UPSET EVENTS AT WASTEWATER TREATMENT PLANTS: IMPLICATIONS FOR MITIGATIVE STRATEGY DEVELOPMENT AND BIOREACTOR MICROBIAL ECOLOGY.

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ABSTRACT

This study consists of three research phases. First, we developed corrective action strategies to mitigate the impact of calcium hypochlorite and cadmium pulse shocks for the Plum Island Wastewater Treatment Plant (WWTP) in Charleston, SC. The corrective action strategies were developed in consultation with industrial consultants and operational personnel from the utility. These strategies were tested using a laboratory scale system, which was constructed and operated similar to the parent facility. Two corrective actions were tested for calcium hypochlorite, while only one strategy was tested for the cadmium at the laboratory scale. This study shows that no corrective action strategies are required for an acute hypochlorite stress. This is due to the fact that hypochlorite is highly reactive and dissipates rapidly on contact with the wastewater matrix, thus causing only low level process deterioration. In fact, implementation of corrective action strategies results in greater process deterioration as compared to the non-intervention approach. The corrective action tested for cadmium stress showed potential for reducing the peak impact of the toxin and allowed for faster process recovery as compared to the unstressed control.

For the second phase, the corrective actions were tested at a pilot scale facility operated at the Plum Island wastewater treatment plant. We tested two different corrective action strategies for cadmium, while only one strategy was tested for hypochlorite during the pilot scale study. Similar to the laboratory scale experiments, we conclude that no mitigative approaches are necessary for an acute hypochlorite stress. Additionally, the implementation of mitigative approaches for the pilot scale cadmium stress events resulted in greater process deterioration as compared to the non-intervention approach. In contrast to the laboratory scale experiments, theoretical effluent blending calculations showed that corrective actions may not reduce the impact of the cadmium stress. This was attributed to the lower intensity of process deterioration caused by the simulated cadmium stress. The pilot scale study shows that prior to implementing a corrective action strategy, the operator should determine the probable extent of process deterioration due to the detected chemical contaminant before deciding if a corrective action is needed. The pilot scale study also evaluated the effectiveness of current sensor technologies towards the upstream detection of influent anomalies and reliable monitoring of process performance during an upset event. Multivariate analysis on the rate of change of influent sensor signals was reliably able to detect the presence of both toxins tested during this study.

For the third phase of this research, we investigated the impact of cadmium stress on the structure and function of bioreactor microbial communities. We observed significant increases in post-stress heterotrophic and autotrophic bacterial respiration rates for the bioreactors subjected to cadmium stress. The higher respiration rates were due to an increase in bacterial abundance in the cadmium stressed reactors. We were also able to show that the increase in bacterial abundance was not due to changes in community structure or due to cadmium induced deflocculation. In fact, this study demonstrates that transient cadmium stress reduces predator abundance within the activated sludge community and this reduction in predator grazing was responsible for the increase in bacterial abundance. This research highlights the importance of higher life forms, specifically eukaryotic microorganisms, in regulating bacterial community dynamics in systems undergoing chemical perturbations.

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Attribution

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Table of contents

List of tables

List of figures

Chapter 3.0

Chapter 4.0

List of abbreviations

amoA	ammonia monooxygenase subunit A
ANOSIM	Analysis of similarity
AOB	Ammonia oxidizing bacteria
CA	Corrective action
CEPT	Chemically enhanced primary treatment
DO	Dissolved oxygen
FSC	Flow short circuiting
HRT	Hydraulic retention time
IC10	10% inhibitory concentration
IC25	25% inhibitory concentration
lpd	liters per day
MGD	Million gallons per day
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NC	Negative control
NDMS	Nondimensionalzed metric scaling
ORP	Oxidation reduction potential
PC	Positive control
PCA	Principal component analyses
PCR	Polymerase chain reaction
Q-PCR	Quantitative polymerase chain reaction
sAOR	Specific ammonia oxidation rate
SBR	Sequencing batch reactors
sCOD	Soluble chemical oxygen demand
SDS	Sludge diversion and storage
sNGR	Specific nitrite generation rate
sOUR	Specific oxygen uptake rate
SR1	Stressed reactor 1
SR2	Stressed reactor 2
SRT	Solids retention time
SVI	Sludge volume index

tCOD	Total chemical oxygen demand
TOC	Total organic carbon
T-RF	Terminal restriction fragment
T-RFLP	Terminal restriction fragment length polymorphism
TSS	Total suspended solids
UC	Unstressed control
UEWS	Upset early warning system
UV-vis	Ultra violet-visible
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

Chapter 1.0

Executive Summary

1.1 Introduction

Wastewater treatment plants (WWTP) are an important pollution control mechanism and represent an interface between urban settlements and the environment. Activated sludge wastewater treatment utilizes a naturally seeded and operationally engineered microbial consortium to remove contaminants from wastewater, and discharges treated effluent into the receiving environment. Carbon, various nitrogen species, and phosphorus are some of the primary contaminants that are regulated by National Pollution Discharge Elimination System (NPDES) limits. However, WWTPs are frequently subject to direct or indirect toxic chemical shock events [1]. Direct shocks may involve purposeful or accidental spills of industrial strength toxins into the collection system. Indirect shocks may be a result of upstream decontamination events, such as decontamination of the drinking water distribution system. Long-term process upsets due to toxic chemical shocks can result in damage to the receiving environment and potentially harm drinking water sources for downstream communities. The toxic upset of treatment plants that include ammonia removal is further complicated by the relatively slow growth rate of the autotrophic bacteria involved in ammonia and nitrite oxidation. The current wastewater treatment infrastructure is ill-prepared to deal with toxic shock inputs due to the (1) absence of

a robust and accurate upstream monitoring infrastructure capable of predicting process upset conditions, and (2) lack of a systematic framework to guide the operator's response during and after a toxic shock event. The mitigation of toxic events requires that operator(s) are aware of the probable process effect(s) of the toxin involved. This work will help the wastewater treatment industry better understand and manage upset events by addressing three aspects:

1. developing and testing corrective action strategies for mitigation of toxic chemical shocks,
2. evaluating the application of current sensor technologies in detecting and monitoring the wastewater treatment processes during an upset event, and
3. understanding the contribution of prokaryotic and eukaryotic communities on the response of bioreactors to toxic shock events.

The choice of toxins for this study was based on the contaminant prioritization framework developed by the Environmental Protection Agency (EPA) in collaboration with the University of Pittsburgh [2]. The toxins selected for this study are:

1. Cadmium: an industrially relevant toxin that impacts activated sludge processes and has been studied previously [3,4]. It is widely used in electroplating applications at high concentrations [5].
2. Calcium hypochlorite: a stable bleaching agent, which has higher free chlorine availability as compared to sodium hypochlorite. It is used widely for water disinfection and in industrial applications such as bleaching of textiles, paper etc.

This dissertation is broken into three parts, and each part constitutes a separate chapter. Each chapter is formatted for submission as a manuscript to the journals noted in the title heading.

1.2 Part 1. Development and testing of corrective action strategies to mitigate toxic chemical shocks to nitrifying activated sludge systems (Chapter 3.0)

The objective of this study was to develop and test corrective action strategies for a conventional completely mixed activated sludge system with nitrification. This work was conducted on laboratory scale experimental systems that were designed and operated similar to the Plum Island WWTP in Charleston, South Carolina, which was the source of the inoculum. An expert panel consisting of utility operators, industrial consultants, and academic researchers developed the corrective action strategies that are tested in this study. The mitigative strategies were organized into a corrective action plan matrix (CAPM) [6]. The CAPM is a multi-tiered decision making tool that addresses the:

1. nature of the contaminant,
2. data available on process effects of the contaminant,
3. plant-specific operational flexibility, and
4. provides instructions for process monitoring during and immediately after a toxic shock event.

Two corrective action strategies were tested for calcium hypochlorite, while one strategy was tested for cadmium. Despite previous concerns about the potency of calcium hypochlorite as a process disrupting agent, it did not result in temporary process upset during several experimental runs and, therefore, would not require a correction action strategy to be implemented. This conclusion was heavily influenced

by the hydraulic buffering provided by primary clarification in the laboratory system evaluated, and is typical of many WWTPs. The high reactivity of hypochlorite with the influent matrix combined with the hydraulic buffering provided by the primary clarifier ensures very low exposure of the activated sludge biomass to the toxin. In fact, corrective actions aimed at upset event mitigation resulted in process deterioration for the duration of their implementation. The process upset that occurred in response to implementing the corrective action strategy was due to specific aspects of the strategies tested. The negative impacts of the corrective action strategies were exhibited only during its implementation and were easily reversed once they were terminated. In contrast, cadmium caused a significant impact on the process performance of the laboratory scale reactors. The impact was clearly exhibited through the inhibition of both organic carbon removal and ammonia oxidation. However, the stressed reactors showed complete process recovery. The corrective action strategy helped reduce the process impact on both carbon removal and ammonia oxidation. Additionally, the corrective action strategy allowed for quicker process recovery. Overall, this study showed that corrective action strategies can mitigate deleterious process effects caused by chemical contaminants. However, the implementation of corrective strategies should be based on evaluating the persistence of the toxin within the treatment system.

1.3 Part 2. Pilot scale evaluation of upset event detection and mitigation approaches for nitrifying wastewater treatment systems (Chapter 4.0)

The objective of this study was to extend the testing of previously developed corrective actions to a pilot scale facility operated at the Plum Island WWTP. A secondary objective of this study was to evaluate the effectiveness of conventional

sensor technologies in detecting an upset event and accurately monitoring the mixed liquor and effluent quality characteristics during the process upset and recovery. Two corrective action strategies were tested for cadmium, while only one strategy was tested for hypochlorite.

The influent pH, ORP, and conductivity sensors were able to successfully detect the presence of both toxins tested. Use of the rate of change in sensor signal as opposed to the raw sensor value lead to better detection of anomalies in influent chemistry. The sensor responses for cadmium were much more reliable, as they accurately predicted toxin presence and persistence in the influent lines. In contrast to cadmium, the pH and ORP sensors returned slowly to pre-stress levels after the preliminary detection of hypochlorite. The conductivity sensor was always a robust sensor because it was able to immediately detect the presence of the toxin and quickly return to pre-stress signal levels upon dissipation of the toxin. The application of multivariate analyses to the rate of change in signals of the three influent sensors was able to identify influent anomalies while effectively masking ambient changes in influent chemistry and the slow return of the pH and ORP signals to pre-stress levels. Despite the fact that one could visibly review the raw responses of these sensors and detect the presence of the toxin, multivariate analysis using influent sensor signals did not have the ability to consistently discriminate the two toxins. This could be due to the variability in influent composition during the study.

The ORP sensor detected the presence of hypochlorite in the aeration basins, while none of the sensors were directly able to detect the presence of cadmium in the aeration basin. The ORP sensor was the best detector of first order changes in

aeration basin chemistry as it was also reliably able to detect the presence of a coagulant used as part of the corrective action strategy. The DO and pH sensors were reliable and robust indicators of the upset and recovery of carbon and ammonia oxidation processes, respectively. Effluent quality monitoring was conducted using a STIP:Scan sensor, which operates on the principle of light absorption in the UV to visible range. The STIP:Scan estimates for nitrate had to be corrected for nitrite interference, but maintained both sensitivity and accuracy. The STIP: Scan estimates of total organic carbon maintained its sensitivity but lost accuracy over the duration of the study.

As mentioned previously, the hypochlorite stress did not require any corrective action as the primary clarifier provided sufficient hydraulic buffering that minimized exposure levels of the activated sludge. Corrective action strategies tested for the mitigation of hypochlorite and cadmium stress during the pilot scale study were not able to reduce the toxin residence time. In contrast to the laboratory scale experiments (Chapter 3.0), theoretical effluent blending calculations showed that corrective actions may not reduce the impact of the cadmium stress. This is attributed to the shorter time frame of cadmium induced process upset. It is recommended that the implementation of corrective action strategies, for even a potent toxin like cadmium, be evaluated on a case-to-case basis.

1.4 Part 3. Investigating the role of bacterial community structure and predator grazing on the recovery of bioreactors exposed to transient cadmium stress (Chapter 5.0)

The goal of this study was to evaluate the impact of cadmium stress on the microbial ecology of a conventional completely mixed activated sludge system with

nitrification. Nitrifying activated sludge reactors were subject to cadmium stress and the upset and recovery of the process was monitored. Biomass kinetic and effluent quality monitoring indicated that the process recovered rapidly, and the recovery was characterized by a significant increase in specific heterotrophic and ammonia oxidizing bacteria (AOB) respiration rates as compared to the unperturbed control. This trend was reproducible for 6 different cadmium experiments involving 12 stressed reactors at the laboratory and pilot scales. Therefore, the observed effect appears to be a generally applicable characteristic of the biomass at the Plum Island WWTP and independent of the scale of operation.

The AOB community was chosen for community level analyses. Terminal restriction fragment length polymorphism (T-RFLP) analysis indicated that the AOB community had low diversity and did not show significant and sustained change in response to the perturbation as compared to the unstressed control. Though the T-RFLP community patterns were able to predict the trends in specific nitrite generation rates for the unstressed reactor, similar predictions could not be made for the perturbed reactor. The increase in specific nitrite generation rates was accompanied by an increase in total bacterial abundance. Additional experiments indicated that the increase in bacterial abundance was a direct result of a decrease in predatory stress due to loss of grazing eukaryotes. Other potential mechanisms for increases in bacterial abundance such as stress-induced deflocculation or a shift in community structure were not responsible for the observed trends. This study was able to show that a cadmium-induced reduction in predator grazing is directly responsible for the increase in bacterial abundance and activity after the initial inhibition.

1.5 References

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Chapter 2.0

Physiological and ecological considerations for heavy metal-related wastewater treatment process upsets

Ameet J. Pinto and Nancy G. Love

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Summary of recent advances

Wastewater treatment plants are subject to toxic stress events, which can result in long-term process deterioration. Heavy metals constitute industrially-relevant toxic stressors that are responsible for wastewater treatment plant upsets. The development of operational strategies that can mitigate treatment processes that are upset by heavy metals necessitates a greater understanding of the kind of process impact(s) that these toxins cause. Process impact studies have primarily focused on toxin inhibition kinetics for different bacterial functional groups within the activated sludge matrix. More recently, efforts have been made to establish chemical toxin-induced microbial stress responses as causal mechanisms for process-level performance effects, and to use this information as the basis for monitoring technologies. For example, oxidative stressors have been shown to cause activated sludge deflocculation, which has been correlated with induction of an antioxidant response called the glutathione-gated

potassium efflux. With the application of advanced molecular biology tools to microbial ecology, there is an increasing interest in the role of microbial community structure and diversity in influencing bioreactor performance during chemical perturbation events. Investigations into bacterial community structure indicate that higher diversity and/or community evenness may result in greater functional stability. In addition to bacterial dynamics, there is a growing recognition that eukaryotes, such as protozoa, may play a key role in regulating bioreactor response to toxic stressors. In this paper, we develop an understanding of the roles that physiological response and ecological interactions play in defining bioreactor performance during chemical perturbation events.

2.1 Introduction

Wastewater treatment plants are subject to chemical upset events [1], which can cause significant process deterioration and pose a direct threat to the receiving environment. Wastewater upset events may involve industrial toxins such as heavy metals [2], organic solvents [3], acids/bases [4] , strong oxidizing agents like bleach [5], hydrophobic liquids [6] and natural causes such as weather related flow events [7]. The mitigation of chemical upset events, especially chemical shocks, requires that the operator understand the probable process effect(s) and fate of the toxin responsible for the upset event. The process impact of a toxin not only involves physiological aspects of microbial stress responses, but also encompasses bacterial community- and trophic level- dynamics involving prokaryotic-eukaryotic interactions. Both elements of biological treatment process response are complex, but

must be better understood in order to fully understand how to best mitigate deteriorating treatment process performance at plants that are exposed to chemical toxins. This review specifically considers heavy metal toxicity and guides the reader through the dynamics of a wastewater treatment bioreactor subjected to heavy metal perturbations. The implications of physiological and ecological reactions to heavy metal perturbations on bioreactor process performance are discussed.

2.2 Mechanisms of heavy metal toxicity

Heavy metals are classified as redox active (e.g., $Cu^{2+/1+}$, $Fe^{3+/2+}$, $Cr^{3+/6+}$) or inactive (e.g., Cd^{2+} and Zn^{2+}). Both redox active and inactive metals result in cellular toxicity by causing oxidative stress [8]. Redox active heavy metals result in stress by generating reactive oxygen species (ROS) through direct redox cycling within the cell [9], while redox inactive metals such as cadmium generate ROS by interfering with the respiratory chain [10] or by inducing excessive release of iron into cells through damage to FeS clusters [11,12]. The accumulation of ROS can result in (1) permeabilization of membranes due to oxidation of lipids, (2) extensive DNA damage, and (3) depletion of critical thiol-containing proteins [8]. The increase in cytoplasmic metal concentrations can also inactivate important thiol- and sulfhydryl-containing proteins and peptides, which act as endogenous radical scavengers; for example, glutathione (GSH) can react with metals to form extremely stable complexes which reduces the available intracellular GSH level [13,14]. Heavy metals may also interact with physiologically relevant cations such as Mg^{2+} and inhibit important metalloenzyme dependent functions [15].

2.3 Microbial stress response to heavy metal stress

With the advent of microarray technology, there has been a significant increase in whole genome transcriptomic analysis of bacterial responses to heavy metals [16-24]. These studies indicate a high degree of overlap between bacterial responses to different types of metals, with common response strategies being metal efflux, detoxification through complexation/precipitation, and oxidative stress response. Metal efflux [15,25,26] is observed through increased expression of genes coding for ATP dependent and chemi-osmotic heavy metal efflux pumps [20,27], while detoxification is carried out through complexation with thiol-containing compounds [11,28], and/or biotransformation of metals to less toxic forms [29]. Additionally, bacteria can also reduce toxicity by inducing extracellular precipitation of heavy metals [20]. Bacteria invest significant amount of metabolic energy towards combating oxidative stress caused by intracellular accumulation of ROS. Oxidative stress responses, which have been reviewed extensively [30,31] are controlled by numerous regulators, of which transcription factors SoxR and OxyR or corresponding homologs are particularly important [32]. Metal binding and ROS-initiated inactivation of thiol-containing proteins/peptides results in the redox imbalance of key molecules, such as GSH. This redox imbalance activates efflux mechanisms such as the glutathione gated potassium efflux (GGKE) [33], which involves the active efflux of potassium from the cell.

2.4 Effect of heavy metal stress on prokaryotic community structure

With the application of advanced biomolecular methods, engineers and scientists have begun uncovering the immense complexity that underlies a wastewater treatment bioreactor. The activated sludge bacterial consortium harbors engineered functional diversity. Typically, heterotrophic bacteria are involved in carbon oxidation, nitrate reduction and phosphate accumulation, and autotrophic bacteria are involved in ammonium and nitrite oxidation [34]. This operationally optimized functional diversity also harbors significant structural diversity [35]. Various biomolecular methods can be used to elucidate taxa-level [36-38] and phylogeny-level [39,40] dynamics of complex microbial communities. With the ability to elucidate structural dynamics, there is increasing interest in investigating the applicability of traditional ecological theories to wastewater bioreactor systems. These theories include but are not limited to resource competition [41-43], island biogeography [44], niche selection [45,46] and neutral assembly [47,48]. A predominant focus of these studies is the application of ecological theories to explain community assembly or diversity for steady state reactor conditions. Interestingly, there have been very few microbial ecology studies that address the impact of toxic stress on the microbial community structure of wastewater treatment bioreactors [49-52].

Bioreactor studies indicate that microbial communities tend to exhibit chaotic trends due to non-linear structural interactions [53], which may include mutualisitc relationships between different functional groups [54] and competitive non-equilibrium [55]. Heavy metal-influenced structuring can result in the enrichment of

metal tolerant bacterial species [56] and development of metal resistance mechanisms [57]. Long-term chronic stress has exhibited a strong potential for microbial community structuring along spatially-segregated contaminant gradients [58-61]. For example, Ranjard et al. [62] report that chronic mercury exposure resulted in the enrichment of Gram negative mercury resistant bacteria, subject to bioavailability of the toxin along spatial gradients. However, unlike natural environmental systems, wastewater treatment plants that are exposed to unplanned loads of heavy metals tend to experience them as acute shock events. Although industrial discharges are regulated by pre-treatment requirements that are designed to protect downstream treatment plants [63], accidental spills do occur and expose bioreactors to acute toxicity.

A key question that has yet to be thoroughly addressed is whether toxic perturbations can cause a discernible change in community structure, in light of the underlying chaotic dynamics. Additionally, if the impact of toxic stress is discernible, then what is the time scale over which this impact is relevant? Principi et al. [50] were able to show that acute perturbations caused a significant change in ammonia oxidizing bacterial community structure for a short term experiment, approximately 24 to 72 hours. They did not evaluate the community structuring patterns over the long term. There is increasing evidence that long term microbial community dynamics may be independent of acute perturbations in completely mixed systems [49,64]. This behavior may persist even in light of strong perturbations such as heavy metal stress [65]. Our studies indicate that structural changes in microbial communities in perturbed reactors are discernible from unperturbed control reactors

over the short term, i.e. 24 to 72 hours. In contrast, the inherent variability associated with unperturbed communities can eliminate any independent clustering that may be associated with the heavy metal stress over the long term (Chapter 5.0).

2.5 Effect of prokaryotic community structure on bioreactor response to heavy metal stress

An important concept that has dominated recent microbial ecology studies is the macro-ecological correlation between biodiversity and ecosystem reliability [66,67] and stability [68]. The application of biomolecular methods in conjunction with various biodiversity indices [69] has revealed significant diversity within the microbial communities found in wastewater bioreactors. Broadly speaking, the definition of microbial diversity in bioreactors is based on clustering the sequence diversity of conserved genes into operational taxonomic units (OTU). Studies indicate that simple OTU diversity [52], community evenness [70], and functional flexibility [71] show a strong correlation with functional stability. Briones et al. [72] review the implications of diversity and microbial community dynamics on process stability, while Botton et al. [73] provide a review on the resilience of microbial systems towards perturbation events.

From the perspective of wastewater bioreactors, the functional stability of microbial groups is measured by the removal of pollutants that are primarily regulated in the effluent, i.e. carbon, ammonia, total nitrogen, and phosphorus. Some microbial communities that are responsible for the removal of these pollutants may show significant phylogenetic and catabolic diversity, such as the organic carbon oxidizing

heterotrophs, while other groups, such as the ammonia and nitrite oxidizing autotrophs, show low diversity [74] and uneven community structure [75]. It would thus be expected that the uneven and low diversity communities would show greater instability to environmental perturbations and/or toxic stress events as compared to heterotrophic communities. Logic thus dictates that increasing the biodiversity of microbial communities, especially for sensitive bacterial groups, can result in greater process stability under perturbed conditions [52]. Hence, the critical question is: "can process design or operational parameters be altered to increase biodiversity?" Common operational and design parameters that can be varied across multiple process configurations include solids retention time (SRT), hydraulic retention time (HRT), and food to microorganism ratio (F:M) or loading rate. Changes in operational conditions such as SRT [43,76] to alter microbial community diversity have yielded contradicting results. There are examples where organic loading rates impact community diversity, with a decrease in organic loading is accompanied by an increase in community diversity [77] and improved community evenness [78]. No published studies were found that elaborated on the effect that HRT has on microbial community structure or diversity. In contrast, if the speculation that community assembly, and by default diversity, is largely driven by stochastic dynamics of bacterial birth, death, and immigration [47] irrespective of environmental conditions is true, then discussions about design-driven diversity optimization and the resulting higher process stability of wastewater treatment systems are moot.

2.6 Beyond the prokaryotic domain

Despite the trends in wastewater treatment research, it is important to realize that prokaryotes are not the sum total of the activated sludge community [79]. Early studies recognized the contribution of eukaryotes in shaping the activated sludge ecosystem [80,81]. Unfortunately, due to the perception of limited functionality, the eukaryotic members of the activated sludge consortium, comprising of protozoa, metazoa, invertebrates, and fungi, have been largely neglected. Indeed, these higher organisms may play a critical role in bioreactor performance dynamics [82,83] and bacterial community structure [84,85] by regulating complex microbial food webs [86].

Protozoa have long been known as good indicators of bioreactor performance [87-89]. There is sufficient evidence to suggest that similar to bacteria, protozoal communities are also adversely impacted by heavy metal stress [90,91]. Studies indicate variable susceptibility of eukaryotes to heavy metal stress in both monoxenic and complex environmental cultures [92-96]. The eukaryotic community can be further inhibited by ammonia accumulation resulting from metal-induced nitrification inhibition [97]. The depletion in protozoal numbers due to heavy metal stress can substantially reduce predator stress on bacteria [98]. Reduction in predator abundance has a strong impact on bacterial biomass in wastewater bioreactors [99,100]. In fact, grazing stress on bacteria may be reduced by non-fatal inhibition of protozoa using sub-lethal concentrations of heavy metals. Hoffman et al. [101] showed that at sub-lethal metal concentrations, grazing activity by ciliated protozoa was markedly reduced without a significant reduction in predator numbers. Under such a scenario,

the lack of grazing activity may be able to generate favorable conditions for bacterial proliferation.

2.7 Impact of environmental conditions on heavy metal toxicity

Environmental conditions control metal bioavailability, and hence play a significant role in regulating microbial inhibition. The impact of heavy metals is largely governed by its speciation under relevant environmental conditions [102]. In biological systems, metals can precipitate into various non-toxic salts [103] and adsorb to biomass [104]. Studies indicate that the soluble metal fraction is the primary bioavailable and toxic form, and correlates with biological inhibition levels [105]. Some studies have indicated that heavy metal toxicity may also correlate with intracellular metal concentrations for certain metals [106]. Metal bioavailability is governed by the matrix pH, with a decrease in pH resulting in increased soluble metal levels. However, lower pH does not necessarily imply greater inhibition of biological function. In fact, Sandrin et al. [107] demonstrated 3-fold lower intracellular cadmium levels, accompanied by a decrease in cadmium toxicity under slightly acidic pH conditions. Worden et al. [108] speculate that this decrease in cadmium toxicity may be largely due to differential gene expression under different pH conditions. They observed higher and faster expression of important stress response genes at slightly acidic pH. Metal bioavailability is also strongly influenced by the presence of various organic constituents in the wastewater matrix.

Humic substances [109] and bacterial extra-polymeric substances (EPS) show a pH dependent ability to sequester soluble metals, with increasing pH resulting in

decreased soluble metal concentrations [110,111]. The EPS of a bacterial floc not only provides excellent sorption based removal of metal pollutants, but also adds a mass transfer barrier that lowers metal exposure [112]. Previous studies have indicated that extracellular exclusion of heavy metals in the EPS matrix provides a survival advantage to bacterial cells that are able to produce EPS material [56]. Some bacterial communities may be able to stimulate EPS production upon exposure to heavy metal stress [113]. EPS-related and mass transfer-governed lowering of metal exposure is also affected by floc size [114] and biofilm structure [115]. Therefore, any evaluation of toxicity should consider environmental parameters that govern metal bioavailability and bacterial response to metal exposure.

2.8 Process effects of heavy metal toxicity as influenced by microbial physiology and ecology

Heavy metal toxicity and associated stress responses can play a significant role in process upset. The expression of oxidative stress responses, metal efflux, and detoxification through complexation/precipitation are associated with significant cellular metabolic energy costs [25]. The impact of energy investment in stress functions is likely accompanied by inhibited growth rates characterized by a decrease in the biosynthesis of proteins, amino acids, [18,19,22], a shift towards starvation [20] and/or anaerobic metabolism, if possible [22], and a decrease in energy production [22]. Lethal metal concentrations can increase biomass death rate by causing metal-induced protein/peptide modifications [31], which compromises the cell's ability to meet minimal maintenance requirements and cause irreversible damage to several critical cellular components due to oxidative stress [8]. The increase in death rate

coupled with inhibited growth results in a decrease in active biomass fractions within the mixed liquor. Additional biomass loss can also occur due to metal induced deflocculation [116]. Bott and Love [117] were able to directly attribute metal-induced deflocculation to the GGKE mechanism. They linked deflocculation to a GGKE-based increase in K^+, which can deteriorate sludge settleability by increasing the monovalent to divalent cation ratio of mixed liquor [118]. The loss of biomass due to inhibited growth rate, increased death rate, and deflocculation can significantly compromise bioreactor performance. The underlying reality of a long-term process upset may include complete washout of the existing biomass, due to the aforementioned reasons.

Heavy metal induced process inhibition has been well studied [2,119]. A majority of the heavy metal inhibition studies review the inhibitory threshold levels for heterotrophic and autotrophic bacteria at relatively high concentrations. However, considering that the bacteria are protected within floc or biofilm structures, sub-lethal levels of heavy metal exposure may only cause short-term process perturbation. Additionally, the hydraulic buffering provided by collection systems, sedimentation and equalization basins located upstream of bioreactors at wastewater treatment plants would most likely result in low-level stressor concentrations despite high initial pulse loads [120]. In contrast to bacteria, eukaryotic grazers may be more susceptible to low level toxin concentrations, as they are not protected by a mass transfer barrier [121]. Additionally, concurrent nitrification inhibition can have a deleterious impact on the predator community [97].

Given that heavy metals often wash out of the system over time, the inhibited bacteria may be able to quickly recovery to pre-stress functional levels once the residual toxin concentration becomes low enough. Upon recovering, the bacterial community faces reduced grazing stress due to a greater degree of inhibition exhibited by predators. Previous studies indicate that a drop in predator abundance or activity results in a significant increase in the live fraction of bacterial biomass, along with a proportionate increase in the biomass activity [99,100]. These studies used low levels of protozoa specific stressors. If the hypothesis of reduced predator grazing due to low-level heavy metal stress is true, then the recovery in bacterial activity must be accompanied by an increase in bacterial biomass. In fact, our work indicates that initial bacterial inhibition in response to low-level acute stress was followed by a significant increase in heterotrophic and autotrophic biomass and a corresponding increase in total activity (Chapter 5.0). Similarly, observations of an increase in bacterial biomass due to a metal-influenced reduction in grazing have also been made in natural systems [122]. To our knowledge, there has been minimal investigation of the impact of stress dependent predator dynamics on bioreactor performance and biomass activity. In fact, these multi-trophic dynamics may be extremely important in wastewater treatment plants that are equipped with significant process redundancy, where a primary mitigative strategy might be flow equalization and dilution to reduce toxin concentration. Under such scenarios, predator population dynamics will have a strong impact on process performance and this impact may even overshadow bacterial physiological and ecological responses.

2.9 Conclusions

It is evident that bioreactor responses to heavy metal stress are a complex function of wastewater chemistry, bacterial physiology, community ecology, and trophic interactions. However, thus far most perturbation studies have emphasized the impact of chemistry and bacterial inhibition on bioreactor performance and kinetics. With the wide-scale application of advanced biomolecular methods, there is growing interest in evaluating perturbation-dependent ecological changes at the community level. As we continue to utilize powerful biomolecular tools to understand the dynamics of perturbed reactors, it is important that we step back and recognize that the players in an activated sludge consortium extend beyond the prokaryotic domain.

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Chapter 3.0

Development and testing of corrective action strategies to mitigate toxic chemical shocks to nitrifying activated sludge systems

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Abstract. Corrective action (CA) strategies were developed for a conventional continuous-flow complete mixed activated sludge system with nitrification. These strategies were tested independently for two different toxins: calcium hypochlorite ($Ca(OCl)_2$) and cadmium. The impact of the chemical shocks and corrective actions were determined by comparing shocked treatment trains to an unstressed control. The acute $Ca(OCl)_2$ shock did not impact process performance, probably due to complete dissipation of the toxin within a short period of time. The CA strategies negatively impacted process performance and resulted in poor effluent quality during the $Ca(OCl)_2$ shock. In contrast, implementation of the CA strategies for cadmium shock showed potential for reducing process impact and enabling rapid process recovery. This work presents a framework for further development and testing of treatment configuration specific CA strategies.

Keywords. upset event, corrective action strategies, calcium hypochlorite, cadmium

3.1 Introduction.

Wastewater treatment plants (WWTP) are an important pollution control interface between urban/rural settlements and the environment. WWTP design and process control strategies include sufficient redundancy to accommodate variations in influent flow, temperature, wastewater composition and organic/inorganic loading (Grady *et al.* 1999, Tchobanoglous *et al.* 2003). However, the introduction of toxic contaminants into the influent stream may disrupt process performance for a sustained period of time (Henriques *et al.* 2007, Love *et al.* 2005, Kelly *et al.* 2004, Dalzell *et al.* 2002, Blum and Speece 1992, Madoni *et al.* 1996). Love and Bott (2000) reported that 64% of the 101 surveyed WWTPs reported an upset event resulting from an upstream chemical spill. Process upset and the resulting poor effluent quality not only pose a direct threat to the immediate environment, but also a danger to the public health. A large number of WWTPs discharge their effluents into surface waters that serve as a drinking water source for downstream communities. With the growing emphasis on water re-use strategies; the danger to public health posed by WWTPs that experience chemical shock events is clearly a concern (Levine and Asano 2004).

The current wastewater treatment infrastructure is ill-prepared to deal with toxic shock inputs due to the (1) absence of a robust and accurate upstream monitoring infrastructure capable of predicting process upset conditions, and (2) lack

of a systematic framework to guide the operator response during and after a toxic shock event. Developments in the contaminant detection field address the use of biosensors (Tan *et al.* 1993, Ivask *et al.* 2004, D'Souza 2001, Tizzard *et al.* 2004, Nielsen *et al.* 2001), as well as liquid and gas phase sensors that can identify chemical anomalies in the influent stream (Shaw *et al.* 2006, Bourgeois *et al.* 2001, Ciosek and Wroblewski 2007). However, the application of these technologies is limited due to problems associated with harsh environmental conditions and sensor signal unreliability originating from loss of accuracy, calibration, and low signal to noise ratios. Additionally, an operational-level corrective action (CA) framework that provides alternatives for mitigating the impacts of chemical shocks, thus enabling rapid process recovery, is lacking. The extent of any operational response would be guided by the quality of toxin-related information, specifically toxin identity and concentration, chemical class and/or solubility/volatility. The utility of this framework would be further enhanced by knowing the probable process effect(s) caused by the toxin. Information about the probable process effects can be obtained from studies that focus on the impact of various contaminants on activated sludge performance (Kelly *et al*. 2005, Henriques *et al*. 2007, Cabrero *et al.* 1998, Hu *et al.* 2004, Juliastuti *et al.* 2003a, Juliastuti *et al.* 2003b, Anthonisen *et al.* 1976, Hu *et al.* 2002, Xie *et al.* 2002). These studies cover a large variety of chemical classes, including heavy metals, solvents, chlorinated organic compounds, as well as inorganic toxins and provide process-level information that would be invaluable to the operator(s). However, situations may arise where the operator(s) may not be aware of the nature or identity of the toxin involved in the upset event. This would

likely initiate a worst case response scenario. The extent and duration of CA implementation would also depend upon the point of upset event detection, specifically (1) remote data from upstream sensors, (2) data from primary effluent lines, and (3) toxin detection due to the initiation of process upset. The development of any CA framework must accommodate the aforementioned factors. Additionally, the developed framework should be experimentally tested to determine the effectiveness of the corrective actions involved.

To this end, this study addresses the development and testing of CA strategies for a conventional complete mixed nitrifying activated sludge system exposed to two different toxins, namely calcium hypochlorite ($Ca(OCl)_2$) and cadmium chloride ($CdCl_2$). The CA strategies were developed in consultation with industrial consultants and operational staff and were based on the (1) location of toxin detection along the wastewater collection and treatment continuum, (2) nature of the toxin as determined by its solubility, and (3) operational flexibility of the target WWTP. The toxins used in this study were selected from a list generated by an EPA-approved contaminant prioritization framework, which scores contaminants based on numerous criteria including toxin potency, availability, persistence, etc. (Casson *et al*. 2009). Calcium hypochlorite is a stable bleaching agent and has a higher amount of free chlorine availability as compared to conventional liquid bleach, i.e. sodium hypochlorite. It is used widely for water disinfection and in industrial applications such as bleaching of textiles, paper etc. The second toxin, cadmium, is a significant industrial chemical with large scale applications in the electroplating industry (John *et al.* 2002, Morrow 2007).

This study involved evaluating the effectiveness of WWTP-specific CA strategies to mitigate calcium hypochlorite and cadmium pulse perturbations at the laboratory scale and determine toxin-specific utility of the developed CA framework. The utility of each CA strategy was evaluated based on two primary criteria, specifically (1) the ability to reduce residence time of the toxin in the treatment system, and (2) the ability to reduce or match the peak impact and/or recovery time for a "non-intervention" control. The ability of any strategy within the CA framework to meet either or both of these criteria would make the strategy a suitable option for mitigative purposes.

3.2 Materials and methods

Experimental set-up. The laboratory scale set-up was located in Blacksburg, VA and consisted of three identical continuous flow complete mixed activated sludge systems. The experimental systems were constructed to mimic the Plum Island WWTP in Charleston, South Carolina, USA. Each treatment train consisted of its own primary clarifier, aeration basin, and secondary clarifier with a volume of 4 liters, 18.5 liters, and 7.5 liters, respectively (Appendix figure A.1). The system was operated at an 8 hour hydraulic retention time (HRT) and a 10 day solids retention time (SRT). During the experimental runs, the negative control (NC) train was not shocked with any toxin. Two other treatment trains, namely the positive control (PC) and corrective action (CA) trains were shocked by adding toxin to the raw sewage upstream of the primary clarifier, while corrective actions were implemented only on the CA train. The reactors were fed raw wastewater collected from a manhole

adjacent to the research shed where the experimental set-ups were based. The influent was supplemented with 150 mg $CaCO_3$/l alkalinity in the form of sodium bicarbonate to provide sufficient buffer against pH changes resulting from nitrification. The influent was also supplemented with 1000 mg/l NaCl to match the salinity levels at the Plum Island WWTP. Prior to every simulated shock event, the reactors were seeded with return activated sludge (RAS) from the full-scale Plum Island plant (shipped overnight) and given 6 HRTs to acclimate to the laboratory-scale system. During the acclimation period, the biomass from all three trains was removed, mixed, and redistributed twice.

Corrective action development and testing. The CA framework was developed during a 2 day workshop whose participants included operational and management personnel from the Plum Island WWTP, industrial consultants, as well as academic researchers. A hypothetical shock event consisting of an eight hour toxic load was used as the basis for the CA formulation. No specific toxin was selected for the formulation process. However, the nature of the toxin, as it affects control decisions, was addressed. Namely, the nature of the toxin was varied from soluble to insoluble/particulate. The fate and progression of the toxin through the WWTP was simulated using a BioWin^{TM} model specific to the Plum Island WWTP. The BioWin^{TM} models were calibrated based on historical process data from the facility.

The Plum Island WWTP has a design capacity of 36 million gallons per day (MGD); however, it generally operates between 8-24 MGD during a normal diurnal cycle. The plant operates two parallel trains consisting of a primary clarifier, aeration train, and a secondary clarifier. The two trains are rated at design capacities of 24

(line A) and 12 MGD (line B). Line A usually treats 56% of the influent flow while line B treats 44 %. The plant has the ability to isolate one line and divert the entire flow through the other. The treatment plant also has the ability to bypass all biological process components (secondary treatment) by diverting flow immediately after the primary clarifiers. Apart from this, the treatment configuration does not have any other process bypassing capabilities. Based on the toxin fate predicted by the BioWin^{TM} model and the available operational flexibility, two corrective action strategies were devised, namely, (1) flow short-circuiting (FSC), and (2) sludge diversion and storage (SDS). For calcium hypochlorite shock events, FSC and SDS strategies were tested, while only the SDS strategy was tested for the cadmium shock events. Cadmium has a strong tendency to precipitate and adsorb to the activated sludge (Fristoe and Nelson 1983), hence the FSC strategy was not tested for the heavy metal. Figure 3.1 depicts the various steps involved in the two corrective actions tested.

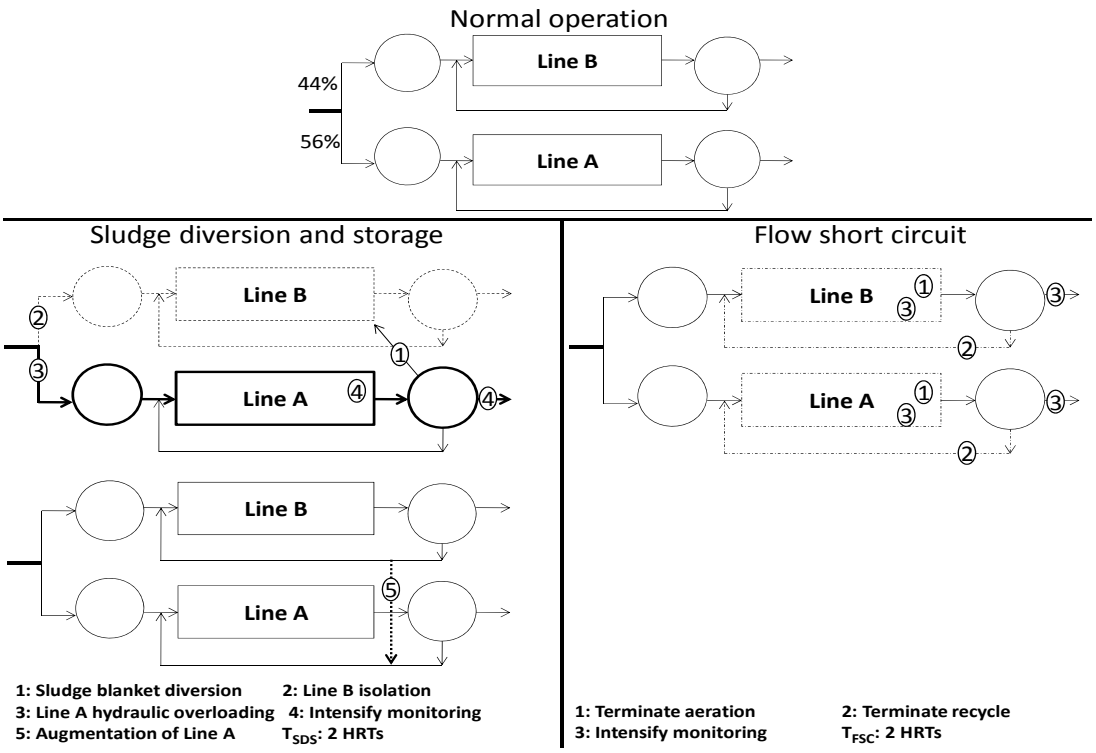

Figure 3.1: Corrective action strategies tested for Plum Island WWTP.

Shock event simulations. The shock events simulated a toxic spill immediately upstream of the Plum Island WWTP. Table 3.1 shows the shocks loads simulated for the experimental investigations. The shock loads represent a spill simulation for the Plum Island WWTP operating at maximum capacity (36 MGD). The simulated spills were scale adjusted for the laboratory experiments. Solid calcium hypochlorite flakes and $CdCl_2$ salt crystals were dissolved in nanopure water immediately before simulating the shock events. The cadmium stock solution was prepared in pH adjusted nanopure water containing 0.5% HNO_3 to ensure complete solubility of the cadmium salt prior to introduction into the influent stream.

Table 3.1: Shock event-corrective action experimental pairing.

Toxin	Shock load simulated	CA strategy	Experimental Replicates	Shock Identifier
Calcium hypochlorite	1382 kgs of $Ca(OCl)_2$	SDS	2	H-SDS_1, H-SDS_2
Calcium hypochlorite	1843 kgs of $Ca(OCl)_2$	FSC	2	H-FSC_1, H-FSC_2
Cadmium	9000 gallon spill of 200 g Cd/l	SDS	2	Cd-SDS_1, Cd-SDS_2

The loads that were simulated reflect typical industrial concentrations for the toxins (Morrow 2007). The hypochlorite shock was increased by 34% from the SDS strategy to the FSC strategy, as the SDS strategy shock simulation failed to register any impact on the process performance. This change does not affect the outcome of the study, as comparisons between stressed and unstressed reactors are made within and not between shock events and/or corrective action strategies.

Sample collection and analyses. Influent was sampled on a daily basis as a composite (100 mL every hour for 24 hours) and was analyzed for total chemical oxygen demand (tCOD) and soluble COD (sCOD), pH, alkalinity, ammonium (NH_4^+), and total suspended solids (TSS). Effluent and mixed liquor grab samples were collected frequently immediately following the chemical shock event, with decreasing frequency after the first 3 days. The effluent was analyzed for sCOD, pH, alkalinity, soluble contaminant, NH_4^+, nitrate (NO_3^-), nitrite (NO_2^-) and TSS. The biomass was analyzed for mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), total contaminant, pH, dissolved oxygen (DO), and sludge volume index (SVI). Sample storage and analyses for tCOD, sCOD, alkalinity, NH_4^+, NO_2^-, were conducted according to standard methods (APHA 1998).

Samples for NO_3^- were filtered through 0.45 µm nitrocellulose filters (09-719-2d, Fisher Scientific) and analyzed immediately using a Dionex DX 120 suppressed conductivity ion chromatograph (IC) with an AS14 anion separation column and AG14 column guard using a 10.0 mM sodium carbonate eluent. The SVI tests were performed in 250-ml graduated cylinders. Hypochlorite presence was monitored by measuring free and total chlorine in the effluent samples. Samples were filtered through 0.45 µm nitrocellulose filters and chlorine concentrations were measured immediately using the N, N- diethyl-p-phenylenediamine (DPD) ferrous titrimetric method (APHA 1998). Samples for cadmium analyses were acidified to pH<2.0 and stored at 4°C until further analyzed. Total cadmium samples were acid digested according to standard methods (APHA 1998). Filtered and digested cadmium samples were analyzed using a Perkin-Elmer flame atomic adsorption spectrophotometer (Perkin Elmer Inc., Wellesley, MA) according to standard methods (APHA 1998). The pH and DO measurements were carried out using an Oakton pH 6 Acorn series model 9708 pH/mV/°C meter with an Accumet pH probe and a YSI model 59 DO meter with a YSI 5905 probe, respectively.

Mixed liquor health was monitored by specific oxygen uptake rate (sOUR) assays. sOUR measurements were conducted as previously described (Henriques *et al*. 2007), with the exception that the assays were conducted in 80 ml glass vials. The oxygen uptake rate (OUR) was determined as the slope of the linear portion of the decreasing DO concentration profile. The sOUR (mg O_2/gVSS.hr) was calculated by dividing the OUR (mg O_2/l.hr) by the MLVSS (gVSS/l). The health of the ammonia oxidizing bacterial (AOB) community, which catalyzes the crucial first step of

nitrification, was also determined by conducting specific ammonium oxidation rate (sAOR) assays. The sAOR assays were conducted similar to the specific nitrate generation rate assays as previously described (Kelly 2005). However, sodium azide was added to decouple two-step nitrification by selectively inhibiting the nitrite oxidizing bacteria (NOB), resulting in nitrite accumulation (Ginestet *et al.* 1998). The optimal dose of sodium azide was determined as the concentration at which maximum sAOR values were observed. The sodium azide dose was optimized at the beginning of each experiment and was consistently found to be 150 µM. The sAOR (mg O_2/gVSS.hr) values were calculated by multiplying the slope of the linear portion of the nitrite accumulation profile over a period of 40-50 minutes (mg NO_2^- as N/l.hr) by the stoichiometric oxygen consumption during the oxidation of NH_4^+ (as N) to NO_2^- (as N) (3.48 mg O_2/mg NO_2^- as N formed) and then dividing by MLVSS (gVSS/l) value.

Data representation. All solids, NO_2^-, NO_3^-, alkalinity, sOUR and sAOR analyses were conducted in duplicates, whereas tCOD, sCOD, NH_4^+, and soluble/total contaminant were analyzed in triplicate. The data points provided in figures represents the average of replicate analyses, while the error bars indicate standard deviations for triplicate measurements and mean absolute difference between measurements, where only duplicate analyses was conducted. A process or kinetic parameter for the shocked system was declared as recovered when its average value was within 10% of or better than the NC value for three consecutive measurements.

