rate during shock 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Positive Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Negative Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)
0.0	3.6 ± 1.1	4.7 ± 1.0	3.0 ± 1.3
1.0	2.7 ± 0.5	6.1 ± 0.5	5.6 ± 2.1
3.0	5.0 ± 3.2	4.0 ± 0.1	6.2 ± 0.7
7.0	6.2 ± 1.8	5.9 ± 2.4	17.8 ± 0.2

Table B-33. Maximum phosphate uptake rate during shock 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Positive Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Negative Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)
0.7	5.1 ± 1.4	7.1 ± 4.6	5.0 ± 1.0
3.0	6.2 ± 0.9	2.3 ± 0.2	4.6 ± 0.9

Table B-34. Maximum phosphate uptake rate during shock 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Positive Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)	Negative Control ($mg \cdot PO_4^{3-} \cdot P/g \cdot MLVSS \cdot hr$)
0.0	6.8 ± 1.0	3.1 ± 0.1	4.2 ± 0.3
1.0	1.5 ± 1.5	6.6 ± 0.9	6.4 ± 4.0
3.0	2.6 ± 0.2	2.7 ± 1.2	4.4 ± 0.1
7.0	12.8 ± 1.9	4.5 ± 0.1	26.2 ± 0.3

Table B-35. Inhibition of maximum phosphate release rate during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (%)	Positive Control (%)	Negative Control (%)
0.7	-22% ± 12%	17% ± 4%	3% ± 2%
3.0	16% ± 11%	-29% ± 6%	29% ± 5%

Table B-36. Inhibition of maximum phosphate release rate during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (%)	Positive Control (%)	Negative Control (%)
0.0	5% ± 2%	56% ± 14%	44% ± 20%
1.0	26% ± 16%	18% ± 5%	40% ± 23%
3.0	21% ± 14%	14% ± 0%	21% ± 4%
7.0	-75% ± 22%	59% ± 24%	65% ± 15%

Table B-37. Inhibition of maximum phosphate uptake rate during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (%)	Positive Control (%)	Negative Control (%)
0.7	-52% ± 33%	-20% ± 18%	6% ± 6%
3.0	-5% ± 2%	-42% ± 5%	26% ± 6%

Table B-38. Inhibition of maximum phosphate uptake rate during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (%)	Positive Control (%)	Negative Control (%)
0.0	64% ± 13%	11% ± 2%	74% ± 5%
1.0	-2% ± 2%	4% ± 2%	41% ± 27%
3.0	28% ± 20%	22% ± 18%	55% ± 18%
7.0	66% ± 57%	92% ± 50%	94% ± 14%

Table B-39. Effluent alkalinity during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot CaCO_3/L$)	Positive Control ($mg \cdot CaCO_3/L$)	Negative Control ($mg \cdot CaCO_3/L$)
0.0	55.4 ± 0.1	73.5 ± 0.0	69.8 ± 0.0
0.7	44.7 ± 0.1	59.9 ± 0.1	148.2 ± 0.9
1.2	51.4 ± 0.4	56.9 ± 0.2	85.2 ± 0.4
1.8	51.3 ± 0.4	59.4 ± 0.4	62.6 ± 0.1
2.7	74.4 ± 0.4	50.8 ± 0.1	106.3 ± 0.1
9.7	154.8 ± 0.4	105.3 ± 0.9	71.4 ± 0.9

Table B-40. Effluent alkalinity during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot CaCO_3/L$)	Positive Control ($mg \cdot CaCO_3/L$)	Negative Control ($mg \cdot CaCO_3/L$)
0.0	93.2 ± 0.1	111.2 ± 0.1	121.7 ± 0.5
1.0	173.9 ± 0.0	98.8 ± 0.1	127.3 ± 0.4
2.0	169.8 ± 0.6	- ± -	108.5 ± 0.4
3.0	168.3 ± 0.0	96.4 ± 0.5	102.8 ± 0.4
4.0	155.1 ± 0.0	98.2 ± 0.5	98.7 ± 0.0
5.0	209.3 ± 0.1	141.4 ± 0.6	134.1 ± 0.1
6.0	209.2 ± 0.5	136.9 ± 0.6	129.6 ± 0.3
7.0	205.3 ± 0.6	144.4 ± 0.6	141.8 ± 0.3
8.0	161.3 ± 0.6	107.3 ± 0.3	116.6 ± 0.5
9.0	170.1 ± 0.1	85.5 ± 0.0	115.0 ± 0.1
10.0	178.1 ± 0.7	99.0 ± 0.4	112.1 ± 0.2
11.0	80.1 ± 0.3	179.6 ± 0.2	117.6 ± 0.2
13.0	83.6 ± 0.0	127.6 ± 0.3	120.2 ± 0.1
15.0	160.4 ± 0.8	89.2 ± 0.1	155.2 ± 0.2
17.0	116.5 ± 0.2	80.4 ± 0.6	154.9 ± 0.3

Table B-41. Composite influent alkalinity during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Influent ($mg \cdot CaCO_3/L$)
0.7 → 1.8	187.3 ± 1.9
1.8 → 2.7	208.0 ± 1.8
8.7 → 9.7	198.6 ± 0.4

Table B-42. Composite influent alkalinity during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Influent ($mg \cdot CaCO_3/L$)
-1 → 0	230.3 ± 0.0
0 → 1	210.6 ± 0.0
1 → 2	224.7 ± 0.0
2 → 3	306.9 ± 0.5
3 → 4	294.7 ± 0.5
4 → 5	305.5 ± 0.0
5 → 6	279.2 ± 1.3
7 → 8	342.2 ± 1.1
8 → 9	245.3 ± 0.2
9 → 10	219.5 ± 0.1
10 → 11	323.5 ± 0.5
12 → 13	223.3 ± 2.7
14 → 15	181.6 ± 0.8
16 → 17	184.3 ± 0.0

Table B-43. Effluent nitrite concentrations during shock event 1 (analyses performed in duplicate).

Days After Shock Event	Corrective Action ($mg \cdot NO_2^- \cdot N/L$)	Positive Control ($mg \cdot NO_2^- \cdot N/L$)	Negative Control ($mg \cdot NO_2^- \cdot N/L$)
0.0	1.5 ± 0.0	0.1 ± 0.0	0.9 ± 0.0
0.7	0.8 ± 0.6	0.0 ± 0.0	0.5 ± 0.1
1.2	1.0 ± 0.1	0.0 ± 0.0	0.8 ± 0.0
1.8	1.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.1
2.2	1.8 ± 0.0	0.4 ± 0.0	0.7 ± 0.0
2.7	1.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
3.2	1.1 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
3.9	0.9 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
4.8	0.6 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
5.8	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
6.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
9.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table B-44. Effluent total suspended solids (TSS) concentrations during shock event 1 (analyses performed in triplicate).

Days After Shock Event	Corrective Action (mg·TSS/L)	Positive Control (mg·TSS/L)	Negative Control (mg·TSS/L)
0.0	241 ± 25	334 ± 61	14 ± 11
0.7	164 ± 7	12 ± 1	5 ± 5
1.2	25 ± 3	29 ± 7	78 ± 9
1.8	12 ± 2	9 ± 4	3 ± 3
2.2	20 ± 9	15 ± 7	27 ± 11
2.7	12 ± 2	10 ± 5	10 ± 4
3.2	17 ± 6	30 ± 2	11 ± 1
3.9	17 ± 1	5 ± 1	3 ± 2
4.8	8 ± 5	39 ± 3	50 ± 1
5.8	1 ± 2	1 ± 1	3 ± 1
6.8	7 ± 2	23 ± 2	1 ± 1
7.7	4 ± 1	5 ± 1	26 ± 4
9.7	2 ± 1	0 ± 0	3 ± 3

Table B-45. Effluent TSS concentrations during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Corrective Action (mg·TSS/L)	Positive Control (mg·TSS/L)	Negative Control (mg·TSS/L)
1.0	9 ± 8	6 ± 5	2 ± 2
2.0	6 ± 2	- ± -	6 ± 4
4.0	27 ± 3	3 ± 0	0 ± 5
5.0	4 ± 3	0 ± 1	6 ± 5
6.0	0 ± 0	0 ± 6	0 ± 2

Table B-46. Composite influent TSS concentrations during shock event 1 (analyses performed in triplicate).