3.3 Results

Hypochlorite toxic shock does not cause process upset. A hypochlorite shock was applied at two different loads, one simulating a 1382 kg ($H\text{-}SDS_1$, $H\text{-}SDS_2$) and one simulating an 1843 kg spill ($H\text{-}FSC_1$, $H\text{-}FSC_2$). For both the chemical shocks, no free or total chlorine was detected in the effluent within 1 to 2 HRTs of when the event occurred. By looking at the positive control (PC) response relative to the negative control, direct impacts due to the presence of the chemical toxin or its byproducts can be determined. Effluent quality from the hypochlorite-exposed reactors showed only small and brief deterioration relative to the unshocked control, possibly because of the rapid dissipation of hypochlorite. For example, there was a small drop in effluent NO_3^- concentrations in the positive control train during $H\text{-}SDS_2$, but there was no concurrent NH_4^+ accumulation (Figure 3.2A and 3.2B). Nitrification was also not impacted in the positive control during the FSC strategy trial (Figure 3.3), and effluent sCOD concentrations were affected to a minor degree relative to the unshocked control in only one of the two scenarios (Figure 3.4). Effluent alkalinity concentrations were consistent with these measurements and showed little to no nitrification inhibition (Appendix tables A.29 and A.41). Furthermore, the hypochlorite shock did not exhibit a discernible impact on the mixed liquor as indicated by sOUR (Appendix tables A.24, A.36, and A.48), MLVSS (Appendix tables A.15, A.27, and A.38) or SVI (Appendix tables A.16, A.28, and A.40) measurements in the PC train. Effluent solids were an unreliable indicator of process performance during the hypochlorite shock because denitrification occurred in the

secondary clarifiers and caused sludge to float for reasons unrelated to the chemical shock event.

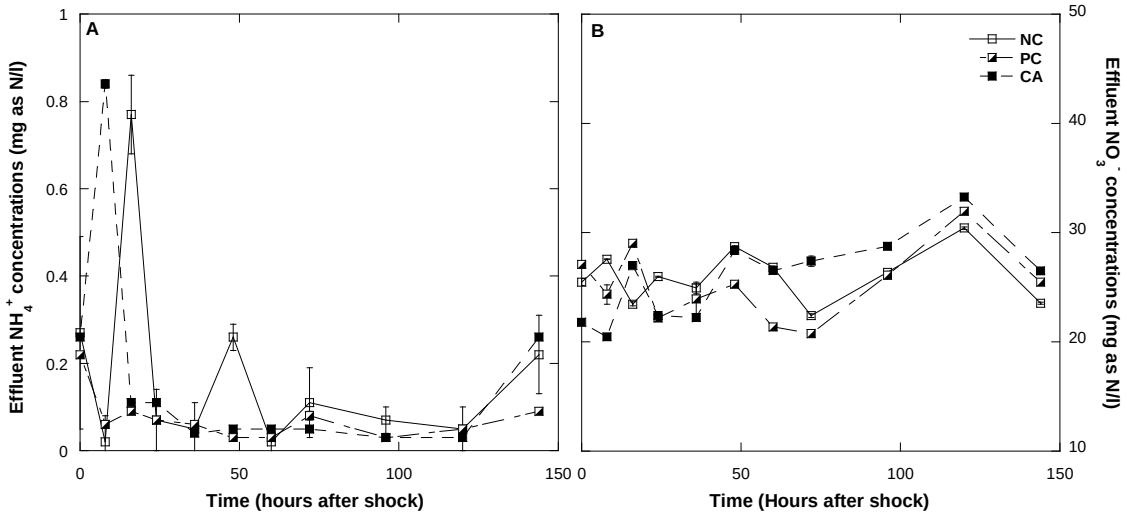

Figure 3.2: Impact of hypochlorite shock and SDS strategy on nitrification.

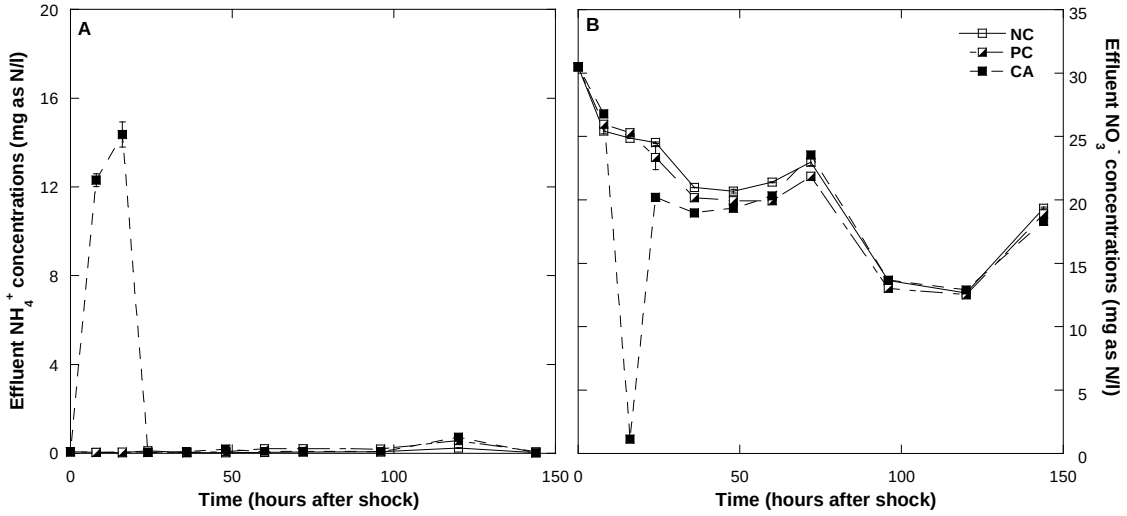

Figure 3.3: Impact of hypochlorite shock and FSC strategy on nitrification.

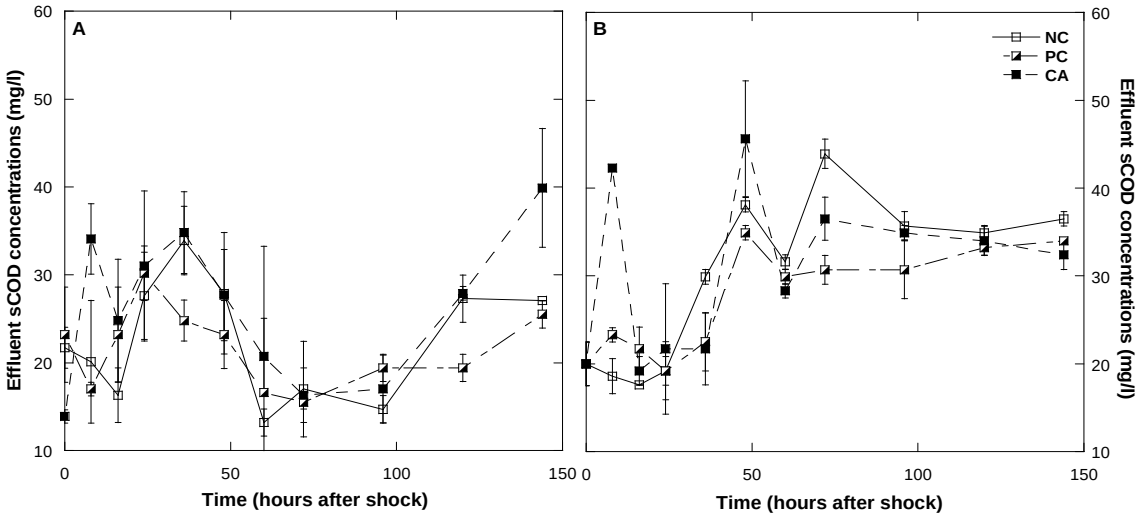

Figure 3.4: Impact of hypochlorite shock and SDS (H-SDS_2) and FSC (H-FSC_2) strategies on sCOD removal.

Corrective actions are detrimental to process performance for hypochlorite shock events. Both FSC and SDS corrective action strategies were evaluated for their ability to mitigate deleterious effects of the hypochlorite shock. While the PC reactors did not show nitrification inhibition, the FSC strategy strongly inhibited nitrification (Figures 3.2 and 3.3) and COD removal (Figure 3.4) in the CA train for the duration over which the CA strategy was implemented. The CA train that was subjected to the SDS strategy had a moderate but significant ($p=0.001$) peak negativeimpact on effluent NO_3^- concentrations. However, peak deterioration of nitrification was significant for the FSC strategy (Figure 3.3A) ($p<0.001$). Ammonia accumulated to almost 15 mg/L as N and was accompanied by a concomitant decrease in effluent NO_3^- concentrations for the CA train (Figure 3.3B). The decrease in NO_3^- concentration could be attributed to (1) shutdown of nitrification and/or (2) a bacterial switch to anoxic respiration. An alkalinity balance was performed for the

train where the FSC strategy was implemented, and results show that both the aforementioned factors may have contributed to low effluent NO_3^- concentrations (Appendix tables A.29 and A.41).

Carbon removal was negatively impacted by both CA strategies to similar degrees. This is in contrast to nitrification which was impacted to different degrees by the CA strategies. The effluent sCOD concentrations for the CA train increased over the first day after the shock by approximately 75% for both CA strategies (Figure 3.4A and 3.4B) and then fluctuated between being worse than, and better than the negative control. Interestingly, the mixed liquor in the CA train, (Figure 3.5B) exerted a strong oxygen demand (33% greater than NC) during the implementation of the FSC strategy. In contrast, the sOUR values for the PC train decreased as compared to both the CA and NC trains (Figure 3.5B). A delayed, but brief decrease in PC sOUR values was seen immediately after the toxic shock (Figure 3.5A). However, this decrease could not be attributed to any deterioration in process performance.

The sOUR assays were conducted by measuring the rate of DO decrease for biomass samples spiked with 100 mg/l of readily biodegradable carbon. The soluble COD and NH_4^+ concentrations in the mixed liquor from the CA train during the implementation of the FSC strategy, were approximately 10 to 20 mg/l higher than for the PC train at t =16 hours. Unlike the sOUR values for the PC and NC trains which reflect carbonaceous respiration, the sOUR analyses for the CA train also included nitrification due to NH_4^+ accumulation. Moreover, the soluble COD may be in the form of readily degradable volatile fatty acids (VFA) due to fermentative processes during the anaerobic storage period. Though VFA analysis was not

conducted to confirm this assumption, the likelihood of VFA presence is supported by the drop in pH in the aeration basin during the implementation of the FSC strategy (Appendix tables A.30 and A.42). Additionally, bacteria can generate a significant amount of internal storage products under anoxic (Gujer *et al.* 1999), as well as anaerobic (Blackall *et al.* 2002) conditions. It is possible that the combination of higher ambient COD and NH_4^+ concentrations, especially as VFAs and internal stored carbon polymers, could lead to higher sOUR rates for the CA train. Both CA strategies did not exhibit reproducible trends for the impact on other mixed liquor characteristics like the MLSS (Appendix tables A.15, A.27, and A.38), MLVSS (Appendix tables A.16, A.28 and A.39), or SVI (Appendix tables A.16, A.28, and A.40). Table 3.2 summarizes the impact of the CA train as compared to the PC train for the hypochlorite shock events in terms of performance at the point of peak impact and in terms of the length of time required to recover. Table 3.2 shows that while the SDS strategy causes short term deterioration in carbon removal processes, the FSC strategy exhibits deleterious effects for both carbon and NH_4^+ removal.

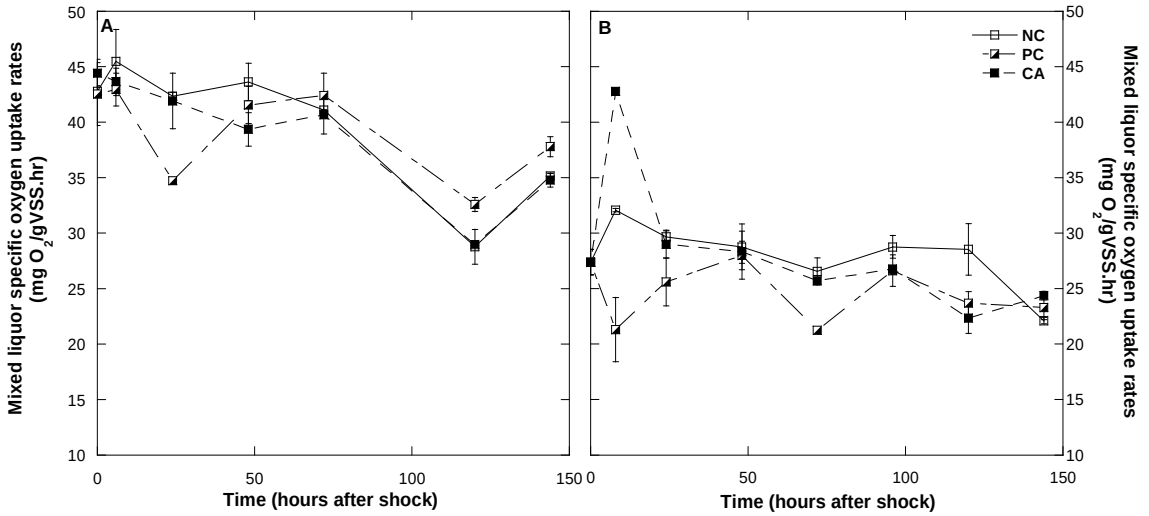

Figure 3.5: sOUR profiles for SDS (H-SDS_2) and FSC (H-FSC_1) strategies during hypochlorite shock events.

Table 3.2: Effectiveness of corrective action strategies for hypochlorite shock events as compared to the positive control.

Corrective action	Peak impact		Recovery time	
	Carbon removal	Nitrification	Carbon removal	Nitrification
SDS	x	NI	-	NI
FSC	x	x	x	x

x: poorer performance, - equal performance, NI: no impact of toxic shock

Cadmium stress causes a significant and sustained impact on process performance. Total cadmium measurements in the aeration basin remained elevated well above levels known to cause inhibition (Madoni *et al*. 1998), even at the end of each experimental run. The final total cadmium levels were between 16 and 20 mg/l for the PC train (Figure 3.6A). The soluble cadmium levels were 10 to 20 times smaller than the total cadmium levels, with peak soluble cadmium levels for the PC train (Figure 3.6B) at 10% of the peak total cadmium levels (Figure 3.6A).

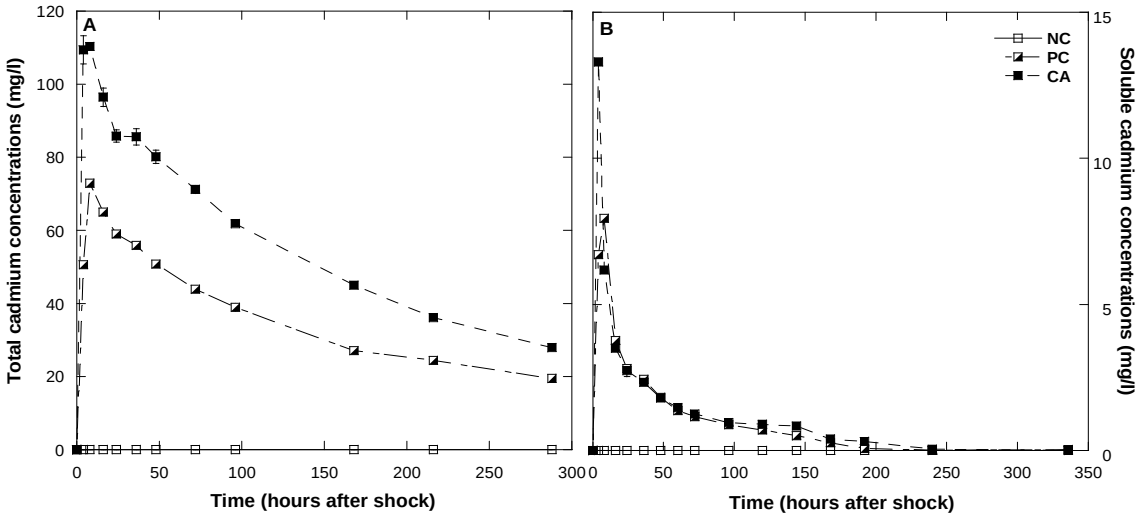

Figure 3.6: Cadmium concentrations in the three treatment trains, indicating total (A) concentrations were higher and sustained as compared to soluble (B) cadmium concentrations for Cd-SDS_1.

The cadmium stress significantly impacted nitrification in the PC train. The effluent NH_4^+ levels peaked at t =16 hours after the shock event, followed by gradual recovery to NC levels at t = 120 hours (Figure 3.7A). Recovery of effluent NH_4^+ levels was accompanied by an accumulation in effluent NO_2^- in the PC train (Appendix tables A.59 andA.75), with peak levels ranging from 5 mg as N/l for Cd-SDS_1 to 16 mg as N/l for Cd-SDS_2. The effluent NO_3^- (Figure 3.7B) and alkalinity concentrations (Appendix tables A.53 and A.69) accurately reflected the inhibition and recovery of the entire nitrification process. Similar to nitrification, carbon removal was also severely impacted by the cadmium shock. The effluent sCOD concentrations were 75 to 430% higher than the NC levels for Cd-SDS_1 and Cd-SDS_2 at the time that peak inhibition occurred. Recovery to NC levels took approximately 120 to 216 hours after the start of the shock event (Figure 3.8A). The sustained effluent sCOD levels were also complimented with high effluent TSS levels for the

PC and CA trains (Figure 3.8B). The effluent solids were approximately 46 mg/l higher than they were in the NC train, while recovery to NC levels took 120 hours after the shock event was initiated.

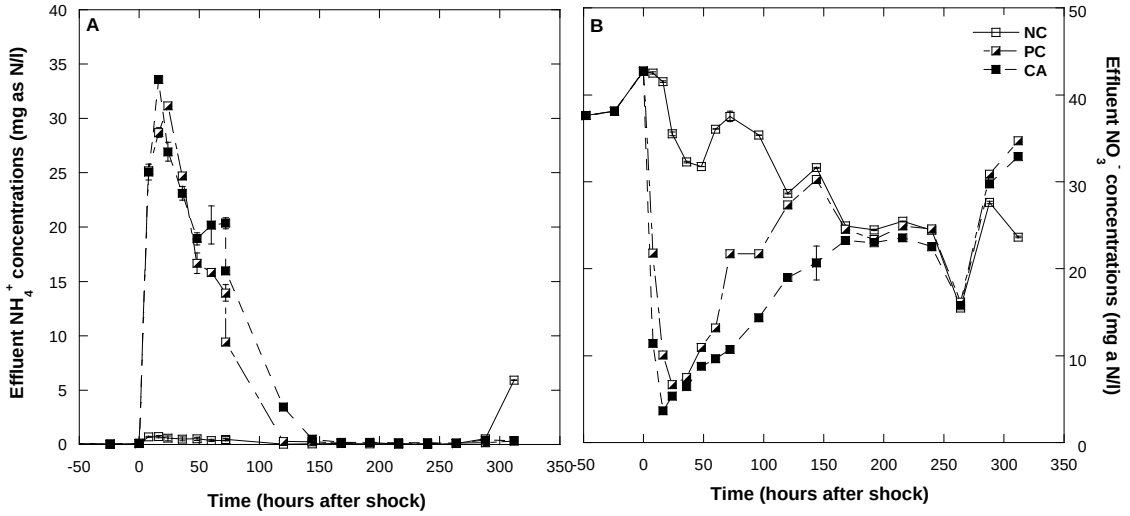

Figure 3.7: Impact of cadmium shock and SDS strategy on nitrification as measured through effluent ammonium-N (A) and nitrate-N (B) concentrations.

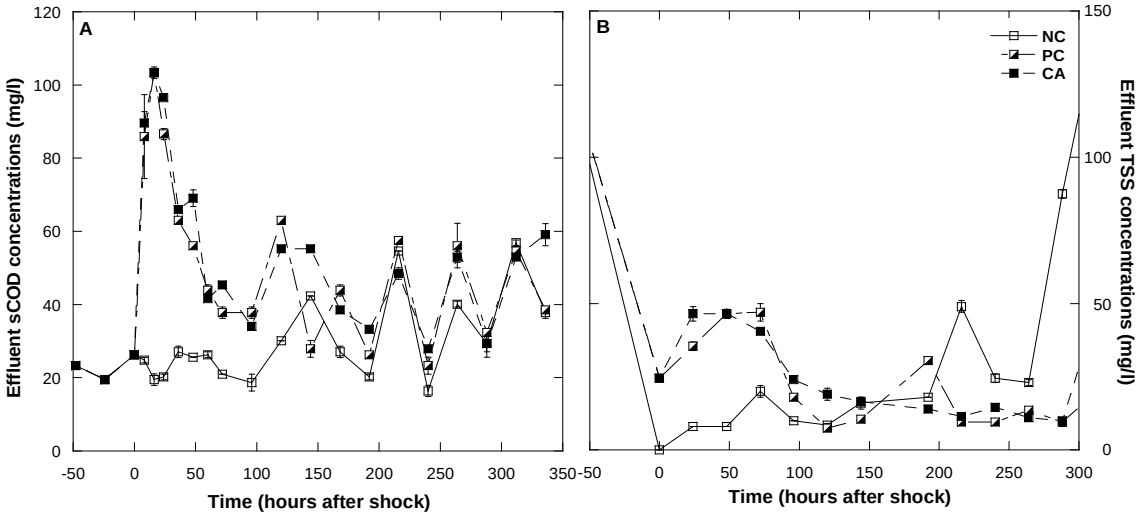

Figure 3.8: Impact of cadmium shock and SDS strategy on (A) effluent sCOD and (B) TSS concentrations.

Kinetic assays provided an insight into the impact of the toxic shock on the heterotrophic bacteria and the AOB community, which is a subset of the autotrophic nitrifying bacteria in the activated sludge consortium. Heterotrophic and AOB

inhibition was near 100% for Cd-SDS_2 (Figure 3.9A and 3.9B). The slowest kinetic value was observed between t= 8 and 16 hours after the shock event was initiated. For Cd-SDS_1, the PC sOUR and sAOR values were completely inhibited (Figure 3.9B). Interestingly, the sOUR and sAOR values recovered rapidly, beginning at t =8 hours and achieving complete recovery at t= 90 to 110 hours after the shock event was initiated. Furthermore, both kinetic indicators showed higher community activity levels after recovery to NC levels were achieved. The sOUR and sAOR values for the PC reactors peaked at 40 to 65% and 115 to 130% above the NC levels for the two cadmium stress events. The MLSS and MLVSS concentrations in the PC control train dropped immediately after the cadmium stress for both shock events (Appendix tables A.50 and A.66). Biomass growth in the PC train was also affected by the cadmium shock, and it took approximately 200 hours for the MLVSS to recover to NC levels for Cd-SDS_2, which was largely due to the recovery of volatile solids as indicated by the MLSS: MLVSS ratio for the shocked reactors. Unlike previous studies (Henriques *et al*. 2007), there was no impact of cadmium shock on the sludge settleability (Appendix tables A.52 and A.68).

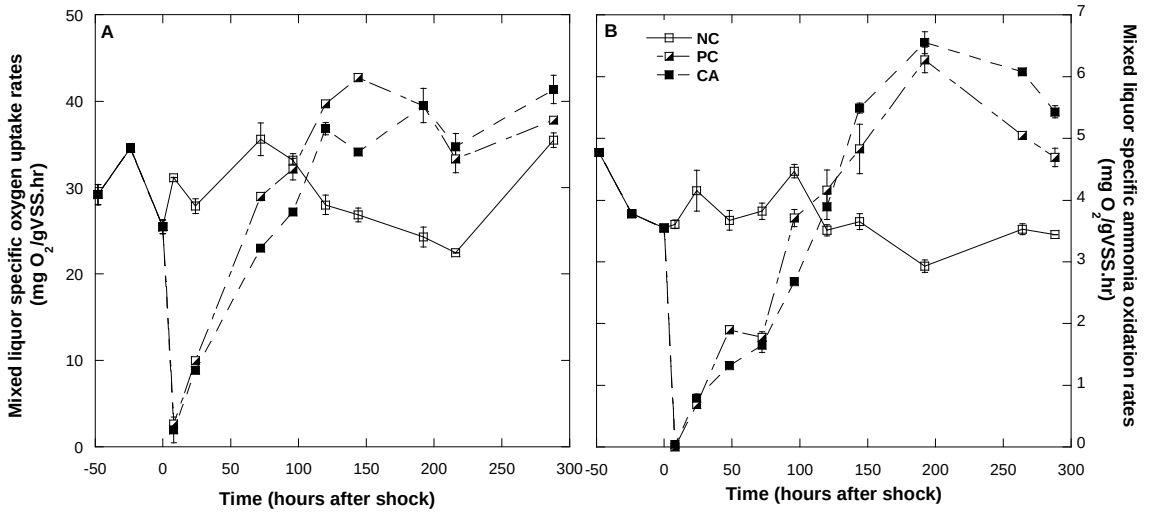

Figure 3.9: Heterotrophic and AOB kinetic response to cadmium shock and SDS strategy. sOUR (A) and sAOR (B) rates are plotted versus time after the initiation of the shock event.

Corrective action trains performed worse than the "no-intervention" approach for cadmium shock events. Due to the increased hydraulic loading outlined in the SDS strategy, the total and soluble cadmium levels were higher in the CA train, as compared to the PC train. The peak total cadmium loads for the CA train were 42% higher than the PC train (Figure 3.6A), while the peak soluble cadmium levels were 67 to 130% higher than the PC train (Figure 3.6B). Effluent NH_4^+ (Figure 3.7A) and NO_2^- concentrations for the CA train were 1 to 3 mg/l greater than were measured in the PC train, and peaked in the CA train approximately 1 HRT sooner than occurred in the PC train. Effluent NO_3^- (Figure 3.7B) and alkalinity concentrations confirmed the long recovery process. The CA train recovery was delayed by 24 to 48 hours as compared to the PC train. The peak impact and recovery times of the cadmium stress on sCOD were very similar between the CA and PC trains, for both toxic shock events (Figure 3.8A). The MLVSS levels indicate that the activated sludge

community in the CA train was negatively impacted as compared to the PC train; however the recovery times for the PC and CA trains were similar. Kinetic assays clearly demonstrate a more negative impact of the toxin on the CA train compared to the PC train. Similar to the PC train, the sAOR inhibition observed for the CA train was close to 100%. However, the AOB community kinetics in the CA train consistently took 24 to 48 hours longer to return to NC levels as compared to the PC train (Figure 3.9B). The sOUR values for the CA train also showed a near 100% inhibition, while recovery to NC levels took 8 to 15 hours longer than the PC train. Table 3.3 summarizes the impact of the CA train as compared to the PC trains for the cadmium shock events.

Table 3.3: Effectiveness of the SDS corrective action strategy for cadmium shock events as compared to the positive control.

Corrective action	Peak impact		Recovery time	
	Carbon removal	Nitrification	Carbon removal	Nitrification
SDS	-	-	-	x

x: poorer performance, - equal performance, NI: no impact of toxic shock

Implementation of CA strategy provides opportunity for reducing effluent NH_4^+ and COD loads during recovery. A contributing aspect of the SDS strategy is the process implication of using anaerobic starvation during the 16 hour storage phase of the strategy. Short term starvation of the isolated biomass is expected to have a minimal effect on the nitrifying bacterial community. Previous work has indicated that ammonia oxidizers can withstand long periods of starvation/idle phases (Morgenroth *et al.* 2000) and should be amenable to sludge storage. However, the

cited study was conducted on activated sludge from sequencing batch reactors (SBRs) and may not translate to a continuous flow reactor, as bacteria may operate under different physiological conditions in batch and continuous flow conditions (Frigon *et al.* 2002). As a result, the reactor configuration may have a strong impact on the sludge activity as influenced by corrective actions.

Assuming the immediate return of the anaerobically starved reactor to NC performance levels, blending the effluent from hydraulically overloaded and isolated trains may lower the apparent impact of the toxin on plant nitrification and carbon removal. This assumption is demonstrated by projecting "what if" scenarios from measured process performance data. The Plum Island WWTP has two parallel trains with capacities of 24 (Train A) and 12 (Train B) MGD. Shock events and CA strategies were implemented under the assumption of a worst case scenario, where influent flows are at 100% of the design capacity. However, the Plum Island WWTP normally operates at 40 to 70% of its design capacity. Normal flow conditions provide additional room for flow and performance manipulations to minimize the poor plant performance after a cadmium shock event. Once the corrective actions on CA train (line A) are terminated, the flow distribution can be adjusted such that line B (NC) operates at maximum capacity of 12 MGD, while the remaining flow is diverted to the CA train. Assuming sufficient sludge inventory in line B to treat a 12 MGD hydraulic loading, changes in effluent loads can be calculated by varying the percent of design flow experienced by the plant. Figures 3.10A and 3.10B reflect the impact of such altered flow distribution on effluent carbon and NO_3^- concentrations for strategy SDS (experiment S5) and assuming 5 different conditions, namely (1)

influent flows at 40% of design capacity (DC), (2) flows at 70% DC, (3) flows at 100% DC, (4) non-intervention as indicated by the PC effluent, and (5) unstressed NC train performance. Figures 3.10A and 3.10B clearly highlight that the benefit of blending effluents increases with lower influent flows.

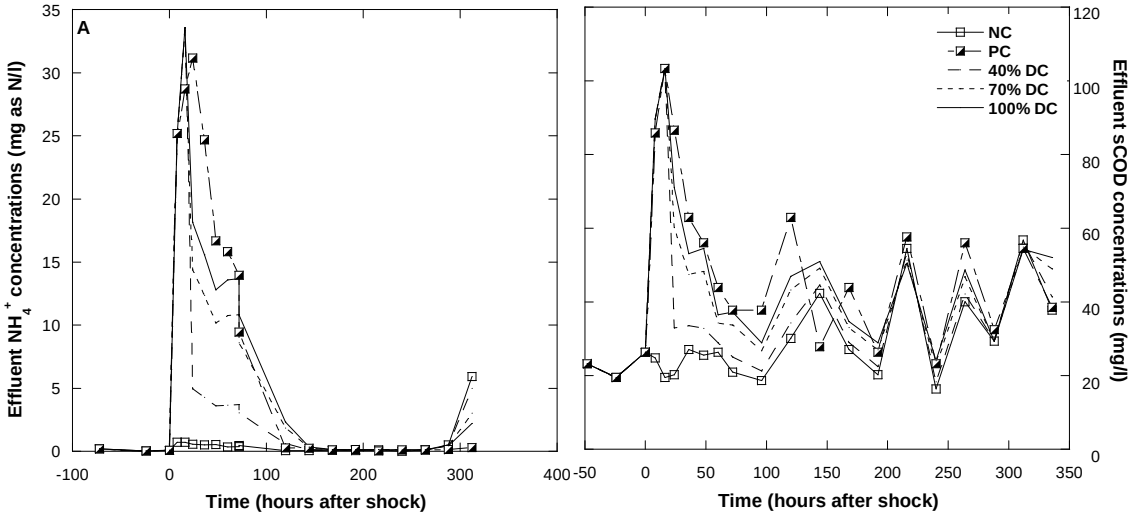

Figure 3.10: Comparing the performance of CA+NC effluent blends for (A) NH_4^+ removal and (B) carbon removal under different flow conditions with the non-intervention approach.

The blending of effluents from the CA and NC train does not reduce the peak impact of the toxic shock on the effluent quality, as compared to the PC train. However, the recovery times decrease significantly with the decrease in influent loading rates. The availability of the isolated train as a component of the CA strategy does provide a significant benefit towards faster recovery.

3.4 Discussion

Key process upset and recovery indicators. Previous studies presented a range of process performance parameters that were impacted by a wide array of toxins

(Henriques *et al*. 2007, Kelly *et al*. 2004). Unlike these previous studies, the key process parameters for this study were largely limited to carbon removal and nitrification. Important process parameters such as effluent TSS did not provide significant information. Previous research has linked oxidative stress related deflocculation to the induction of the GGKE stress response (Bott and Love 2004). The stress induced efflux of K^+ can increase the monovalent to divalent cation (M:D) ratio, which damages the activated sludge floc structure (Higgins and Novak 1997) and increases effluent TSS concentrations. The influent for this study was supplemented with 1000 mg/l of NaCl to simulate salinity levels at the Plum Island WWTP. This high background salt concentration would have likely reduced the impact of stress induced K^+ efflux on sludge settleability and effluent TSS. Additionally, the operation of a nitrifying continuous-flow systems caused denitrification in the secondary clarifiers, resulting in floating sludge and the loss of solids which was unrelated to the chemical stress or corrective action. This biomass loss was accounted for while maintaining the system SRT; however, the effluent solids concentration could not be reliably used to assess process performance in the experimental systems. The continuous flow systems caused various settling and hydraulic artifacts that were unrelated to the toxic shocks simulated or the corrective actions implemented. Due to the low ambient concentrations of carbon and nutrients in the aeration basin, activated sludge in the experimental system was constantly subject to famine conditions. As a result, filamentous bacteria proliferated and affected the sludge settling characteristics in some instances. This effect could also potentially have masked any impact of the toxic shock or corrective action on the

sludge settling characteristics. However, effluent quality parameters such as nitrogen species, alkalinity, and sCOD levels, as well as biomass kinetics proved to be effective and sufficient indicators of process upset and recovery.

Mitigation of hypochlorite shock events. The primary toxicity arising from calcium hypochlorite is due to the formation of hypochlorous acid. In aqueous solutions, there is equilibrium between hypochlorite ion (OCl^-) and hypochlorous acid (HOCl). Of the two, hypochlorous acid is the primary bacteriocide (Albrich and Hurst 1982). Hypochlorous acid causes a wide variety of bacterial stresses. These include but are not limited to oxidative stress (Chesney *et al.* 1996), bacterial growth inhibition (McKenna and Davies 1988), membrane permeability damage (Venkobachar *et al.* 1977), and inhibition of transport mechanisms (Barrette *et al.* 1989). In wastewater treatment systems, free chlorine or hypochlorous acid is known to primarily cause deflocculation by affecting adhesion properties of floc formers (Mascarenhas *et al.* 2004) and by inducing the GGKE stress response (Wimmer and Love 2004). Sustained chlorine exposure has also shown a significant drop in bacterial viability (Ramirez *et al.* 2000).

Despite the heavy hypochlorite shock load simulated during this study, the actual hypochlorite exposure was likely very low. The presence of the primary clarifier upstream of the aeration basin results in slow dissipation of the toxin into the aeration basin (Pinto *et al*. 2007). Additionally, literature reports that chlorine dissipates almost immediately when in contact with the wastewater matrix (Neethling *et al.* 1987, Qualls and Johnson 1983). Therefore, the amount of active toxin in the primary effluent is probably even lower, in the case of hypochlorite. This assumption

is supported by the fact that no free or total chlorine was detected in the PC and CA train effluents after 1-2 HRTs, suggesting that high hypochlorite reactivity also resulted in a short system residence time. Hence, the application of the CA strategy did not provide any benefit towards reducing the system residence time of hypochlorite for the Plum Island WWTP, as compared to the PC train.

In such a scenario, it would be ideal if the operator does not enact any CA strategy. In fact, this study suggests that the implementation of the CA strategies results in poorer process performance in response to hypochlorite shock. The ability of the operator to discern the appropriateness of CA strategy implementation is dependent on his/her ability to know the identity of the toxin. Under most scenarios involving accidental toxin spills, the information available to the operator may be limited to the detection of anomalous influent chemistry. Under such a scenario, it is recommended that the operator proceed with caution. Though the deleterious effects of the CA strategy were limited to its implementation, it is possible to conceive of situations under which the process upsets from the CA strategy may be a result of biological inhibition, rather than operational changes. For example, hypochlorous acid has a pKa of 7.54. As the pH of the system decreases below 7.5, the amount of hypochlorous acid available increases very rapidly. Under such conditions, hypochlorous acid may react with organic and inorganic constituents in the wastewater matrix to generate other chlorinated by products, such as monochloramines and chlorinated organic compounds (Pehlivanoglu-Mantas and Sedlak 2006, Mitch *et al.* 2003, Lazarova *et al.* 1998, Rebhun *et al.* 1997). Hypochlorous acid is highly reactive and has a very small half time in the wastewater

matrix (Neethling et al. 1987, Qualls and Johnson 1983). However, the chlorinated byproducts, which have significant toxic properties, are more stable than hypochlorous acid. The formation of these toxic byproducts can be significant when large amounts of free chlorine are available in the influent matrix, such as may occur during a toxic shock event. In order to minimize direct or indirect toxicity of hypochlorous acid, the primary goal of an effective mitigative strategy would be to ensure maintenance of a well buffered system with pH> 7.5.

However, the implementation of the FSC strategy resulted in conditions that could result in an increase in hypochlorous acid concentrations. The anaerobic isolation of activated sludge in the presence of untreated influent lead to a drop in the aeration basin pH due to VFA formation. The drop in pH in the aeration basin increases the likelihood of hypochlorous acid toxicity towards the activated sludge. Additionally, the accumulation of NH_4^+ during the implementation of the FSC strategy can induce the formation of chloramines. The combination of higher hypochlorous acid and NH_4^+/carbon accumulation during the FSC strategy could result in toxic effects for the biomass, thus extending the negative impact of the FSC strategy beyond its active implementation. It is recommended that implementation of a CA strategy should be accompanied by increased monitoring intensity. The monitoring should not only focus on identifying the toxin involved, but also evaluating changes in operational conditions, such as aeration basin pH levels, which may result from the CA strategy implementation.

Mitigation of cadmium shock events. It is clear that cadmium has a strong impact on process performance in the PC and CA trains. Its adverse effects include DNA

damage (Hartwig 1994), interference with the electron transport chain (Surowitz *et al.* 1984), and induction of the GGKE response (Bott and Love 2004, Gillam *et al.* 2005). The impact of cadmium toxicity on WWTP process performance has been well documented (Love *et al*. 2005). It is known to impact carbon (Henriques *et al*. 2007) (Figure 3.8A) and nitrogen removal (Kelly *et al*. 2004) (Figure 3.7), result in deflocculation (Neufeld 1976, Bott and Love 2002) (Figure 3.8B), as well as inhibit heterotrophic (Henriques *et al*. 2007, Madoni *et al.* 1999) (Figure 3.9A) and autotrophic respiration rates (Hu *et al*. 2004, Madoni *et al.* 1999) (Figure 3.9B). Unlike other heavy metals like copper, which exhibits a rapid impact on nitrification, cadmium toxicity requires 12 to 24 hours to display its maximum inhibition potential (Hu *et al*. 2004, Kelly 2005).

Cadmium toxicity is largely governed by its bioavailability. Cadmium can form otavite ($CdCO_3$) at pH 8.0 in high alkalinity systems; otavite is highly insoluble in water ($K_{sp} = 10^{-12}$). Cadmium also shows a strong potential for sorption to the biomass at ambient pH levels, 6 to 8, in activated sludge systems (Fristoe and Nelson 1983). Sequestration of the cadmium into the biosorbed form is accompanied by a decrease in toxicity levels (Zarnovsky *et al.* 1994). Cadmium speciation is not limited to the divalent cation, otavite, and biosorbed form, but also includes other species such as $CdOH^+$, $CdPO_4^-$, and CdOHCl, to name a few. Previous research has shown that soluble cadmium (Cd^{2+}) is the predominant toxic form and its concentrations correlate with inhibition levels seen in activated sludge systems (Hu *et al*. 2002). Studies suggest that in addition to bioavailability, microbial toxicity is also governed by physiological changes that may be induced by environmental changes. For

example, the inhibitory impacts of cadmium are lower under low pH conditions (Sandrin and Maier 2002). Worden *et al*. (2009) showed that the impact of pH on toxicity was due to differential gene expression, with higher and faster expression of important stress response genes occurring under slightly acidic pH conditions. Hence, it is important to consider both (1) cadmium speciation and (2) changes in microbial physiology subject to relevant environmental conditions while formulating appropriate mitigative actions.

The goal of an effective CA strategy should be to (1) minimize bioavailability of cadmium and (2) reduce system residence time. It is clear that the remedial scenarios did not achieve either of the two goals, as the residence times and peak levels for soluble cadmium were longer and higher in the CA train. Instead of relying on hydraulic overloading for toxin washout, an alternative strategy would have been to improve sequestration of the toxin in the primary clarifier either through precipitation or sorption. The pH induced precipitation could be achieved through the use of agents such as lime or sulfides. The shortcoming of this approach would be the impact of the increased pH or sulfide concentration on process performance. An alternative strategy would be to augment the primary influent with waste biosolids from the WWTP. This would allow for sorption based removal of cadmium, followed by removal of cadmium laden biomass in the grit chambers and the primary clarifier tank. However, both of these approaches would necessitate significant operational maneuvering, additional chemical storage and/or operational flexibility which may not be possible in the short-term. The choice of the SDS strategy was due to ease of implementation within the existing infrastructure at the Plum Island WWTP. In light

of the failure of the SDS strategy to reduce toxin exposure, the merits of the strategy should be considered based on the observed process effects.

Despite poorer performance of the CA train as compared to the PC train, the availability of an isolated treatment train as a part of the SDS strategy can be used to reduce the recovery time of the entire treatment system after the toxic shock, as compared to a no-intervention approach. This is due to the blending of effluents from the hydraulically overloaded and isolated trains. In addition to reducing recovery times, effluent blending also helped reduce the impact on process deterioration. Process deterioration was determined by calculating the decrease in effluent NO_3^- concentrations and the increase in effluent sCOD concentrations over a period of 1 SRT following the toxic shock event. Analysis of the effluent sCOD and NO_3^- concentrations highlight the significant advantage of the SDS strategy at the laboratory scale. Figure 3.11A highlights the decreasing impact of the toxic shock on carbon removal and nitrification performance in the entire treatment system under the assumption of varying the influent flow rates. Error bars represent the absolute difference in percent deterioration of process performance between the two independent experimental events, Cd-SDS_1 and Cd-SDS_2, as compared to the NC train performance. Figure 3.11B exhibits the fact that the SDS strategy can reduce recovery times, especially during WWTP operation at less than the design capacity. Error bars indicate the absolute differences between the recovery times for the effluent blend from the CA and NC trains as compared to the PC train for the two independent experiments, Cd-SDS_1 and Cd-SDS_2. The Plum Island WWTP typically operates at 40 to 70% of its design capacity. An evaluation of overall process

deterioration clearly indicates the benefits of implementing the SDS corrective actions strategy, especially with respect to carbon removal.

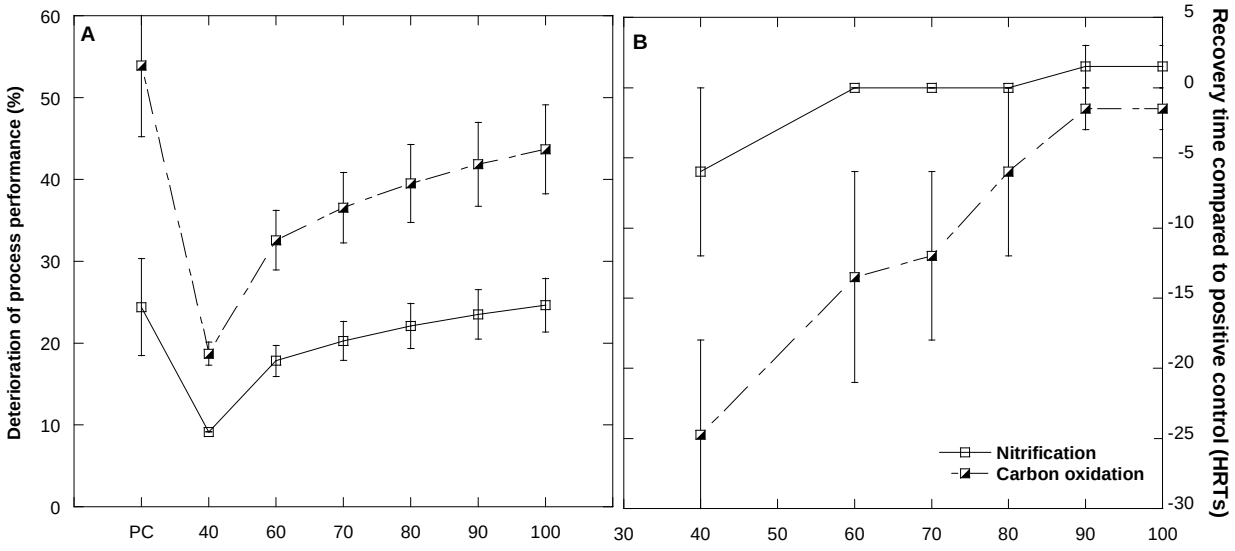

Figure 3.11: The impact of flow blending and variable influent loadings on nitrification and carbon oxidation: (A) percent process deterioration and (B) recovery times compared to the positive control. The abscissa reflects flow as a percent of design capacity between 40 to 100%. PC label in figure 3.11A reflects the positive control train or the non-intervention approach.

3.5 Significance and limitations.

The remedial interventions explored in this study address two completely different toxins. Though causal mechanisms for apparent activated sludge inhibition may vary significantly for the wide array of toxins relevant to WWTP upset, the two toxins tested in this study can be broadly classified as (1) a strictly soluble toxin with a large reactivity matrix which includes biologically active and inert materials and (2) a speciation-dominated toxin with reactivity targeted towards biologically active components of the treatment system. Such categorization of toxins may help the operator determine the appropriate remedial intervention approach. Unfortunately, under most situations, WWTP operators do not have the luxury of knowing toxin

identity or even chemical class. Upstream event detection systems, though promising, still largely suffer on account of signal unreliability and challenges associated with full-scale application. There is a research being conducted in this arena (Shaw *et al*. 2006, Ciosek and Wroblewski 2007); however, the ability of these sensors to reliably determine the chemical classification of toxins in real time seems unlikely in the immediate future.

The critical component of upset event detection and mitigation is the time scale of toxin detection and speed of CA strategy implementation. The operator must act solely on toxin detection data. At the same time, it is apparent from this study that the inappropriate application of CA strategies may impair process performance, as observed in the case of hypochlorite. Hence, all corrective actions should be immediately followed by the initiation of operational response validation protocols. Tools such as respirometry (Spanjers *et al.* 1996) or other toxicity sensors (Dalzell *et al.* 2002, Nasha *et al.* 2005, Tizzard *et al*. 2004) will prove to be an important component of the validation framework. Immediately after the implementation of CA strategies, the operator should collect influent samples for a toxicity assay. Based on the almost complete dissipation of hypochlorite in the primary clarifier, it is recommended that on-site toxicity assays be conducted on the primary effluent. Based on the output of the toxicity assay, if the operator determines that the primary effluent does not pose an eminent threat to process integrity, CA actions can be terminated. This will reduce the impact of the CA strategy on the deterioration of process performance. On the other hand, if the toxin is deemed to be a threat to the biology of the WWTP, the operator's actions may avert long term process upset.

3.6 Conclusions.

The results of this study indicate that:

1. Acute hypochlorite shock events do not pose a significant risk to WWTP performance and, hence, do not require remedial intervention. Inappropriate implementation of CA strategies for toxins like hypochlorite can adversely impact process performance.
2. Process upset during the CA implementation was due to specific aspects of the strategy tested. The negative impacts of the CA strategy were exhibited only during its implementation and were easily reversed upon termination of the mitigative intervention.
3. The adverse impact of the CA strategy for the hypochlorite shock event demonstrates the need to implement an operational response validation procedure through the use of rapid toxicity assays.
4. Cadmium shock severely impacted process performance. It impacted AOB and heterotroph kinetics, carbon removal, nitrification, and overall biomass growth rates.
5. Implementation of CA strategies for a cadmium shock event helps reduce process impact and recovery time. Under normal flow conditions, the SDS strategy should be able to reduce process impact on carbon removal and nitrification, and should also allow for quicker process recovery as compared to no intervention.