Days After Shock Event	Influent (mg·TSS/L)
0.7 → 1.8	131 ± 19
1.8 → 2.7	229 ± 42
2.7 → 3.9	127 ± 4
3.9 → 4.8	1244 ± 17
4.8 → 5.8	101 ± 3
5.8 → 6.8	174 ± 22
6.8 → 7.7	196 ± 10
7.7 → 8.7	391 ± 14
8.7 → 9.7	417 ± 0

Table B-47. Composite influent TSS concentrations during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Influent (mg·TSS/L)
0 → 1	28 ± 3
1 → 2	19 ± 3
2 → 3	657 ± 11
3 → 4	205 ± 7
4 → 5	108 ± 6
5 → 6	191 ± 13
7 → 8	489 ± 21
8 → 9	145 ± 9
9 → 10	658 ± 16
13 → 14	488 ± 14

Table B-48. Composite influent volatile fatty acid (VFA) concentrations during shock event 2 (analyses performed in quadruplicate).

Days After Shock Event	Acetic Acid (mg·COD/L)	Propionic Acid (mg·COD/L)	Isobutyric Acid (mg·COD/L)	Butyric Acid (mg·COD/L)
-1 → 0	35.0 ± 4.2	11.8 ± 2.4	1.0 ± 0.7	1.9 ± 0.8
0 → 1	33.4 ± 0.4	15.2 ± 0.2	1.0 ± 0.0	2.3 ± 0.1
1 → 2	30.6 ± 0.5	17.9 ± 0.5	0.9 ± 0.1	2.4 ± 0.1
2 → 3	65.2 ± 1.4	47.3 ± 1.4	1.7 ± 0.1	4.1 ± 0.1
4 → 5	88.1 ± 1.2	82.8 ± 1.0	2.6 ± 0.1	8.9 ± 0.4
6 → 7	80.3 ± 1.2	70.0 ± 1.0	2.3 ± 0.0	7.1 ± 0.1
8 → 9	74.9 ± 2.6	71.0 ± 3.7	2.2 ± 0.4	6.4 ± 0.4
9 → 10	70.5 ± 1.0	56.0 ± 0.8	1.7 ± 0.1	6.3 ± 0.2
13 → 14	175.5 ± 1.6	207.7 ± 1.7	5.7 ± 0.2	36.4 ± 0.3

Table B-49. Composite influent total Kjeldahl nitrogen (TKN) concentrations during shock event 2 (analyses performed in duplicate).

Days After Shock Event	Composite Influent (mg·TKN/L)
-1 → 0	49.0 ± 0.0
3 → 4	38.1 ± 0.9
6 → 7	52.2 ± 0.5
9 → 10	39.0 ± 0.0

Appendix C

PURI Experimentation

Initial phosphate uptake/release inhibition (PURI) experiments were conducted with the addition of 50 mg·CH_3COO^-/L and 5 mg·P/L. During these experiments, pH was unadjusted. The objective of this kinetic measurement was to characterize the potential EBPR performance of the mixed liquor in its existing state, even if the PAOs would benefit from an increase in pH. Figures C-1, C-2, and C-3 show the results of the preliminary PURI experimentation, illustrating the negative impact of hypochlorite on phosphate release and uptake.