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Chapter 4.0

Pilot-scale evaluation of upset event detection and mitigation approaches for nitrifying wastewater treatment systems

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Abstract. The wastewater treatment industry lacks a systematic framework of corrective actions aimed at mitigating upset events resulting from toxic chemical shocks. Such a framework is essential to ensure rapid and calibrated responses to unexpected upset events. This study presents a pilot-scale evaluation of corrective actions developed to mitigate shock events involving hypochlorite and cadmium. The experimental systems were equipped with influent, process, and effluent monitoring sensors including pH, ORP, conductivity, dissolved oxygen, and a UV-vis absorbance based STIP:Scan sensor for estimating effluent nitrate and organic carbon concentrations. The influent sensor suite was successfully able to detect the presence of both toxins; however sensor response patterns varied significantly for both toxins. One corrective action was tested for the hypochlorite stress, while two independent corrective actions were tested for the cadmium stress event. This paper presents a comparative evaluation of the effectiveness and utility of the tested corrective actions, and discusses the reliability of real time

process data in assisting operators during the implementation of remedial intervention strategies.

Keywords. Cadmium, corrective action, hypochlorite, sensors

4.1 Introduction

Wastewater treatment plants (WWTPs) are subject to influent variability caused by inherent changes in wastewater quality and loads. At some utilities, the intentional and unintentional introduction of toxic chemicals into the treatment plant can cause serious problems for downstream biological processes. These upset events can result in the inhibition and washout of microbial communities that are important contributors to good activated sludge performance, which is evaluated at all plants based on the degree of organic carbon oxidation; at many plants, performance evaluations also consider ammonium oxidation (nitrification). Indeed, process failures can be exacerbated by the fact that functionally important bacteria, such as nitrifiers, are slow growing (Grady et al. 1999) and also highly susceptible to inhibition by chemical toxins (Anthonisen et al. 1976; Hockenbury and Grady 1977; Kelly et al. 2004). Process upsets can result in effluent quality that exceeds discharge limits for an extended period of time, especially if nitrification is an important performance metric.

The mitigation of direct (specifically targeted at the WWTP) and indirect (enters the collection system as a consequence of an upstream malfunction or direct attack on an upstream system) shock inputs of toxins to WWTPs requires that operators know the probable process effects from the contaminant(s) and the appropriate operational corrective actions (CA's) to enact in order to minimize or mitigate the impact of the

shock event. Information on the likely process effect(s) and the appropriate corrective actions to take that will keep the treatment process functional is largely unavailable at this time. A comprehensive strategy to address such upset events would require (1) an upset early warning system (UEWS) (Love and Bott 2000; Shaw et al. 2006) to detect the presence of toxins upstream of the WWTP, (2) a detailed understanding of source-cause-effect relationships that correlate process changes to the type of toxin present (Henriques et al. 2007; Kelly et al. 2004; Love et al. 2005), and (3) a framework for implementing corrective action strategies that can guide operators on how to mitigate contaminant-related process effects and facilitate rapid recovery (Pinto et al. 2007).

There have been significant developments in real-time water quality monitoring technology in the wastewater industry (Alex et al. 2003; Bourgeois et al. 2001). Online sensors have been developed that can estimate water parameters in relatively harsh oxygenated and reduced environments. These sensors include dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, conductivity and ultraviolet (UV)-based nutrient sensors that measure effluent nitrate (NO_3^-)/nitrite (NO_2^-) and carbon concentrations. The traditional application of real-time sensing is largely limited to process monitoring and control (Olsson et al. 2005). The vast amount of real-time data produced by these sensors is extremely useful for automated and continuous process control, and further equips operators with the ability to enforce operational feed-back loops wherever manual control is necessary. Often, a combination of signals are used to inform a WWTP-specific calibrated model to enable aeration, feeding, and recycle controls (Nielsen et al. 2001). A second application for using sensor technologies at WWTPs is to detect anomalies in wastewater composition upstream of the plant.

Previous work has shown that conventional and next-generation upstream sensor installations have the ability to detect the presence of toxins in the collection system (Ciosek and Wroblewski 2007; Shaw et al. 2006). While application of real-time sensing holds great promise for developing monitoring and response protocols for WWTPs that are vulnerable to chemical shock events, complex process control depends heavily on reliable sensor signals. Little is known about the accuracy, shift, response time, and long-term reliability of sensor signals that would be used in such an application. Although significant work has been directed toward minimizing the impact of sensor drift and measurement error towards process control (Rieger et al. 2003; Rieger et al. 2004), similar efforts have not been made to address reliability of sensor performance when used to detect toxins in wastewater.

This paper couples previous work on developing CA strategies that are designed to help operators respond to chemical toxin shock events at WWTPs (Chapter 3.0) with a pilot scale assessment of those CA strategies using real-time sensors. In this paper, we show that current sensor technology (1) can reliably detect anomalous influent chemistry, and (2) can be effectively used to monitor upset and recovery of WWTPs subject to toxin exposure. Additionally, we evaluate the effectiveness of two different CA strategies for the mitigation of hypochlorite and cadmium upset events.

4.2 Materials and methods

Experimental set-up. Experimental work was conducted at the Plum Island WWTP in Charleston, SC. The Plum Island WWTP has two trains, each with a capacity of 24 MGD (line A) and 12 MGD (line B), operating in parallel. The plant is operated at an 8

hour total hydraulic retention time (HRT) and a 10-day solids retention time (SRT). The pilot-scale system simulated Line A and was operated identical to the parent facility. The pilot-scale set-up was fed with primary effluent from the Plum Island WWTP. The choice of primary effluent as compared to raw effluent was based on the logistics of providing an influent supply line to the experimental set-up. The pilot-scale experimental system consisted of 3 identical continuous-flow complete mixed activated sludge systems, each consisting of a primary clarifier, an aeration basin, and a secondary clarifier. Each reactor line had a design capacity of 260 liters per day (lpd). The system was fed from the primary effluent line at the Plum Island WWTP. Two of the three lines received a simulated shock of a chemical toxin, while the third reactor line was used as the negative control (NC). Of the two shocked reactors, CA strategies were implemented on one, the CA train, while the other train was used as a positive control (PC). The experimental set-up was equipped with a suite of sensors that monitored influent chemistry, aeration basin conditions, as well as effluent quality. Sensors for this study were provided by Endress+Hauser (Greenwood, IN). A list of the sensors used for this study is presented in Table 4.1. This table also lists the points of sensor installation along the treatment systems. These points of installation are indicated in the pilot plant schematic shown in Figure 4.1.

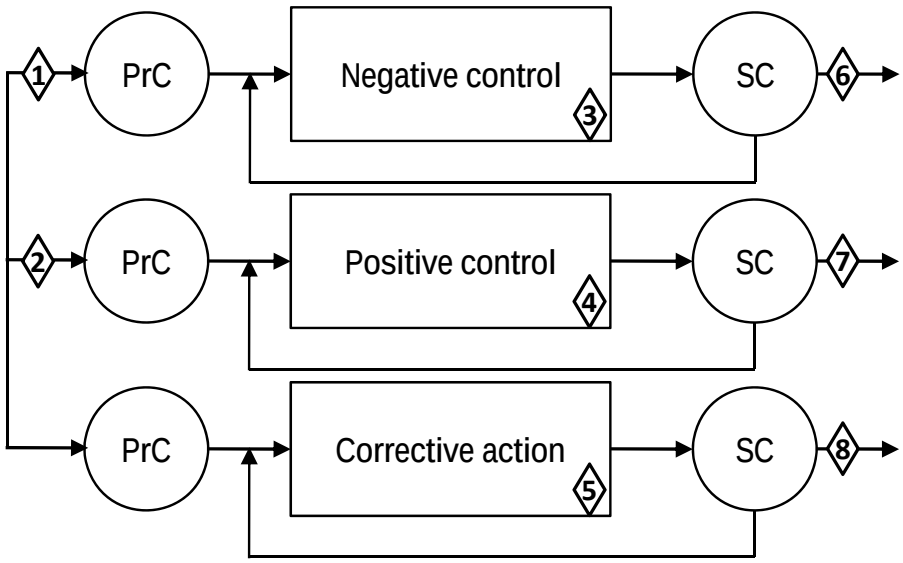

Figure 4.1: Schematic of points of sensor installations for the pilot-scale system. PrC: primary clarifier, SC: secondary clarifier.

Table 4.1: A list of sensors used for the pilot-scale study.

Total number of sensors	Parameter	Point of installation
5	pH	1, 2, 3, 4, 5
5	ORP	1, 2, 3, 4, 5,
3	Dissolved oxygen (DO)	3, 4, 5
2	Influent conductivity	1,2
1	Nitrate and total organic carbon	6, 7, 8

Shock event simulation. All the reactors were seeded from the Plum Island aeration basin outflow 16 hours prior to each shock event. Shock event simulations were carried out using either calcium hypochlorite ($Ca(OCl)_2$) or cadmium chloride ($CdCl_2$) as the chemical toxins. The choice of these toxins was based on a contaminant prioritization framework developed by the US Environmental Protection Agency (USEPA) (Casson 2009). The shock events were simulated spills equivalent to 1843 kgs of solid $Ca(OCl)_2$ or 5,000 gallons of 200 g/l cadmium received into the Plum Island WWTP when operating at its maximum design flow capacity, 36 million gallons per day (MGD). The $Ca(OCl)_2$ solid was dissolved in nanopure water immediately before simulating the shock

event. The toxin loads were chosen to simulate realistic spills of a decontamination agent and electroplating media (Morrow 2007).

Corrective action strategy. Corrective actions were implemented once the toxin was detected by upstream sensors. CA strategies were based on a CA plan matrix (CAPM) that was previous developed and assessed at the laboratory-scale using mixed liquor from the Plum Island facility (Chapter 3.0). The CAPM is a multi-tiered decision making tool that is organized based on (1) the nature of the toxin, (2) the data available on process effects caused by the toxin, (3) plant specific operational flexibility, and (4) instructions for process monitoring during and immediately after a toxic shock event (Pinto et al. 2007). The two corrective actions tested in this study were (1) chemically enhanced primary treatment (CEPT) and (2) sludge diversion and storage (SDS).

For the CEPT strategy, a portion of the biomass was partially isolated from the influent stream and stored anaerobically for 1 HRT. During this period the exposed portion of the treatment system was hydraulically overloaded to flush the contaminant from the treatment system, simulating the complete diversion of flow through Line A while isolating Line B from the influent. Additionally, an easily accessible coagulant was added into the influent stream to enhance the flocculation and settling of insoluble toxin in the primary clarifier. For this study, ferrous chloride ($FeCl_2$) was used as the coagulant of choice because the Plum Island WWTP uses it to achieve odor control in the headworks. The treatment plant is equipped to introduce up to 4000 gallons of $FeCl_2$ into the influent stream, and this capacity was considered in selecting a pilot-plant dosing of 9 ml/min for a period of 10 minutes. After 1 HRT, normal process operations were resumed and the exposed portion of the treatment system was reinforced by seeding it with

anaerobically stored biomass from the isolated train. Biomass storage in the isolated train was simulated by diverting the secondary clarifier sludge blanket from the corrective action train to a separate side stream reactor for anaerobic storage. Additionally, primary sludge was removed immediately following the termination of the CA strategy to avoid reintroducing any toxin that was not irreversibly sequestered. The SDS strategy was identical to the CEPT approach, except that coagulant was not added to the primary clarifier, and the CA was applied over 2 HRTs. Only the SDS strategy was applied for the $Ca(OCl)_2$ shock events, while both strategies were evaluated for cadmium . Table 4.2 presents a summary of the shock experiments conducted and the corrective actions tested.

Table 4.2: Shock event-corrective action experimental pairing.

Toxin	Shock load simulated	CA strategy	Replicates	Shock Identifier
Hypochlorite	1843 kgs of $Ca(OCl)_2$	SDS	2	$H\text{-}SDS_1$, $H\text{-}SDS_2$
Cadmium	5000 gallon spill of 6% $Ca(OCl)_2$	CEPT	2	$C\text{-}CEPT_1$, $C\text{-}CEPT_2$
Cadmium	5000 gallon spill of 200 g Cd/l	SDS	2	$C\text{-}SDS_1$, $C\text{-}SDS_2$

Sampling protocol and analyses. The pilot-scale systems were sampled at a decreasing frequency following each shock event. The experimental runs were concluded after all three trains exhibited consistent and comparable performance, as indicated by the effluent quality. The stressed reactors were considered to have recovered once the effluent quality was better than or within 10 percent of the quality parameters of the NC reactor.

Extant mixed liquor specific oxygen uptake rate (sOUR) assays (Grady et al. 1996) were conducted by terminating the influent and air supply to the aeration basin and measuring the drop in DO concentration for 5-6 minutes. The slope of the decreasing DO concentration was divided by the mixed liquor volatile suspended solids (MLVSS) concentration to generate calculated sOURs. Total organic carbon (TOC) and nitrate-N concentrations were measured using a STIP:Scan, which uses light absorption (200 to

480 nm) to quantify both (Endress+Hauser, Inc). Interference in the STIP:Scan outputs for TOC and nitrate-N were evaluated for each toxin, the coagulant, and nitrite (NO_2^-). As previously reported, NO_2^- showed a strong linear interference with nitrate measurements (Kelly et al. 2004) and was independent of NO_3^- concentrations. The positive error between what STIP:Scan reported and actual NO_3^- values was determined to be 1.766±0.065 mg/L NO_2^--N (Appendix figure B.10). All reported nitrate concentrations are adjusted for nitrite interference using this correction factor. Accuracy of the STIP:Scan instrument was checked on a daily basis by estimating the TOC and NO_3^- concentrations for manufacturer provided standard solutions. The NO_3^- measurements retained both accuracy and precision, while the TOC estimates lost accuracy but maintained their precision during the course of the experiment. In order to correct for the loss of TOC accuracy, the analysis of each sample was preceded and followed by estimating TOC concentrations for clean tap water. Sample TOC concentrations were calculated as the difference between the measured values of the sample and clean tap water. Mixed liquor suspended solids (MLSS), volatile suspended solids (MLVSS), total/soluble contaminant, sludge volume index (SVI), influent/effluent ammonium (NH_4^+), and effluent NO_2^- were measured according to standard methods (APHA 1998). Table 4.3 summarizes sample collection and analysis for influent, effluent and mixed liquor matrices, provides monitoring frequency, and lists the protocol number for standard methods used.

Table 4. 3: Pilot-scale monitoring schedule

Matrix	Test	Day 0	Day 1	Days 2-3	Day 4 onwards
Effluent	Soluble toxin[a]	-	3x	2x	1x
	TOC	Every 2 hours per reactor train			
	pH	Continuous			
	TSS[b]	1x	1x	1x	1x
	Ammonium[c]	1x	3x	2x	1x
	Nitrite	1x	3x	2x	1x
	Nitrate	Every 2 hours per reactor train			
Influent	TOC	Every 2 hours per reactor train			
	Ammonium[c]	1x	1x	1x	1x
	TSS[b]/VSS[d]	1x	1x	1x	1x
	pH	Continuous			
	ORP	Continuous			
	Conductivity	Continuous			
Mixed Liquor	SVI[e]	1x	1x	1x	1x
	SOUR	1x	2x	1x	1x
	sAOR	1x	2x	1x	1x
	MLSS[b]/MLVSS[d]	1x	1x	1x	1x
	Dissolved oxygen	Continuous			
	pH	Continuous			
	ORP	Continuous			

[a] Method 3111B
[b] Method 2540D
[c] Method 4500F
[d] Method 2540D
[e] Method 2710D

Sensor data processing. Sensors were thoroughly cleaned and calibrated once every day according to manufacturer's instructions (Endress + Hauser, Inc). Data was filtered to remove time points during sensor clean up and calibration periods. Filtered data from each monitoring environment, such as influent, aeration basin, and effluent, were aligned manually along a common time series with the initiation of the toxic shock fixed at t=0. Gaps occurred in aligned data sets due to the removal of time points when sensor

cleaning was performed and were filled with values equal to the last measured time point, to facilitate discriminate analysis in accordance with recommended practices (Olsson et al. 2005). Sensor data was normalized by dividing data points with the maximum range recorded for each sensor signal for the duration of the experiment. This allowed each sensor signal to have equal weighting when used for multivariate analysis. The rate of change in normalized sensor signals was calculated and the rates were used to perform a principal component analysis (PCA) of the influent data (Rosen and Olsson 1998). Use of normalized rate of change data ensured that variance in principal component scores did not show greater bias towards any single sensor measurement. Principal components were calculated using the statistical software package, PAST (Hammer et al. 2001).

4.3 Results and Discussion

Toxin detection in primary effluent. The mitigation of upset events requires rapid operational intervention. The appropriate question for an operator facing an imminent process upset is whether any remedial intervention is essential. Additionally, the implementation time frame of any corrective action must be small enough to accommodate a response to unexpected upset events. This time frame requirement not only necessitates logistical simplicity in the actions proposed, but also entails effective early detection and continuous monitoring abilities that can inform the operator of the process state of the treatment plant. In this study, pH, ORP, and conductivity sensors were employed in the influent lines to help identify anomalous influent chemistry (Shaw et al. 2006). The choice of these sensors was based on recommendations from a previous

study (Shaw et al. 2006), which evaluated the effectiveness of these influent parameters in identifying an array of chemical toxins.

Independent examination of the raw signals produced by the three influent sensors shows that each was able to discriminate between the two toxins based on the nature of their response curves (Figure 4.2). The pH, ORP, and conductivity sensors showed a positive spike for the hypochlorite shock, while the pH sensor exhibited a negative spike after cadmium was introduced. Despite rapid detection of both toxins, the return of the pH and ORP signals to pre-stress levels took much longer for the hypochlorite shock than the cadmium shock. The pH and ORP sensors in the PC influent line required between 5 and 13 hours to return to NC trends for the hypochlorite shock, while both signals returned to pre-stress levels within 5 hours of the cadmium shock (Appendix figures B.11, B.12, B.23, B.24, B.35, B.36, B.49, B.50, B.63, and B.64). The conductivity sensor was the most robust and reliable among the three sensors used for influent monitoring. It consistently provided stable signals and maintained calibration for the duration of the study, despite encountering biofouling at the sensor surface. Additionally, the rapid rate at which the conductivity signal responded to the presence of the toxin was followed by a quick return to pre-shock conditions, and indicates the reliability of the conductivity sensor in successfully detecting both the beginning of a chemical shock and its dissipation from the influent line. The toxin was delivered over a period of 10 to 15 minutes, and the conductivity signal for hypochlorite shock returned to its baseline levels within 0.17 hours (10 minutes) while the cadmium induced signal took approximately 0.58 hours (35 minutes) to return to unstressed levels. This high level of responsiveness

gives operators an important tool that signals the duration of a shock event, and informs the CA strategy that is ultimately implemented.

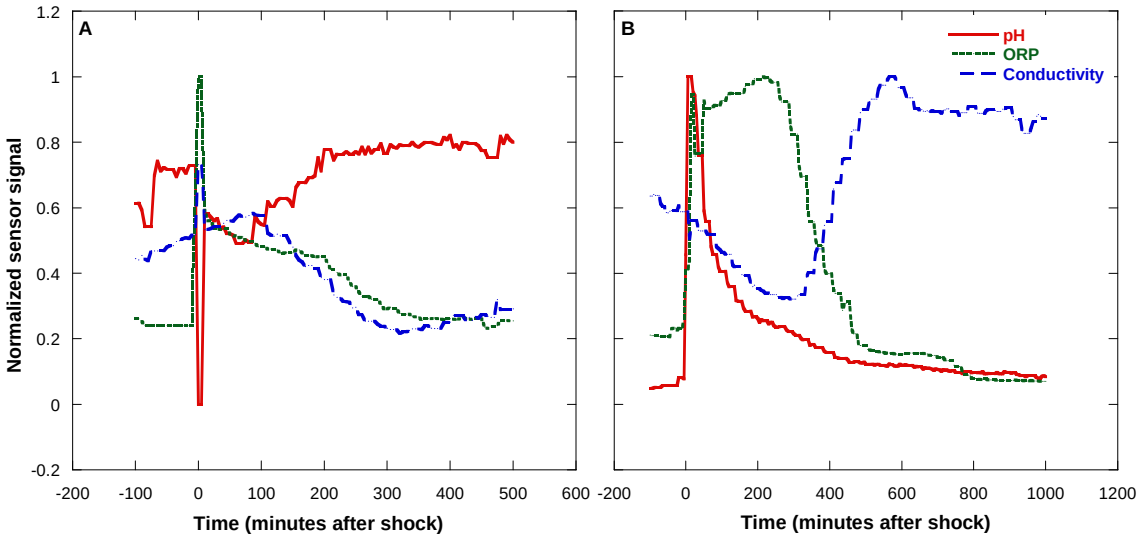

Figure 4.2: Recovery time for raw sensor signals normalized to experimental range for the (A) C-SDS$_1$ and (B) H-SDS$_1$.

The Plum Island WWTP experiences large, but gradual, variations in influent conductivity due to infiltration of seawater into the collection system. In addition, the ORP signal shows a significant drift due to the collection and sloughing of debris on its surface. Prior studies have shown that a persistent problem with influent sensors can be the low signal to noise ratio, which requires extensive data clean up (Shaw et al. 2006). Changes due to sloughing of debris on the sensor surfaces may result in a false positive signal that indicates the presence of an influent anomaly, while low signal to noise ratios may hide potential upset events. To improve our ability to identify sensor signal outliers that could indicate an important change in wastewater composition, the rate of change in sensor response was used rather than raw values.

The rate of sensor signal change dampened the natural inherent variations observed for all three sensors and highlighted rapid changes that could be directly

attributed to anomalous influent chemistry. By conducting PCA on the normalized rate of signal change from all three sensors together, we were able to discriminate between time points where the toxin was detected as compared to pre-and post-stress data (Figure 4.3). Figure 4.3 shows that PCA detected anomalies in influent composition due to the presence of either toxin when the normalized rate of change in sensor signal data were used (Figure 4.3A and 4.3B) but not when the normalized raw data were used (Figure 4.3C and 4.3D). The use of normalized rate of change data was also able to eliminate the problem observed with the raw pH and ORP data where the sensors were slow in returning to baseline signals. Therefore, manipulating normalized raw data by calculating the rate of change and applying the modified data to PCA proved to be a powerful tool in reliably detecting the presence and persistence of the toxins tested here. However, PCA was not able to distinguish between the two toxins based on 6 independent experiments. Though, each sensor signal was independently able to identify the presence of the toxins tested during this study, the range of chemical variations captured by the pH, ORP, and conductivity sensors will increase the probability of capturing a greater array of influent anomalies than can be detected by a single sensor alone.

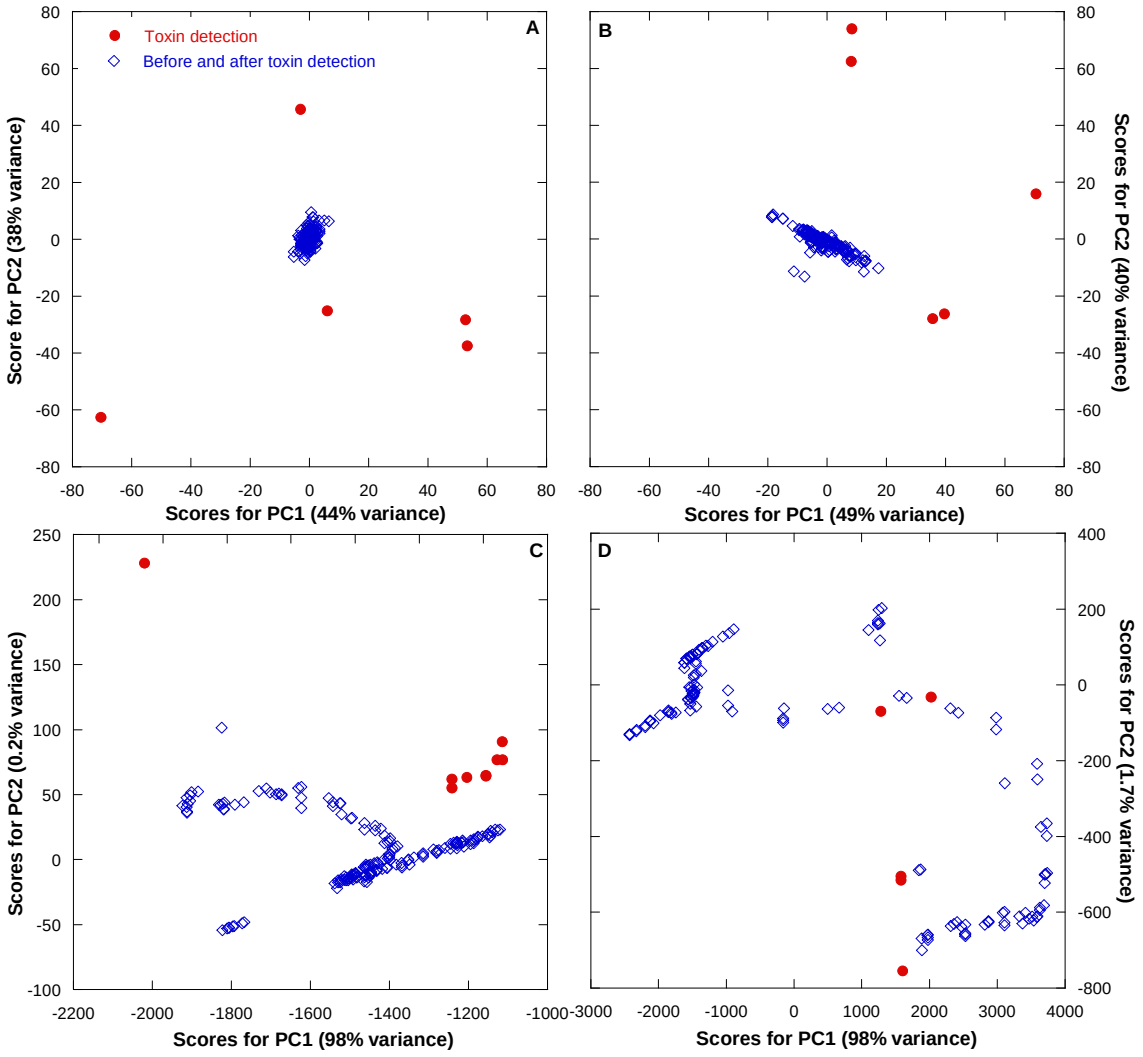

Figure 4.3: Principal component analysis in pH, ORP, and conductivity sensors for the (A, C) cadmium shock and (B, D) hypochlorite shock. Figures (A) and (B) presents a PCA conducted on normalized rate of change of sensor signals. Figures (C) and (D) presents a PCA conducted on raw sensor signals.

Upset event monitoring and mitigation for hypochlorite stress. Hypochlorite dissolves to form hypochlorous acid, which is the primary toxicant. Sustained exposure to hypochlorous acid has been shown to correlate with a significant drop in bacterial viability (Ramirez et al. 2000). Hypochlorous acid causes a wide variety of bacterial stresses, which include oxidative stress (Chesney et al. 1996), bacterial growth inhibition

(McKenna and Davies 1988), membrane permeability damage (Venkobachar et al. 1977), and inhibition of transport mechanisms (Barrette et al. 1989). In wastewater treatment systems, free chlorine or hypochlorous acid is known to cause deflocculation by affecting adhesion properties of floc-forming bacteria (Mascarenhas et al. 2004) and by inducing the glutathione-gated potassium efflux (GGKE) stress response (Wimmer and Love 2004). Previous research has shown that hypochlorite dissipates extremely rapidly when in contact with influent organic material (Qualls and Johnson 1983). Additionally, chlorine has a short system residence time when introduced into the activated sludge matrix (Neethling et al. 1987). In the current study, the aeration basin biomass was exposed to free chlorine only briefly. The magnitude of ORP change and rate of change in aeration basin ORP imply that, indeed, the hypochlorite concentration dissipated rapidly (Figure 4.4). The retention time and hydraulic profile of the primary clarifier allowed enough time for the hypochlorite to react with the influent matrix, and dampened the concentration of toxin entering the aeration basin (Pinto et al. 2007).

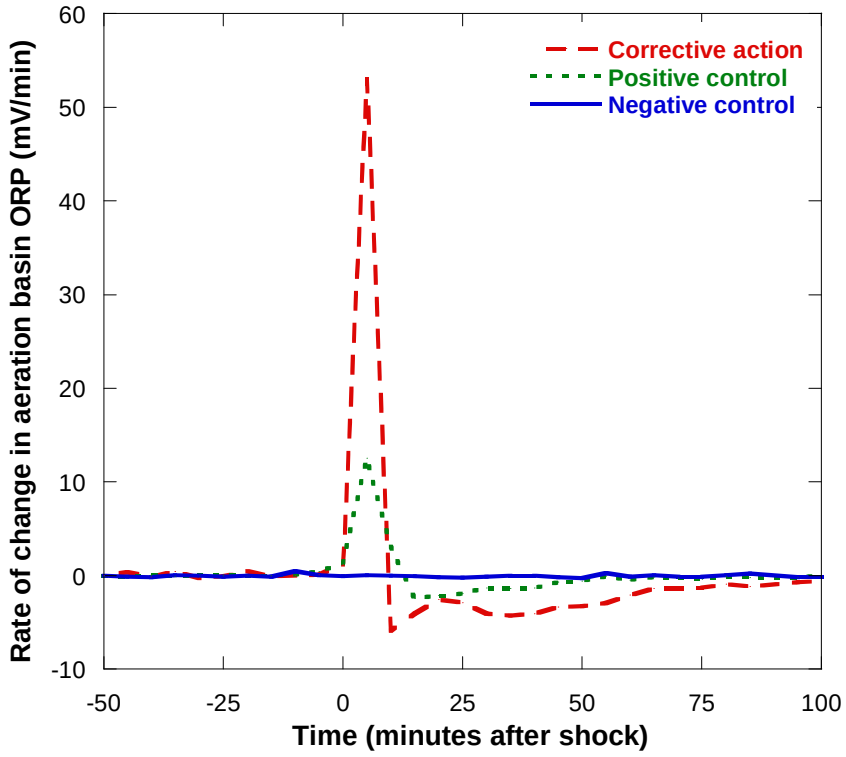

Figure 4.4: Detection of hypochlorite in the aeration basin by the ORP sensors in the CA and PC trains relative to the NC train.

The $Ca(OCl)_2$ stress experiments did not correlate with indicators of deteriorating, overall process performance or maximum specific activities of heterotrophic and autotrophic biomass (Appendix figures B.14 to B.34). This is probably due to the low hypochlorite concentrations and probable short-term exposure time in the aeration basin. In fact, the most significant impact on process performance occurred in the CA train, implying that the SDS strategy of hydraulic overloading and biomass storage lead to measurable stress not seen with the hypochlorite. Specifically, the CA train experienced decreased effluent nitrate-N as compared to the PC train (Figure 4.5); this observation was corroborated by a small amount of ammonium-N accumulation (Appendix figure B.26) and significant nitrite-N accumulation (Appendix figures B.15 and B.28). The extant sOUR assays showed that these process performance effects were not due to

inhibited biomass, since sOURs for the hydraulically overloaded CA train increased relative to the positive and negative controls approximately 8 hours after the chemical shock (Figure 4.5). This increase can be attributed to higher ambient ammonium-N, nitrite-N and TOC concentrations present in the CA train relative to the PC and NC trains (Appendix figures B.15, B.26, B.28, and B.29). Additionally, there was no significant impact of the hypochlorite shock or the SDS strategy on mixed liquor concentrations or effluent suspended solids concentrations in the PC and CA reactors. Hence, the acute hypochlorite shock did not generate a significant impact on process performance. While some performance impacts were observed for the CA train, they were small and rapidly recovered. Based on these observations, it is recommended that no CA strategy be employed for the mitigation of acute hypochlorite stress.

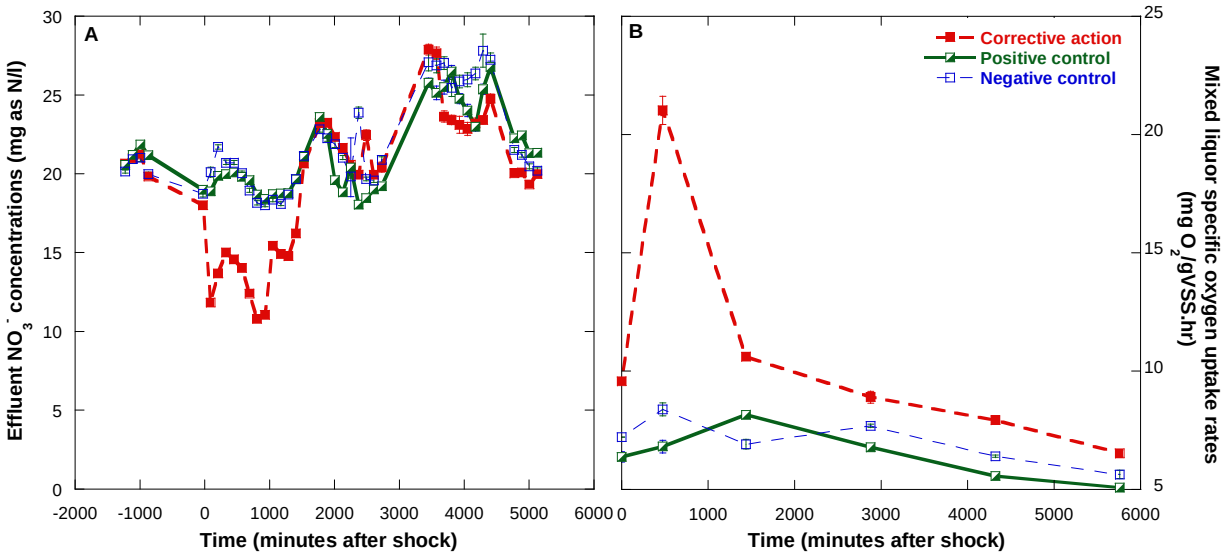

Figure 4.5: Impact of the hypochlorite shock and corrective action on the (A) effluent nitrate and (B) mixed liquor sOUR levels.

Upset event monitoring and mitigation for cadmium stress. Cadmium is a significant industrial chemical with large scale applications in the electroplating industry (John et al. 2002; Morrow 2007). Its adverse biological effects include DNA damage (Hartwig

1994), interference with the electron transport chain (Surowitz et al. 1984), and induction of the glutathione gated potassium efflux response (Bott and Love 2004; Gillam et al. 2005). Additionally, cadmium toxicity is largely governed by its bioavailability. Previous research has shown that soluble cadmium (Cd^{2+}) is the predominant toxic form and it correlates with inhibition levels seen in activated sludge systems (Hu et al. 2002; Hu et al. 2003; Kelly et al. 2004). Unlike hypochlorite, cadmium stress had a strong impact on process performance as exhibited by an increase in effluent TOC and decrease in effluent nitrate-N concentrations in the PC train (Figure 4.6). Though short lived, the process inhibition was clearly exhibited by poor effluent quality and slower biomass kinetics (Appendix figure B.59). Of the two different corrective actions tested, specifically the SDS and CEPT strategies, both were aimed at (1) minimizing biomass exposure to the toxin, and (2) mitigating the process impact of the toxin. Table 4.4 highlights the advantages and drawbacks of the two corrective action strategies.

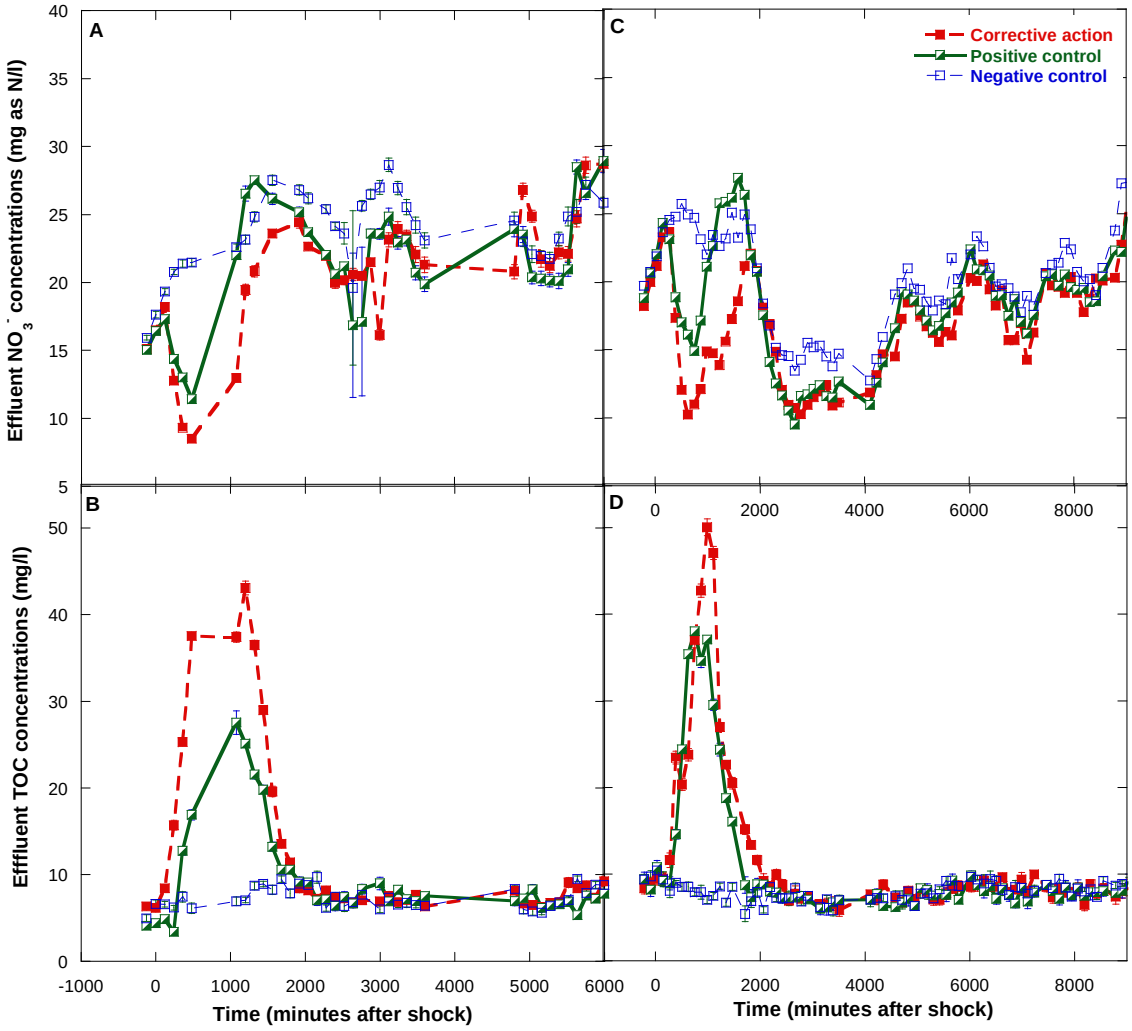

Figure 4.6: Impact of cadmium stress and corrective actions on effluent nitrate and effluent TOC concentrations for the (A, B) SDS and (C, D) CEPT strategies

Table 4.4: Potential benefits and drawbacks of the CEPT and SDS Strategies.

Strategy	Actions	Benefits	Drawback
SDS	Hydraulic overloading of the CA train for 2 HRTs.	High loading reduces the system residence time of the toxin.	Sustained anaerobic starvation of the isolated train could reduce biomass viability.
	Augmentation of the toxin-exposed biomass with toxin-free biomass.	Toxin-free biomass provides additional carbon and nitrogen removal efficiency which is lost through inhibition of toxin shocked biomass.	Reduce biomass content of the anaerobically starved biomass, thus reduce the carbon and nitrogen removal ability of isolated train.
CEPT	Hydraulic overloading of the CA train for 1 HRT.	Reduces anaerobic starvation period of the isolated biomass. Faster resumption of normal WWTP operation protocol.	Reduces the rate at which toxin is flushed from the treatment system.
	Addition of coagulant to settle cadmium precipitate in the primary clarifier.	Reduces carryover of cadmium precipitate into the aeration basin. If carried into the aeration basin, the cadmium precipitate could increase soluble and bioavailable toxin concentrations in the aeration basin.	Coagulant could have an inhibitory impact on the biomass.
	Remove cadmium-ladened primary sludge after 1 HRT.	Reduces potential for carryover of settled cadmium precipitate.	Additional step of removing primary sludge could take a primary clarifier offline and increase carbon and nitrogen loading to the aeration basin for the duration of this procedure.

A key advantage of the CEPT strategy is that the addition of coagulant and removal of primary sludge would likely reduce the intensity of cadmium exposure in the aeration basin. However, this strategy did not reduce the difference in soluble effluent cadmium concentrations between the PC and CA trains for the pilot-scale shock events as compared to the SDS strategy (Appendix figures B.38, B.52, B.66 and B.81). Hence, it is clear that the addition of $FeCl_2$ did not provide any advantage towards cadmium retention in the primary clarifiers. This observation should not rule out the cadmium removal benefits of other, more potent coagulants such as $FeCl_3$ and alum. $FeCl_2$ was chosen in this study, due to easy availability and onsite capability of introducing it into the influent stream. Similarly, the extended period of hydraulic overloading dictated by the SDS strategy also did not reduce the toxin residence time in the treatment train. Hence, the beneficial aspects of both corrective action strategies are limited to the mitigation of process effects. Analyses of peak deterioration in effluent quality and time taken to return to NC levels provide an important insight into the performance of the two CA strategies. Table 4.5 summarizes the recovery times and peak inhibition of TOC removal and nitrate-N formation for the PC and CA trains for both corrective action strategies.

Table 4.5: Comparing peak cadmium shock impact on and recovery times of effluent TOC and nitrate-N concentrations. Values presented are the average (± difference) of two independent experimental runs for each CA strategy.

Parameter	TOC		Nitrate	
	Peak impact factor	Recovery time (hours)	Peak impact factor	Recovery time (hours)
CEPT strategy				
PC train (PC)	3.45±1.7	31.8±1.0	1.35±0.3	13.8±6.9
CA train (CA)	10.9±9.5	37.1±9.0	1.55±0.1	25.1±11
Difference (CA-PC)	7.45 ±7.8	5.3±8.0	0.2±0.2	11.3±4.1
SDS strategy				
PC train (PC)	4.8±0.9	34.9±1.8	1.4±0.1	16.9±2.3
CA train (CA)	6.5±0.9	39.3±3.9	1.6±0	30.2±2.3
Difference (PC-CA)	1.7±1.8	4.4±2.1	0.15±0.1	13.3±0

Both corrective action strategies implemented during this study resulted in greater process deterioration as compared to the no-intervention approach, as simulated by the PC train. In the case of the hypochlorite shock event, the deterioration in process performance was associated with an increase in hydraulic loading to the CA train. In comparison, the CA train for the cadmium stress showed poorer performance due to a combination of increased hydraulic loading as well as toxin loading. Table 4.5 clearly highlights that the SDS and CEPT strategies had a similar impact on process performance, except for the greater deterioration in TOC removal for the CEPT strategy. The lower TOC removal efficiency for the CEPT strategy could be due to (1) $FeCl_2$ interference in STIP: Scan based TOC estimates or (2) inhibition of TOC removal due to $FeCl_2$ addition. Independent $FeCl_2$ controls showed that the addition of the coagulant did not have any effect on TOC removal or STIP:Scan-based TOC analyses. Therefore, the SDS strategy outperformed the CEPT strategy with respect to the peak impact on TOC removal.

The absence of CA strategy benefits should not be interpreted to rule out the importance of corrective actions, such as the ones proposed in this study. The

duration of the process upset and recovery following the cadmium stress was less than 48 hours for all four cadmium shock events. The extent of the process damage may be an important parameter in determining the validity of the developed CA strategies. Prior laboratory scale experiments indicated that similar CA strategies were able to reduce the overall impact of the cadmium stress (Chapter 3.0). This reduced impact was obtained by calculating how flow manipulation could be used to maximize the influent flow through the isolated train (Line B) and bleed the remaining flow through the inhibited treatment (Line A). Altering the influent flow distribution after the termination of the corrective action resulted in significant improvements in effluent quality, especially under flow conditions below the design capacity. However, such theoretical flow manipulations and effluent blending estimations did not exhibit any significant advantages for the pilot-scale experiments described in this paper. The duration of cadmium-associated process upset for the pilot-scale study was significantly lower, by 6 to 8 days, as compared to the laboratory scale experiments (Chapter 3.0). If the pilot-scale cadmium stress events had resulted in greater process disruption, then the theoretical manipulation of flow between the two parallel treatment trains would have likely made the CA strategies a much more attractive option. As a result, it can be argued that corrective actions would be appropriately implemented after assessing the imminent process damage posed by the detected upset event.

Process monitoring sensors maintained their accuracy and sensitivity for the duration of the study, except for the STIP:Scan sensor. Based on analysis of manufacturer provided standards, STIP:Scan estimated TOC values lost their

accuracy during the course of the study, while maintaining both precision and sensitivity. The pH and DO sensors were good indicators of ammonium-N and carbon oxidation inhibition. The pH sensor signal served as a real time indicator of nitrification upset and recovery (Appendix figure 97) and the DO sensor reflected the overall biomass inhibition and recovery. The DO sensor patterns reflected the pattern observed with measured TOC (Appendix figure 98). Similar to the hypochlorite shock events, the ORP sensor was a rapid indicator of changes in aeration basin chemistry and detected cadmium shock, $FeCl_2$ addition, and hydraulic overloading required by the SDS and CEPT strategies (Figures B.93, B.94).

4.4 Conclusions

Early detection and appropriate remedial intervention may help with the successful mitigation of upset events. This study shows that the application of conventional sensors such as pH, ORP, and conductivity in the influent line can successfully detect anomalies in the influent matrix. Multivariate analysis can be used to determine normal process states and anomalous states; however, such an analysis is not likely to distinguish the type of chemical toxin being detected. The effectiveness of the corrective action depends on the nature of the toxin and the extent of imminent process damage. No corrective actions are required for hypochlorite, a highly reactive and soluble oxidative toxin, which has a short system residence time. The need for corrective actions for toxins with long system residence time, such as cadmium, should be assessed on a case by case basis. Corrective actions should only be implemented if influent anomaly detection indicates long term process damage.

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Chapter 5.0

Investigating the role of bacterial community structure and predator grazing on the recovery of bioreactors exposed to transient cadmium stress.

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Abstract.

This study investigates the impact of transient cadmium stress on the structure and function of a microbial community in an activated sludge system at two different operational scales. The impact of toxic stress at the bioreactor process, microbial kinetics, community structure, and multi-trophic interaction scales is examined. Following complete process recovery, there was a significant increase in the respiration rates of both heterotrophic and autotrophic bacteria present in the mixed liquor. Though a community-fingerprinting approach provided substantial insight into competitive interactions and the toxin sensitivity of select taxa, it was unable to explain the kinetic trends observed with the perturbed community. The post-stress increase in respiration rates correlated with the post-stress increase in overall bacterial

abundance. The increase in bacterial abundance was not related to cadmium stress induced changes in community structure, but strongly correlated with the elimination of protozoal predatory stress. Independent non-inhibitory tests confirmed that the decrease in predatory stress directly correlates with increased bacterial abundance. This paper highlights the importance of expanding the investigative boundaries of the microbial ecology of bioengineered systems to include trophic level interactions, especially under perturbation scenarios.

Keywords. Toxic perturbation/microbial kinetics/ammonia oxidizer/community structure/trophic interactions/predator

5.1 Introduction.

The activated sludge wastewater treatment process represents an engineered ecosystem with well-defined functional demands. Consistent with the vulnerabilities exhibited by any ecosystem, activated sludge systems experience frequent breakdowns resulting from an array of unpredictable environmental deviations, including chemical shock events (Henriques *et al.*, 2007; Hu *et al.*, 2002; Kelly *et al.*, 2004; Zarnovsky *et al.*, 1994). Though toxin-related wastewater process upsets are common (Love and Bott, 2000), microbial ecology studies of bioreactor systems exposed to toxic perturbations are limited (Principi *et al.*, 2006; Principi *et al.*, 2009; Saikaly, 2005).