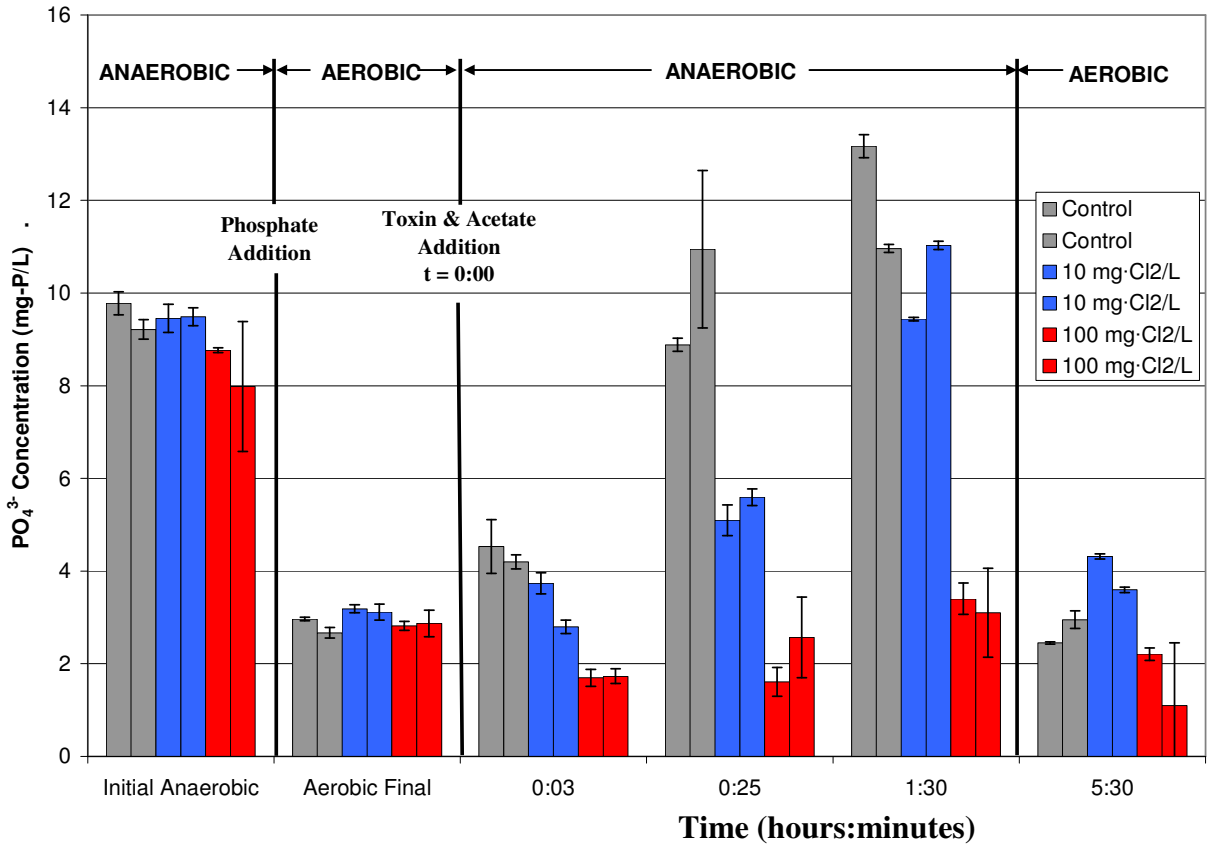


Figure C-1. Graph of soluble orthophosphate during initial phosphate uptake/release inhibition (PURI) experiment. A calcium hypochlorite concentration of 10 mg·Cl_2/L resulted in significant inhibition of phosphate release and uptake.