Bioreactor microbial ecology studies are primarily geared towards evaluating the transference of macro-ecological principles (Ayala-del-Rio *et al.*, 2004; Curtis

and Sloan, 2006; Fernandez *et al.*, 1999; Fernandez *et al.*, 2000; Woodcock *et al.*, 2007) to bioengineered microbial systems. Ecological principles that govern microbial community structure and function in non-perturbed bioreactors may be significantly different from perturbed communities (Wootton, 1998). Community interactions within the activated sludge consortium are not only governed by competitive (Tilman, 1987), symbiotic (Moran *et al.*, 2009), and communicative (March and Bentley, 2004) interactions of functionally relevant microorganisms, but they are also influenced by traverse trophic landscapes (Konopka, 2009). In this paper, we evaluate the impact of a transient perturbation with a model toxin, cadmium (Cd^{2+}), on the microbial ecology of an activated sludge system. Cadmium is industrially important (John *et al.*, 2002; Morrow, 2007) and its impact on bacterial (Chandran and Love, 2008; Zarnovsky *et al.*, 1994) and eukaryotic microorganisms (Madoni *et al.*, 1999, Hoffman and Atlas, 1987) has been investigated previously, making it a reasonably well characterized toxin. This paper will answer two key questions:

1. Can bacterial structural dynamics accurately predict community function for chemically perturbed systems?
2. What is the impact of predator grazing on microbial community structure and function in perturbed systems?

In addressing these questions, we restricted the bacterial community analyses to ammonia oxidizing bacteria (AOB) and the eukaryotic community analysis to ciliated bacterivorous protozoa. Constrained phylogeny (Purkhold *et al.*, 2000) and limited diversity in bioreactor systems (Juretschko *et al.*, 1998) makes the AOB community

an attractive target for microbial ecology studies (Ahn *et al.*, 2008; Akarsubasi *et al.*, 2009; Coskuner *et al.*, 2005; Fierer *et al.*, 2009; Wittebolle *et al.*, 2009). Ciliated protozoa are important bacterivorous predators and are important indicators of biomass health in the activated sludge matrix (Madoni *et al.*, 1999).

5.2 Materials and Methods

Continuous flow experimental systems. Laboratory- and pilot-scale systems were operated in accordance with the Plum Island Wastewater Treatment Plant in Charleston, SC, which served as the source of the inoculum used for this study. Each independent experiment consisted of one unstressed control (UC) and two stressed reactor trains, specifically SR1 and SR2. In order to mimic the hydraulics of toxin flow through a wastewater treatment system, each treatment train consisted of a primary clarifier, aeration basin, and secondary clarifier. The total volume of the each reactor train at the laboratory- and pilot-scale was 30 and 260 liters, respectively. Both systems were operated at an 8-hour hydraulic retention time (HRT) and 10-day solids retention time (SRT) and fed raw wastewater. The inoculum used in this study was acclimated to high salt concentrations due to sea water infiltration into the collection system. The influent for laboratory scale experiments was supplemented with 1000 mg/l of NaCl in order to simulate ambient salt concentrations, while the pilot scales experiments were conducted at the Plum Island WWTP and hence, did not require supplementation. A total of four independent single stress experiments were conducted at both scales of operation. A fifth experiment at the laboratory-scale involved the application of a second cadmium stress after complete recovery of SR1

and SR2, as indicated by effluent quality and biomass health, following the first cadmium stress. Table 5.1 summarizes the experimental schedule and the cadmium loads applied to the stressed reactors.

Table 5.1: Cadmium stress experimental strategy. Stresses were simulated as an acute shock load of cadmium. Each replicate experiment consisted of two stressed and one unstressed reactor.

Experiment type	Operational scale	First cadmium stress load (mg/gVSS)		Second cadmium stress load (mg/gVSS)		Number of replicates
		SR1	SR1	SR1	SR2	
Single stress	Laboratory	96	120	N/A	N/A	2
	Pilot	37.5±5*	54±6*	N/A	N/A	2
Repeat stress	Laboratory	96	120	101	138	1

*loads are presented as: average of replicate experiments ±absolute difference between replicate experiments.
N/A: Not applicable

Sequencing batch reactor systems. Sequencing batch reactor (SBR) experiments were conducted to quantify the impact of predator grazing on bacterial communities under perturbation scenarios. The parent SBR system was seeded with inoculum from the Plum Island WWTP and allowed to acclimate for 1 SRT. Similar to the continuous flow laboratory scale experiments, the influent was supplemented with 1000 mg/l NaCl. The parent SBR was operated at a six hour cycle with a five hour react time, 30 minute settling time, and 15 minutes each of fill and decant periods. The biomass from the parent SBR was then distributed into eight 800 ml SBRs which were operated identical to the parent SBR. The eight smaller SBRs were subjected to four different treatments: (1) control, (2) 10% inhibitory concentration of cadmium (IC_{10}), (3) 25% inhibitory concentration of cadmium (IC_{25}), and (4) salt spike with a high pulse load of NaCl to selectively stress the predator community (Moussa *et al.*,

2005; Salvado *et al.*, 2001). Prior to the re-distribution of biomass into the smaller SBRs, respirometric inhibition assays (Henriques et al 2007) were conducted to estimate IC_{10} and IC_{25}, which were determined to be 9.5 and 19 mg cadmium/gVSS, respectively. Based on prior recommendations (Moussa *et al.*, 2005), the salt spike was added to raise the total NaCl concentration to 5.0 g/l. Though such high salt concentrations can potentially induce deflocculation by affecting the monovalent to divalent cation ratio (Higgins and Novak, 1997), no significant increase in effluent total suspended solids concentration was observed. This could be attributed to the fact that inoculum used for this study has a history of high ambient salt concentration.

Chemical analyses. Influent was characterized daily for soluble and total chemical oxygen demand (COD) and ammonium (NH_4^+) content. Effluent samples were characterized multiple times each day for soluble COD, NH_4^+, nitrite (NO_2^-), nitrate (NO_3^-), alkalinity, total (TSS) and volatile suspended solids (VSS) according to standard methods (APHA, 1998), with a few exceptions. Immediately after the toxic shock, all analyses were conducted three times a day for the first day, twice each day from the second through fourth day, followed by once every day until the end of the experiment.

Effluent total organic carbon and NO_3^- for the pilot-scale experiments were estimated by measuring light absorption in the UV-visible spectrum using a STIP:Scan probe (Endress+Hauser Inc). STIP:Scan based NO_3^- estimates were corrected for NO_2^- interference. Biomass samples were analyzed for mixed liquor (MLSS) and mixed liquid volatile suspended solids (MLVSS), and total and soluble cadmium concentrations according to standard methods (APHA, 1998).

Kinetic analyses. Biomass respiration rates were determined for general heterotrophic bacteria as well as the AOB community. Briefly, fresh biomass samples were aerated for 5 to 6 minutes to increase DO levels and transferred to 80 ml bottles. Samples were spiked with 100 mg/l of soluble COD that was composed of a blend of carbohydrates, proteins and organic acids, as described previously (Henriques *et al.*, 2007). The intrinsic specific oxygen uptake rate ($sOUR_I$) (Grady *et al.*, 1996) was estimated by dividing the rate of oxygen depletion by the sample MLVSS. The rate of ammonia oxidation by AOB was determined by estimating specific nitrite generation rate (sNGR). sNGR assays were conducted by transferring 100 ml of freshly collected biomass into a well mixed and aerated reactor. Biomass samples were spiked with 9.8 mg/l sodium azide and 30 mg NH_4^+ as N/l. Sodium azide dose was optimized to selectively inhibit nitrite-oxidizing bacteria (NOB) (Ginestet *et al.*, 1998) and was consistently found to be 9.8 mg/l for the inoculum used in this study (Appendix Figures C.1 and C.2). Nitrite accumulation in the sNGR vessels was monitored at 10-minute intervals for a period of 50 minutes. The rate of NO_2^- (mg NO_2^- as $N.l^{-1}.hr^{-1}$) accumulation was normalized to MLVSS to obtain sNGR (mg NO_2^- as $N.gVSS^{-1}.hr^{-1}$).

DNA extraction. Four milliliters of biomass was collected and centrifuged at 8000 xg for 2 minutes. The supernatant was discarded and the biomass pellets were stored at -80°C until they were further analyzed. DNA was extracted using the UltraClean Soil DNA extraction kit (MoBio Inc, Carlsbad, CA) according to the manufacturer's instructions, with modifications to improve DNA yield. Prior to the use of the extraction kit, biomass pellets were treated with lysozyme (6 mg/ml) and proteinase

K (1 mg/ml) and incubated at 37°C for 30 minutes (Regan *et al.*, 2003). This was followed by vortexing for 2 minutes in 1M NaCl (Chan and Goodwin, 1995). Next, the samples were incubated at 70°C for 30 minutes after addition of solution S1 containing SDS (provided with UltraClean Soil DNA extraction kit), prior to further processing. Extracted DNA was aliquoted into several DNAase free sterile tubes and stored at -80°C until further processing was done. DNA quantity and purity was determined spectrophotometrically by measuring absorbance at 260 nm/280 nm using Nanodrop ND1000 (Nanodrop Technologies, Wilmington, DE). The average mass of DNA extracted from 4 ml of activated sludge was approximately 16.2 ± 5.6 µg.

Quantitative PCR. Quantitative PCR (Q-PCR) was conducted by targeting the 16S rRNA gene. Primer sets Eub338f /Univ518r (Einen *et al.*, 2008; Ovreas *et al.*, 1998) and CTO189F/RT1r (Hermansson and Lindgren, 2001; Kowalchuk *et al.*, 1997) were used to target general eubacterial and AOB specific 16S rDNA, respectively (Appendix table C.25). Q-PCR assays were conducted using a 25 µl volume and Power SYBR Green Chemistry (Applied Biosystems, Foster City, CA) on an Eppendorf Realplex^2 thermocycler (Eppendorf North America). A stock solution of plasmid that contained an insert from the 16S rRNA gene of *Nitrosomonas europaea* ATCC 19718 and was prepared specifically for this study was serial diluted over a range of 10^8 to 10^2 copies per µl and used as a standard. Samples were serial diluted thrice and each dilution was analyzed independently. Each cycle was followed by a melting curve analysis. Melting curves were evaluated to ensure the absence of primer-dimers and unspecific product formation. Q-PCR efficiency for the AOB-

specific and general bacterial 16S rRNA targets was 94±1.7% and 98±3.0%, respectively.

Clone libraries. Clone libraries were generated using DNA extracts of sludge inoculum from the parent facility. AOB specific clone libraries were generated by targeting both the 16S rRNA and ammonia monooxygenase subunit A (*amoA*) genes, to ensure appropriate phylogenetic characterization of ammonia oxidizers. The absence of ammonia oxidizing archae (AOA) was confirmed by testing samples with crenarchael *amoA* primer set Arch-amoAF/Arch-amoAR (Beman *et al.*, 2006). Polymerase chain reaction (PCR) was conducted using primer set Eub338f/Nso1225r (Siripong and Rittmann, 2007) for AOB 16S rRNA and *amoA*-1F/*amoA*-2R (Nicolaisen and Ramsing, 2002; Rotthauwe *et al.*, 1997) for the *amoA* gene (Appendix table C.25). Cloning was done using the pGEM®-T Easy Vector System (Promega, Madison, WI) according to manufacturer's instructions. Colonies were picked and grown overnight in LB medium, and plasmids were extracted using a MiniPrep plasmid extraction kit (Qiagen Inc, Valencia, CA). Plasmid inserts were sequenced at the University of Michigan DNA sequencing core (Ann Arbor, MI) using the chain terminator sequencing protocol on an Applied Biosystems Model 3730 XL sequencer. A total of 60 and 105 colonies were sequenced for the 16S rRNA and *amoA* gene targets, respectively. Rarefaction analysis was done using Analytic Rarefaction 1.3 (http://www.uga.edu/strata/software/) to ensure the optimum recovery of sequences from the clone library. The 16S rRNA based sequences were classified using Naïve Bayesian rRNA classifier V 2.0 (Wang *et al.*, 2007) with a confidence threshold of 80%, to identify and eliminate non-AOB sequences from any

further phylogenetic analyses. Sequences for 16S rRNA were checked for chimeras using Mallard (Ashelford *et al.*, 2005) and aligned using ClustalW (Thompson *et al.*, 1994) algorithm in Molecular and Evolutionary Genetic Analysis (MEGA) software (Takamura *et al.*, 2007). Chimera detection for *amoA* sequences was done by comparing sequences to nearest BLAST matches (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using Mallard. Phylogenetic trees were constructed using MEGA with neighbor joining (NJ) algorithm (Saitou and Nei, 1987) and the Jukes Cantor nucleotide substitution model. The tree was subject to 1000 bootstrap trials with a consensus cutoff of 50%.

Terminal Restriction Fragment Length Polymorphism (T-RFLP). T-RFLP was used to generate microbial community fingerprints (Liu *et al.*, 1997). AOB specific T-RFLP analysis was conducted for the laboratory- and pilot-scale experiments, while the community fingerprints were also determined for the general bacterial community for the SBR experiments. Forward primer Eub338f was labeled with fluorescent marker 6-FAM at the 5' primer end, while Nso1225r (Wagner *et al.*, 1998) and 1492r (Lane, 1991) were used as reverse primers for AOB specific and general bacterial PCR (Appendix table C.25). The preliminary choice of enzymes for AOB specific T-RFLP was based on Enzyme Resolving Power Analysis (ERPA) (Shyu *et al.*, 2007). The list of AOB specific high resolution enzymes were analyzed in conjunction with 16S rRNA sequences from the clone library using the Restriction Enzyme Online Picker (REPK) (Collins and Rocap, 2007). In-silico digests indicated that enzyme Taq1 (T/CGA) was able to generate a maximum number of unique fragments. For general bacterial T-RFLP, the choice of enzymes was limited to ERPA

analysis. Taq1 and Rsa1 (GT/AC) were chosen as appropriate restriction enzymes. PCR reactions for T-RFLP were conducted in duplicate in a 25 µl volume. The replicate PCR products were combined and purified using a PCR purification kit (Qiagen, Valencia, CA). The purified product was then digested in duplicate for each enzyme. Each digestion reaction consisted of 7 µl of purified PCR product and 10 units of the respective enzyme and digested at 65 or 37 °C for 5 hours according to manufacturer's instructions (New England Biolabs, Ispswich, MA). Digested products were analyzed on an ABI 3730 XL DNA sequencer at the University of Michigan DNA sequencing core.

T-RFLP data analysis. Fragment analyses files were analyzed using Peak Scanner V1.0 (Applied Biosystem, Foster City, CA) using a threshold of 50 fluorescence units. Replicate profiles were standardized using the constant baseline thresholding method (Dunbar *et al.*, 2001) and consensus profiles were derived and aligned from standardized replicate profiles using T-Align (Smith *et al.*, 2005). Drifts in fragment sizes were corrected by comparing sample electropherograms to synthetic controls that consisted of a mix of fragments that have known sizes. T-RFLP fragments retained in the final profiles were checked for AOB specificity, by comparing with in-silico digests generated using the Virtual Digest tool (Shyu *et al.*, 2007), which used the SILVA rRNA project database (Pruesse *et al.*, 2007) and TAP-TRFLP in silico analysis (Marsh *et al.*, 2000). T-RFs that were unambiguously aligned with non-AOB sequences were eliminated from further analysis. AOB have only one copy of the *rrn* operon per genome (Aakra *et al.*, 1999); hence, the relative abundance of AOB

specific 16S rRNA T-RFs were used to track the changes relative to AOB taxa abundance.

Model relating T-RF dynamics to AOB kinetics. T-RF abundances were used to predict the relative changes in estimated sNGR values. The measured sNGRs can be modeled (see Equation 1) by using the conventional Monod-type saturation model, assuming that a constant amount of nitrite is generated for every mole of ammonium oxidized (α = constant) (Chandran and Smets, 2000), and assuming that a simple inhibition relationship exists to account for cadmium inhibition.

$$sNGR = \frac{1}{X_{AOB}} \frac{d\left(S_{NO_2^-}\right)}{dt} = -\alpha \frac{1}{Y_{AOB}} \left(\frac{\mu_{\max} \times S_{NH}}{K_{NH} + S_{NH}} \right) \left(\frac{1}{1 + \frac{S_{Cd}}{K_{I,Cd}}} \right) \qquad \text{eqn (1)}$$

where:

α =	Stoichiometric ratio of the amount of nitrite formed per amount of ammonium consumed
X_{AOB}=	AOB cell abundance (mg VSS.l^{-1})
S_{NO2-}	NO_2^- concentration (mg NO_2^- as N.l^{-1})
μ_{max}=	Maximum specific growth rate (mg VSS formed per day/mg VSS in system)
K_{NH}=	Half saturation coefficient for ammonium oxidation (mg NH_4^+ as N.l^{-1})
S_{NH}=	NH_4^+ concentration (mg NH_4^+ as N.l^{-1})
S_{Cd}=	Soluble cadmium concentration (mg.l^{-1})
$K_{I,Cd}$=	Inhibition coefficient (mg.l^{-1})
Y_{AOB}=	AOB yield (mg VSS.mg N^{-1})

Since sNGR assays were conducted under intrinsic, and not in situ conditions, i.e. $S_{NH} >> K_{NH}$, eqn (1) becomes,

$$sNGR = \frac{1}{X_{AOB}} \frac{d\left(S_{NO_2^-}\right)}{dt} = -\alpha \frac{\mu_{\max}}{Y_{AOB}} \left(\frac{1}{1 + \frac{S_{Cd}}{K_{I,Cd}}} \right) \qquad \text{eqn (2)}$$

The relative change in sNGR over time can be determined by normalizing with sNGR at t=0, as follows:

$$REL(sNGR_{T=t}) = \frac{\sum_{j=1}^{n}\left[\mu_{\max}^{j} R_j \left(\frac{1}{1+\frac{S_{Cd}}{K_{I,Cd}^{j}}}\right)\right]_{T=t}}{\sum_{j=1}^{n}\left[\mu_{\max}^{j} R_j \left(\frac{1}{1+\frac{S_{Cd}}{K_{I,Cd}^{j}}}\right)\right]_{T=0}} \qquad \text{eqn (3)}$$

Where, j =1…n is the observed T-RF and R_j is the relative abundance of the j^{th} T-RF in the community. For the unstressed AOB communities equation 3 becomes:

$$REL(sNGR_{T=t}) = \frac{\sum_{j=1}^{n}\left[\mu_{\max}^{j} R_j\right]_{T=t}}{\sum_{j=1}^{n}\left[\mu_{\max}^{j} R_j\right]_{T=0}} \qquad \text{eqn (4)}$$

Adding R_j into this analysis allows for the variations in individual T-RF abundance within the AOB community to be incorporated. Equation 4 was solved by minimizing the error between experimentally observed and model predicted REL(sNGR) to obtain the μ_{max}^{j}. The μ_{max}^{j} values obtained for the unstressed controls were used in equation 3 to solve for $K_{I,Cd}^{j}$ for the stressed reactors.

The model assumed a constant yield coefficient for the AOB community under stress. However, recent single species studies with *N. europaea* indicate significant downregulation of genes involved in carbon fixation (Park and Ely, 2008), which might reduce yield under cadmium stress. In order to use a consistent model, we maintained the assumption of constant yield. Additionally, the model did not

account for the change in AOB 16S rRNA numbers. The sNGR values were normalized to the MLVSS and inclusion of variation of AOB abundance would require obtaining a ratio of autotrophic to heterotrophic biomass. This exercise necessitates assumptions about either bacterial genome size or amount of DNA per bacterial cell (Loisel *et al.*, 2006) or an average *rrn* copy number per genome to convert DNA quantities or 16S rRNA copy numbers to cell numbers. However, bacterial genome sizes (Cole and Saint-Girons, 1999), cell DNA content (Akerlund *et al.*, 1995) and *rrn* copy number per genome (Klappenback *et al.*, 2001) can vary significantly depending on species identity and cell growth phase. Though it may be reasonable to make assumptions about either of the aforementioned factors to relate qPCR-based data to sNGR kinetics in unperturbed bioreactors or environmental conditions, this assumption may not be valid under situations with significant community function and structure altering perturbation. In order to maintain model consistency, we did not account for variations in AOB abundance but sought to capture the sNGR trends for both the perturbed and unperturbed systems using the same model.

Statistical analysis. T-RFLP profile similarities/dissimilarities were determined along the sampling time-series using the Bray-Curtis index (Bray and Curtis, 1957). Analysis of similarity (ANOSIM) was performed to quantify the T-RFLP profile pair-wise similarities with 1000 permutations (Clarke, 1993). The time series progression of the community T-RFLP profiles was visualized by non-metric dimensionless scaling (NDMS) using pair-wise Bray-Curtis similarity estimates. All statistical

analyses were done using Paleontological Statistics (PAST) software (Hammer *et al.*, 2001).

Microscopic analyses. Freshly collected activated sludge samples were thoroughly mixed before a 100 μl sample was collected. The sample was spotted on a glass slide and mounted with a glass coverslip. For laboratory- and pilot-scale experiments, the protozoal community was monitored qualitatively on an AxioSkop 2 (Carl Ziess Microimaging Inc, Thornwood, NY), while an AxioObserver microscope was used for the SBR experiments. Protozoal communities were observed at 40x magnification. For the SBR experiments, a subset of the protozoal community, specifically bacterivorous ciliated protozoa, were quantified. The bacterivorous protozoa were further categorized into free swimming, crawling, and sessile ciliates and were manually counted (Madoni, 1994). Protozoal quantification was conducted in duplicate for each sample.

5.3 Results.

Process performance recovery from cadmium stress was followed by enhanced reaction kinetics for the continuous flow systems. Despite the heavy cadmium pulse load applied to the reactor systems, the presence of the primary clarifier upstream of the bioreactor caused slow toxin dissipation and thus resulted in lowered total cadmium concentrations in the aeration basin. The biosorption of cadmium and precipitation as otavite (Fristoe and Nelson, 1983) within the reactor system resulted in sustained total cadmium concentrations, which did not correlate with process recovery. Rather, the soluble cadmium concentrations correlated very well with

process upset and recovery, as defined by effluent ammonium concentrations (Figure 5.1C), sNGRs (Figure 5.1D) and to a lesser extent, by organic carbon oxidation (Figure 5.1B). The upset and recovery times were consistent with the differential shock load application to SR1 and SR2 (Figure 5.1). Nitrification inhibition was confirmed by both increased effluent ammonium concentrations and decreased sNGR profiles immediately following each of the shock events (Figures 5.1C and 5.1D). However, in contrast to previous studies with cadmium (Kelly *et al.*, 2004), process and kinetic recovery was more rapid (3 days at a load of 120 mg cadmium/g VSS for this study as opposed to 11 days at 42 mg cadmium/gVSS in the study by Kelly *et al.* (2004)).

Interestingly, complete process recovery was followed by an increase in the sNGR values for the stressed reactors as compared to the unstressed bioreactor. The post-recovery increase in sNGR values was reproducible for laboratory-scale experiments were cadmium stress was repeated (Figure 5.1D). Interestingly, the degree of nitrification inhibition and time over which process deterioration was observed were both smaller during the second stress event relative to the first (Figure 5.1C), and there was no observable impact on COD removal during the second stress event (Figure 5.1B). In the case of ammonia oxidation, the extent of ammonia accumulation during the second repeated stress event was 40 to 60% less than it was during the first stress event, and the duration of the accumulation was 24 hours versus 72 hours after the first cadmium stress. The significant reduction in the impact of cadmium stress on SR1 and SR2 during the second stress event indicates increased resistance and resilience of the recovered microbial community. The post-stress

increase in sNGR values was observed for six cadmium stress experiments, involving 12 stressed reactors at both laboratory- and pilot-scale. Hence, this phenomenon of increased respirations rates is consistent at different bioreactor scales.

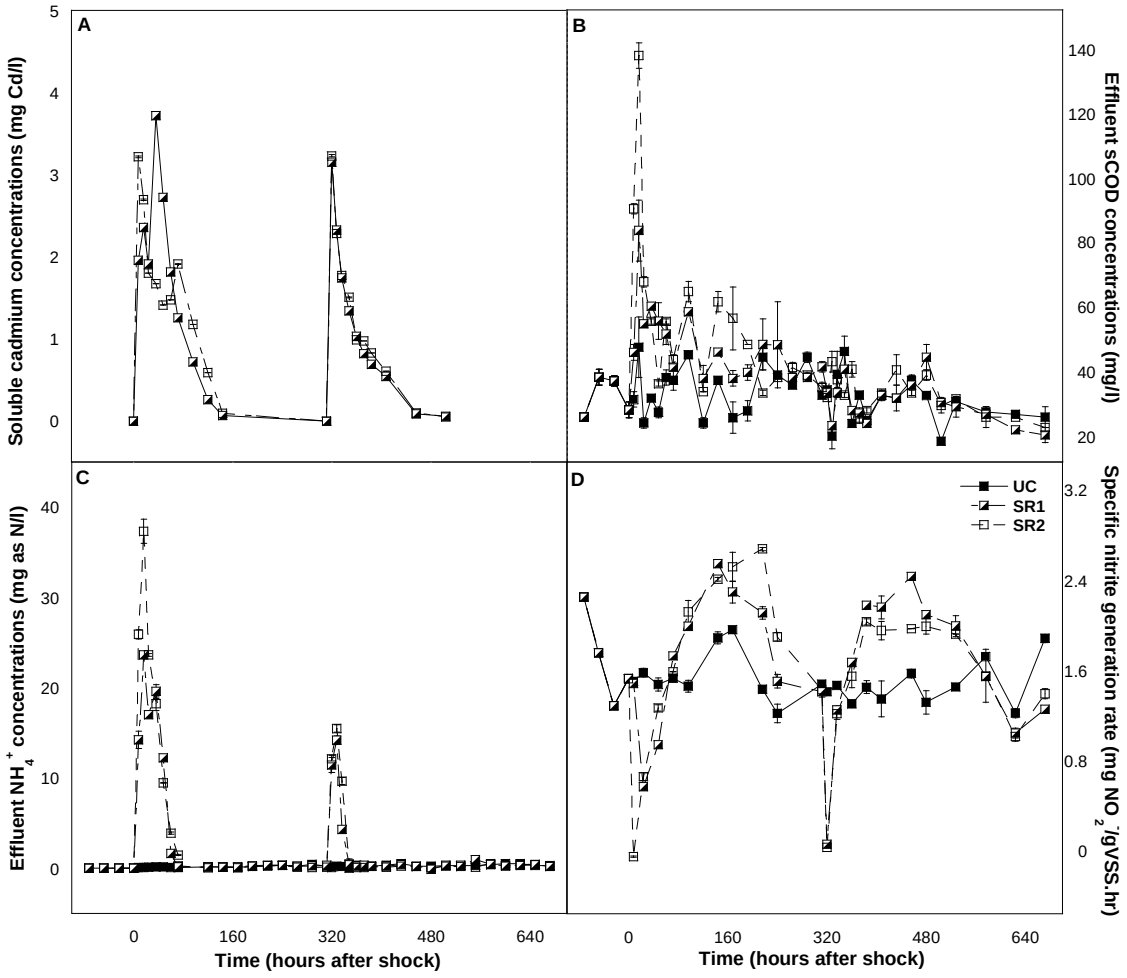

Figure 5.1: (A) Soluble cadmium, (B) effluent sCOD, (C) effluent NH_4^+-N, and (4) sNGR for UC, SR1, and SR2 for the laboratory-scale repeat cadmium stress experiment.

Ammonia oxidizing bacterial community exhibited limited diversity. Previous research has shown that high salinity bioreactor conditions tend to exhibit low diversity (Moussa *et al.*, 2006). Consistent with this observation, both 16S rRNA and

amoA gene based phylogeny highlight the limited diversity of the AOB community. Approximately 83% of sequences were classified as belonging to the genus *Nitrosomonas*. The primers used for the 16S rRNA based AOB clone library included one general bacterial and one Betaproteobacteria AOB specific primer set. As a result, the clone library sequences consisted of non-AOB sequences as well. Approximately 3.4 % of the sequences belonged to the families *Methylophilaceae* and *Gallionellaceae*, and 13.6% were unclassified Betaproteobacteria. Unclassified and non-AOB sequences were eliminated from further phylogenetic analysis. Consistent phylogenetic clustering was seen for 16S rRNA and *amoA* sequences. The majority of the sequences closely aligned with the *N. marina* cluster, while small fractions aligned with *N. europaea/Nitrosococcus mobilis* and *Nitrosospira* clusters (Figure 5.2A and 5.2B).

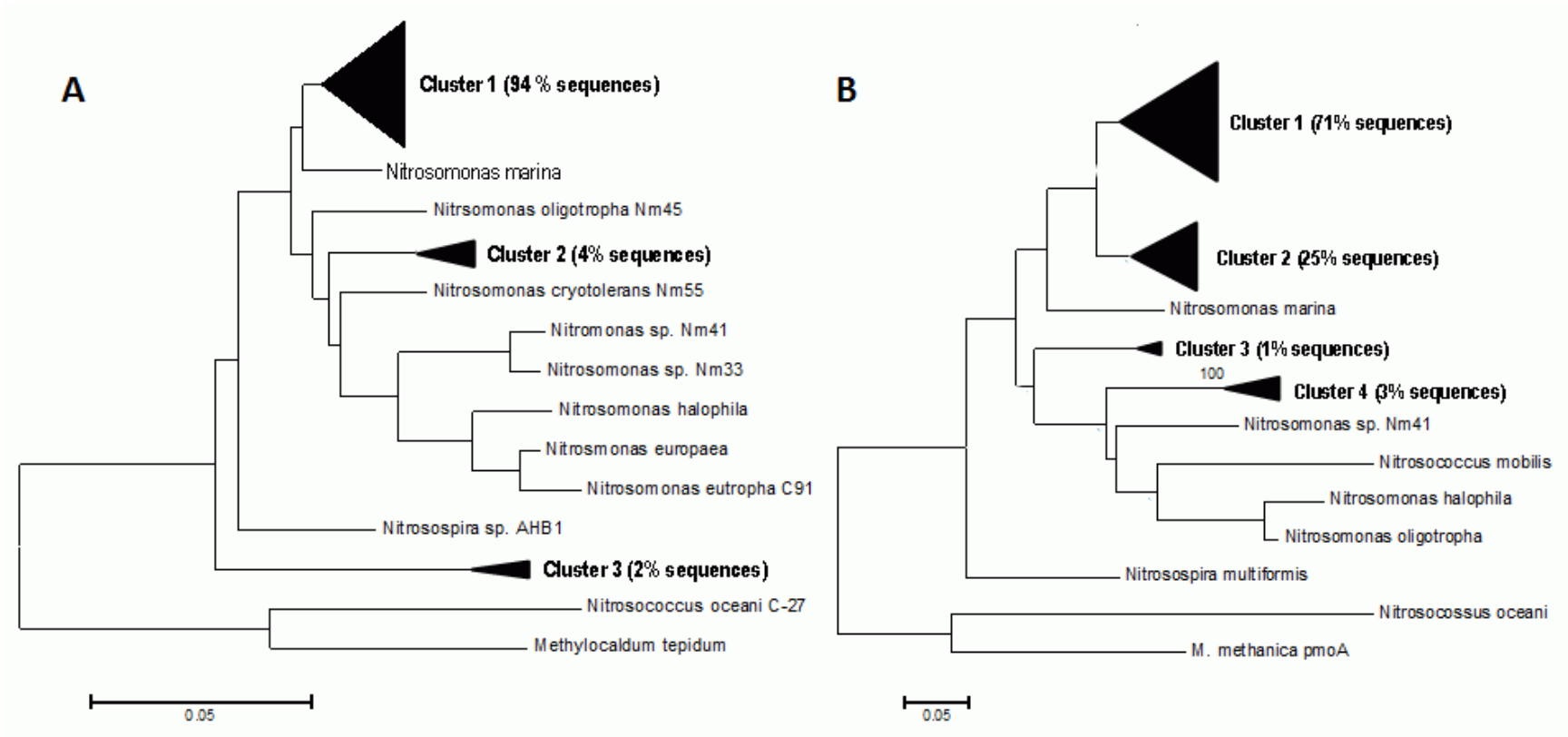

Figure 5.2: (A) 16S rRNA and (b) *amoA* gene based phylogeny of ammonia oxidizing bacteria in the activated sludge inoculum used for this study.

Cadmium stress did not cause significant changes in AOB community structure. Table 5.2 lists the possible AOB-specific T-RF's generated by the primer set and enzyme used in this study, and highlights the T-RF's observed during the experimental phase. Again, T-RF's exhibiting unambiguous non-AOB phylogeny were eliminated from this analysis. Consistent with the unevenness exhibited in the clone library, the AOB community was largely dominated by a single T-RF measuring 106 bp (T_{NM1}), which belonged to the *N. marina* cluster based on clone library analysis. Other T-RFs observed in this study were T_{NM2} (382 bp) associated with the *N. marina* cluster, and T_{NSP1} (485 bp) and T_{NSP2} (679 bp) associated with the *Nitrosospira* cluster.

Table 5.2: Phylogenetic affiliations for forward T-RF's generated using the Taq1 enzyme using TAP-TRFLP analysis. Fragments observed in this study are indicated by an asterisk.

AOB phylogenetic affiliation	Taq1 T-RF length (bp)
N. europaea/Nc. mobilis	613, 615, 623-627, 630, 909
N. oligotropha	106, 151, 382
N. marina	106* (T_{NM1}), 382* (T_{NM2})
N. cryotolerans	106
N. communis	625
Nitrosospira cluster	207, 480, 485* (T_{NSP1}), 492, 611, 615, 621-625, 628, 631, 679* (T_{NSP2}), 901, 906
*T-RF's observed during this study	

Linear regression analysis of the change in relative abundance of T-RFs with respect to each other exhibited significant correlations for all experiments, for stressed and unstressed reactors at both operational scales (Figure 5.3). The strong correlations indicate the presence of inherent competitive interactions between the different T-RF's in the experimental systems, independent of any perturbation. The competitive behavior exhibited showed that an increase in *N. marina* associated fragments was accompanied by a decrease in *Nitrosospira* associated T-RFs. This was also seen in the stressed reactors (Appendix Tables C.22, C.23, C.24, C.25, and C.26). The primary difference between the stressed and unstressed reactors was the higher

sensitivity of fragment T_{NSP1} to the cadmium stress, with a sharp decrease upon exposure to cadmium followed by recovery upon washout of the heavy metal. However, this difference was not enough to cluster cadmium stressed communities independently from the unstressed biomass (ANOSIM R statistic, R<0.5, p>0.05, for all pair wise comparisons).

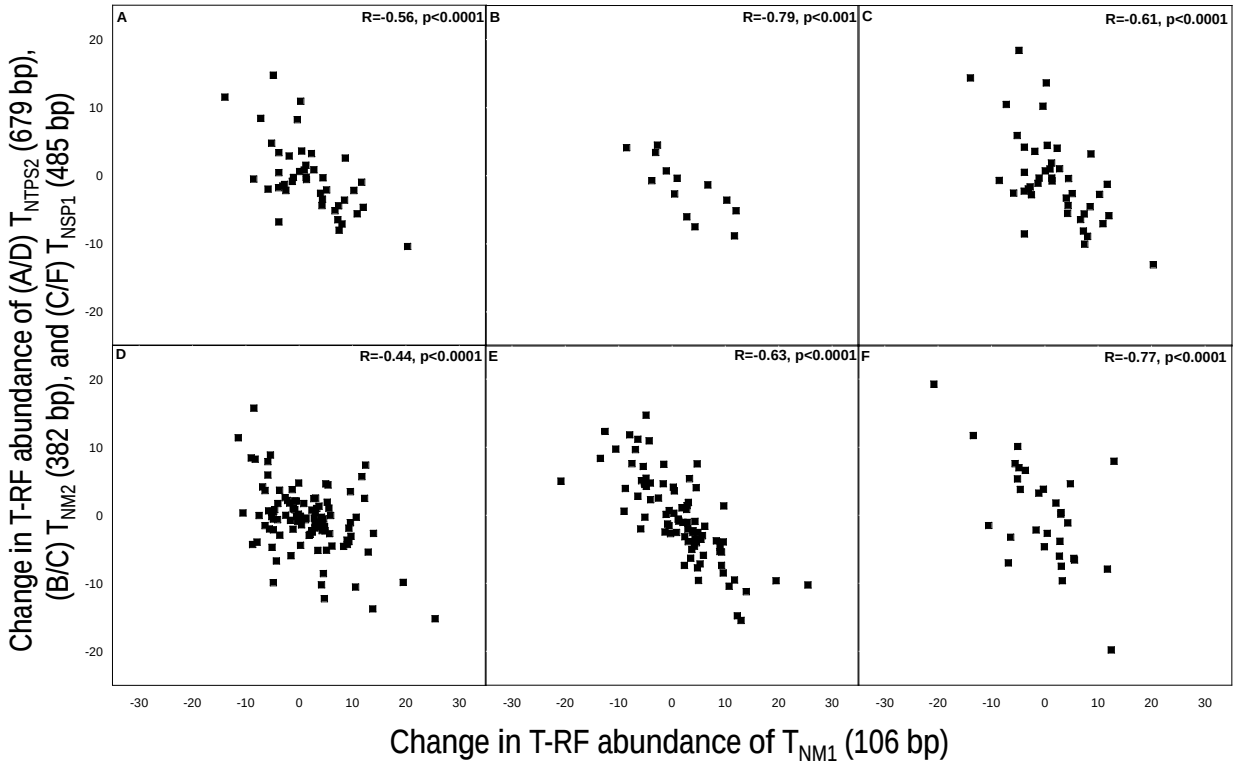

Figure 5.3: Linear regression analyses for the major T-RF's observed in this study for the unstressed (A, B, C) and stressed (D, E, F) reactors: T_{NM1} vs. (A/D) T_{NSP2}, (B/E) T_{NM2}, and (C/F) T_{NSP1}. Data points include both laboratory- and pilot-scale experiments and highlight the presence of similar competitive interactions in both stressed and unstressed reactors irrespective of operational scale.

The kinetic model only predicts sNGR trends for the unstressed reactors. A model was used to determine if the T-RF dynamics can be used to predict the experimentally observed sNGR trends. For simplicity, we reduced the $\mu_{\max}^{j}$ matrix to only include *N. marina* and *Nitrosospira*, which were the most prevalent among the species identified for this study. We observed

consistently good model fits for the UC reactors operated at both the laboratory- and pilot-scale (Figure 5.4). The calculated ratio of $\mu_{\max}^{NM} / \mu_{\max}^{NSP}$ for the unstressed reactors from five independent experiments, at 2 different scales of operation was 1.28±1.1. The calculated ratio is similar to the ones presented in the literature (Prosser, 1989) for kinetic comparisons between *N. marina* and *Nitrosospira*. This relatively low ratio, may explain the ability of both T_{NM1} and T_{NSP2} to coexist. The dominance of *N. marina* could be attributed to the high ambient salt concentrations in the reactors (Whang *et al.*, 2009) and/or substrate limited conditions in the continuous flow reactors. Early studies have indicated that *N. marina*-like AOB tend to dominate highly oligotrophic marine environments, and hence may have half-saturation co-efficient values lower than *Nitrosospira* (Burrell *et al.*, 2001; Glover, 1985; Prosser, 1989).

In contrast to the UC reactors, the model fit poorly to the sNGR trends in the stressed reactors (Figure 5.4). Furthermore, the estimated ratio of inhibition coefficients did not exhibit consistent trends towards higher or lower susceptibility of *N. marina* or *Nitrosospira* T-RF's to soluble cadmium concentrations, with $K_{I,Cd}^{NM} / K_{I,Cd}^{NSP}$ varying between 0.08-3.7. This analysis shows that T-RF dynamics cannot be used to predict AOB kinetics under perturbation scenarios.

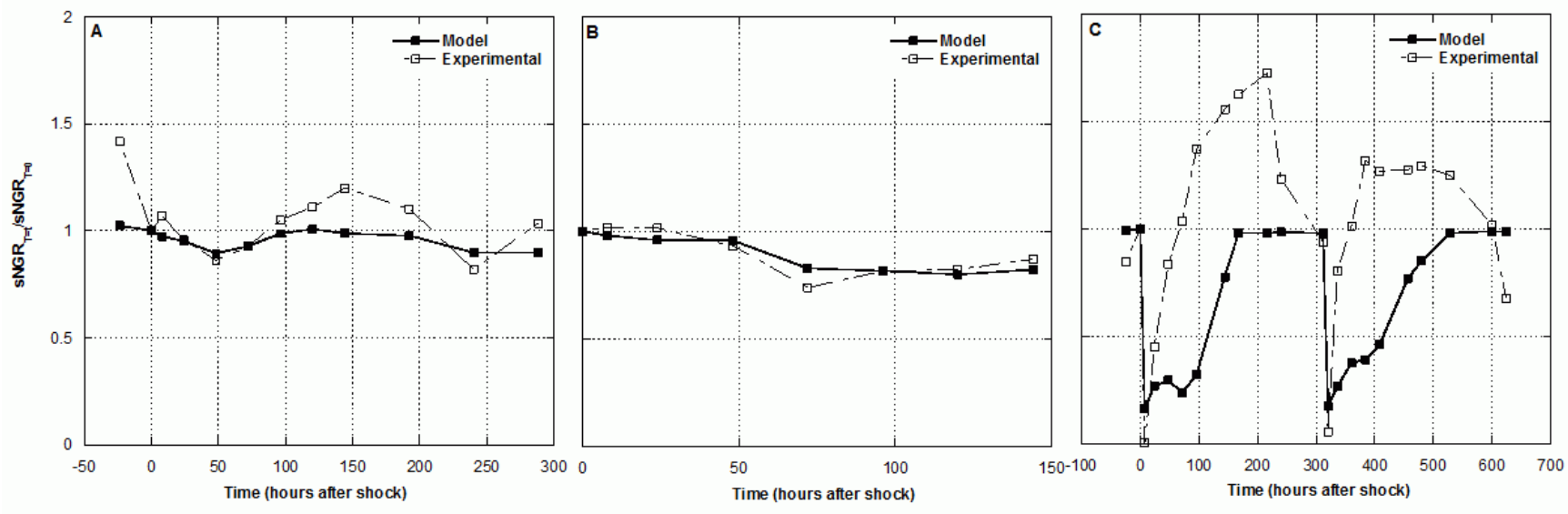

Figure 5.4: Comparing the predicted sNGRT=t/sNGRT=0 ratio to experimentally observed values for (A) one laboratory-scale unstressed control reactor, (B) one pilot-scale unstressed control reactor, and (C) laborartory scale cadmium stressed reactor exposed to repeated stress events.

Recovery was followed by an increase in bacterial abundance. The post-recovery spikes in the sNGR rates were accompanied by an increase in the total abundance of the AOB community. In fact, during the process recovery phase, the total AOB abundance measured as 16S rRNA gene copies/ng DNA correlated with the sNGR values for datasets compiled from three laboratory-scale experiments ($R= 0.87$, $p<0.0001$) and two pilot-scale experiments ($R = 0.86$, $p< 0.001$). Interestingly, this increase in sNGR and AOB abundance was also accompanied by an increase in sOUR and total bacteria abundance numbers, implying that the underlying mechanism for the observed trends was not specific to any particular functional group. Higher post-stress heterotrophic sOURs (Appendix Tables C.1, C.3, C.5, C.7, and C.21) and sNGR (Appendix Tables C.2, C.4, C.6, C.8, and C.22) levels, and heterotrophic bacterial and AOB abundance levels (Appendix Tables C.25, C.26, C.27, C.28, and C.29) were observed for five independent experiments and 10 independent cadmium stressed reactors.

Increase in bacterial abundance was due to cadmium-related inhibition of predator grazing. The increase in general bacterial 16S rRNA copy number could be a result of a change in community structure due to cadmium inhibition or toxicity, cadmium-induced deflocculation, a decrease in predator grazing, or some combination of all three. Short-term SBR experiments were performed to evaluate which of these three causes were most responsible for the post-stress community behavior observed in the flow through treatment systems. The SBR exposed to the highest cadmium concentration (IC_{25}) exhibited a shift in total bacterial community structure (Appendix Figures C.3 and C.4). Additionally, cadmium induced deflocculation caused effluent biomass and mixed liquor to mix, as indicated by a Bray-Curtis based ANOSIM comparison (Table 5.3). Higher ANOSIM R values indicate greater dissimilarities between the two communities being compared. This indicates that the change in community structure was not

due to indirect mechanisms such as deflocculation, but likely due to the direct impact of cadmium on the biomass. The decrease in similarity between the cadmium stressed reactors and the control reactors did not correlate with changes in AOB (for Rsa1 T-RFLP profiles, R=-0.59, p=0.09; for Taq1 T-RFLP profile, R=0.18, p=0.64) or total bacterial (for Rsa1 T-RFLP profiles, R=-0.69, p=0.04; for Taq1 T-RFLP profile, R=-0.29, p=0.45), 16S rRNA copy numbers.

Table 5.3: ANOSIM R values and statistical significance for pairwise comparisons between MLSS and effluent biomass communities in SBRs for Taq1 and Rsa1 restriction digests. Higher ANOSIM R values indicate greater dissimilarity between the bacterial communities in the MLSS and effluent biomass.

Reactor	Taq1 digest electropherograms		Rsa1 digest electropherograms	
	ANOSIM R	p	ANOSIM R	p
Control	0.56	0.001	0.92	0.001
IC_{10}*	0.33	0.023	0.23	0.054
IC_{25}*	0.19	0.062	0.23	0.062
Salt spike#	0.58	0.001	0.51	0.001

*Deflocculation increased similarity between effluent and mixed liquor biomass communities as indicated by lower R values at reduced significance levels.
High ANOSIM R values indicate the absence of deflocculation in the salt spike reactor.

The increase in bacterial abundance was clearly not affected by the shift in community structure, but was strongly impacted by the reduction in predator grazing due to the cadmium stress. The impact of cadmium stress on the eukaryotic community is apparent from microscope images. The cadmium stress caused significant morphological changes for several members of the eukaryotic community, which could be a result of oxidative stress-mediated cell membrane damage or metal bioaccumulation (Figure 5.5). The decrease in bacterivorous predator abundance strongly correlated with the increase in both AOB and general bacterial 16S rRNA copy number abundance for the SBR experiments (Figure 5.6), and the recovery of grazers was accompanied by a proportionate decrease in bacterial quantities. Similar to the cadmium stressed reactors, the salt spike reactor showed a significant increase in both total and AOB bacterial abundance as compared to the control (Figures 5.6B and 5.6C). The changes in predator

abundance (Figure 5.6D) correlated strongly with the changes in total bacterial 16S rRNA copy numbers (R =-0.93, p<.0001). In contrast, the changes in AOB abundance showed slightly lower sensitivity to the drop in predator abundance (R = -0.67, p=0.015). These correlations were observed for both, the cadmium and salt stress reactors, and clearly indicate that the observed increase in sOUR and sNGR rates and bacterial abundance were due to a reduction in predator stress.