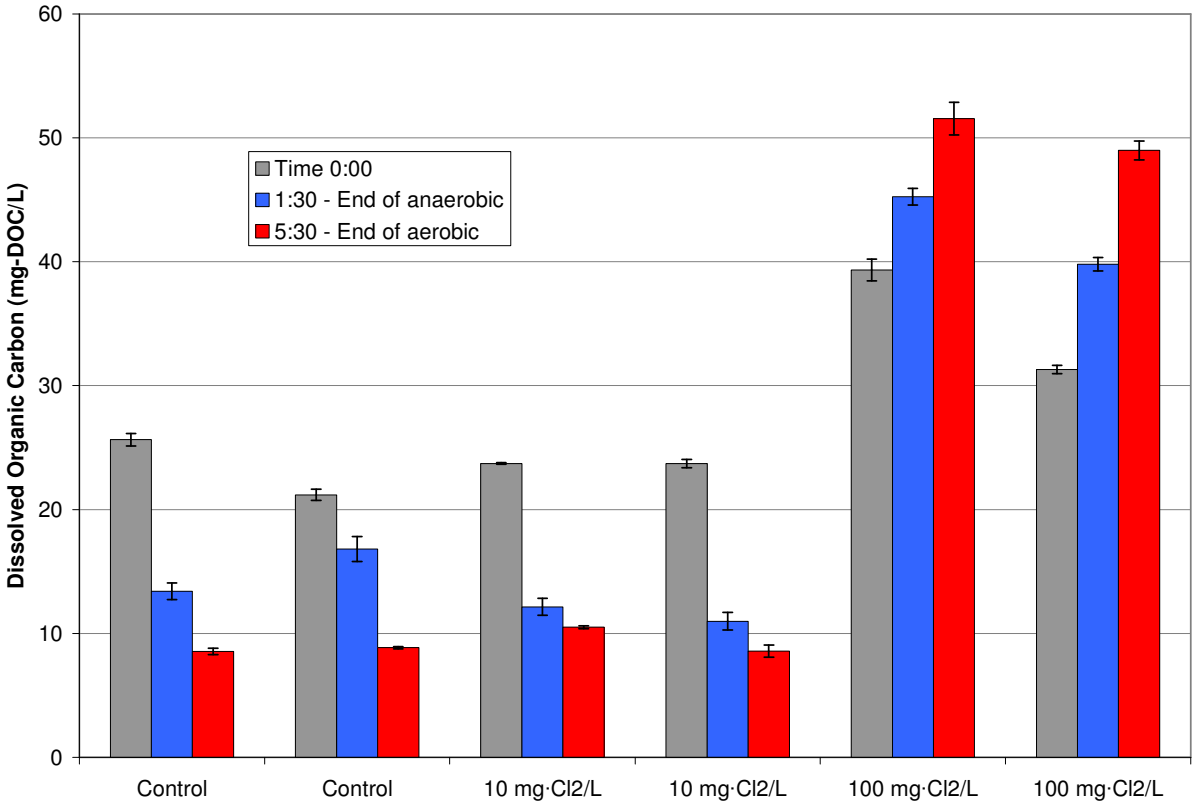
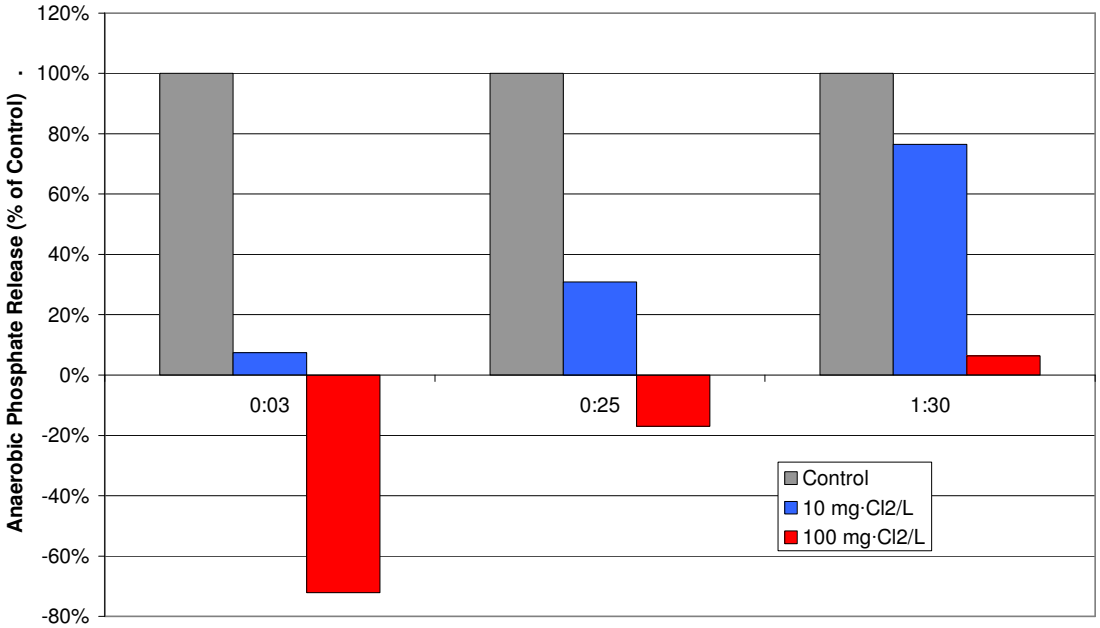


Figure C-2. Graph of dissolved organic carbon during initial PURI experiment. A calcium hypochlorite concentration of 10 mg•Cl_2/L appeared to negatively impact the aerobic utilization of organic carbon more than the anaerobic utilization. 100 mg-Cl_2/L of calcium hypochlorite may have caused the leakage of intracellular material, resulting in increased DOC concentrations.



† 100 mg·Cl_2/L data is from one biological run with triplicate PO_4^{3-} analysis. Control and 10 mg·Cl_2/L data is from duplicate biological runs with triplicate analysis of each biological run.

Figure C-3. Relative anaerobic phosphate released by biomass inhibited by calcium hypochlorite.

In preparation for enriched culture PAO experiments, a media recipe was adapted from Martin et al., 2006, and personal communication with Katherine McMahon (Professor, University of Wisconsin – Madison). For future phosphate uptake/release rate measurements, it is recommended that the following nutrient concentrations be achieved prior to rate measurement (Table C-1). Carbon sources should be added last, and under fully anaerobic conditions. The rate of phosphate uptake and release is significantly affected by the mixed liquor pH. It is recommended that pH be maintained between 7.3-7.5 to maintain optimal conditions for PAOs.

Table C-1. Target nutrient concentrations for future phosphate uptake/release rate experiments.

Carbon Feed	CH_3COO^-	185 mg/L
	Yeast Extract	8.6 mg/L
	Casamino acids	31 mg/L
Nutrient Feed	KCl	117 mg/L
	NH_4Cl	119 mg/L
	$MgCl_2$-$6H_2O$	219 mg/L
	$MgSO_4$	7.0 mg/L
	$CaCl_2$-$2H_2O$	60.7 mg/L
	H_3BO_3	0.063 mg/L
	$ZnSO_4$-$7H_2O$	0.313 mg/L
	KI	0.016 mg/L
	$CuSO_4$-$5H_2O$	0.063 mg/L
	$CoCl_2$-$6H_2O$	0.063 mg/L
	Na_2MoO_4-$2H_2O$	0.031 mg/L
	$MnSO_4$-H_2O	0.350 mg/L
	$FeSO_4$-$7H_2O$	0.564 mg/L
	Allylthiourea	4.0 mg/L
	NaH_2PO_4-H_2O	62.38 mg/L (14 mg·PO_4-P/L)

Additionally, it is recommended that the frequency of sampling points be increased. For maximum uptake/release rate experiments (versus uptake/release capacity experiments), the duration of the experiment could be reduced to allow for this increased sampling frequency. It is also recommended that samples be stored, unacidified, in hydrochloric acid-washed glassware at -20 °C.

Appendix D

Laboratory Reactors

Three identical, laboratory-scale, 5-stage Bardenpho systems were constructed and housed in a remote storage shed adjacent to a municipal sanitary sewer system manhole. Operational parameters such as recycle rates and hydraulic retention times (HRTs) were designed to mimic train A1 of the Long Creek Water Resources Reclamation Facility (WRRF) in Gastonia, North Carolina.