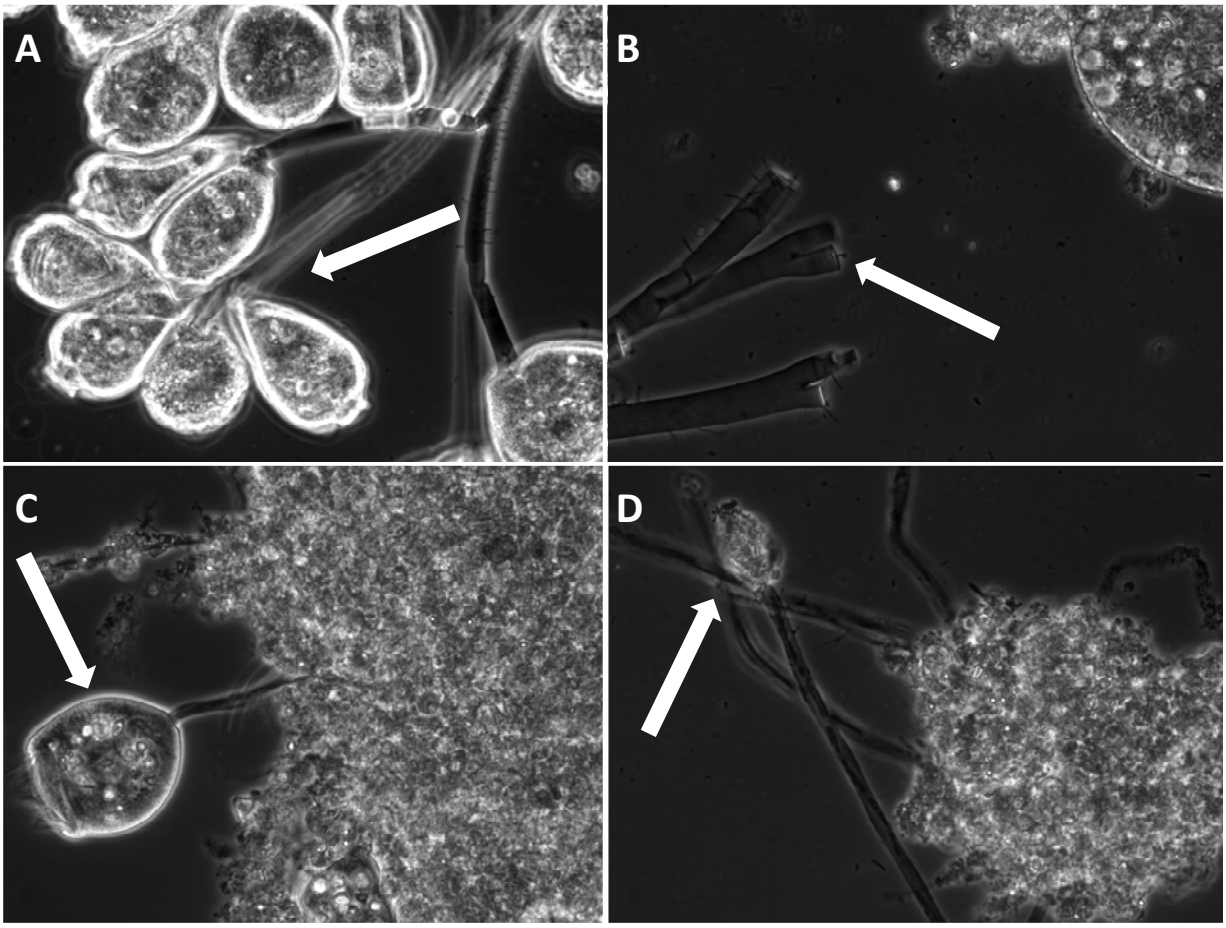

Figure 5.5: Impact of cadmium stress on the eukaryotic community. Photos highlight sessile ciliates from unstressed (A, C) and stressed (B, D) bioreactor communities. Heavy metal stress results in (B) decapitation and (D) morphological damage to ciliated heads.

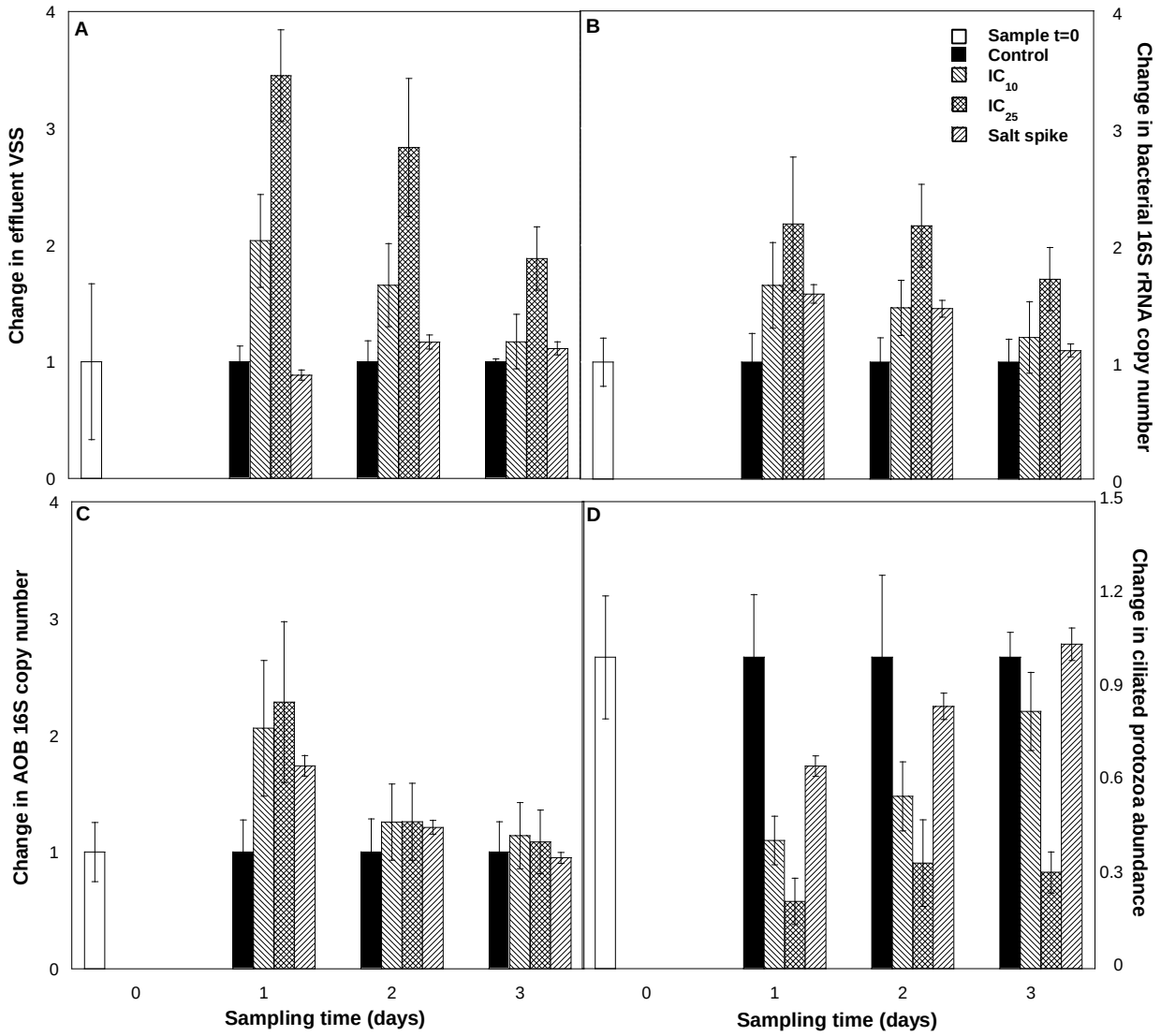

Figure 5.6: Changes in (A) effluent VSS, (B), total bacteria 16S rRNA copy number, (C) AOB specific 16S rRNA copy number, and (D) ciliated protozoal abundance normalized to the control reactor. All results are from SBR experiments. Changes are reported for the control, IC_{10}, IC_{25}, and salt spike reactors over time after cadmium stress was imposed. Sample at t=0 is identical for all four reactors.

5.4 Discussion.

The impact of cadmium stress on the bioreactor microbial community was evident throughout the process upset and recovery. However, we repeatedly documented an increase in

the kinetic potential of both the AOB and the general bacterial community after complete process recovery was achieved (Figure 5.1D). We tested for two potential mechanisms that could be responsible for the observed increase in kinetics, specifically (1) change in community structure and (2) an increase in the total cell abundance. A change in community structure could result in the increased dominance of certain taxa with higher substrate utilization kinetics. On the other hand, the increase in bacterial abundance could result from: (1) substrate accumulation during cadmium stress which would allow for greater bacterial growth after cadmium wash out and/or (2) the elimination of predatory stress, thus creating favorable conditions for bacterial proliferation. Cadmium stress may also induce differential gene expression, especially for genes coding for respiration-related proteins. For example, Radniecki *et al*. (2009) have shown that cadmium stress induces increased expression of genes coding for the ammonia monooxygenase (AMO) protein in *N. europaea*. This increased expression could be associated with higher energy requirements to fulfill stress response related functions, such as the increased expression the mercuric reductase (*merA*) gene which codes for proteins involved in contaminant detoxification (Radniecki *et al.*, 2009). Hence, the increased sNGR and sOUR rates could be due community structure changes due to cadmium exposure, as well as changes in gene expression levels of the stressed bacteria.

In order to simplify the target community matrix, we focused on AOB-specific community level dynamics to determine if a structural investigation limited to the changes in bacterial community structure could adequately explain the observed kinetic trends. T-RFLP fingerprinting indicated inherent competitive dynamics between the major AOB specific T-RF's observed in this study in the presence and the absence of cadmium stress (Figure 5.3). This indicates the presence of strong ambient community structuring forces. We were also able to use

the T-RF dynamics to model the observed sNGR trends. However, the model was not able to predict the sNGR values for the perturbed systems. The model caveats presented in the material and methods section indicate the difficulty of translating an identical model between the perturbed and unperturbed systems. Unraveling community structural dynamics does not capture the complexity of bacterial cellular- and community-level responses to perturbations. Hence, though a model based purely on structural variations in bacterial community can reasonably predict kinetic trends under steady state conditions, the same is not true for perturbed systems.

Trends in post-stress kinetics and 16S rRNA copy abundance were not specific to the AOB community, but were also observed for general bacteria. This implies that the observed post-stress trends in community kinetics and abundance are not specific to a certain sub-group of the stressed activated sludge consortium, and the mechanisms responsible for this trend might be general in nature. We were able to show that cadmium stress causes a decrease in predator abundance, which causes the observed increase in bacterial abundance. The simple inhibition of predators through the use of a salt spike was able to simulate the increase in AOB and total bacterial 16S rRNA copy number without inducing deflocculation or changes in community structure. The strong correlations between the changes in predator abundance and total bacterial 16S rRNA copy number further prove the direct impact of protozoal predators on bacterial prey. Predator inhibition can also be caused by sub-lethal but inhibitory cadmium concentrations (Hoffman and Atlas, 1987) or due to the accumulation of NH_4^+ caused by AOB inhibition (Puigagut *et al.*, 2005). Unlike bacteria which are protected within floc structures, eukaryotic microorganisms may be more vulnerable to low concentrations of cadmium simply due to higher levels of exposure (Macdonald *et al.*, 2008). Though bacterial inhibition was observed during this study, it was relatively short lived and Q-PCR data did not reveal a significant decline in 16S

rRNA copy numbers. Moussa *et al*. (2005) reported that predator elimination results in a significant increase in the live fraction of activated sludge flocs, and is accompanied by a significant increase in activity. Increases in nitrification rates upon elimination of protozoal stress have also been reported by Lee and Welander (1994). However, the previous studies (Moussa *et al*., 2005; Lee and Welander, 1994) used chemicals with the express purpose of predator inhibition. In contrast, this study conclusively shows that the bacteria can proliferate due to cadmium-related reduction in grazing stress, despite initial inhibition due to a universal toxic agent. Similar observations of an increase in bacterial biomass due to metal-induced reductions in predators have also been speculated in natural systems (Hedrick *et al.*, 2009).

In contrast to total bacteria, the AOB 16S rRNA copy numbers showed a slightly weaker correlation with total ciliated protozoal abundance. This is likely due to the fact that among the unlike the entire bacterial pool, AOB tend to exist primarily as part of the floc structure; therefore, predatory pressures, originating from ciliated protozoa, on AOB may be relatively less than they are on heterotrophic members of the community. This trend is consistent with observations by Madoni (Madoni, 1994), who reported a greater abundance of crawling and stalked ciliates in nitrifying plants, as compared to swimming ciliates. In the current study, linear regression shows that AOB abundance is much more sensitive to crawling ciliates ($R=-0.69$, $p<0.01$) and stalked ciliates ($R=-0.7$, $p<0.01$) which can feed on floc structure, as compared to free swimming ciliates ($R=-0.46$, $p=0.14$) which exclusively feed on dispersed floc material. In future studies, quantification of other eukaryotes such as testate amoeba, which show a higher abundance in nitrifying plants, may aid in strengthening correlations between changes in AOB and predator abundance (Madoni, 1994).

Despite the important role of eukaryotes in regulating ecosystem microbial food webs (Sherr and Sherr, 2002), there has been a dearth of studies focused on multi-trophic level interactions in engineered systems. The application of predator community dynamics in wastewater treatment systems has been limited to qualitative assumptions about biomass health (Madoni, 1994). Additionally, this study indicates that higher trophic levels may give quick estimates of the population trends associated with bacteria. This study highlights the importance of understanding the predator community dynamics in wastewater treatment bioreactors, especially under perturbed conditions. To our knowledge, to date there has been no prior demonstration of toxic perturbation-induced bacterial upset and recovery under the impact of diminished predator capacity.

Two conclusions can be made from this study. First, bacterial community structure cannot be used to predict bioreactor functional performance under perturbation conditions. Second, trophic interactions, such as presence or absence of grazing, play an important role in regulating bacterial community abundance and function. A great challenge in the application of the advanced microbiological tools in the wastewater treatment industry has been the slow methodological pace and the requirement of microbiological technical expertise, which impedes their wide scale application. However, it is likely that practitioners may be able to use tools like flow cytometry (Boddy *et al.*, 2001) or high throughput microscopy (Pamp *et al.*, 2009) in conjunction with image processing algorithms (Ginoris *et al.*, 2007) focused on the eukaryotic community in activated sludge systems to predict bacterial abundance.

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Chapter 6.0

Engineering significance

Wastewater treatment plants (WWTP's) are frequently subject to upset events involving toxic chemical contaminants. Exposure to chemical contaminants can cause severe process deterioration and threaten the receiving environment for the WWTP. The mitigation of upset events requires that the operator know the probable process effect(s) of the toxins involved. Additionally, the operator must be able to respond quickly to implement the corrective action strategies, while being cognizant of the limitations and opportunities afforded by the operational flexibility of the WWTP. The wastewater treatment industry currently lacks a systematic framework of response protocols for the mitigation of toxic chemical shocks. To this end, we developed a corrective action plan matrix for the Plum Island WWTP located in Charleston, SC. The corrective actions considered the nature of the contaminant, data available on the process effects of the contaminants, plant specific operational flexibility, and instructions for process monitoring during and immediately after a toxic shock event. These corrective actions were developed in consultation with industrial consultants and operational personnel with extensive knowledge of the Plum Island WWTP.

We tested the corrective actions at the laboratory and pilot scales. Interestingly, the corrective actions tested did not have a universal mitigative effect. In fact, the corrective actions resulted in greater process deterioration as compared to the non-intervention approach for the hypochlorite stress events. In contrast to hypochlorite, the mitigative approaches did show potential for mitigating process impact and reducing recovery times for the laboratory scale simulation of cadmium stress. However, these beneficial aspects were limited in cases where the cadmium induced process upset was not severe. This study clearly highlights that the inappropriate application of corrective actions can actually result in greater process deterioration as compared to a non-intervention approach; emphasizing that the corrective actions should be implemented with caution. The implementation of mitigative responses should be accompanied by a constant evaluation of the impact of both, the chemical contaminant and corrective action, on process performance. The ability to conduct rapid assessments will require that the operator be provided with the tools and education to interpret real time data being collected by online sensors.

An important judgment that the operator must make prior to the implementation of corrective action strategies is the magnitude of the threat posed by the detected upset event. In this study we show that a combination of conventional sensors such as pH, ORP, and conductivity can help identify influent anomalies. The application of a simple multivariate analyses technique such as principal component analyses (PCA) on the rate of change of sensor signal can easily help identify outliers. The challenge of determining the magnitude of threat can be met by identifying the chemical contaminant(s) detected and/or predicting the process impact of the detected influent anomaly. Previous research

has developed source-cause-effect matrices for an array of contaminant classes by identifying process impacts and identifying causal mechanisms for the observed process effects. Given the existing information in the literature, a major knowledge gap in the area of upset event detection and mitigation is the absence of a reliable approach to accurately identify and classify unknown chemical contaminant(s) in the influent matrix. An alternative approach to direct toxin identification would be to identify the contaminant class, based on mode of toxicity, through the application of biosensors. Biosensors may be used to determine the mechanisms of toxicity that can be attributed to the contaminant. For example, biosensors may be applied to determine toxicity mechanisms of the contaminant such as oxidative stress, damage to cell membrane integrity, etc. This study provides a good framework for the development and implementation of corrective actions, while providing opportunities for next generation upset event detection technologies to assist in the decision making process.

A second major contribution of this work is the elucidation of microbial community dynamics of a stressed bioreactor. Through repeated transient cadmium stress experiments, we were able to show that process recovery was followed by significant increase in bacterial respiration rates, as well as bacterial abundance. Bacteria in the activated sludge ecosystem experience constant ambient stress due to predator grazing. In the event of a toxic perturbation, bacterial dynamics are governed by tradeoffs between growth due to reduction in ambient stress levels and inhibition due to the presence of the foreign stressor. This study shows that despite the presence of an inhibitor such as cadmium, the reduction in predator grazing is sufficient enough to result in an increase in bacterial abundance and activity.

There is a growing emphasis on unraveling microbial community dynamics in the activated sludge system. Microbial ecology studies and experimental approaches primarily tend to focus on functionally important bacteria, such as nitrifiers, denitrifiers, etc. A primary challenge in the application of the advanced microbiological tools in the wastewater treatment industry has been the slow methodological pace and the requirement of microbiological technical expertise, which impedes their wide scale application. Despite this methodological barrier, there is a significant dearth of studies that focus on the community dynamics of eukaryotes, which are easily visible and routinely monitored by wastewater operators. By demonstrating the presence of strong correlations between ciliated protozoa and total bacterial abundance in perturbed systems, we were able to highlight the important role played by eukaryotic microorganisms in regulating bacterial community behavior. Future research should thus focus on understanding specific interactions between grazing microorganisms and functionally important bacteria. The ability to reveal specific eukaryotic-bacteria relationships in the activated sludge system will allow practitioners to quantify bacterial abundance and activities by making observations about different eukaryotic microorganisms. The application of advanced ecological theories and microbiological tools within the activated sludge ecosystem should focus on their primary objective, which is to aid the operator in maintaining a functionally stable wastewater treatment plant.

APPENDICES

List of figures for appendices

Appendix A

Appendix B

Appendix C

List of tables for appendices

Appendix A

Appendix C

Appendix A

Data in support of Chapter 3.0

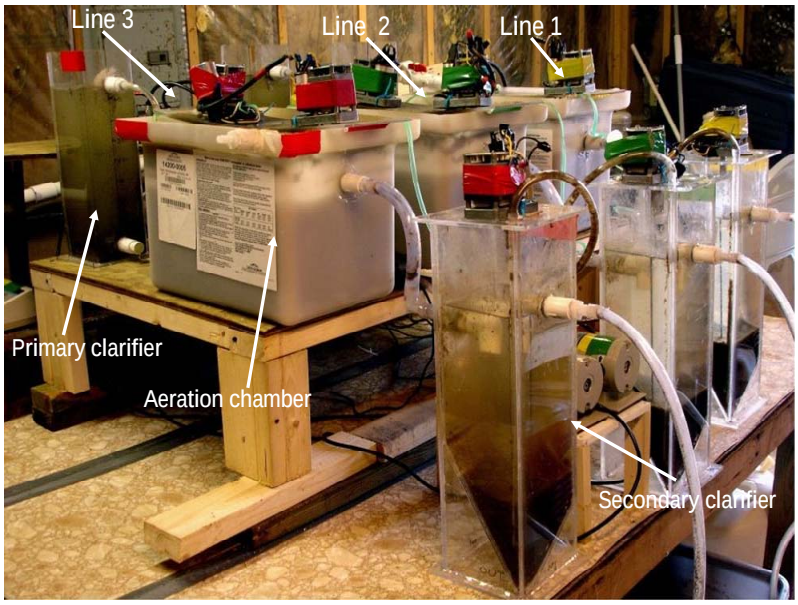

Figure A.1: Laboratory-scale Plum Island Setup Showing Primary Clarifier, Aeration Train, and Secondary Clarifier.

Table A.1: Effluent solids for H-SDS$_1$

Effluent total suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	24	6.36	46.25	6.01	27	12.73
0.33	7.5	0.71	14.5	1.41	43.5	1.41
0.67	13.5	1.41	44.75	5.3	57.75	1.06
1	45.25	1.06	73.75	1.06	49.5	1.41
1.5	308.5	0	35.25	1.06	61	0.71
2	63.75	1.06	19.25	1.77	30.25	1.06
2.5	30	0.71	36	10.61	83	14.14
3	20.5	0.71	23	0.71	11.75	1.06
4	19.25	2.47	21.75	3.18	27.5	0.71
6	28.25	0.35	23.25	1.06	47.5	0
7	8.5	0.71	0	0	21.5	0
8	11.5	1.41	16	1.41	11.25	1.06
9	18.25	2.47	31.75	10.25	5	1.41

10	19	0	23.25	2.47	20.75	1.06
11	34.75	2.47	49.75	1.06	26.75	1.06
12	58	6.36	54	0.71	77.5	4.24
13	62	0.71	60.75	3.89	55.25	6.72

Table A.2: MLSS for H-SDS$_1$

Mixed liquor suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	2710	197.99	2455	35.36	2665	120.21
1	2530	84.85	2405	162.63	2470	14.14
2	2470	127.28	2165	49.5	2720	0
3	2810	0	2350	56.57	2370	42.43
4	2360	0	1965	63.64	2275	35.36
5	2905	233.35	1750	0	3000	98.99
6	1855	7.07	1690	0	1990	14.14
7	2255	63.64	2160	14.14	2055	7.07
8	2380	42.43	2120	28.28	2330	28.28
9	2340	70.71	2245	7.07	2295	7.07
10	2215	21.21	2245	35.36	2205	7.07
11	2415	35.36	2425	21.21	2355	35.36
12	2325	7.07	2275	35.36	2020	28.28
13	1480	0	1825	35.36	1765	35.36

Table A.3: MLVSS for H-SDS$_1$

Mixed liquor volatile suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	2045	63.64	1945	35.36	2100	0
1	1965	49.5	1890	56.57	1985	7.07
2	1975	7.07	1755	63.64	2155	35.36
3	1710	14.14	1945	49.5	1970	56.57
4	1820	0	1570	42.43	1865	7.07
5	1975	63.64	1640	0	1975	63.64
6	1585	63.64	1555	7.07	1755	7.07
7	1920	56.57	1825	21.21	1770	28.28
8	2105	21.21	1845	7.07	2065	21.21
9	1970	28.28	1940	0	2050	14.14
10	1965	21.21	1965	21.21	1850	14.14
11	2060	28.28	2005	7.07	2040	14.14
12	2045	35.36	1990	28.28	1815	7.07
13	1320	0	1600	28.28	1595	35.36

Table A.4: SVI for H-SDS$_1$

Sludge volume index			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	ml/g	ml/g	ml/g
0	91.51	97.76	88.56
1	82.21	81.05	84.21
2	77.73	71.26	70.59
3	59.79	61.76	81.01
4	74.58	67.51	77.36
5	52.32	59.78	56
6	86.25	45.33	80.4
7	74.5	72.36	73.97
8	70.59	58.39	61.8
9	61.54	58.37	69.72
10	72.23	62.75	72.56
11	76.19	72.56	71.34
12	65.38	64.54	71.29
13	97.3	67.33	77.05

Table A.5: Effluent alkalinity for H-SDS$_1$

Effluent alkalinity						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	36	0.13	34.5	0.4	39.1	0.27
0.33	22.37	0.27	66.08	12.89	134.89	0.13
0.67	14.85	0.27	119.38	9.84	91.09	0.4
1	11.28	0.27	11.19	0.13	62.32	0.66
1.5	6.77	0.27	6.11	0.13	12.88	0.13
2	7.52	0	7.43	0.13	20.4	0.13
2.5	8.27	0	6.11	0.13	12.88	0.13
3	7.52	0	5.64	0	10.34	0
4	5.55	0.13	6.67	0.13	6.02	0.27
5	5.83	0	6.02	0.53	7.24	0.13
6	9.87	0.13	9.17	0.07	14.81	0.07
7	5.08	0.53	5.55	0.13	5.92	0.13
8	4.79	0.13	5.36	0.13	7.43	0.13
9	6.11	0.13	6.39	0.27	6.86	0.4
10	4.7	0	4.98	0.13	6.2	0.27
11	47.47	0.13	41.27	0.13	49.07	0.27
12	60.91	0.8	46.72	0.13	56.12	1.73
13	39.48	0.27	34.78	0	33.28	0.27

Table A.6: Mixed liquor pH for H-SDS$_1$

Mixed liquor pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	6.84	6.3	6.1
1	6.56	6.49	7.6
2	6.45	6.4	7.29
3	6.21	6.17	7.15
4	6.14	6.06	6.41
5	6.08	6.03	6.53
6	6.06	5.84	6.22
7	5.95	5.87	6.1
8	5.79	5.74	5.78
9	5.86	5.81	5.89
10	6.11	5.98	6.29
11	5.63	5.6	5.67
12	5.56	5.18	5.44
13	5.62	5.63	5.71

Table A.7: Mixed liquor DO for H-SDS$_1$

Mixed liquor DO			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	mg/l	mg/l	mg/l
0	6.3	6.3	6.1
1	6	5.5	6.3
2	7.5	6.4	7.2
3	6.1	5.8	5.8
4	6.6	6.5	6.3
5	7.1	6.7	6.5
6	5.7	5.4	5.5
7	6.06	5.76	5.6
8	5.91	5.82	5.08
9	6.1	5.2	4.9
10	5.64	5.36	5.18
11	5.1	5.1	5
12	6.4	6.4	6.5
13	5.6	5.3	5.7

Table A.8: Effluent pH for H-SDS$_1$

Effluent pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	6.69	6.73	6.73
0.33	6.9	6.46	7.39
0.67	6.38	6.27	7.13
1	6.21	6.08	7.06
1.5	5.95	5.83	6.19
2	5.92	5.91	6.45
2.5	6	5.74	6.22
3	5.85	5.88	6.14
5	5.82	5.91	5.93
6	6.06	6.05	6.33
7	5.6	5.73	5.67
8	5.6	5.76	5.89
9	5.69	5.8	5.79
10	5.61	5.69	5.79
11	7.04	6.95	6.95
13	7.08	6.94	6.93

Table A.9: Effluent ammonium-N for H-SDS$_1$

Effluent ammonia						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	1.22	0.03	1.91	0.00	2.47	0.00
0.33	0.90	0.07	21.13	0.19	18.65	0.34
0.67	1.26	0.00	1.25	0.00	11.65	0.13
1	2.25	0.05	1.90	0.06	8.75	0.15
1.5	2.43	0.16	1.94	0.08	0.73	0.06
2	1.97	0.03	2.14	0.06	2.18	0.00
2.5	1.54	0.05	1.55	0.05	0.86	0.00
3	2.09	0.00	2.30	0.06	1.70	0.05
4	1.58	0.11	1.53	0.03	1.10	0.03
5	1.39	0.03	1.64	0.03	0.71	0.03
6	0.24	0.03	0.69	0.07	0.40	0.00
7	4.12	0.03	4.63	0.18	3.89	0.11
8	7.16	0.37	8.52	0.22	7.23	0.37
9	3.96	0.16	5.25	0.10	4.45	0.06
10	3.19	0.05	4.61	0.06	3.73	0.09
11	0.44	0.03	0.28	0.00	0.43	0.03
12	0.32	0.00	0.16	0.00	0.17	0.00

13	0.26	0.03	0.38	0.07	0.25	0.03

Table A.10: Effluent nitrate-N for H-SDS$_1$

Effluent nitrate						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	8.90	0.05	34.58	0.00	35.47	0.00
0.33	10.79	0.04	15.08	0.17	9.07	0.05
0.67	18.60	0.08	15.06	0.39	14.74	1.50
1	41.35	1.40	21.78	0.57	9.65	0.02
1.5	31.31	0.24	25.58	0.08	31.46	0.04
2	34.27	0.99	21.55	0.44	14.21	0.01
2.5	37.83	0.09	31.44	0.10	32.19	0.00
3	32.30	0.07	39.68	0.41	24.53	0.45
4	34.18	0.11	15.49	0.64	32.01	0.13
5	30.83	1.07	15.80	0.06	11.13	0.06
6	10.10	0.79	34.31	0.19	7.67	0.16
7	17.57	0.24	12.30	0.01	13.80	0.00
8	11.04	0.23	34.36	0.17	10.78	0.26
9	30.00	0.04	12.83	0.03	34.59	0.30
10	21.14	0.54	25.19	0.17	24.08	0.28
11	30.49	0.44	18.56	0.46	29.15	0.00
12	17.30	0.00	23.07	0.00	19.21	0.00
13	26.23	0.18	0.00	0.00	11.97	0.12

Table A.11: Effluent soluble COD for H-SDS$_1$

Effluent chemical oxygen demand						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.25	25.06	0.76	29.62	0.76	32.66	2.28
0.5	24.3	1.52	28.1	2.28	29.62	2.28
0.75	35.7	3.8	25.06	3.8	31.9	1.52
1	30.38	4.56	27.34	13.67	33.42	0
1.5	41.01	3.04	26.58	3.8	32.66	0.76
2	19.75	3.04	25.82	1.52	31.14	6.84
2.5	24.3	12.15	22.03	0.76	25.82	0
3	22.78	0	36.46	18.23	24.3	1.52
4	26.58	0.76	31.9	10.63	22.4	4
5	8	2.4	20.8	4	22.4	2.4
6	20	10	7.2	4.8	11.2	0.8
7	20	10	13.6	1.6	16.8	4.8
8	20	10	13.6	1.6	16.8	4.8

9	14.4	2.4	7.2	1.6	18.4	0
11	6	6	13.6	1.6	12.8	0.8
13	0	0	2.4	1.6	2.4	3.2

Table A.12: Mixed liquor sOUR values for H-SDS$_1$

Mixed liquor specific oxygen uptake rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
0.00	34.29	1.01	33.95	0.49	32.08	0.04
1.00	33.94	1.2	29.27	1.52	33.43	2.47
2.00	15.57	0.29	15.07	0.02	21.08	0.59
3.00	15.82	0.61	7.23	0.06	3.95	0.43
5.00	9.65	0.36	10.29	0.23	25.63	0.89
8.00	12.58	0.18	12.23	0.14	14.45	0.98
10.00	10.01	0.36	12.24	0.41	10.3	0.26
12.00	35.46	0.94	28.64	0.28	34.2	0.57
13.00	27.99	0.24	22.41	0.5	24.43	0.11

Table A.13: Effluent TSS for H-SDS$_2$

Effluent total suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.0	32.60	3.86	32.60	3.86	32.60	3.80
1.0	22.95	2.69	28.54	5.24	18.60	4.50
2.0	38.36	8.35	29.66	6.32	22.65	3.60
3.0	45.32	2.68	26.65	8.20	19.36	6.54
4.0	22.68	6.22	25.38	5.46	32.36	6.45
5.0	38.36	4.32	33.92	5.45	22.65	6.33
6.0	33.00	5.67	22.98	2.67	38.29	5.43

Table A.14: MLSS for H-SDS$_2$

Mixed liquor suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	2100.00	14.14	2200.00	56.57	2235.00	63.64
1	2270.00	28.28	2270.00		2290.00	70.71
2	2120.00	42.43	1845.00	106.07	2360.00	42.43
3	2145.00	63.64	1745.00	63.64	2160.00	70.71
4	2390.00	42.43	2155.00	120.21	2655.00	21.21
5	2835.00	7.07	2110.00	84.85	2705.00	63.64

6	2825.00	21.21	2380.00	14.14	2715.00	49.50

Table A.15: MLVSS for H-SDS$_2$

Mixed liquor volatile suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	1795	31.81981	1805	24.74874	1865	10.6066
1	1820	28.28427	1830		1950	14.14214
2	1770	21.2132	1580	49.49747	2000	28.28427
3	1823	27.04683	1483.25	27.04683	1836	30.05204
4	1985	38.89087	1800	14.14214	2120	14.14214
5	2315	3.535534	1700	21.2132	2210	7.071068
6	2335	3.535534	2000	7.071068	2225	24.74874

Table A.16: SVI for H-SDS$_2$

Sludge volume index			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	ml/g	ml/g	ml/g
0	95.24	90.91	89.49
1	98.68	88.11	94.32
2	116.98	108.40	91.53
3	123.08	123.78	111.11
4	130.54	115.08	102.45
5	126.98	132.70	133.09
6	138.76	131.09	132.60

Table A.17: Effluent alkalinity for H-SDS$_2$

Effluent alkalinity						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	111.72	0.13	112.67	0.54	116.95	0.13
0.33	148.77	0.54	131.86	1.07	135.28	0.54
0.67	140.60	0.27	136.61	0.27	163.21	0.27
1	208.81	0.00	210.14	1.61	209.00	0.00
1.5	304.00	1.07	306.85	0.81	307.04	1.07
2	164.54	1.07	163.97	0.81	164.16	1.07
2.5	183.54	0.27	180.12	0.54	184.11	0.27
3	201.97	0.81	203.30	0.00	198.17	0.81
4	65.36	0.00	131.67	0.81	131.48	0.00

5	242.06	1.07	67.64	0.54	65.36	1.07
6	255.17	0.81	247.19	0.27	255.17	0.81

Table A.18: Mixed liquor pH for H-SDS$_2$

Mixed liquor pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	7.19	7.2	7.39
0.33	7.29	7.31	7.33
0.67	7.18	7.37	7.3
1	7.63	7.58	7.6
1.5	7.53	7.48	7.49
2	7.88	7.8	7.85
2.5	7.63	7.6	7.62
3	7.45	7.41	7.42
4	7.38	7.35	7.38
5	7.01	6.99	6.97
6	7.61	7.59	7.62

Table A.19: Mixed liquor DO for H-SDS$_2$

Mixed liquor DO			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	mg/l	mg/l	mg/l
0	4.3	4.4	5.7
0.33	3.1	3.7	4.2
0.67	1	4.6	3.8
1	5.3	5.2	5.5
1.5	4.7	4.5	4.8
2	5	5.1	5.3
2.5	4.9	4.5	5.2
3	4.4	5.3	5.7
4	4.9	4.7	5.5
5	4.3	4.2	5
6	3.4	3.7	4.5

Table A.20: Effluent pH for H-SDS$_2$

Effluent pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	7.1	7.11	7.19

0.33	7.23	7.23	7.23
0.67	7.25	7.31	7
1	7.4	7.47	7.47
1.5	7.4	7.41	7.41
2	7.73	7.74	7.75
2.5	7.32	7.35	7.34
3	7.52	7.53	7.57
4	7.28	7.31	7.32
6	7.52	7.54	7.54

Table A.21: Effluent ammonium-N for H-SDS$_2$

Effluent ammonia						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	0.27	0.00	0.22	0.00	0.26	0.00
0.33	0.02	0.00	0.06	0.00	0.84	0.01
0.67	0.77	0.02	0.09	0.00	0.11	0.00
1.00	0.07	0.00	0.07	0.00	0.11	0.00
1.50	0.05	0.00	0.06	0.00	0.04	0.00
2.00	0.26	0.00	0.03	0.00	0.05	0.00
2.50	0.02	0.00	0.03	0.00	0.05	0.00
3.00	0.11	0.00	0.08	0.01	0.05	0.00
4.00	0.07	0.00	0.03	0.00	0.03	0.00
5.00	0.05	0.00	0.05	0.00	0.03	0.00
6.00	0.22	0.00	0.09	0.00	0.26	0.00

Table A.22: Effluent nitrate-N for H-SDS$_2$

Effluent nitrate						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	25.47	0.32	27.11	0.28	21.79	0.13
0.33	27.58	0.02	24.35	0.88	20.46	0.04
0.67	23.47	0.18	29.02	0.05	26.98	0.13
1.00	25.97	0.04	22.19	0.21	22.40	0.10
1.50	24.94	0.53	23.94	1.37	22.23	0.14
2.00	28.73	0.04	25.27	0.11	28.40	0.39
2.50	26.83	0.14	21.37	0.08	26.52	0.16
3.00	22.41	0.17	20.78	0.12	27.41	0.47
4.00	26.37	0.03	26.10	0.33	28.75	0.10
5.00	30.43	0.11	31.97	0.21	33.25	0.15
6.00	23.54	0.09	25.47	0.03	26.50	0.01

Table A.23: Effluent soluble COD for H-SDS$_2$

Effluent chemical oxygen demand						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	21.7	2.32	23.2	5.42	13.9	0.77
0.33	20.1	6.97	17.0	0.77	34.1	4.01
0.67	16.3	3.10	23.2	5.42	24.8	6.97
1.00	27.6	4.95	30.2	3.10	31.0	8.52
1.50	33.9	3.88	24.8	2.32	34.8	4.65
2.00	27.9	6.93	23.2	3.87	27.7	5.19
2.50	13.2	1.55	16.6	16.65	20.7	4.35
3.00	17.0	5.42	15.5	0.77	16.3	3.10
4.00	14.7	1.55	19.4	1.55	17.0	3.87
5.00	27.3	2.68	19.4	1.55	27.9	0.77
6.00	27.1	0.00	25.5	1.55	39.9	6.75

Table A.24: Mixed liquor sOUR values for H-SDS$_2$

Mixed liquor specific oxygen uptake rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$
0	42.79	0.49	42.54	2.85	44.43	1.26
0.25	45.5	2.87	42.95	1.47	43.65	1.22
1	42.35	0.36	34.72	0.28	41.93	2.51
2	43.63	1.68	41.56	1.68	39.35	1.52
3	41.1	0.03	42.41	2.01	40.68	1.73
5	28.78	1.57	32.58	0.63	28.97	0.12
6	35.17	0.21	37.79	0.89	34.78	0.62

Table A.25: Effluent TSS for H-FSC$_1$

Effluent total suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	33.20	6.20	33.20	6.00	33.20	6.00
1	14.00	2.80	38.00	5.70	45.00	1.40
2	7.50	0.70	12.50	3.20	17.00	8.50
3	12.50	3.20	29.00	2.80	4.00	4.20
4	16.80	2.10	32.50	2.60	16.50	1.83
5	15.30	3.50	16.80	1.90	12.24	9.60
6	28.60	6.50	19.60	5.20	22.34	3.20

Table A.26: MLSS for H-FSC$_1$

Mixed liquor suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.0	2170.00	28.28	2170.00	28.28	2170.00	28.28
0.3	1660.00	84.85	2000.00	35.36	2265.00	21.21
1.0	2355.00	63.64	2310.00	240.42	2315.00	49.50
2.0	2460.00	70.71	2145.00	49.50	2205.00	49.50
3.0	1980.00	56.57	2425.00	106.07	2250.00	70.71
4.0	2180.00	113.14	1645.00	35.36		
5.0	2260.00	42.43	1710.00	0.00	2075.00	21.21

Table A.27: MLVSS for H-FSC$_1$

Mixed liquor volatile suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.0	1815.00	45.96	1815.00	45.96	1815.00	45.96
0.3	1370.00	84.85	1710.00	28.28	1870.00	14.14
1.0	1965.00	31.82	1920.00	91.92	1895.00	10.61
2.0	2070.00	56.57	1825.00	3.54	1900.00	14.14
3.0	1683.00	24.04	2061.25	45.08	1912.50	30.05
4.0	1825.00	24.75	1365.00	3.54	1765.00	24.75
5.0	1805.00	10.61	1415.00	45.96	2220.00	346.48

Table A.28: SVI for H-FSC$_1$

Sludge volume index			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	ml/g	ml/g	ml/g
0.0	81.11	81.11	81.11
1.0	125.30	100.00	91.83
2.0	78.05	78.32	87.07
3.0	92.93	65.98	85.33
4.0	84.40	82.67	
5.0	81.42	79.53	88.67
6.0	76.35	72.93	86.38

Table A.29: Effluent alkalinity for H-FSC$_1$

Effluent alkalinity						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	198.30	2.14	198.30	2.14	198.30	2.14
0.33	219.90	0.00	218.93	0.97	323.04	0.00
0.67	243.83	0.58	246.75	1.95	397.96	0.58
1	244.81	0.39	251.62	0.58	256.48	0.39
1.5	259.21	0.39	265.05	1.17	267.58	0.39
2	267.53	0.60	271.35	0.40	266.72	0.60
2.5	251.22	0.40	256.05	2.01	255.25	0.40
3	253.84	1.41	250.82	1.21	256.66	1.41
4	205.53	0.60	210.16	0.40	141.92	0.60
5	141.11	0.20	149.77	0.81	165.07	0.20
6	162.25	0.81	167.68	0.60	162.25	0.81

Table A.30: Mixed liquor pH for H-FSC$_1$

Mixed liquor pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	7.93	7.74	7.82
0.33	7.77	7.8	7.49
0.67	7.78	7.84	7.55
1	7.79	7.94	7.85
1.5	7.8	7.88	7.88
2	7.85	7.91	7.93
2.5	7.78	7.85	7.85
3	7.77	7.84	7.84
4	7.61	7.66	7.66
5	7.49	7.55	7.52
6	7.37	7.44	7.41

Table A.31: Mixed liquor DO for H-FSC$_1$

Mixed liquor DO			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	mg/l	mg/l	mg/l
0	6.9	5.6	5.85
0.33	7	6.4	0.1
0.67	6.2	6.4	0.2
1	5.9	6.2	6.3

1.5	6.6	6.5	6.6
2	6.35	6.6	6.6
2.5	6.1	6.1	6.1
3	6.3	6	5.9
4	5.9	6.1	6
5	6	6	5.9
6	5.6	5.7	5.6

Table A.32: Effluent pH for H-FSC$_1$

Effluent pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	7.59	7.64	7.66
0.33	7.63	7.67	7.53
0.67	7.63	7.75	7.61
1	7.65	7.74	7.65
1.5	7.71	7.74	7.78
2	7.75	7.8	7.84
2.5	7.66	7.73	7.75
3	7.69	7.75	7.74
4	7.5	7.55	7.55
6	7.29	7.31	7.31

Table A.33: Effluent ammonium-N for H-FSC$_1$

Effluent ammonia						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	0.06	0.00	0.06	0.00	0.06	0.00
0.33	0.03	0.01	0.03	0.00	12.30	0.29
0.67	0.02	0.00	0.04	0.00	14.36	0.56
1.00	0.10	0.00	0.03	0.00	0.03	0.00
1.50	0.02	0.00	0.03	0.00	0.06	0.01
2.00	0.02	0.00	0.06	0.00	0.18	0.00
2.50	0.03	0.00	0.21	0.01	0.06	0.00
3.00	0.04	0.00	0.21	0.01	0.08	0.01
4.00	0.07	0.00	0.19	0.01	0.07	0.01
5.00	0.230549	0.010708	0.56	0.02	0.72	0.01
6.00	0.024702	0.00	0.067002	0.001908	0.022458	0.001047

Table A.34: Effluent nitrate-N for H-FSC$_1$

Effluent nitrate						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	30.50	0.15	30.50	0.15	30.50	0.15
0.33	25.44	0.23	25.95	0.12	26.79	0.04
0.67	24.86	0.02	25.30	0.07	1.13	0.00
1.00	24.51	0.06	23.34	0.95	20.20	0.05
1.50	20.97	0.01	20.16	0.04	18.97	0.03
2.00	20.69	0.15	19.93	0.03	19.35	0.00
2.50	21.39	0.03	19.92	0.01	20.31	0.11
3.00	22.99	0.30	21.85	0.30	23.53	0.16
4.00	13.62	0.06	13.02	0.01	13.65	0.01
5.00	12.65	0.00	12.52	0.01	12.89	0.04
6.00	19.36	0.06	18.89	0.11	18.30	0.05

Table A.35: Effluent soluble COD for H-FSC$_1$

Effluent chemical oxygen demand						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	21.7	2.32	23.2	5.42	13.9	0.77
0.33	20.1	6.97	17.0	0.77	34.1	4.01
0.67	16.3	3.10	23.2	5.42	24.8	6.97
1.00	27.6	4.95	30.2	3.10	31.0	8.52
1.50	33.9	3.88	24.8	2.32	34.8	4.65
2.00	27.9	6.93	23.2	3.87	27.7	5.19
2.50	13.2	1.55	16.6	16.65	20.7	4.35
3.00	17.0	5.42	15.5	0.77	16.3	3.10
4.00	14.7	1.55	19.4	1.55	17.0	3.87
5.00	27.3	2.68	19.4	1.55	27.9	0.77
6.00	27.1	0.00	25.5	1.55	39.9	6.75

Table A.36: Mixed liquor sOUR values for H-FSC$_1$

Mixed liquor specific oxygen uptake rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$
0	27.39	1.15	27.39	1.15	27.39	1.15
0.33	32.08	0.15	21.32	2.9	42.79	0.02
1	29.68	0.6	25.62	2.17	28.99	1.23
2	28.74	1.46	27.99	1.28	28.34	2.48

3	26.56	1.21	21.26	0.06	25.69	0.18
4	28.77	1.02	26.64	1.43	26.77	0.07
5	28.54	2.33	23.68	1.05	22.35	1.38
6	22.11	0.3	23.3	1.11	24.37	0.38

Table A.37: Effluent TSS for H-FSC$_2$

Effluent total suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	12.3	1.26	12.3	1.26	12.3	1.26
1	3.5	0.5	11.5	2.5	20.5	3.5
2	11	1	28	2	27	3
3	33	2	28	1	18.5	2.5
4	7.5	1.5	33.5	0.5	17	3
5	5.5	1.5	16.5	1.5	17	3
6	6	1	18	4	6.5	3.5

Table A.38: MLSS for H-FSC$_2$

Mixed liquor suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.0	1675.00	35.36	1675.00	35.36	1675.00	35.36
0.3	0.00	0.00	2135.00	120.21	1665.00	63.64
1.0	1735.00	7.07	2320.00	70.71	2200.00	70.71
2.0	1840.00	0.00	2110.00	28.28	2210.00	42.43
3.0	1825.00	7.07	2065.00	35.36	2060.00	98.99
4.0	1875.00	7.07	2335.00	21.21	2070.00	28.28
5.0	2215.00	63.64	2510.00	42.43	2265.00	49.50
6.0	2240.00	14.14	2345.00	21.21	2210.00	56.57

Table A.39: MLVSS for H-FSC$_2$

Mixed liquor volatile suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.0	1370.00	10.00	1370.00	10.00	1370.00	10.00
0.3	0.00	0.00	1775.00	65.00	1320.00	20.00
1.0	1460.00	10.00	1890.00	45.00	1755.00	35.00
2.0	1625.00	45.00	1780.00	50.00	1890.00	40.00
3.0	1535.00	35.00	1725.00	15.00	1700.00	90.00
4.0	1515.00	15.00	1840.00	40.00	1655.00	35.00

5.0	1850.00	10.00	2130.00	50.00	1930.00	20.00
6.0	1780.00	10.00	1915.00	35.00	1835.00	5.00

Table A.40: SVI for H-FSC$_2$

Sludge volume index			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	ml/g	ml/g	ml/g
0.0	128.47	128.47	128.47
1.0	109.59	105.82	113.96
2.0	86.15	85.39	80.42
3.0	104.23	83.48	108.24
4.0	105.61	108.70	106.34
5.0	99.46	97.65	99.48
6.0	107.87	100.26	100.27

Table A.41: Effluent alkalinity for H-FSC$_2$

Effluent alkalinity						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.0	259.2	2.8	259.2	2.8	259.2	2.8
0.3	258.8	0.4	272.8	0.8	367	1
0.7	270.2	0.2	268	0.4	278.8	0.4
1.0	275	3	277.2	0.4	280.2	3
1.5	257.8	3	257	1	262.8	1.2
2.0	238.8	1.2	237	1	235.6	0.4
2.5	156.6	0.6	164.6	0.2	159.8	0.2
3.0	192.8	0.8	197.6	1.6	190.8	0.8
4.0	236.4	1.6	235.2	0	237.6	0.4
5.0	220	0	219.4	0.2	221.4	0.2
6.0	219	0.2	225.2	0.8	230	0.4