Table D-1. Hydraulic retention time (HRT) and surface overflow rate (SOR) comparison between Long Creek train A1 and the laboratory-scale reactor system.

Parameter	Typical Operation		Shock Event	
	Long Creek A1	Laboratory-Scale	Long Creek A1	Laboratory-Scale
Primary Clarifier SOR (gal/ft^2/day)	706	99	2820	454
Primary Clarifier HRT (hrs)	2.03	2.01	0.51	0.44
Anaerobic Zone HRT (hrs)	1.42	1.50	0.31	0.33
Anoxic Zone 1 HRT (hrs)	0.95	1.00	0.21	0.22
Aerobic Zone 1 HRT (hrs)	4.80	4.83	1.05	1.06
Anoxic Zone 2 HRT (hrs)	1.60	1.75	NA	NA
Aerobic Zone 2 HRT (hrs)	0.50	0.73	NA	NA
Secondary Clarifier SOR (gal/ft^2/day)	354	91	1415	417
Secondary Clarifier HRT (hrs)	7.11	1.97	1.78	0.43

Table D-2. Flow rate comparison between Long Creek train A1 and the laboratory-scale reactor system.

System	Normal Operation	Shock Event
Long Creek A1	3.5 MGD	16 MGD
Laboratory-Scale	72 L/day (50 mL/min)	330 L/day (229 mL/min)

Figure D-1. Laboratory-scale reactors - primary clarifiers and anaerobic, first anoxic, and first aerobic reactors.

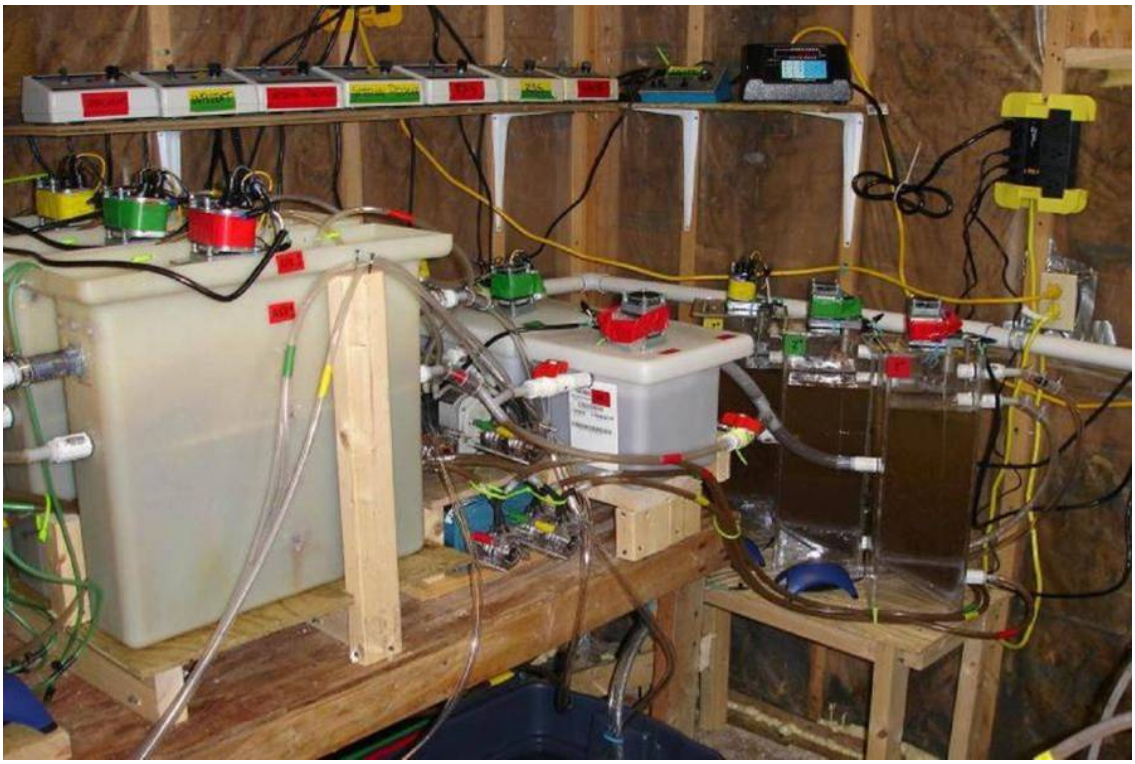


Figure D-2. Laboratory-scale reactors – first aerobic, second anoxic, and second aerobic reactors, followed by secondary clarifiers.