Table A.42: Mixed liquor pH for H-FSC$_2$

Mixed liquor pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	7.74	7.76	7.73
0.33	-	7.81	7.46
0.67	7.81	7.81	7.86
1	7.91	7.93	7.97
1.5	7.68	7.7	7.72

2	7.82	7.87	7.92
2.5	7.71	7.76	7.76
3	7.56	7.6	7.62
4	7.72	7.74	7.77
5	7.65	7.68	7.71
6	7.77	7.74	7.74

Table A.43: Mixed liquor DO for H-FSC_2

Mixed liquor DO			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	mg/l	mg/l	mg/l
0	5.7	6.2	5.9
0.33	0	6.2	0.1
0.67	6.4	6.5	6.5
1	6.3	6.4	6.3
1.5	5.6	5.8	5.8
2	5.6	5.9	5.9
2.5	5.6	5.8	5.6
3	6.2	6.5	6.5
4	6.2	6.1	6.2
5	5.9	6	5.9
6	6.1	5.9	5.8

Table A.44: Effluent pH for H-FSC_2

Effluent pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
0	7.62	7.7	7.72
0.33	0	7.79	7.52
0.67	7.75	7.75	7.8
1	7.83	7.85	7.89
1.5	7.56	7.6	7.63
2	7.72	7.77	7.82
2.5	7.46	7.48	7.52
3	7.54	7.54	7.61
4	7.6	7.62	7.64
5	7.55	7.58	7.6
6	7.64	7.63	7.64

Table A.45: Effluent ammonium-N for H-FSC$_2$

Effluent ammonia						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	0.04	0.01	0.04	0.01	0.04	0.01
0.33	0	0	0.03	0.01	13.94	0.68
0.67	0.04	0.01	0	0	0.06	0.01
1	0.04	0	0.01	0	0.02	0.01
1.5	0.08	0	0.04	0.01	0.03	0
2	0.04	0	0.02	0	0.05	0.01
2.5	0.07	0.01	0.04	0	0.05	0.01
3	0.03	0	0.04	0	0.02	0
4	0.04	0	0.05	0.01	0.03	0
5	0.01	0	0.01	0	0.01	0
6	0.01	0.01	0.01	0	0.03	0

Table A.46: Effluent nitrate-N for H-FSC$_2$

Effluent nitrate						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	26.81	0.05	26.81	0.05	26.81	0.05
0.33			22.43	0.03	4.38	0.00
0.67	21.21	0.04	22.19	0.04	18.27	0.01
1.00	20.32	0.06	20.45	0.11	19.07	0.01
1.50	26.58	0.26	27.17	0.00	26.08	0.00
2.00	29.18	0.03	30.01	0.01	29.88	0.01
2.50	23.08	0.08	23.32	0.02	23.28	0.02
3.00	23.40	0.02	23.14	0.12	23.79	0.07
4.00	29.86	0.02	21.83	0.03	30.38	0.00
5.00	26.24	0.02	26.46	0.01	26.18	0.02
6.00	28.42	0.02	26.04	0.02	25.56	0.01

Table A.47: Effluent soluble COD for H-FSC$_2$

Effluent chemical oxygen demand						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0.00	20.0	2.47	20.0	2.47	20.0	2.47
0.33	25	3.65	23.3	0.82	42.3	0.00
0.67	17.6	0.00	21.7	2.47	19.2	1.65
1.00	19.2	1.65	19.2	3.29	21.7	7.41

1.50	29.9	0.82	22.5	3.29	21.7	4.12
2.00	38.1	0.82	34.9	0.82	45.6	6.59
2.50	31.6	0.82	29.9	0.82	28.3	0.82
3.00	43.9	1.65	30.7	1.65	36.5	2.47
4.00	35.7	1.65	30.7	3.29	34.9	0.82
5.00	34.9	0.82	33.2	0.82	34.0	1.65
6.00	36.5	0.82	34.0	0.00	32.4	1.65

Table A.48: Mixed liquor sOUR levels for H-FSC$_2$

Mixed liquor specific oxygen uptake rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
0	20.8	0.66	20.8	0.66	20.8	0.66
0.33	0	0	28.28	0.16	55.12	0.04
1	27.01	0.9	25.04	0.15	25.73	0.76
2	27.55	0.9	26.82	2.14	25.95	0.49
3	35.04	0.29	32.46	0.94	31.85	0.93
4	29.09	0.13	28.69	1.41	24.51	1.1
5	23.82	0.11	28.08	0.53	28.41	1.44
6	26.16	0.86	23.41	0.97	25.42	0.48

Table A.49: Effluent TSS for Cd-SDS$_1$

Effluent total suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3.0	40	0	40	0	40	0
0.0	5	0	25	5.66	25	5.66
0.7	0	0	0	0	93	4.24
1.0	6.5	0.71	41	1.41	54	0
2.0	6.5	0.71	52.5	4.95	52.5	4.95
3.0	19	5.66	48.5	3.54	46	1.41
4.0	6.5	2.12	17	0	22.5	2.12
5.0	14.5	0.71	12	0	24.5	0.71
6.0	12	2.83	8.5	0.71	16	1.41
8.0	15	1.41	31	4.24	9.5	3.54
9.0	52.5	3.54	3	1.41	7	0
10.0	29.5	0.71	9.5	0.71	14	1.41
11.0	30	0	16.5	0.71	12.5	0.71
12.0	105	2.83	10	1.41	7	0
13.0	167	1.41	19	0	53	4.24

Table A.50: MLSS for Cd-SDS$_1$

Mixed liquor suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	1690	14.14	1690	14.14	1690	14.14
-2	1700	14.14	1700	14.14	1700	14.14
-1	1975	35.36	1975	35.36	1975	35.36
0	2565	0	2565	0	2565	0
0.33	2230	14.14	2005	49.5	1940	28.28
1	2240	14.14	1745	106.07	1765	7.07
2	2315	7.07	1805	21.21	1835	35.36
3	2105	35.36	1835	49.5	1885	63.64
4	2053.5	19.09	1680	28.28	1650	14.14
5	2075	77.78	1755	7.07	1620	0
6	2050	14.14	1870	28.28	1790	14.14
8	1635	49.5	1605	7.07	1570	0
9	1440	42.43	1715	35.36	1585	7.07
10	1480	14.14	1675	63.64	1570	84.85
11	1300	14.14	1655	21.21	1630	28.28
12	1095	21.21	1570	70.71	1525	35.36
13	785	21.21	1470	28.28	1475	7.07
14	0	0	1280	0	1570	42.43

Table A.51: MLVSS for Cd-SDS$_1$

Mixed liquor volatile suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	1405	5	1405	5	1405	5
-2	1445	5	1445	5	1445	5
-1	1685	35	1685	35	1685	35
0	2085	5	2085	0	2085	0
0.33	1900	20	1650	20	1505	5
1	1895	5	1395	65	1380	10
2	1980	10	1475	25	1490	10
3	1835	25	1535	15	1505	35
4	1763.5	13.5	1405	15	1350	10
5	1760	60	1415	15	1265	5
6	1795	5	1610	0	1500	20
8	1410	0	1350	10	1315	15
9	1260	10	1455	5	1320	0
10	1270	10	1390	10	1310	40

11	1095	25	1395	5	1355	35
12	940	10	1360	10	1270	10
13	675	15	1255	5	1230	0
14	0	0	1110	20	1330	0

Table A.52: SVI for Cd-SDS$_1$

Sludge volume index			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	ml/g	ml/g	ml/g
-3	104.14	104.14	104.14
-1	101.27	101.27	101.27
0	74.85	74.85	74.85
1	92.86	119.2	117.85
2	82.94	110.8	104.63
3	91.21	113.35	101.86
4	81.81	109.52	101.82
5	84.82	113.96	108.64
6	81.95	100.53	93.85
8	102.75	109.66	107.01
9	116.67	116.62	105.99
11	104.62	120.85	83.44
12	168.04	122.29	120.66
13	244.59	141.5	130.17
14	0	312.5	107.01

Table A.53: Effluent alkalinity for Cd-SDS$_1$

Effluent alkalinity						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	174.93	1.42	174.93	1.42	174.93	1.42
-2	138.24	0.41	138.24	0.41	138.24	0.41
-1	171.48	0.41	171.48	0.41	171.48	0.41
0	151.42	0.61	151.42	0.61	151.42	0.61
0.33	163.17	0.61	254.79	1.01	305.87	1.82
0.67	163.98	0.2	344.89	1.11	397.9	1.42
1	200.67	0	393.24	2.03	399.52	0.61
1.5	215.27	0.41	383.1	1.62	395.67	3.65
2	210.81	0	346.82	1.42	365.67	1.22
2.5	0	0	294.73	0.41	321.08	2.03
3	136.62	0.41	272.63	1.01	306.69	0.2
4	201.08	1.22	277.09	1.42	323.51	0

5	218.31	0.2	226.42	0.61	251.55	0.2
6	217.5	0.61	219.93	1.01	225.61	0.2
8	228.04	1.01	226.82	0.2	227.02	0.41
9	240	0.41	250.13	0.81	250.74	0.2
10	236.96	0.2	238.78	0.41	246.48	1.22
11	286.21	0.41	287.23	0.2	287.83	0
12	208.98	0.2	200.67	0	192.36	0.2
13	216.08	1.22	176.55	0.2	171.69	0.2
14	235.74	0.61	185.47	1.01	162.77	1.01

Table A.54: Mixed liquor pH for Cd-SDS$_1$

Mixed liquor pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
-3	7.47	7.47	7.47
-2	7.29	7.29	7.29
-1	7.44	7.44	7.44
0	7.47	7.47	7.47
0.33	7.39	8.05	8.25
0.67	7.34	8.16	8.28
1	7.44	8.07	8.18
2	7.41	7.92	8.06
2	7.43	7.84	8.01
3	7.23	7.75	7.96
4	7.56	7.80	8.09
5	7.58	7.57	7.79
6	7.48	7.48	7.62
7	7.70	7.70	7.80
8	7.62	7.63	7.78
9	7.52	7.52	7.69
10	7.68	7.66	7.86
11	7.74	7.77	7.92
12	7.46	7.44	7.56
13	7.44	7.30	7.46
14	7.49	7.24	7.36

Table A.55: Mixed liquor DO for Cd-SDS$_1$

Mixed liquor DO			
	Negative control	Negative control	Negative control
Days	Average	Average	Average
	mg/l	mg/l	mg/l
-3	5.60	5.60	5.60
-2	4.80	4.80	4.80

-1	5.70	5.70	5.70
0	6.20	6.20	6.20
0.33	4.60	6.80	6.60
0.67	4.50	6.80	6.90
1	4.50	6.40	6.70
2	4.50	5.50	6.20
2	4.30	5.30	6.30
3	4.50	5.60	6.40
4	5.10	5.50	6.60
5	4.60	4.50	6.00
6	4.40	3.70	5.20
7	5.40	5.00	6.30
8	5.10	5.00	6.00
9	5.10	4.70	6.00
10	5.10	5.00	5.80
11	5.40	5.20	6.30
12	3.60	3.20	5.00
13	4.30	3.40	5.00
14	4.60	2.70	4.50

Table A.56: Effluent pH for Cd-SDS$_1$

Effluent pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
-3	7.39	7.39	7.39
-2	7.25	7.25	7.25
-1	7.39	7.39	7.39
0	7.35	7.35	7.35
0.33	7.35	8.02	8.24
0.67	7.30	8.15	8.27
1	7.41	8.05	8.17
2	7.38	7.88	8.00
2	7.39	7.78	7.93
3	7.19	7.67	7.89
4	7.52	7.69	8.00
5	7.52	7.51	7.64
6	7.44	7.44	7.52
7	7.64	7.67	7.70
8	7.55	7.58	7.73
9	7.42	7.49	7.66
10	7.60	7.66	7.83
11	7.64	7.74	7.87
12	7.40	7.41	7.47
13	7.34	7.22	7.36
14	7.37	7.18	7.29

Table A.57: Effluent ammonium-N for Cd-SDS$_1$

Effluent ammonia						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	0.2	0.07	0.2	0.07	0.2	0.07
-1	0.04	0.04	0.04	0.04	0.04	0.04
0	0.08	0.04	0.08	0.04	0.08	0.04
0.33	0.72	0.04	25.19	0.16	25.06	0.73
0.67	0.73	0.1	28.7	0.45	33.57	0.04
1	0.57	0.23	31.17	0.27	26.94	0.86
1.5	0.51	0.19	24.69	0.11	23.11	0.63
2	0.53	0.15	16.68	0.95	18.93	0.56
2.5	0.37	0.09	15.83	0.23	20.2	1.75
3	0.38	0.04	13.95	0.75	20.36	0.52
3	0.47	0.09	9.44	0.12	16	0.16
5	0.05	0.01	0.28	0.04	3.47	0.06
6	0.07	0.02	0.25	0.07	0.49	0.02
7	0.08	0.04	0.12	0.05	0.19	0.14
8	0.06	0.01	0.15	0.04	0.19	0.03
9	0.11	0.01	0.03	0.16	0.15	0.05
10	0.01	0.17	0.1	0.07	0.17	0.05
11.00	0.11	0.03	0.13	0.02	0.09	0.24
12.00	0.52	0.03	0.14	0.26	0.39	0.11
13.00	5.92	0.03	0.3	0.05	0.36	0.04

Table A.58: Effluent nitrate-N for Cd-SDS$_1$

Effluent nitrate						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3.00	40.50	0.00	40.50	0.00	40.50	0.00
-2.00	37.60	0.00	37.60	0.00	37.60	0.00
-1.00	38.13	0.00	38.13	0.00	38.13	0.00
0.00	42.72	0.06	42.72	0.06	42.72	0.06
0.33	42.50	0.13	21.81	0.06	11.41	0.10
0.67	41.52	0.11	10.05	0.02	3.67	0.09
1.00	35.52	0.21	6.66	0.01	5.36	0.01
1.50	32.27	0.10	7.49	0.01	6.45	0.02
2.00	31.76	0.01	10.95	0.04	8.76	0.01
2.50	36.07	0.01	13.20	0.00	9.67	0.10
3.00	37.52	0.59	21.72	0.09	10.72	0.06
4.00	35.36	0.01	21.72	0.09	14.37	0.01
5.00	28.65	0.05	27.33	0.09	18.99	0.03

6.00	31.63	0.05	30.26	0.01	20.65	1.94
7.00	24.94	0.08	24.55	0.04	23.25	0.22
8.00	24.47	0.08	23.39	0.02	23.00	0.11
9.00	25.48	0.00	24.92	0.10	23.57	0.08
10.00	24.43	0.09	24.61	0.00	22.54	0.44
11.00	15.49	0.00	16.17	0.00	15.77	0.00
12.00	27.62	0.13	30.88	0.03	29.79	0.00
13.00	23.65	0.10	34.72	0.18	32.90	0.04

Table A.59: Effluent nitrite-N for Cd-SDS$_1$

Effluent nitrite						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3.00	2.09	0.00	2.09	0.00	2.09	0.00
-2.00	0.07	0.00	0.07	0.00	0.07	0.00
-1.00	0.12	0.00	0.12	0.00	0.12	0.00
0.00	0.23	0.22	0.23	0.22	0.23	0.22
0.33	0.48	0.00	0.00	0.00	0.00	0.00
0.67	0.18	0.00	0.00	0.00	0.00	0.00
1.00	0.04	0.01	0.00	0.00	0.00	0.00
1.50	0.41	0.05	0.00	0.00	0.00	0.00
2.00	0.46	0.02	0.00	0.00	0.00	0.00
2.50	0.46	0.01	0.00	0.00	0.00	0.00
3.00	0.39	0.03	0.32	0.00	0.34	0.08
4.00	0.46	0.01	0.58	0.00	0.50	0.02
5.00	0.32	0.00	0.86	0.01	0.60	0.02
6.00	0.48	0.01	1.68	0.01	5.44	0.30
7.00	0.00	0.00	0.58	0.02	1.04	0.07
8.00	0.00	0.00	0.51	0.00	0.86	0.08
9.00	0.34	0.01	0.83	0.01	0.90	0.00
10.00	0.26	0.00	0.66	0.04	0.62	0.00
11.00	0.00	0.00	0.66	0.00	0.62	0.00
12.00	4.96	0.04	3.23	0.01	6.09	0.00
13.00	8.74	0.03	6.17	0.04	8.79	0.01

Table A.60: Effluent soluble COD for Cd-SDS$_1$

Effluent chemical oxygen demand						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
-2.00	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-1.00	23.3	0.76	23.3	0.76	23.3	0.76
0.00	19.5	0.00	19.5	0.00	19.5	0.00
0.33	26.3	0.76	26.3	0.76	26.3	0.76

0.67	24.8	0.76	85.9	11.45	89.7	3.05
1.00	19.5	1.53	103.4	1.53	103.4	0.00
1.50	20.2	0.76	86.6	1.53	96.5	0.76
2.00	27.1	1.53	63.0	0.76	66.0	0.76
2.50	25.6	0.00	56.1	0.00	69.1	2.29
3.00	26.3	0.76	43.9	1.53	41.6	0.76
4.00	21.0	0.00	37.8	1.53	45.4	0.00
5.00	18.7	2.29	37.8	1.53	34.0	0.76
6.00	30.1	0.00	63.0	0.76	55.3	0.76
7.00	42.4	0.00	27.9	2.29	55.3	0.76
8.00	27.1	1.53	43.9	1.53	38.5	0.76
9.00	20.2	0.76	26.3	0.76	33.2	0.00
10.00	54.6	0.00	57.6	0.00	48.5	1.53
11.00	16.4	1.53	23.3	2.29	27.9	0.76
12.00	40.1	0.76	56.1	6.10	53.0	1.53
13.00	29.4	3.82	32.4	0.76	29.4	2.29
14.00	56.9	0.76	54.6	1.53	53.0	0.00

Table A.61: Mixed liquor sOUR levels for Cd-SDS$_1$

Mixed liquor specific oxygen uptake rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
-3	36.02	0.62	36.02	0.62	36.02	0.62
-2	29.19	1.16	29.19	1.16	29.19	1.16
-1	34.58	0.50	34.58	0.50	34.58	0.50
0	25.47	0.81	25.47	0.81	25.47	0.81
0.33	31.15	0.02	2.62	0.07	1.95	1.48
1	27.85	0.87	9.94	0.04	8.85	0.28
3	35.59	1.88	28.96	0.43	23.00	0.36
4	33.17	0.75	32.14	1.26	27.18	0.38
5	27.99	1.13	39.67	0.02	36.81	0.71
6	26.84	0.80	42.75	0.04	34.10	0.02
8	24.28	1.13	39.47	0.22	39.49	1.98
9	22.45	0.02	33.32	1.61	34.73	1.50
12	35.49	0.83	37.83	0.02	41.36	1.63
14			34.97	0.59	40.69	1.53

Table A.62: Mixed liquor sAOR levels for Cd-SDS$_1$

Mixed liquor specific ammonia oxidation rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
-2	4.77	0.00	4.77	0.00	4.77	0.00

-1	3.78	0.04	3.78	0.04	3.78	0.04
0	3.55	0.00	3.55	0.00	3.55	0.00
0.33	3.61	0.07	0.00	0.02	0.04	0.01
1	4.15	0.33	0.69	0.06	0.79	0.07
2	3.67	0.16	1.90	0.06	1.32	0.03
3	3.82	0.13	1.78	0.09	1.65	0.12
4	4.47	0.11	3.71	0.14	2.68	0.03
5	3.51	0.09	4.16	0.33	3.89	0.20
6	3.65	0.13	4.83	0.40	5.49	0.08
8	2.93	0.10	6.27	0.21	6.55	0.18
11	3.53	0.09	5.05	0.01	6.08	0.01
12	3.44	0.00	4.69	0.15	5.43	0.10
13	0.00	0.00	5.17	0.07	5.07	0.03

Table A.63: Total cadmium levels for Cd-SDS$_1$

Total cadmium levels				
	Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l
0.00	0.00	0.00	0.00	0.00
0.17	50.61	0.65	109.41	3.86
0.33	72.89	0.43	110.31	0.95
0.67	64.99	0.43	96.46	2.54
1.00	59.03	0.43	85.84	1.68
1.50	55.92	0.69	85.58	2.22
2.00	50.74	0.78	80.14	1.86
3.00	43.88	0.00	71.21	1.04
4.00	38.96	1.01	61.88	0.43
7.00	27.05	0.69	45.05	0.69
9.00	24.46	0.65	36.11	0.65
12.00	19.53	0.00	27.95	0.43

Table A.64: Soluble cadmium levels for Cd-SDS$_1$

Soluble cadmium levels				
	Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l
0.00	0.00	0.00	0.00	0.00
0.17	6.70	0.00	13.30	0.09
0.33	7.95	0.05	6.17	0.04
0.67	3.77	0.04	3.50	0.05
1.00	2.80	0.05	2.73	0.20
1.50	2.44	0.04	2.34	0.00
2.00	1.82	0.00	1.80	0.00

2.50	1.36	0.00	1.46	0.01
3.00	1.16	0.00	1.25	0.00
4.00	0.88	0.00	0.95	0.00
5.00	0.70	0.00	0.89	0.00
6.00	0.50	0.00	0.84	0.00
7.00	0.27	0.00	0.39	0.00
8.00	0.07	0.00	0.31	0.00
10.00	0.02	0.00	0.05	0.00
14.00	0.03	0.00	0.02	0.00

Table A.65: Effluent TSS for Cd-SDS_2

Effluent total suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2.0	20	4.24	20	4.24	20	4.24
-1.0	24.5	0.71	24.5	0.71	24.5	0.71
0.0	5	0	16	5.66	16	5.66
0.7000	11.5	2.12	38.5	2.12	50.5	3.54
1.0	6	1.41	21	4.24	61	0
2.0	5	0	11.5	2.12	25	1.41
3.0	9	1.41	16.5	0.71	15.5	2.12
4.0	5	1.41	9.5	0.71	16.5	0.71
5.0	5	1.41	7	1.41	14.5	0.71
6.0	7	1.41	17	1.41	11.5	0.71
7.0	39	1.41	61	2.83	11	0
8.0	150	0	36.5	0.71	40.5	2.12
9.0	149.5	0.71	21	0	18.5	0.71
10.0	89	1.41	29.5	0.71	17.5	2.12
11.0	51	4.24	32	1.41	27	1.41
12.0	65.5	0.71	43.5	0.71	31.5	0.71
13.0	0	0	0	0	38.5	2.12

Table A.66: MLSS for Cd-SDS_2

Mixed liquor suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2	1605	7.07	1605	7.07	1605	7.07
-1	1650	70.71	1650	70.71	1650	70.71
0	2565	0	2565	0	2565	0
0.33	1980	14.14	1825	7.07	1500	0
1	2050	28.28	1565	49.5	1435	21.21
2	1745	190.92	1600	28.28	1745	148.49

3	1785	35.36	1635	7.07	1605	7.07
4	1720	0	1650	14.14	1585	49.5
5	1865	35.36	1620	56.57	1675	21.21
6	2110	42.43	1600	28.28	1650	0
7	1795	7.07	1605	35.36	1710	14.14
8	1875	21.21	1610	42.43	1565	7.07
9	1720	0	1525	7.07	1515	77.78
10	1215	7.07	1435	35.36	1615	7.07
11	1165	7.07	1505	21.21	1670	14.14
12	1160	28.28	1300	14.14	1485	35.36
13	1465	148.49	1500	127.28	1345	35.36

Table A.67: MLVSS for Cd-SDS_2

Mixed liquor volatile suspended solids						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2	1355	5	1355	5	1355	5
-1	1410	30	1410	30	1410	30
0	2085	5	2085	0	2085	0
0.33	1695	5	1470	0	1190	10
1	1730	20	1285	25	1145	25
2	1455	155	1320	0	1440	140
3	1560	20	1365	15	1300	20
4	1545	15	1430	60	1320	20
5	1600	20	1335	25	1340	0
6	1835	35	1370	0	1380	20
7	1590	20	1380	20	1440	10
8	1615	5	1330	10	1280	0
9	1440	10	1330	0	1330	50
10	1100	0	1265	5	1400	10
11	1020	10	1275	5	1390	20
12	1010	20	1140	0	1255	5
13	1215	55	1235	55	1120	20

Table A.68: SVI for Cd-SDS_2

Sludge volume index			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
	ml/g	ml/g	ml/g
-2	119.63	119.63	119.63
-1	116.36	116.36	116.36
0	77.97	77.97	77.97

1	113.17	122.68	105.92
2	128.37	120	96.28
3	147.9	112.54	104.67
4	125.58	121.21	111.04
5	120.11	118.52	100.3
6	147.87	107.5	101.82
8	418.13	109.32	112.46
9	316.28	99.67	116.17
11	260.94	90.37	86.23
12	303.45	110.77	91.58
13	300.34	90.67	101.12

Table A.69: Effluent alkalinity for Cd-SDS_2

Effluent alkalinity						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2.0	223	0.4	223	0.4	223	0.4
-1.0	187	0.8	187	0.8	187	0.8
0.0	206	1.2	206	1.2	206	1.2
0.3	227	0.2	275	0.2	314	0.2
0.7	252	1.0	362	1.2	400	0.0
1.0	269	0.8	391	0.2	402	1.4
1.5	257	0.0	379	0.4	383	0.4
2.0	252	0.2	359	1.0	377	0.8
2.5	206	0.8	335	0.6	359	0.4
3.0	189	0.2	314	0.6	357	0.4
4.0	198	0.2	255	0.2	327	0.2
5.0	197	0.2	194	0.8	261	0.6
6.0	186	0.6	190	0.0	209	0.2
7.0	200	0.2	201	0.4	198	0.2
8.0	245	1.8	242	0.8	242	0.4
9.0	253	0.8	247	1.4	256	0.6
10.0	281	1.4	274	0.4	278	0.4
11.0	281	1.2	273	0.8	272	0.0
12.0	227	0.4	219	0.2	220	0.6
13.0	237	0.2	235	0.6	232	0.8

Table A.70: Mixed liquor pH for Cd-SDS_2

Mixed liquor pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
-2	7.54	7.54	7.54

-1	7.60	7.60	7.60
0	7.54	7.54	7.54
0.33	7.49	7.97	8.15
0.67	7.54	8.08	8.18
1	7.64	8.08	8.28
2	7.70	8.11	8.20
2	7.62	8.04	8.18
3	7.48	7.94	8.11
3	7.42	7.80	8.05
4	7.46	7.61	7.98
5	7.42	7.42	7.78
6	7.35	7.37	7.55
7	7.29	7.40	7.51
8	7.51	7.55	7.60
9	7.47	7.52	7.64
10	7.62	7.62	7.69
11	7.60	7.68	7.76
12	7.50	7.53	7.60

Table A.71: Mixed liquor DO for Cd-SDS_2

Mixed liquor DO			
	Negative control	Negative control	Negative control
Days	Average	Average	Average
	mg/l	mg/l	mg/l
-2	5.40	5.20	6.10
-1	5.00	5.00	6.00
0	5.20	4.80	5.80
0.33	7.70	7.20	5.50
0.67	4.70	7.20	7.70
1	4.50	6.10	7.20
2	5.10	6.10	6.50
2	5.60	6.50	6.90
3	3.70	5.50	6.20
3	4.00	5.20	6.20
4	4.20	4.80	6.20
5	3.60	4.10	5.70
6	3.40	3.40	5.20
7	0.00	0.00	0.00
8	4.60	4.80	5.30
9	4.10	4.40	5.20
10	4.50	4.40	4.90
11	4.50	4.90	5.90
12	4.00	4.50	5.00

Table A.72: Effluent pH for Cd-SDS_2

Effluent pH			
	Negative control	Positive control	Corrective action
Days	Average	Average	Average
-2	7.48	7.48	7.48
-1	7.58	7.58	7.58
0	7.54	7.54	7.54
0.33	7.47	7.96	8.13
0.67	7.51	8.08	8.17
1	7.59	8.04	8.24
2	7.66	8.06	8.10
2	7.61	7.99	8.12
3	7.45	7.87	8.04
3	7.38	7.72	7.96
4	7.41	7.51	7.85
5	7.40	7.36	7.64
6	7.35	7.32	7.44
7	7.30	7.44	7.48
8	7.47	7.48	7.50
9	7.42	7.50	7.61
10	7.55	7.61	7.68
11	7.55	7.64	7.72
12	7.43	7.47	7.54

Table A.73: Effluent ammonium-N for Cd-SDS_2

Effluent ammonia						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2	6.14	0.18	6.14	0.18	6.14	0.18
-1	0.22	0.01	0.22	0.01	0.22	0.01
0	0.06	0.03	0.06	0.03	0.06	0.03
0.33	0.01	0.01	11.11	0.07	18.45	0.47
0.67	0.01	0.02	18.27	0.54	19.11	0.08
1	0.08	0.11	18.97	0.41	21.59	0.57
1.5	0	0.02	13.12	0.77	16.71	0.2
2	0.07	0.04	12.19	0.08	15.51	0.32
2.5	0.05	0.03	14.82	0.69	20.98	0.08
3	0.05	0.05	13.39	0.04	18.53	0.28
4	0.01	0.03	8.04	0.09	14.41	0.33
5	0.12	0.08	0.46	0.07	8.81	0.55
6	0.18	0.03	0.72	0.08	0.2	0.01
7	0.12	0.01	0.34	0.05	0.64	0.08

8	0.08	0.01	0.28	0.01	0.52	0.06
9	0.08	0.01	0.06	0.05	0.17	0.06
10	0.13	0.02	0.9	0.19	0.15	0.06
11	0.07	0.01	0.42	0.01	0.43	0.09
12	0.01	0.03	0.63	0.01	0.41	0.02
13	0.06	0.07	0.14	0.08	0.08	0.02

Table A.74: Effluent nitrate-N for Cd-SDS_2

Effluent nitrate						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2.00	18.79	0.00	18.79	0.01	18.79	0.01
-1.00	29.28	0.04	29.28	0.04	29.28	0.04
0.00	25.75	0.02	25.75	0.02	25.75	0.02
0.33	24.44	0.07	12.80	0.03	7.12	0.58
0.67	22.05	0.03	5.06	0.02	1.68	0.01
1.00	19.42	0.00	3.80	0.01	3.01	0.00
1.50	20.89	0.05	5.09	0.01	3.93	0.52
2.00	22.54	0.21	7.47	0.02	5.37	0.07
2.50	29.96	0.34	10.17	0.13	7.56	0.03
3.00	31.49	0.11	14.16	0.11	8.95	0.10
4.00	32.93	0.02	19.30	0.01	11.98	0.01
5.00	30.87	0.00	20.25	0.00	14.12	0.02
6.00	34.76	0.01	21.86	0.11	15.51	0.01
7.00	30.32	0.04	25.90	0.09	18.81	0.00
8.00	19.23	0.03	13.63	0.03	12.28	0.03
9.00	18.20	0.03	18.04	0.01	16.21	0.03
10.00	15.35	0.20	16.39	0.04	15.83	0.18
11.00	22.34	0.00	18.22	0.00	19.67	0.13
12.00	20.37	0.29	11.95	0.04	13.50	0.09
13.00	21.28	0.11	20.54	0.04	22.25	0.00

Table A.75: Effluent nitrite-N for Cd-SDS_2

Effluent nitrite						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2.00	7.36	0.00	7.36	0.01	7.36	0.01
-1.00	4.84	0.01	4.84	0.01	4.84	0.01
0.00	0.66	0.01	0.66	0.01	0.66	0.01
0.33	0.00	0.00	0.00	0.00	0.00	0.00
0.67	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00	0.00	0.00

1.50	0.00	0.00	0.00	0.00	0.00	0.00
2.00	0.00	0.00	0.00	0.00	0.61	0.03
2.50	0.70	0.02	0.78	0.02	0.97	0.01
3.00	0.80	0.01	1.29	0.02	1.22	0.03
4.00	0.64	0.01	6.88	0.01	2.85	0.00
5.00	0.50	0.01	13.50	0.01	9.17	0.01
6.00	0.00	0.00	14.87	0.04	17.52	0.02
7.00	0.00	0.00	4.42	0.03	13.35	0.00
8.00	0.00	0.00	5.08	0.01	7.07	0.02
9.00	0.00	0.00	1.02	0.00	1.24	0.03
10.00	0.00	0.00	1.31	0.01	1.10	0.02
11.00	1.04	0.00	7.57	0.00	6.82	0.03
12.00	4.16	0.08	15.81	0.01	14.13	0.06
13.00	0.39	0.05	3.13	0.08	1.79	0.03

Table A.76: Effluent soluble COD levels for Cd-SDS_2

Effluent chemical oxygen demand						
	Negative control		Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-2.00	43.2	0.00	43.2	0.00	43.2	0.00
-1.00	28.8	1.60	28.8	1.60	28.8	1.60
0.00	36.8	1.60	36.8	1.60	36.8	1.60
0.33	33.6	0.00	80.8	4.00	49.6	38.40
0.67	31.2	2.40	122.4	0.80	148.0	0.80
1.00	42.4	0.80	165.6	4.00	168.8	0.80
1.50	39.2	0.80	77.6	0.80	92.0	4.00
2.00	35.2	0.00	60.0	13.60	97.6	4.80
2.50	31.2	2.40	48.0	0.00	52.0	0.80
3.00	29.6	4.00	56.0	3.20	64.0	8.00
4.00	34.4	2.40	48.8	0.80	52.8	9.60
5.00	28.0	0.80	48.8	2.40	35.2	0.00
6.00	39.2	4.00	57.6	1.60	50.4	0.80
7.00	27.2	0.00	42.4	0.80	62.4	1.60
8.00	53.6	2.40	52.8	0.00	51.2	3.20
9.00	23.2	2.40	32.0	3.20	28.8	0.00
10.00	22.4	1.60	36.8	1.60	31.2	2.40
11.00	11.2	8.00	44.0	0.80	40.8	0.80

Table A.77: Mixed liquor sOUR levels for Cd-SDS_2

Mixed liquor specific oxygen uptake rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$

-2.0	36.75	0.93	36.75	0.93	36.75	0.93
-1.0	33.96	1.70	33.96	1.70	33.96	1.70
0.0	22.19	0.35	22.19	0.35	22.19	0.35
0.3	30.83	0.35	2.06	0.10	0.25	0.15
1	30.82	1.13	9.32	0.26	4.77	0.84
2	32.00	0.78	21.00	0.14	16.58	0.71
3	31.35	1.81	28.73	2.44	27.37	1.34
5	29.65	0.17	40.91	0.80	38.98	1.02
6	32.01	0.36	44.93	0.66	42.17	1.26
7	21.36	1.09	28.98	0.63	46.77	3.52
9			38.57	2.62	33.09	2.95
11	30.82	1.53	32.42	0.59	30.77	0.00
13	22.40	0.27	33.60	0.36	42.64	2.25

Table A.78: Mixed liquor sAOR levels for Cd-SDS_2

Mixed liquor specific ammonia oxidation rates						
	Negative control		Positive control		Corrective action	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
-1	6.32	0.04	6.32	0.04	6.32	0.04
0	4.46	0.01	4.46	0.01	4.46	0.01
0.33	4.78	0.07	0.00	0.00	0.00	0.00
1	4.27	0.11	0.92	0.01	0.84	0.16
2	3.85	0.11	2.12	0.14	1.33	0.01
3	4.14	0.07	2.96	0.08	1.99	0.19
4	4.69	0.03	5.37	0.00	3.00	0.01
5	4.95	0.10	8.22	0.01	4.69	0.00
6	5.35	0.01	12.39	0.71	10.98	0.49
7	4.92	0.34	9.68	0.23	10.89	0.45
9	3.66	0.12	6.30	0.03	6.28	0.01
11	4.62	0.06	4.73	0.00	4.52	0.18
13	4.48	0.20	4.08	0.07	6.85	0.13

Table A.79: Total cadmium levels for Cd-SDS_2

Total cadmium levels				
	Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l
0.00	0.00	0.00	0.00	0.00
0.33	78.72	2.77	115.36	4.93
0.67	80.66	0.43	107.72	1.86
1.00	74.70	1.87	96.33	0.95
2.00	62.79	1.28	94.13	1.82
4.00	41.94	0.43	70.95	0.55

6.00	34.43	0.85	58.64	0.35
8.00	25.36	0.43	43.62	0.35
11.00	18.89	0.00	34.56	0.35
13.00	16.69	0.35	28.86	0.35

Table A.80: Soluble cadmium levels for Cd-SDS_2

Soluble cadmium levels				
	Positive control		Corrective action	
Days	Average	Stdev	Average	Stdev
	mg/l	mg/l	mg/l	mg/l
0.00	0.00	0.00	0.00	0.00
0.17	8.63	0.08	19.63	0.09
0.33	7.49	0.00	9.83	0.05
0.67	5.09	0.05	4.87	0.00
1.00	3.61	0.00	3.26	0.09
1.50	2.79	0.04	2.88	0.05
2.00	2.79	0.04	2.88	0.05
2.50	1.62	0.00	1.62	0.00
3.00	1.40	0.00	1.45	0.01
4.00	1.02	0.00	1.05	0.00
5.00	0.71	0.00	0.99	0.00
6.00	0.40	0.00	0.89	0.00
7.00	0.11	0.00	0.53	0.00
8.00	0.06	0.00	0.14	0.00
9.00	0.05	0.00	0.07	0.00
10.00	0.06	0.00	0.06	0.00
11.00	0.07	0.00	0.07	0.00
12.00	0.06	0.00	0.06	0.00
13.00	0.05	0.00	0.07	0.00

Appendix B

Data in support of Chapter 4.0

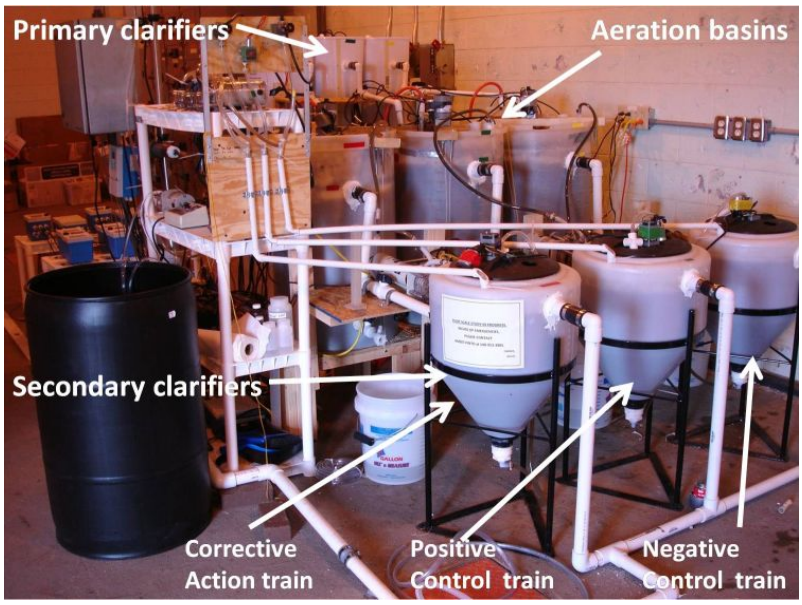

Figure B.1: Pilot plant set-up showing primary clarifier, aeration basin, and secondary clarifier.

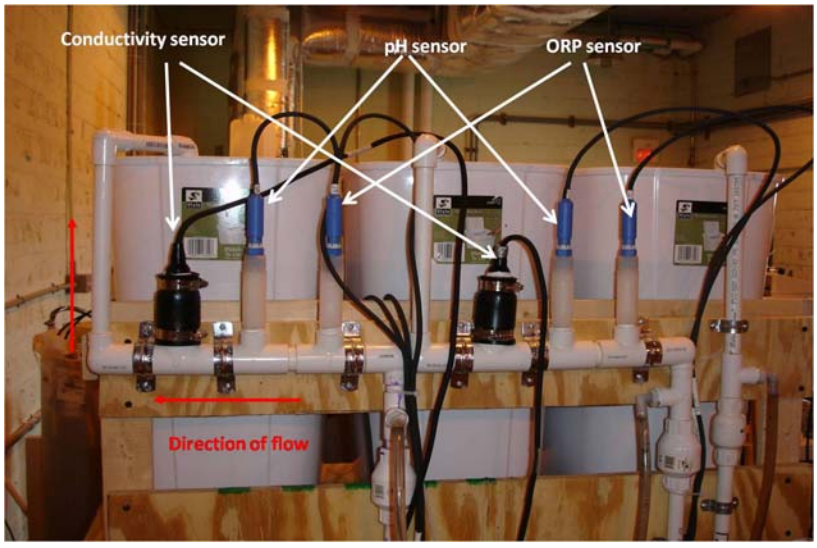

Figure B.2: Sensors installed in the influent lines for NC and PC reactor trains.

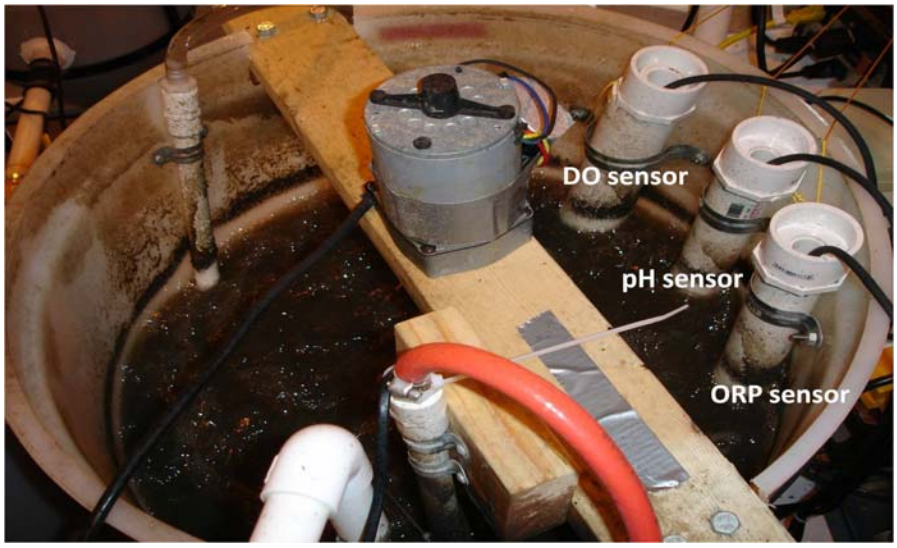

Figure B.3: DO, pH, and ORP sensors were installed in all three aeration basins.

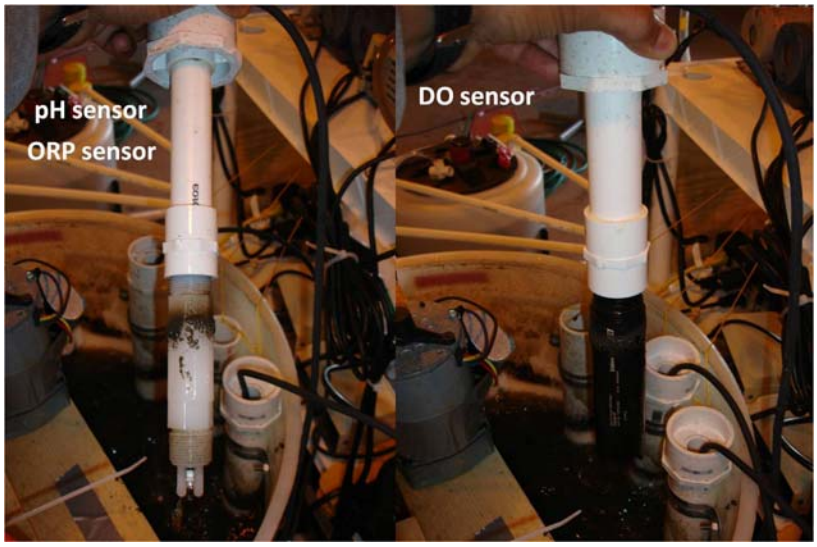

Figure B.4: DO, pH, and ORP sensor mounting hardware for installation inside aeration basins

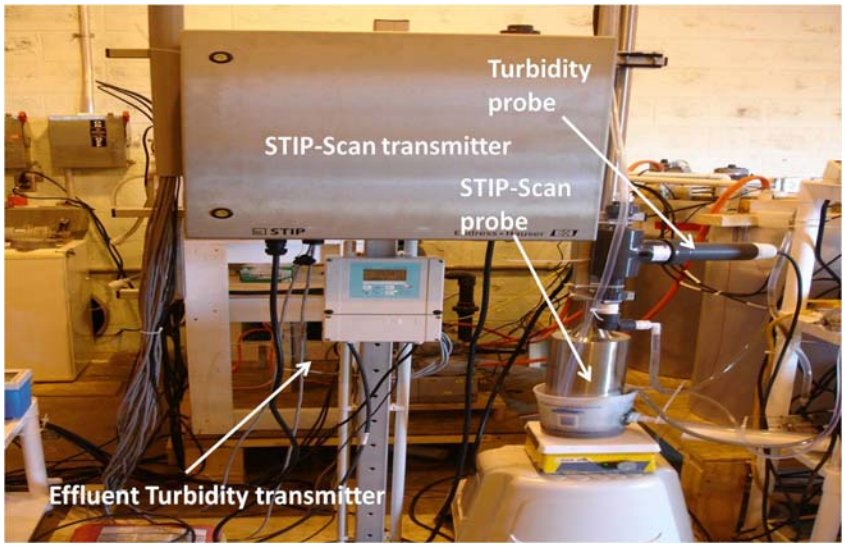

Figure B.5: STIP:Scan online nutrient analyzer was installed on the effluent line.

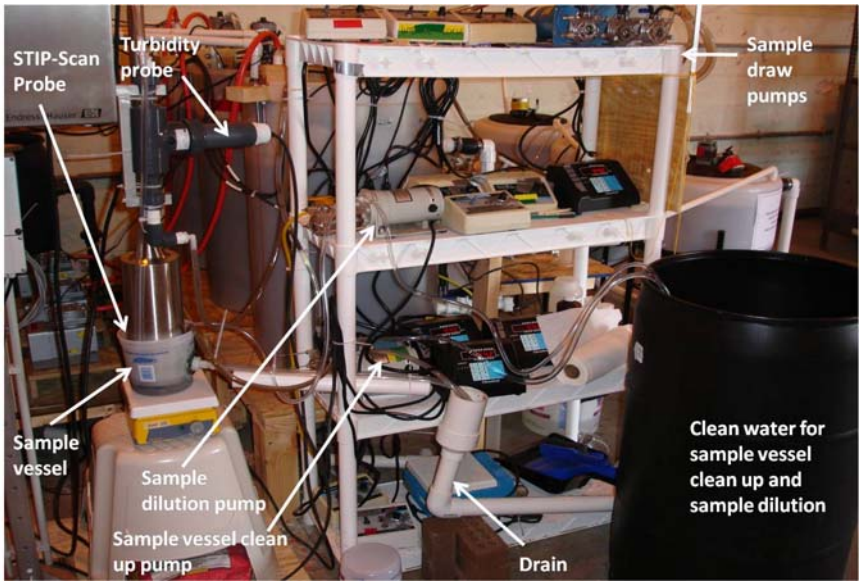

Figure B.6: Sample withdraw system constructed to allow analysis of three different reactor effluents by one STIP:Scan unit.