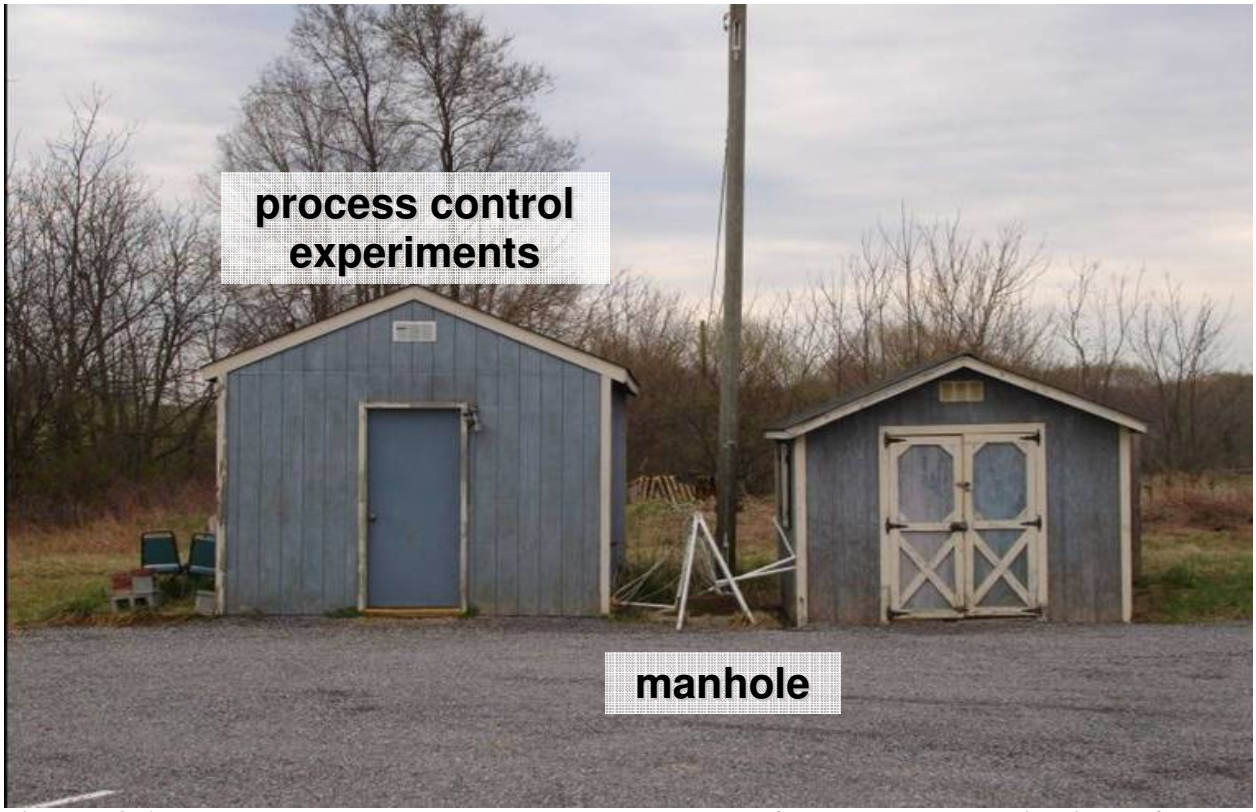


Figure D-3. A remote shed housed the laboratory-scale reactors. Influent was pumped daily directly from an adjacent sewer line.