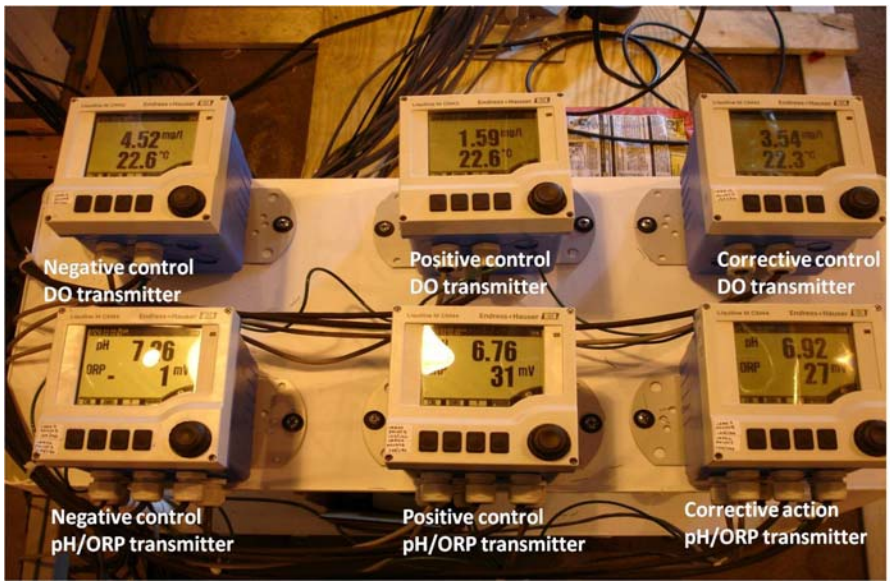

Figure B.7: Transmitters for the pH, DO, and ORP sensors installed inside the aeration basins.

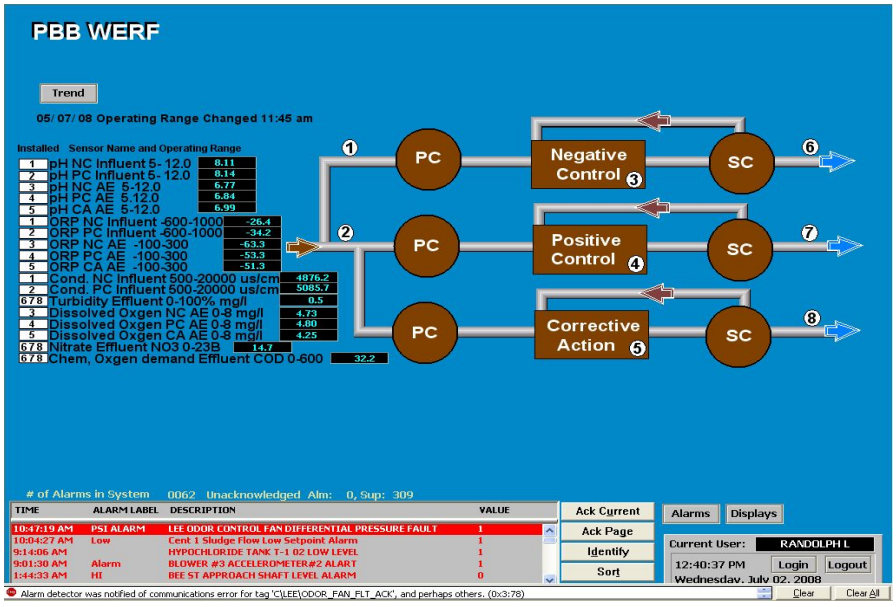

Figure B.8: SCADA interface for monitoring the pilot scale system at the Plum Island WWTP.

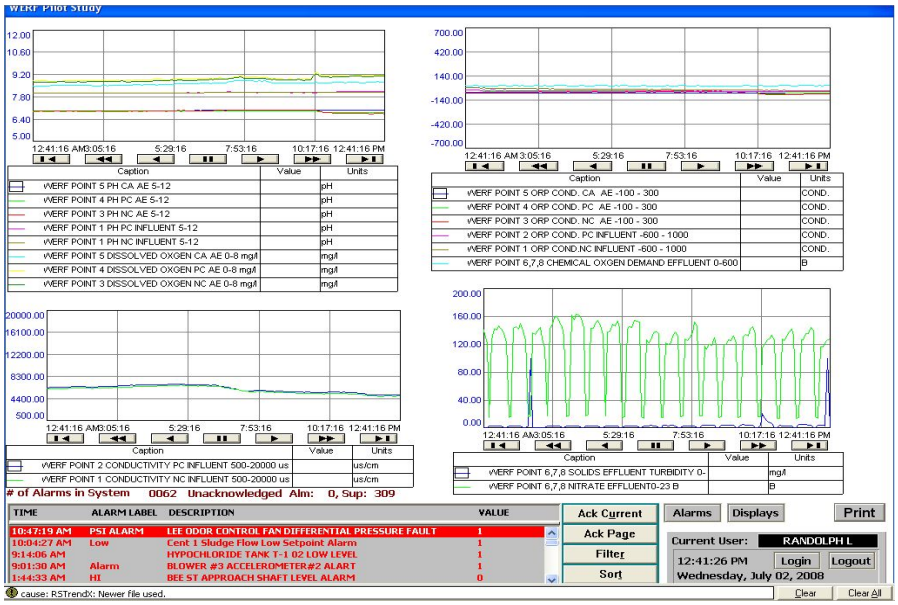

Figure B.9: Trending software for the SCADA interface of the pilot plant system.

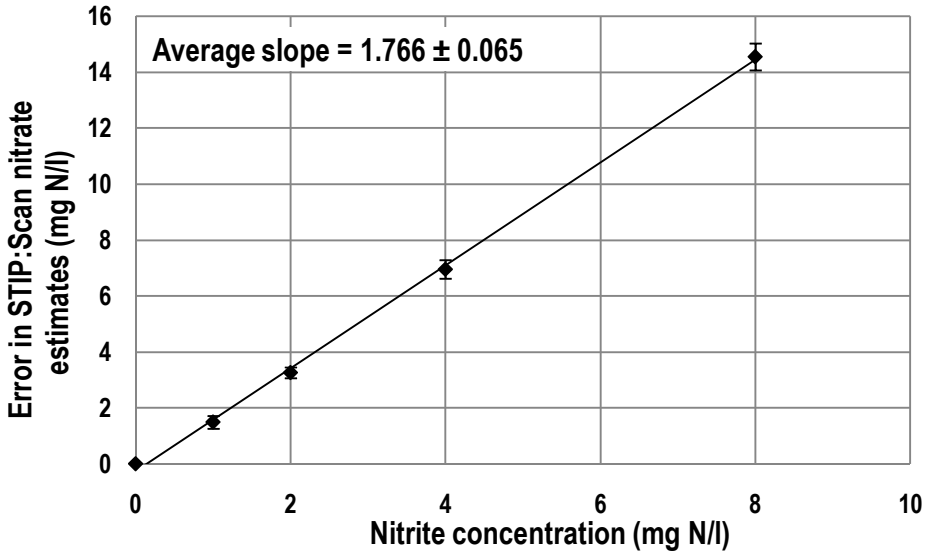

Figure B.10: Error in STIP: Scan nitrate estimate due to nitrite interference. Data points represent an average of nitrite concentrations at three different nitrate concentrations.

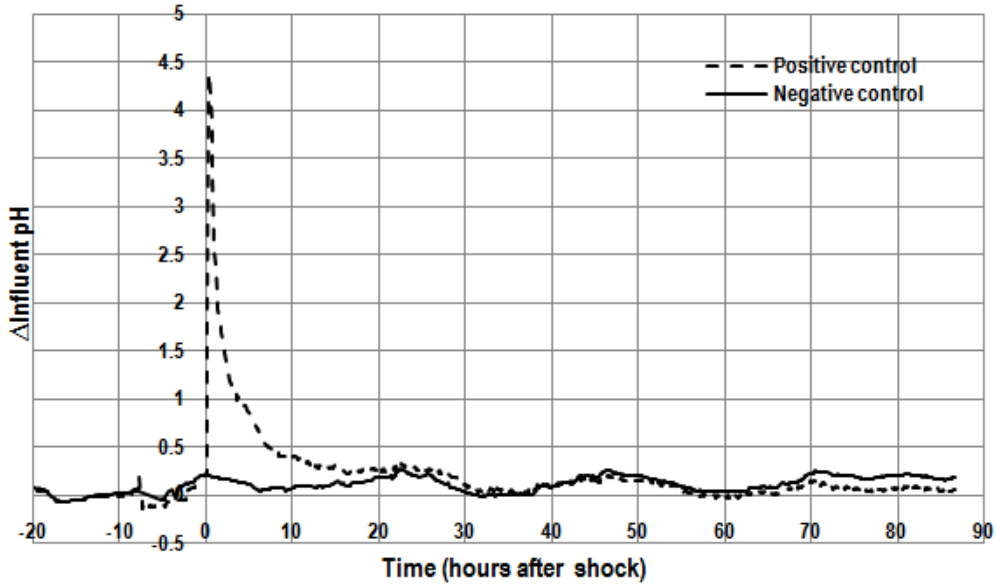

Figure B.11: Change in influent pH for H-SDS$_1$

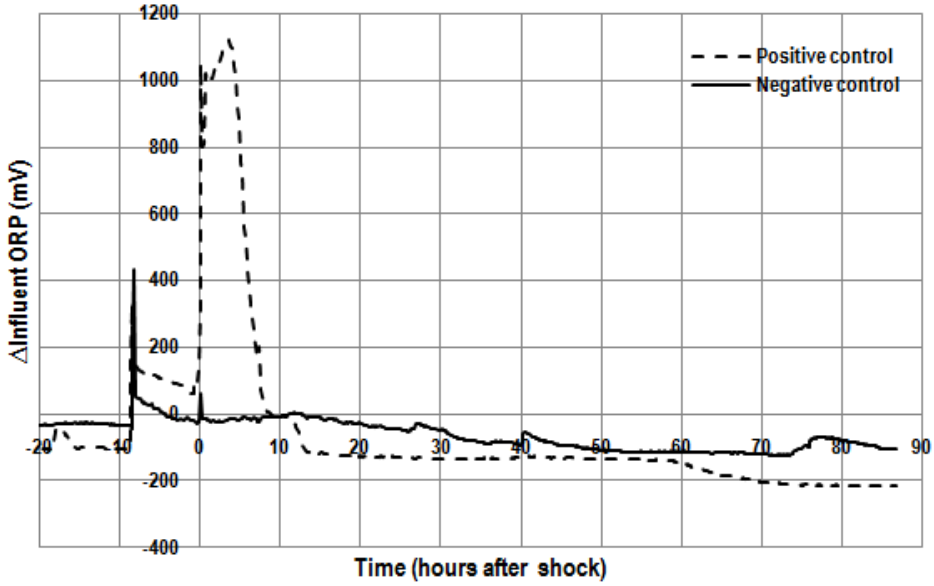

Figure B.12: Change in influent ORP for H-SDS$_1$

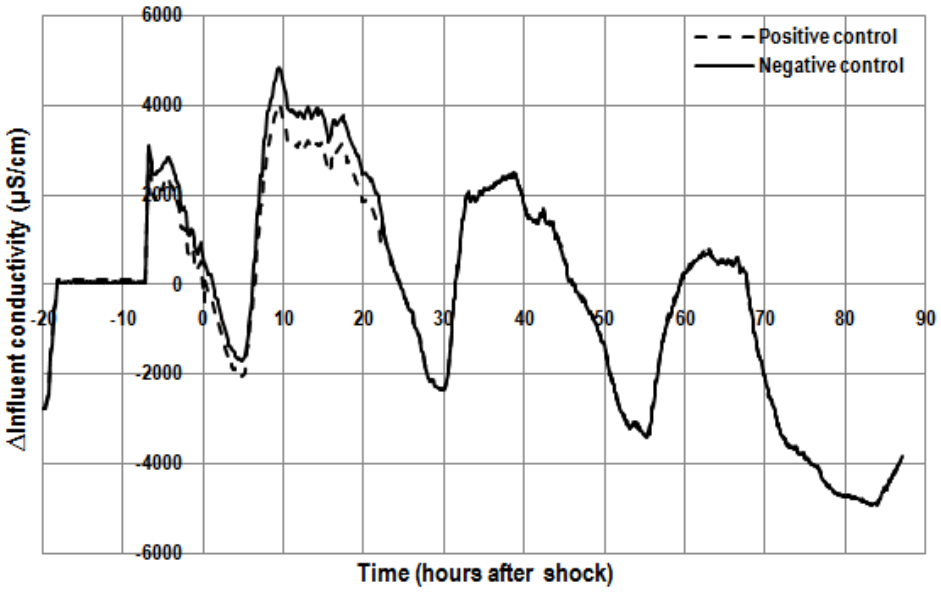

Figure B.13: Change in influent conductivity for H-SDS$_1$

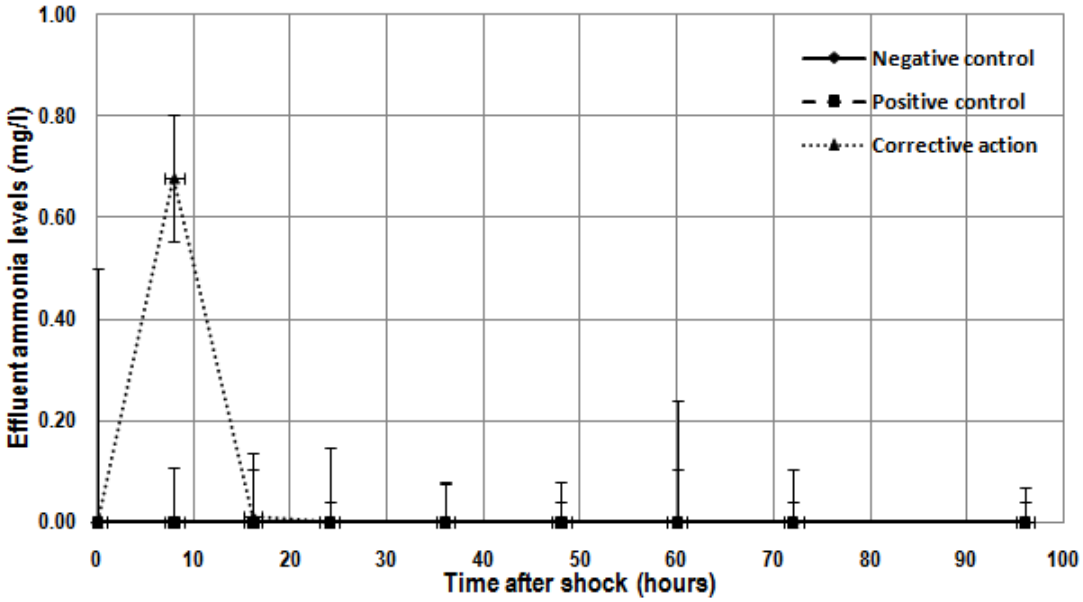

Figure B.14: Effluent ammonium-N levels for H-SDS$_1$

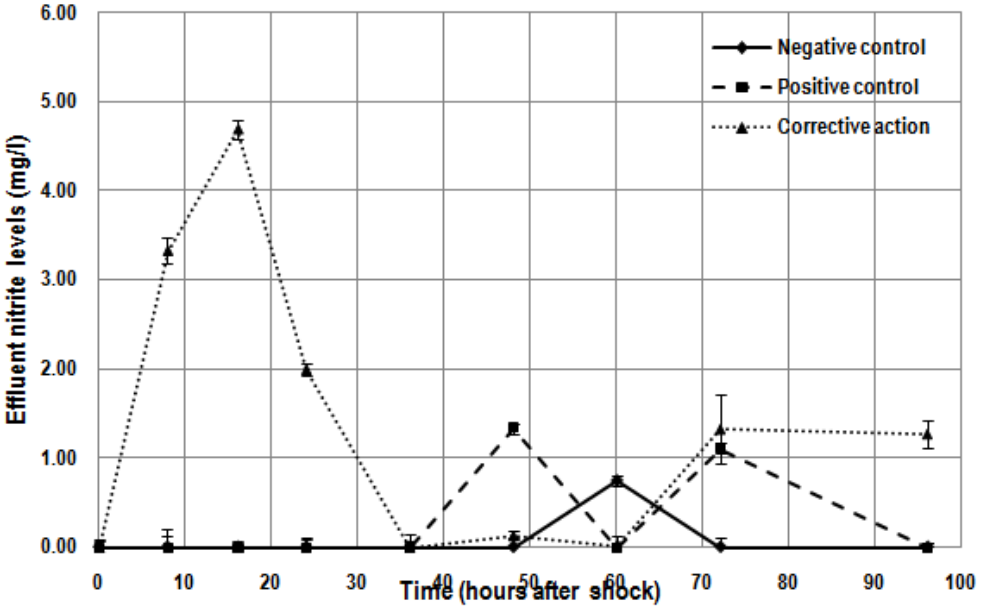

Figure B.15: Effluent nitrite-N for H-SDS$_1$

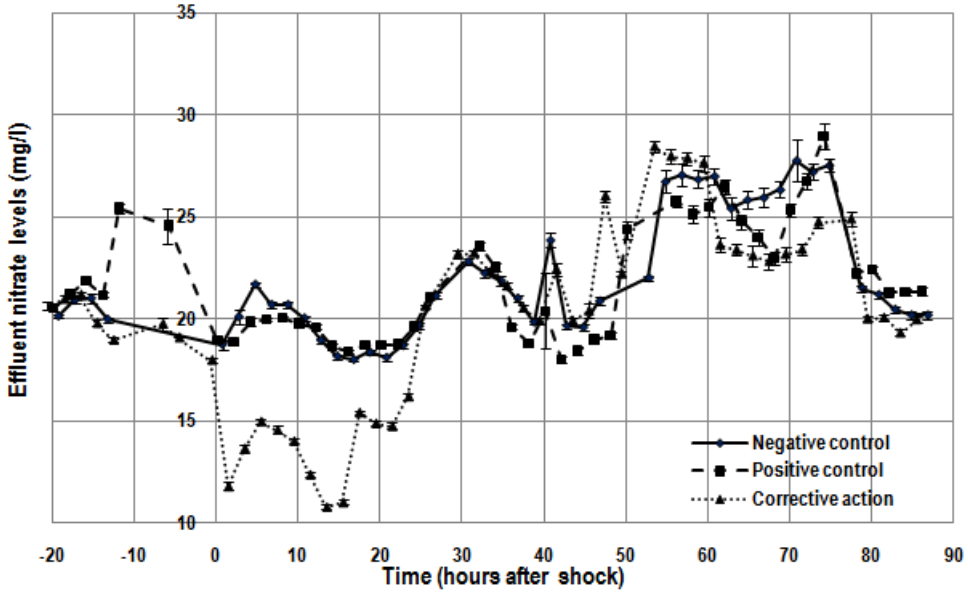

Figure B.16: Effluent nitrate-N for H-SDS$_1$

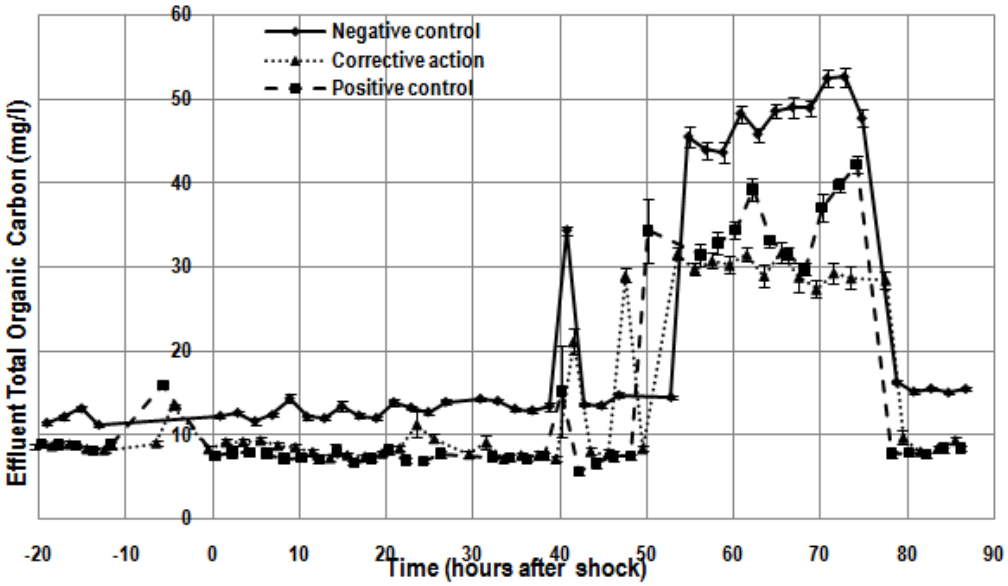

Figure B.17: Effluent TOC levels for H-SDS$_1$

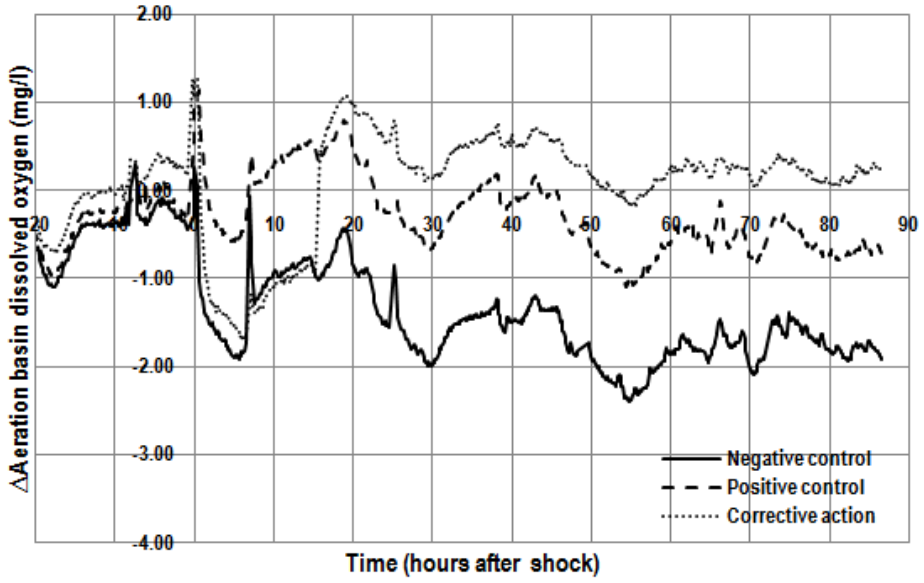

Figure B.18: Change in aeration basin DO levels for H-SDS$_1$

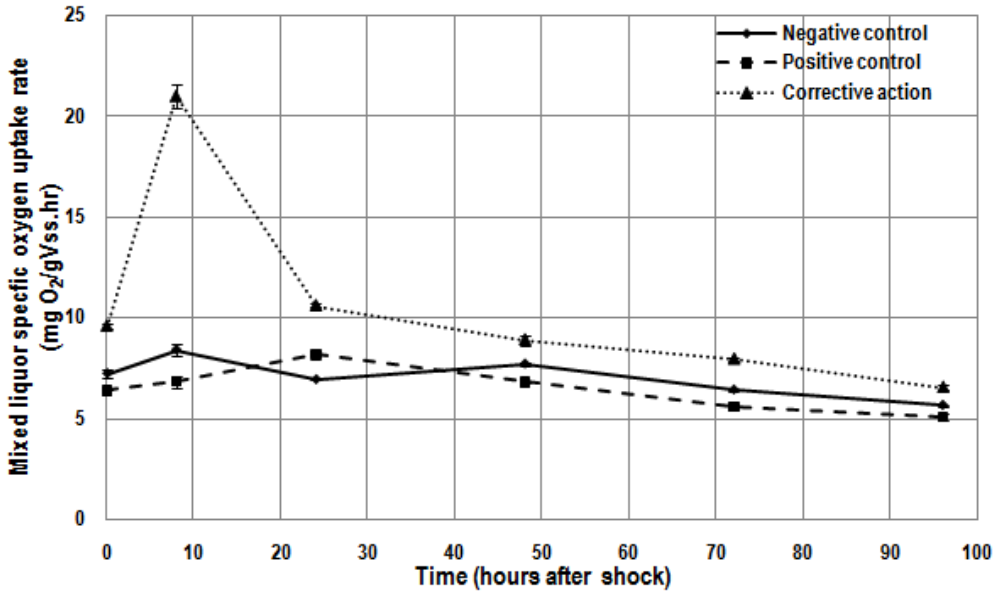

Figure B.19: Mixed liquor sOUR levels for H-SDS$_1$

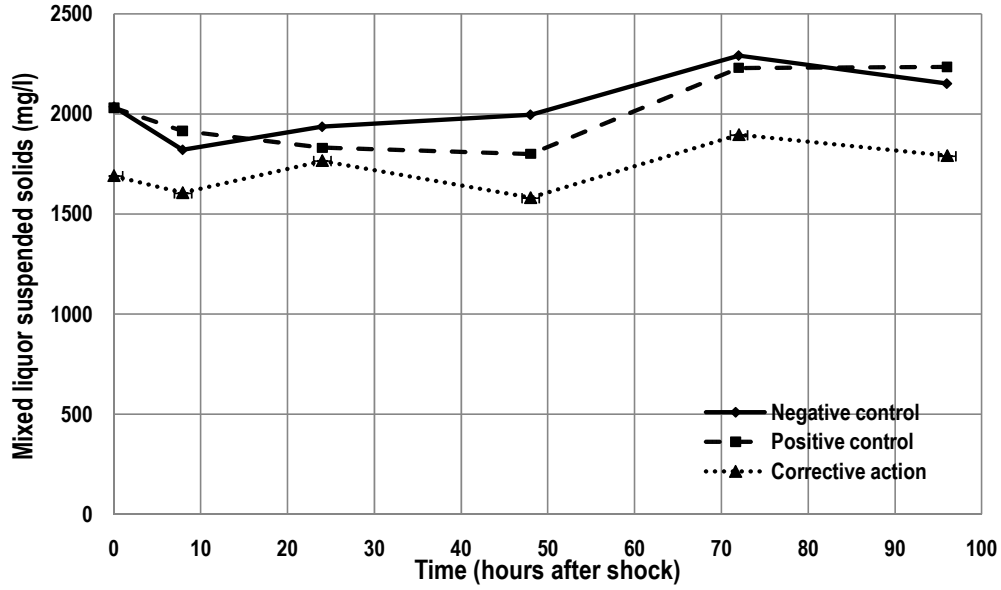

Figure B.20: Mixed liquor suspended solid levels for H-SDS$_1$

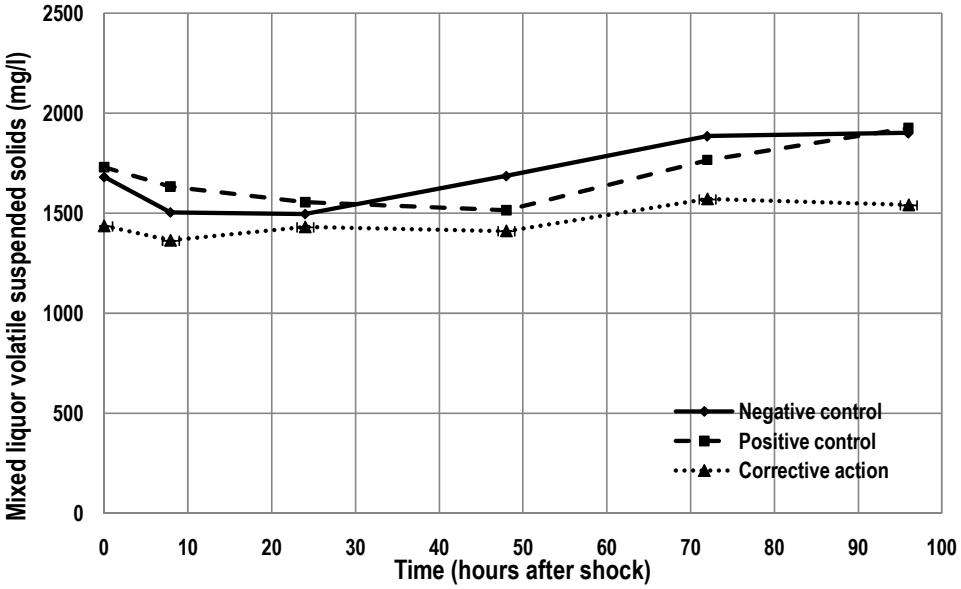

Figure B.21: Mixed liquor volatile suspended solid levels for H-SDS$_1$

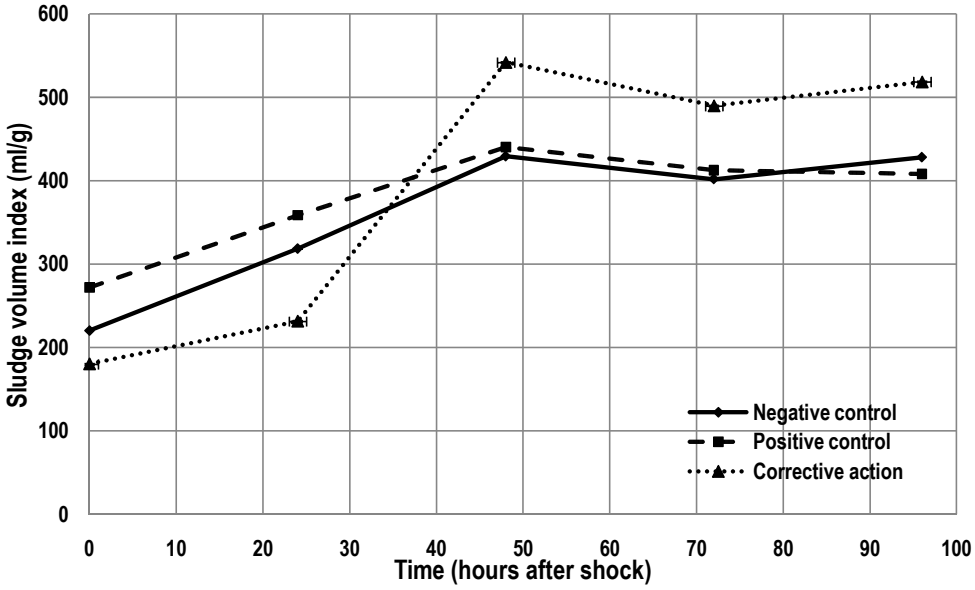

Figure B.22: Sludge volume index levels for H-SDS$_1$

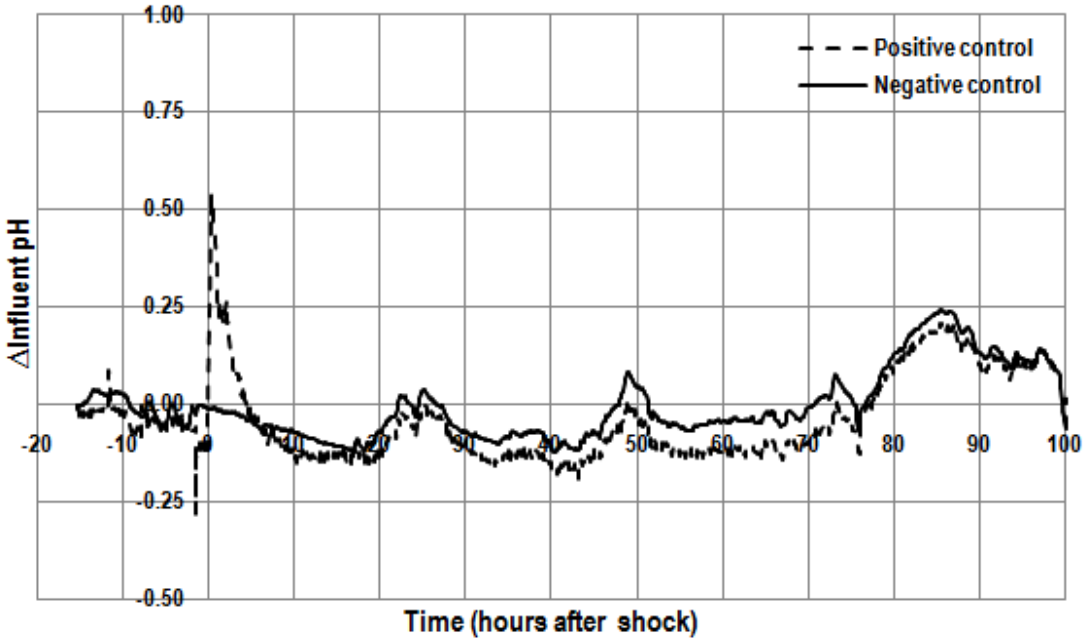

Figure B.23: Change in influent pH for H-SDS$_2$

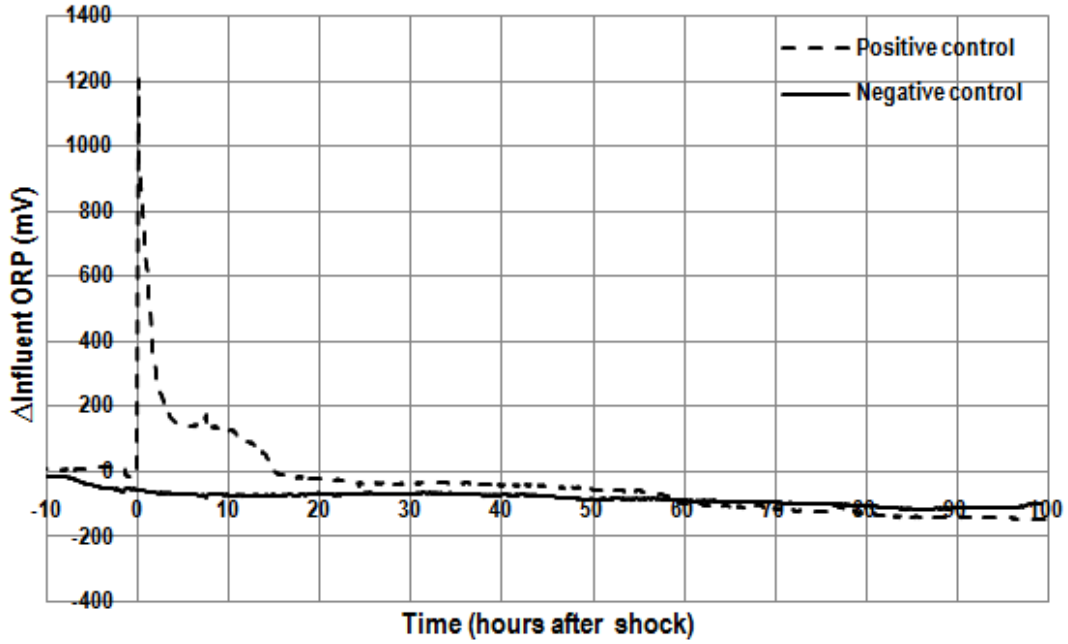

Figure B.24: Change in influent ORP for H-SDS$_2$

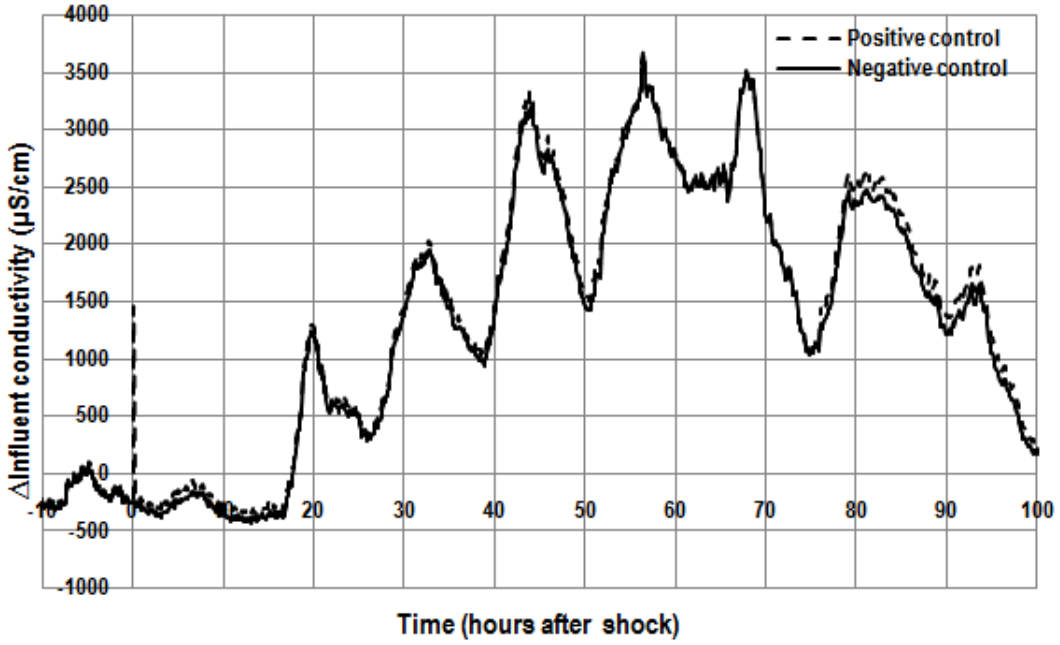

Figure B.25: Change in influent conductivity for H-SDS_2

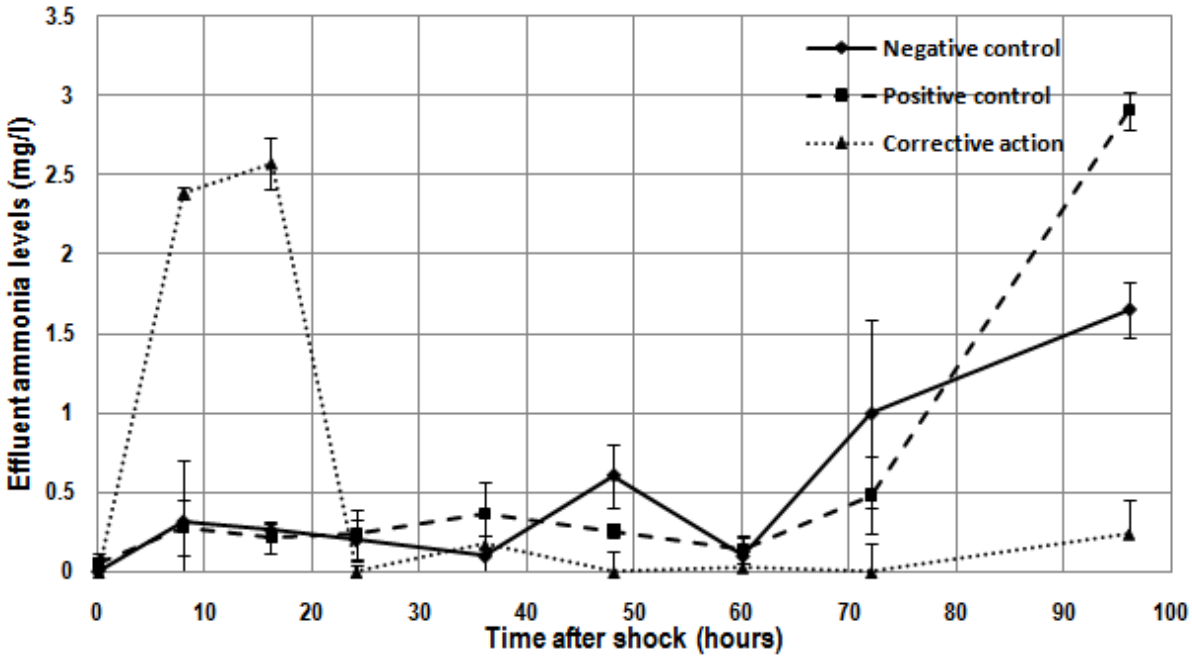

Figure B.26: Effluent ammonium-N levels for H-SDS_2

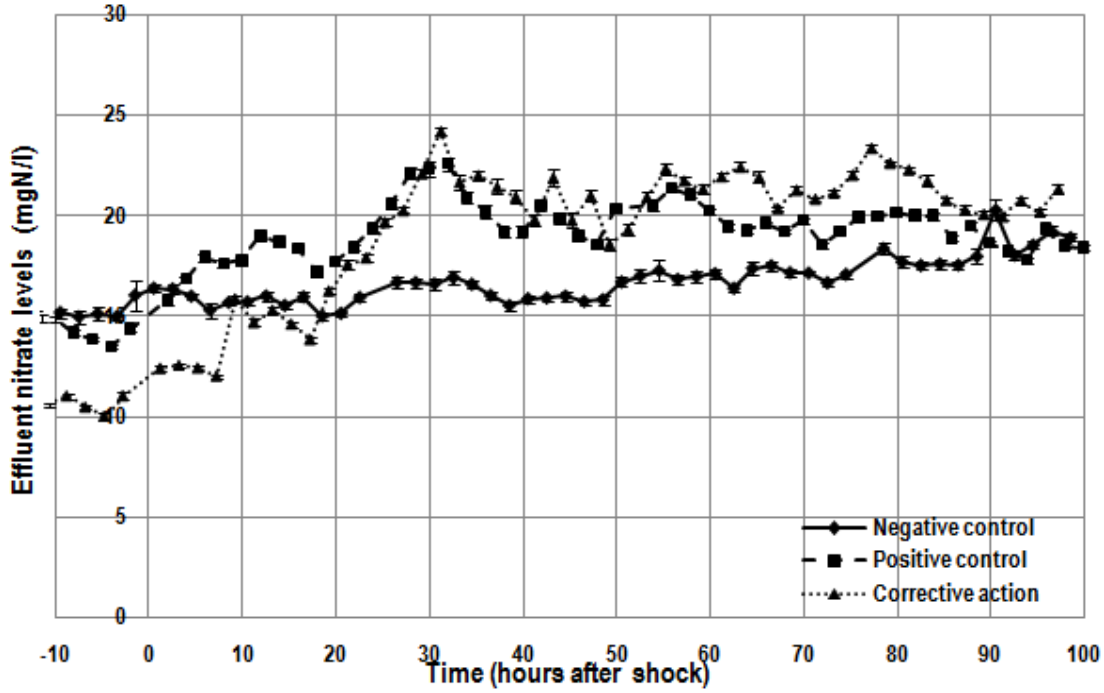

Figure B.27: Effluent nitrate-N levels for H-SDS$_2$

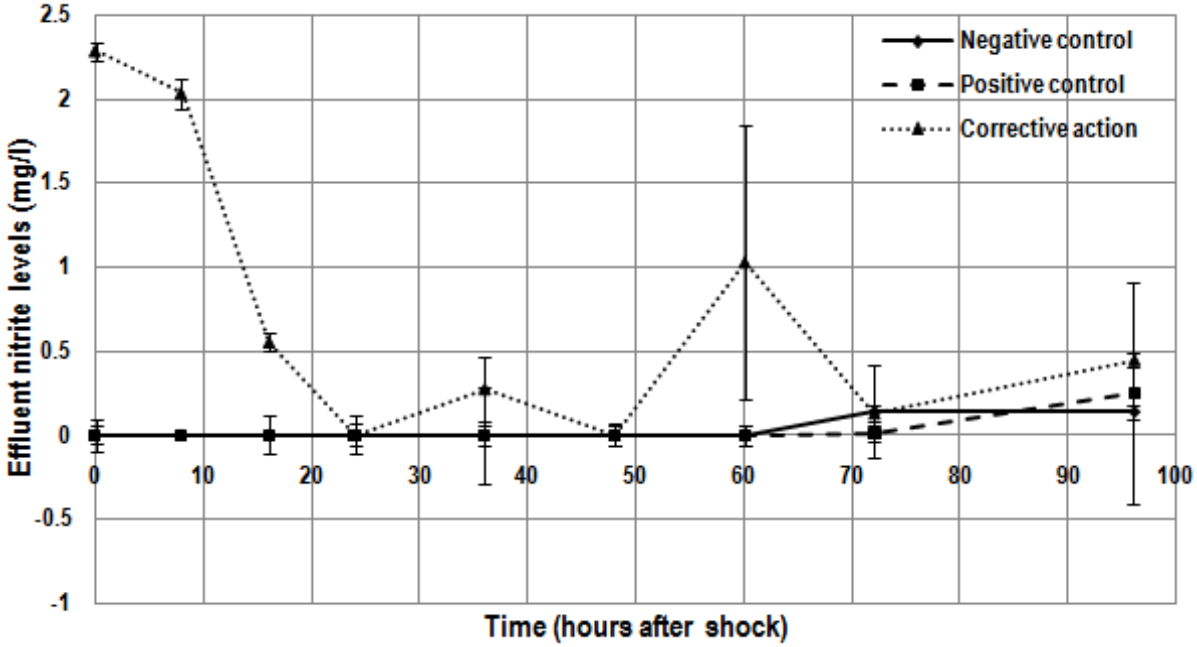

Figure B.28: Effluent nitrite-N for H-SDS$_2$

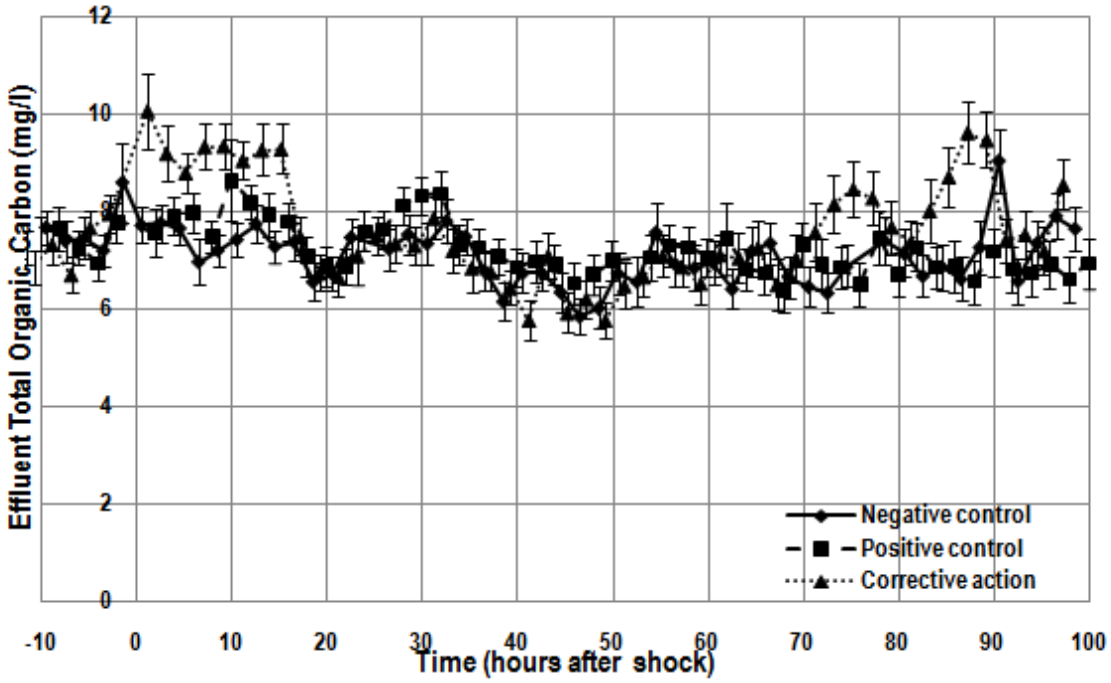

Figure B.29: Effluent TOC levels for H-SDS$_2$

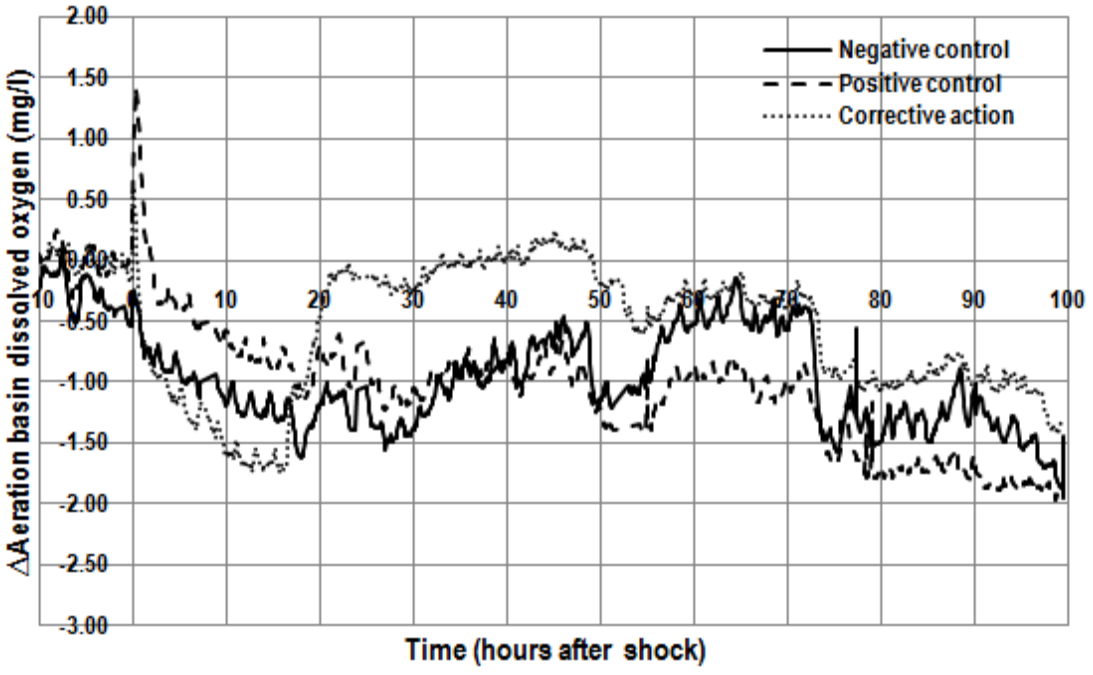

Figure B.30: Aeration basin ORP levels for H-SDS$_2$

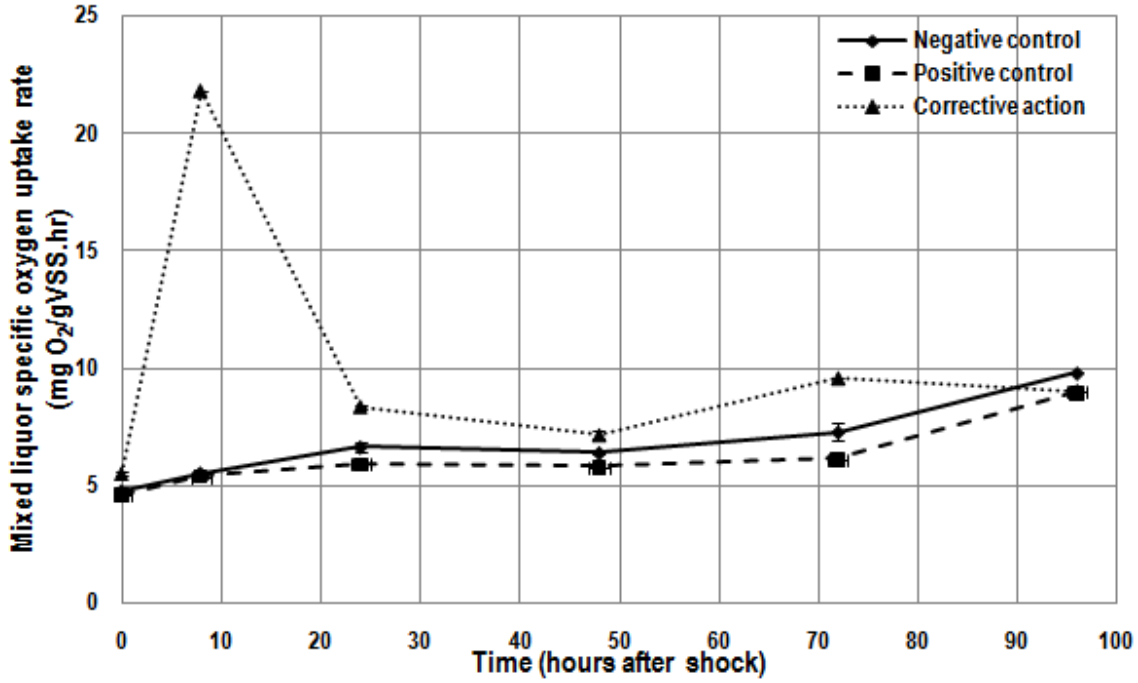

Figure B.31: Mixed liquor sOUR levels for H-SDS$_2$

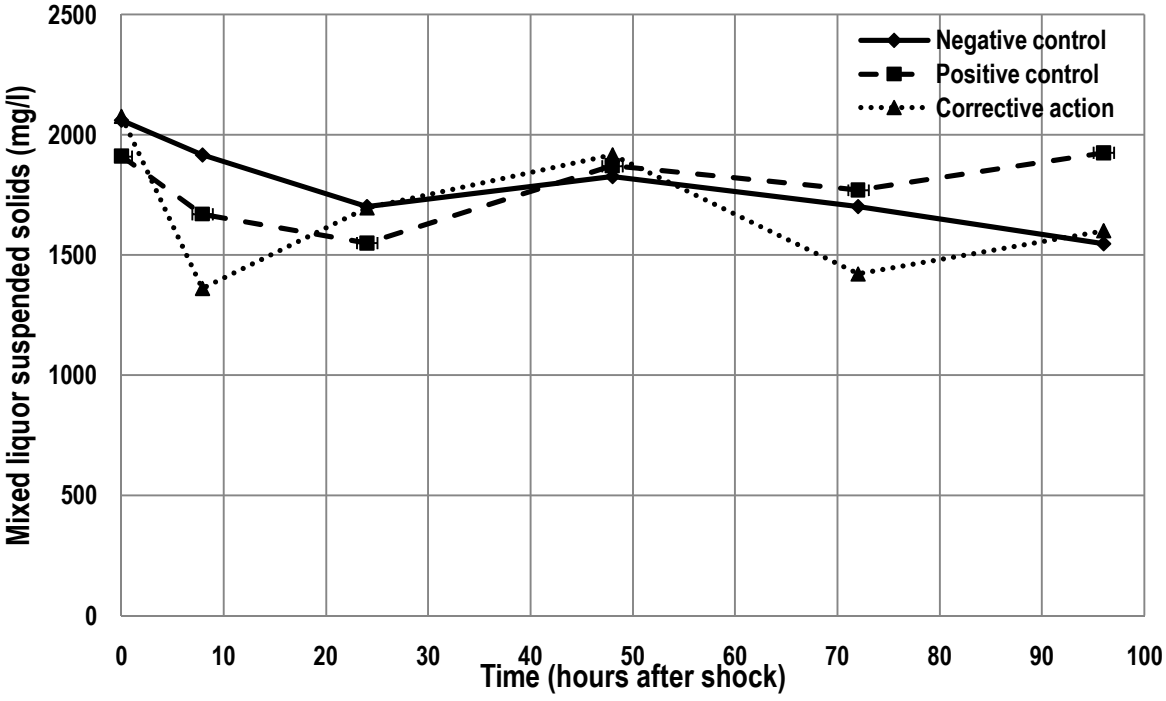

Figure B.32: Mixed liquor suspended solid levels for H-SDS$_2$

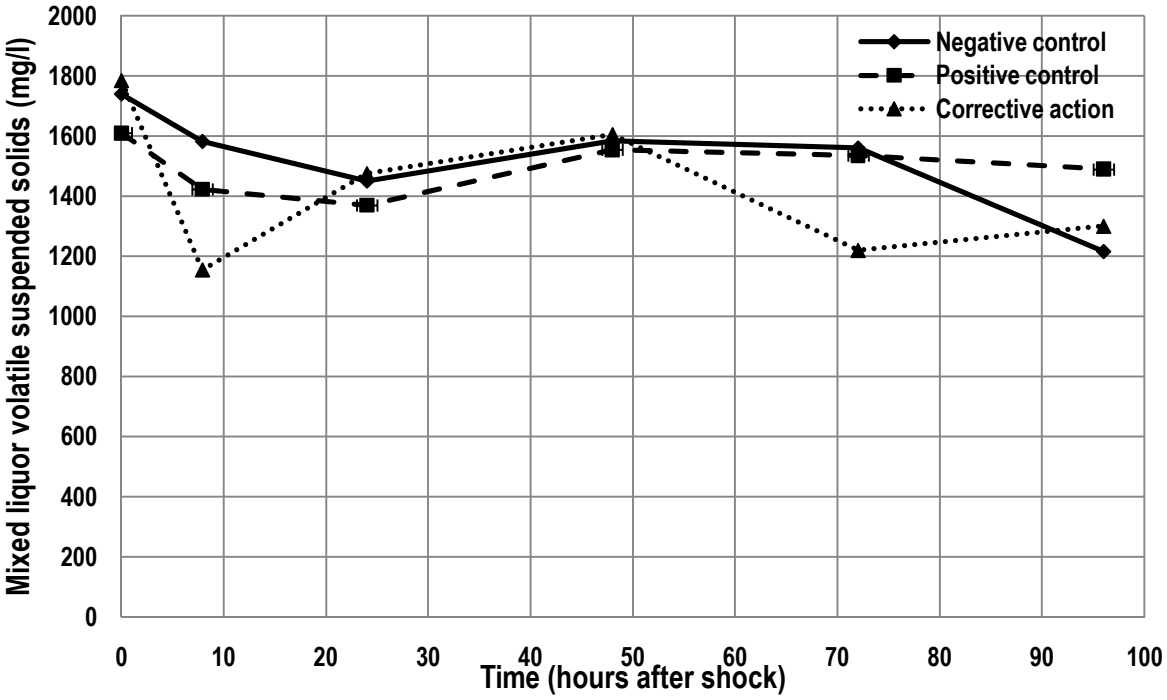

Figure B.33: mixed liquor volatile suspended solid levels for H-SDS_2

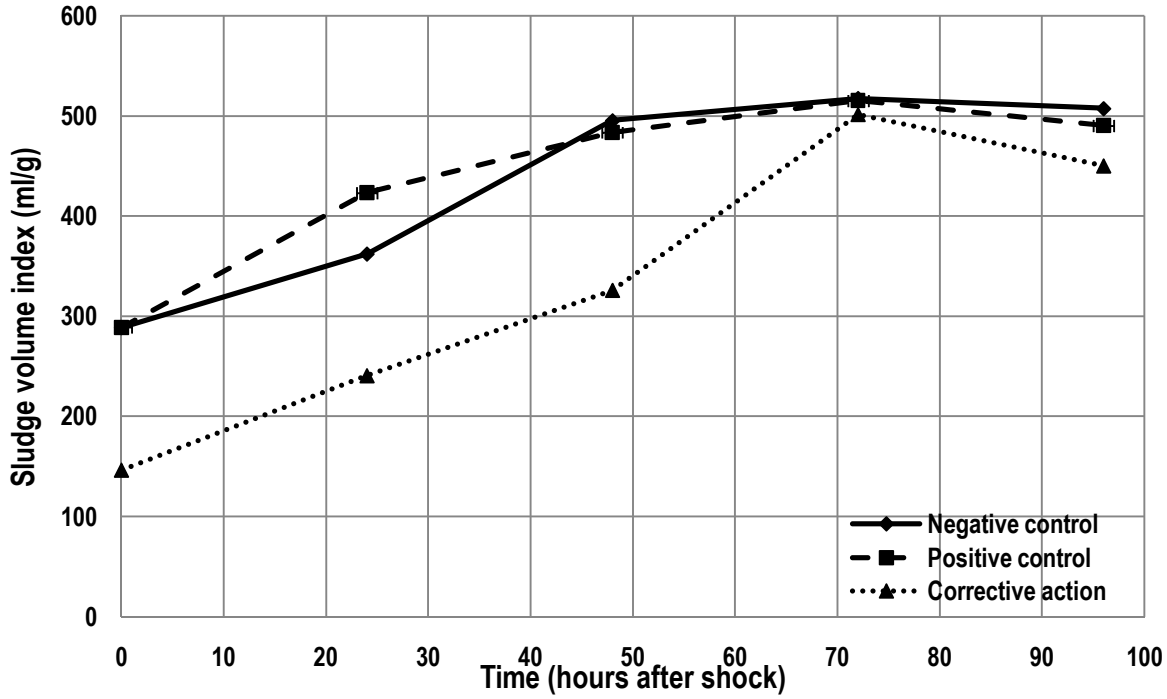

Figure B.34: Sludge volume index levels for H-SDS_2

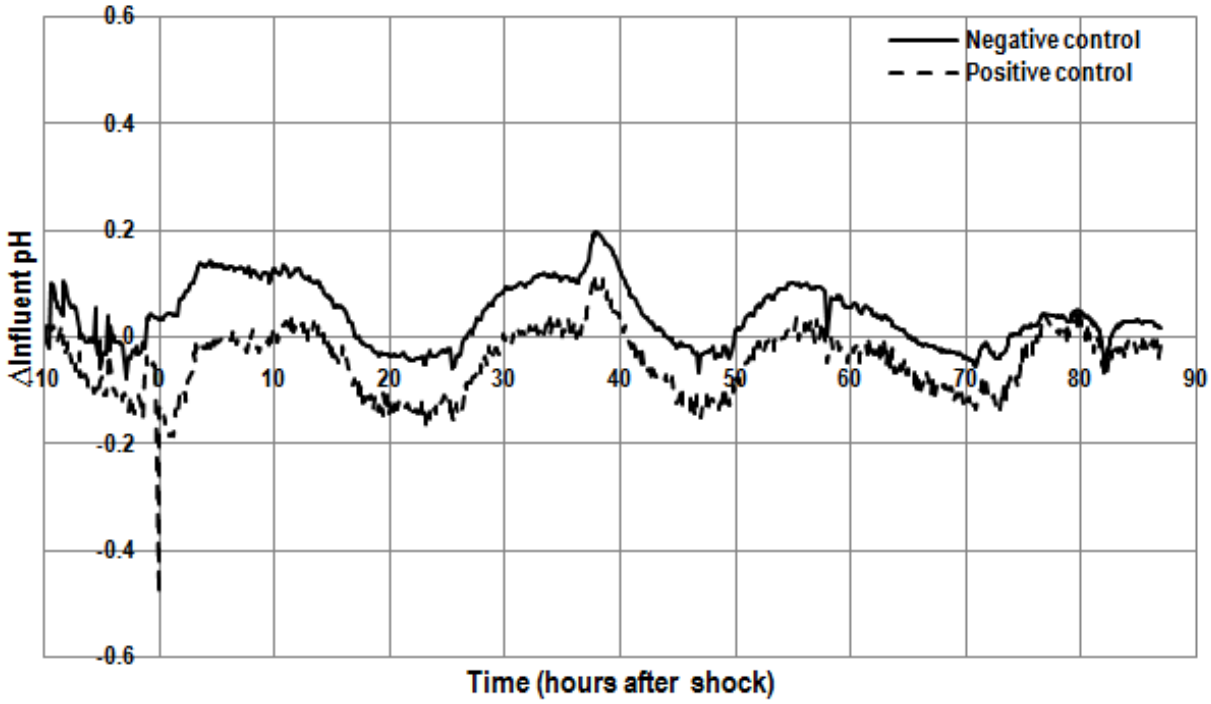

Figure B.35: Change in influent pH for C-CEPT$_1$

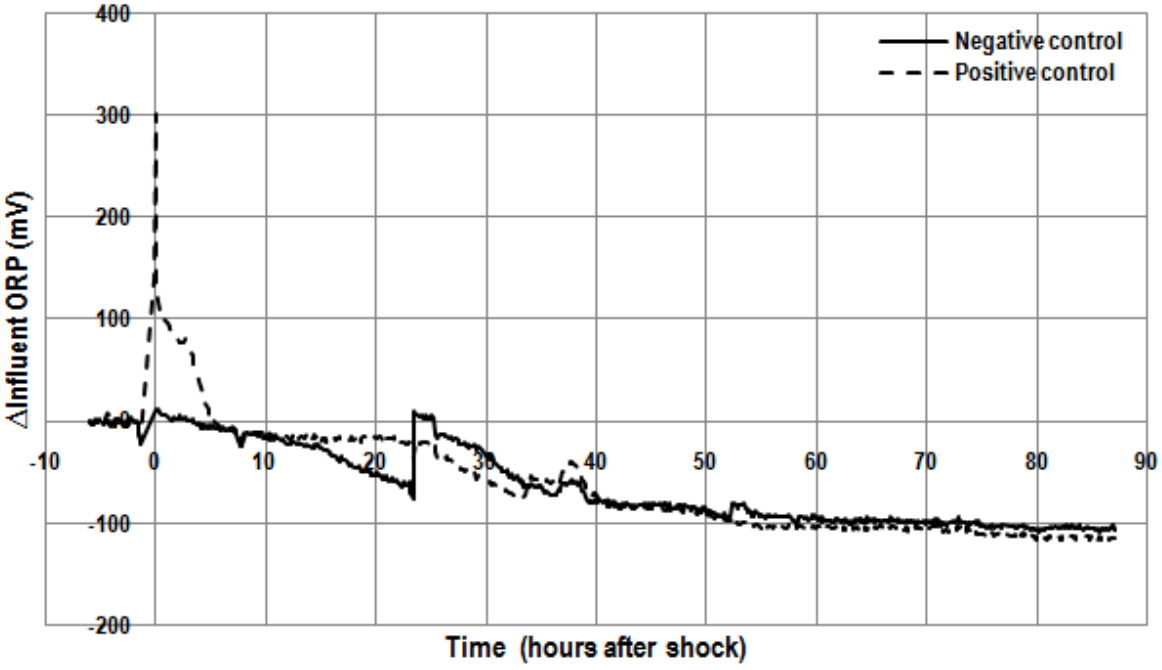

Figure B.36: Change in influent ORP for C-CEPT$_1$

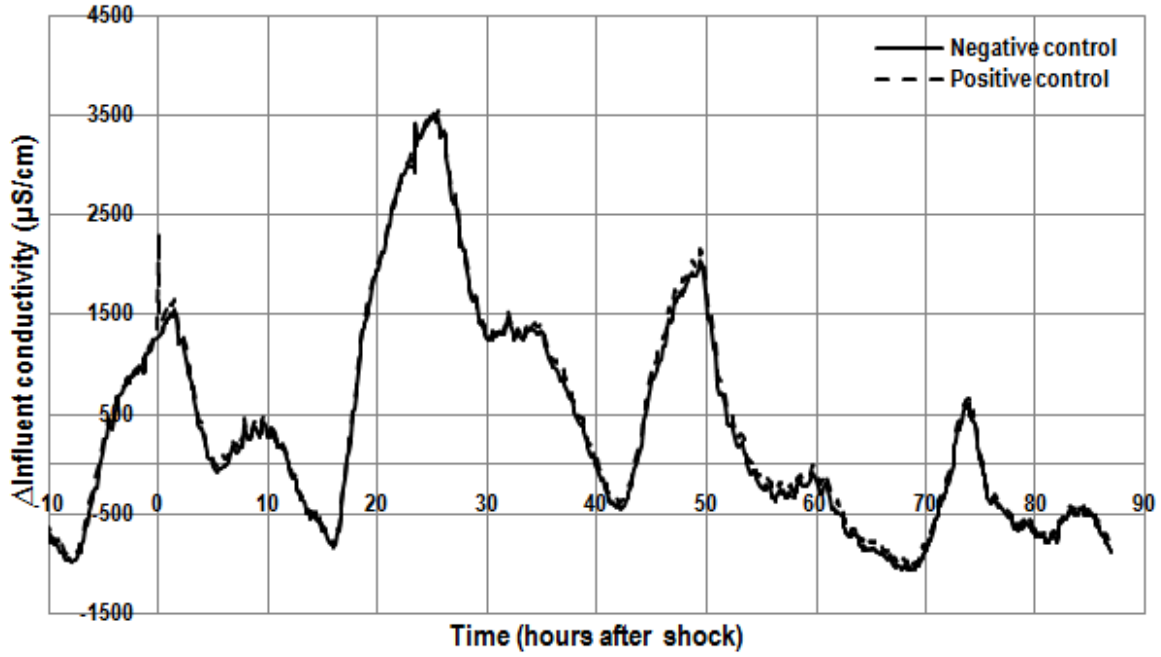

Figure B.37: Change in influent conductivity for C-CEPT$_1$

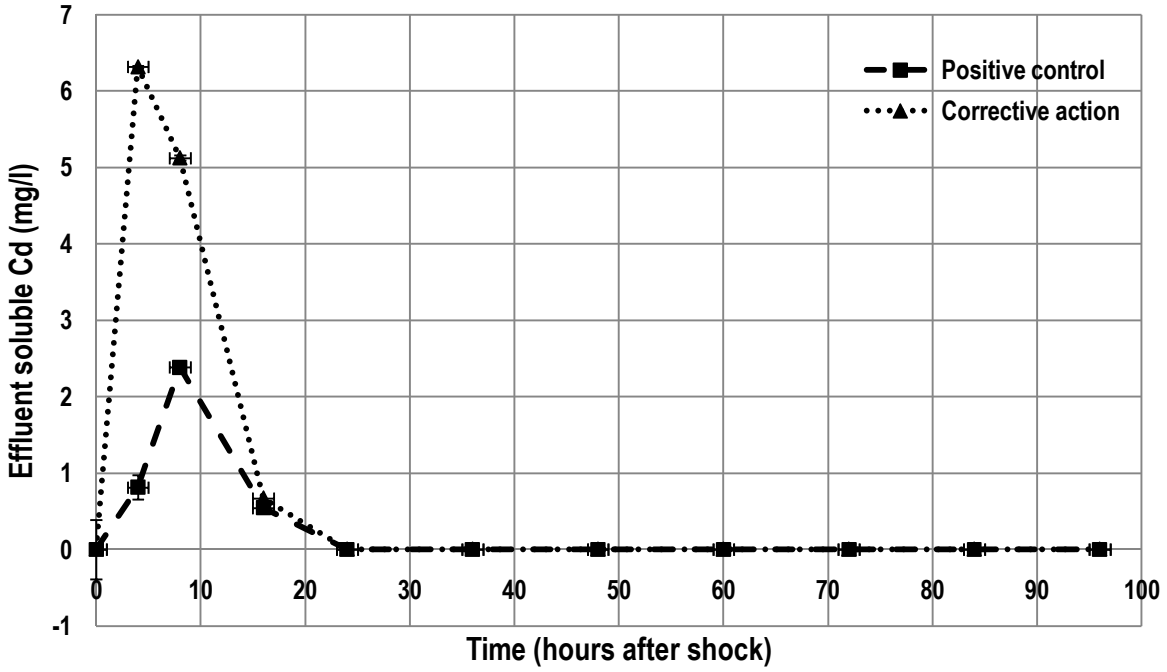

Figure B.38: Effluent soluble cadmium levels for C-CEPT$_1$

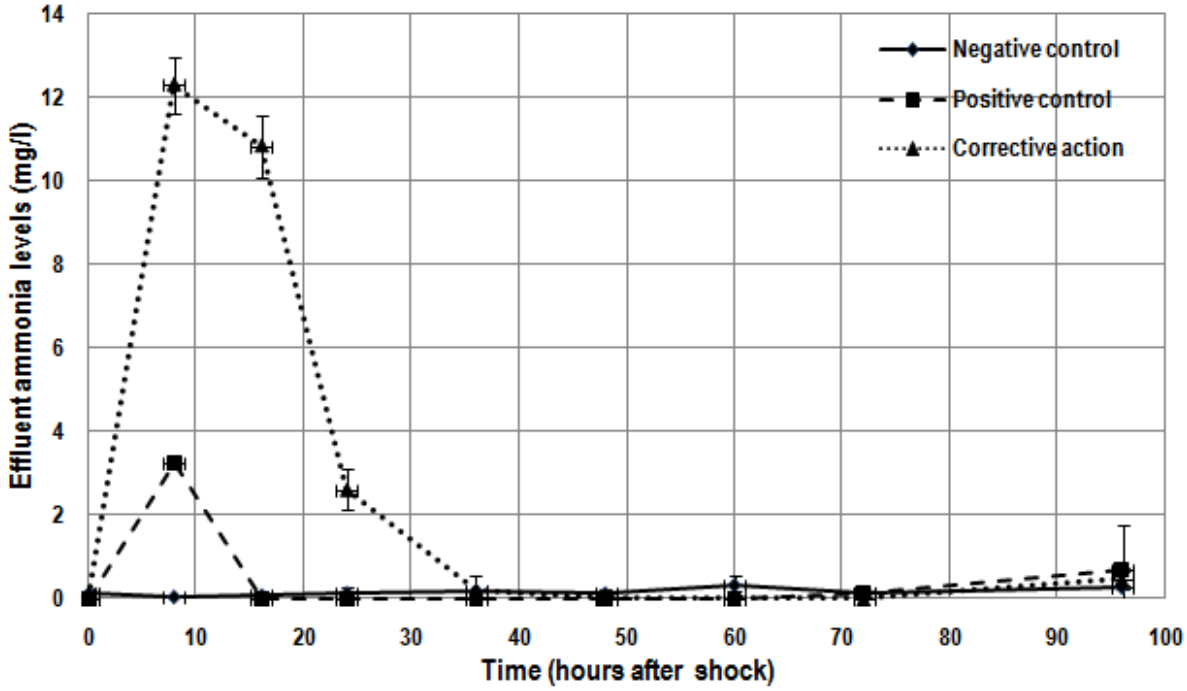

Figure B.39: Effluent ammonium-N levels for C-CEPT$_1$

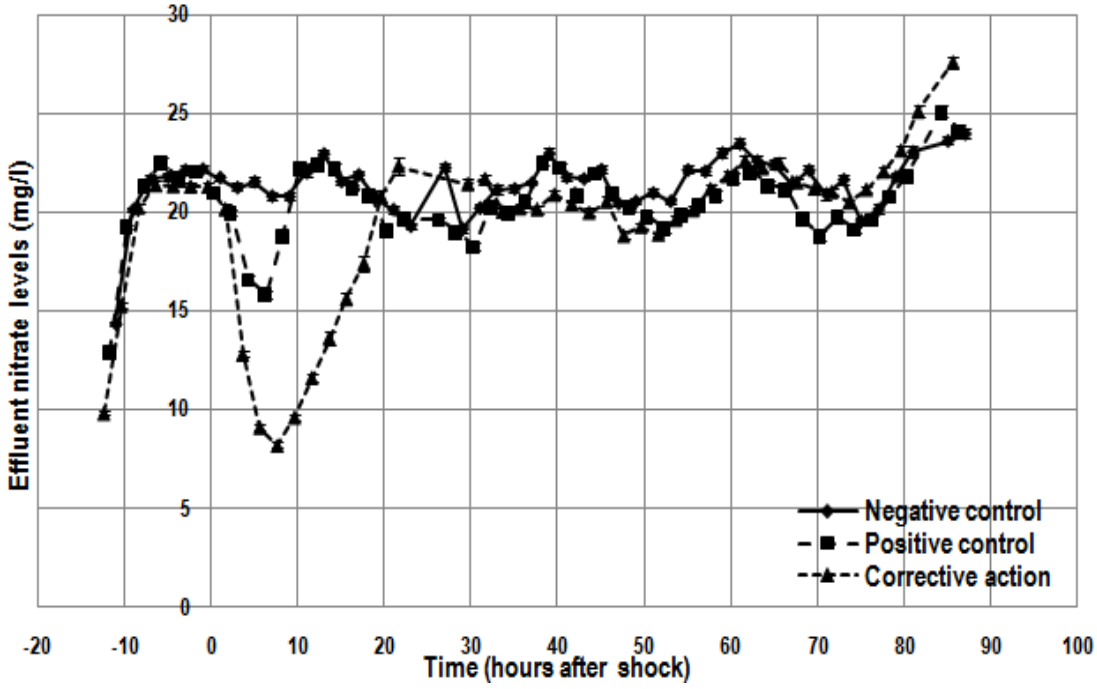

Figure B.40: Effluent nitrate-N levels for C-CEPT$_1$

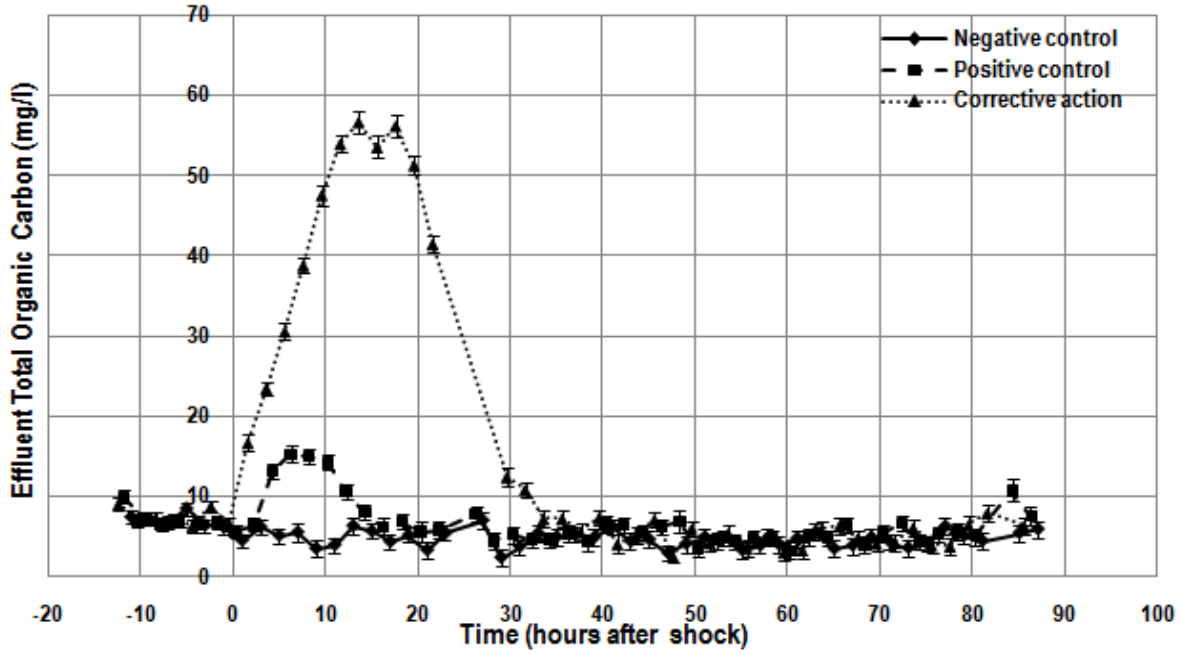

Figure B.41: Effluent TOC levels for C-CEPT$_1$

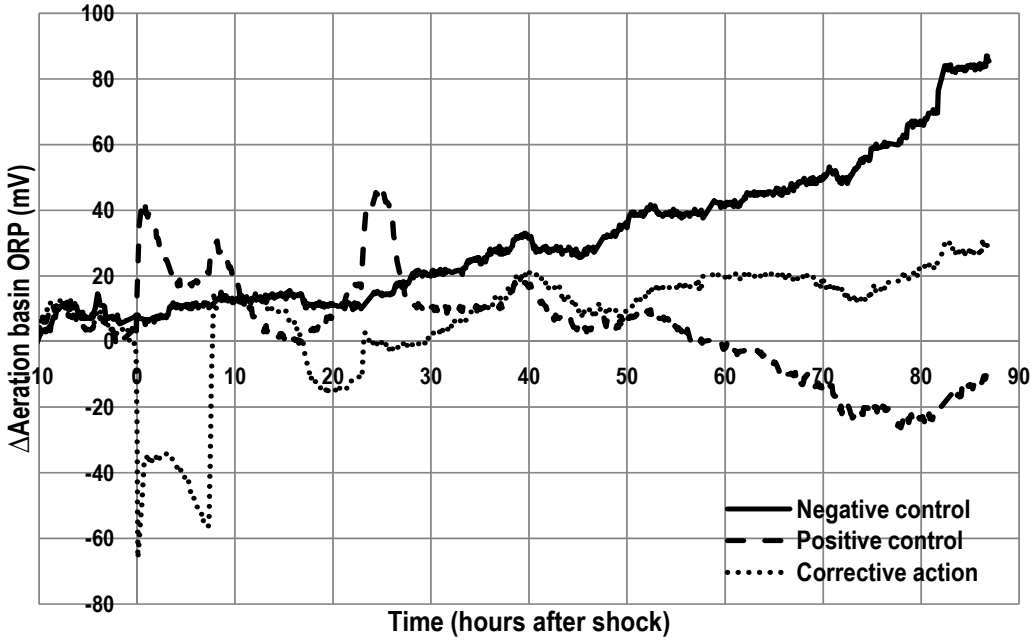

Figure B.42: Change in aeration basin ORP for C-CEPT$_1$

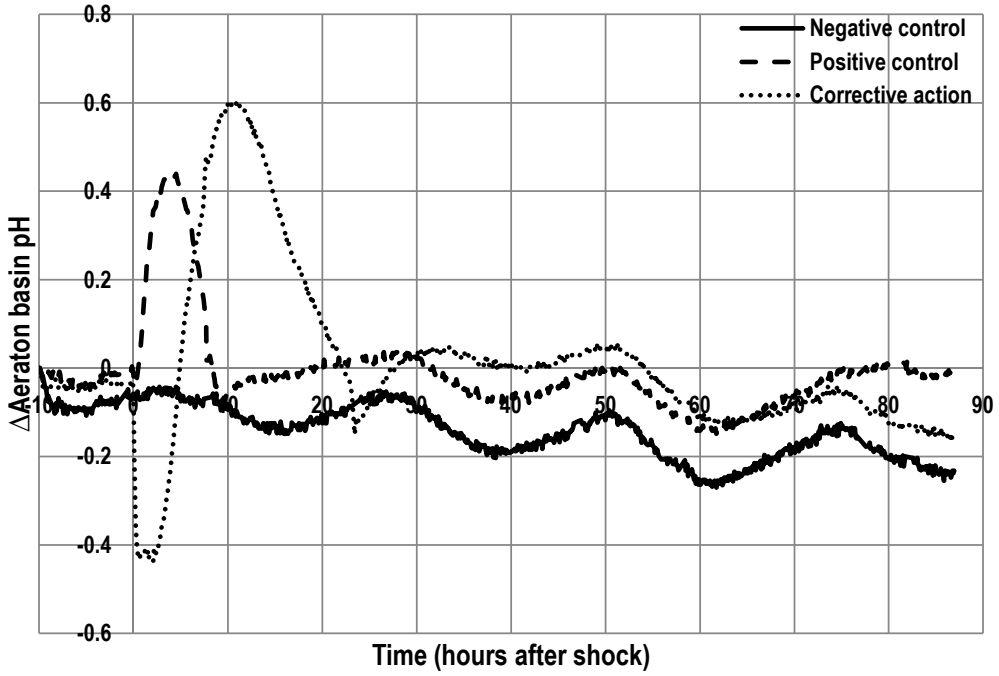

Figure B.43: Aeration basin pH levels for C-CEPT$_1$

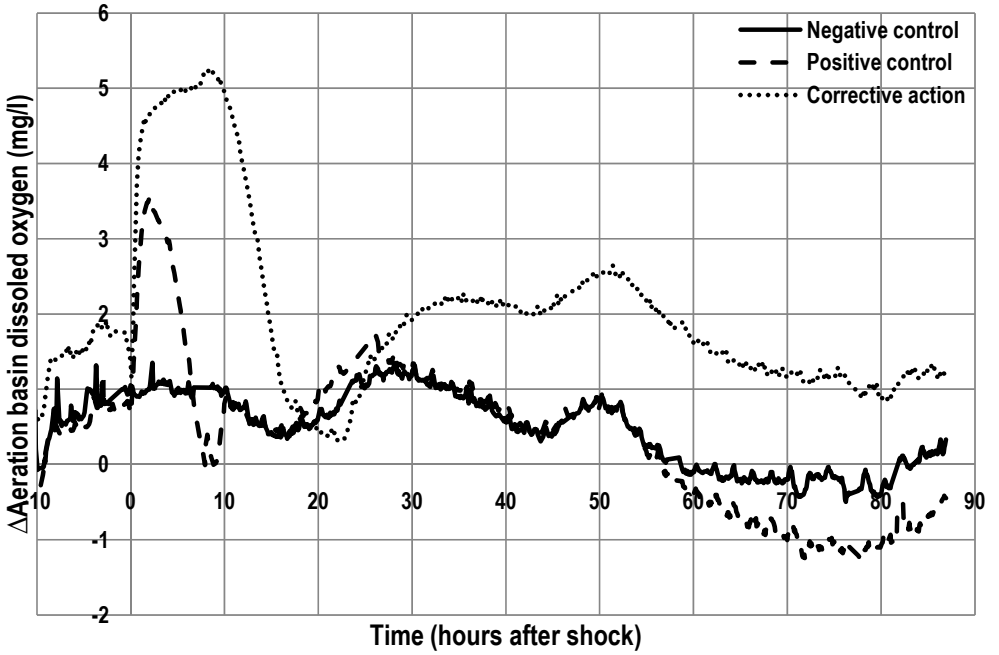

Figure B.44: Change in aeration basin DO levels for C-CEPT$_1$

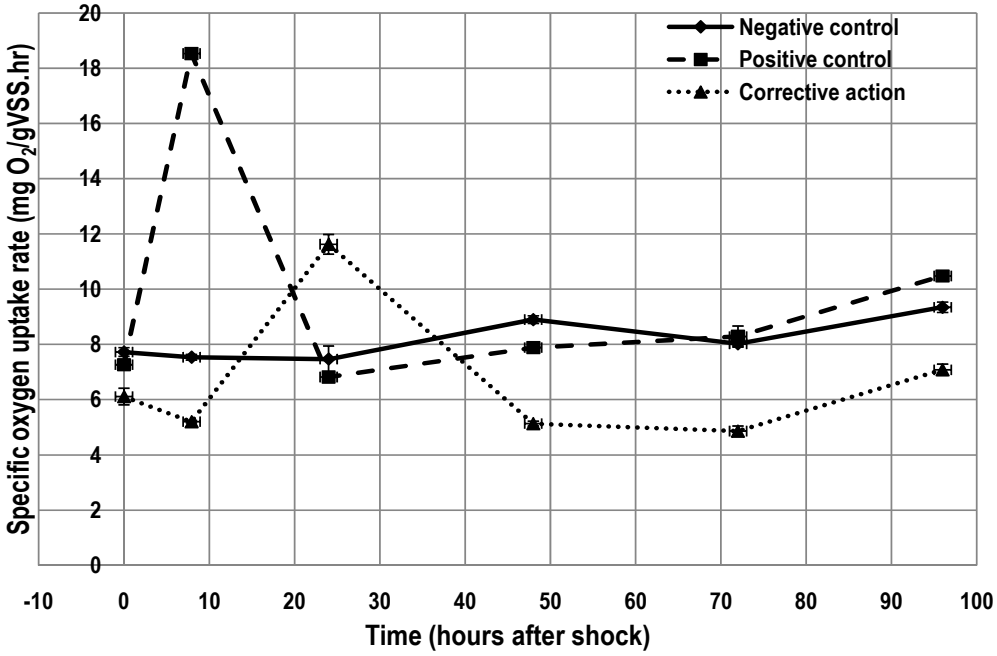

Figure B.45: Mixed liquor sOUR kevels for C-CEPT$_1$

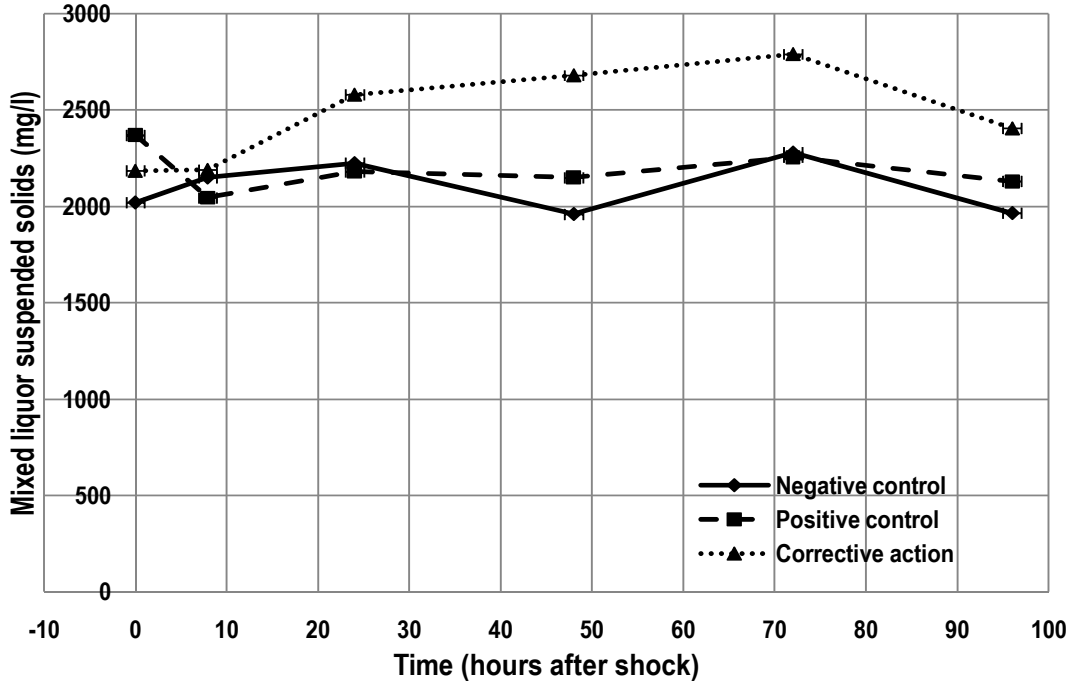

Figure B.46: Mixed liquor suspended solid levels for C-CEPT$_1$

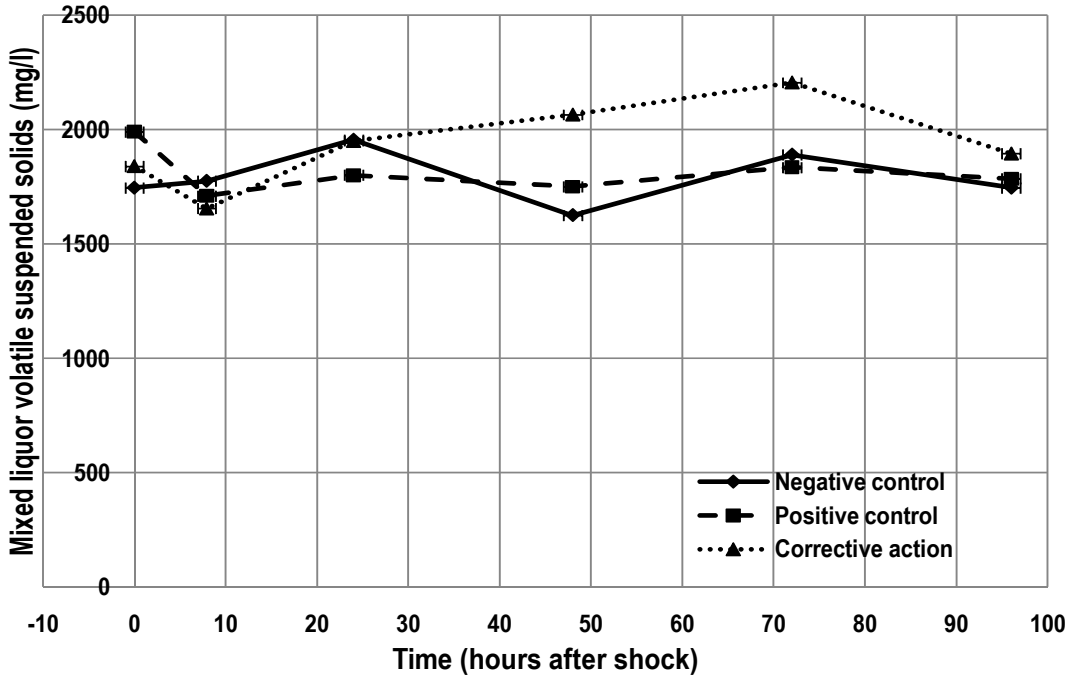

Figure B.47: Mixed liquor volatile suspended solid levels for C-CEPT$_1$

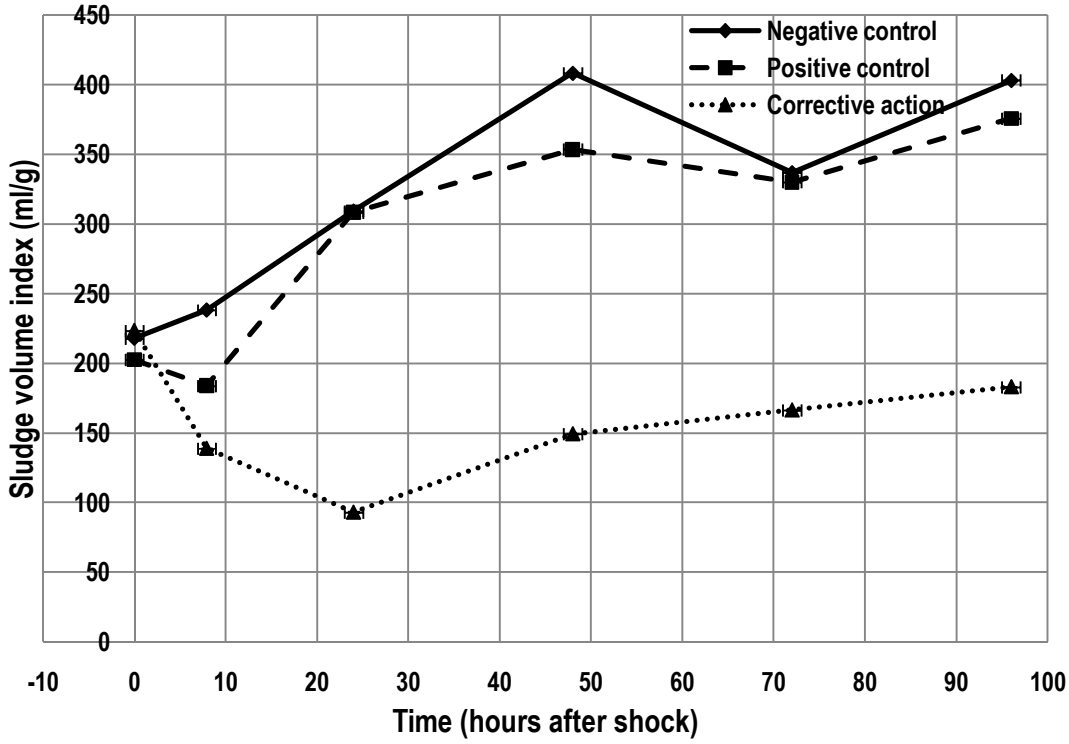

Figure B.48: Sludge volume index levels for C-CEPT$_1$

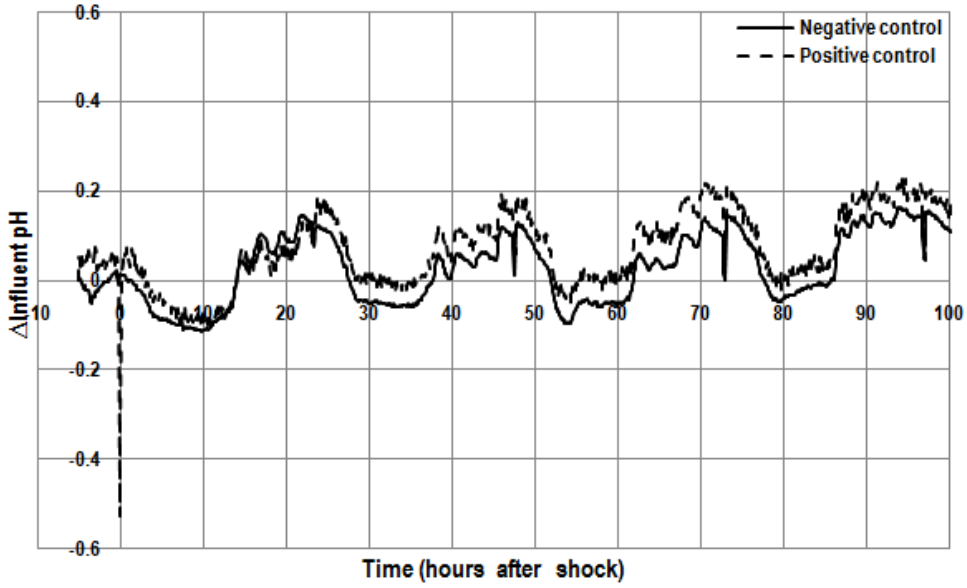

Figure B.49: Change in influent pH for C-CEPT$_2$

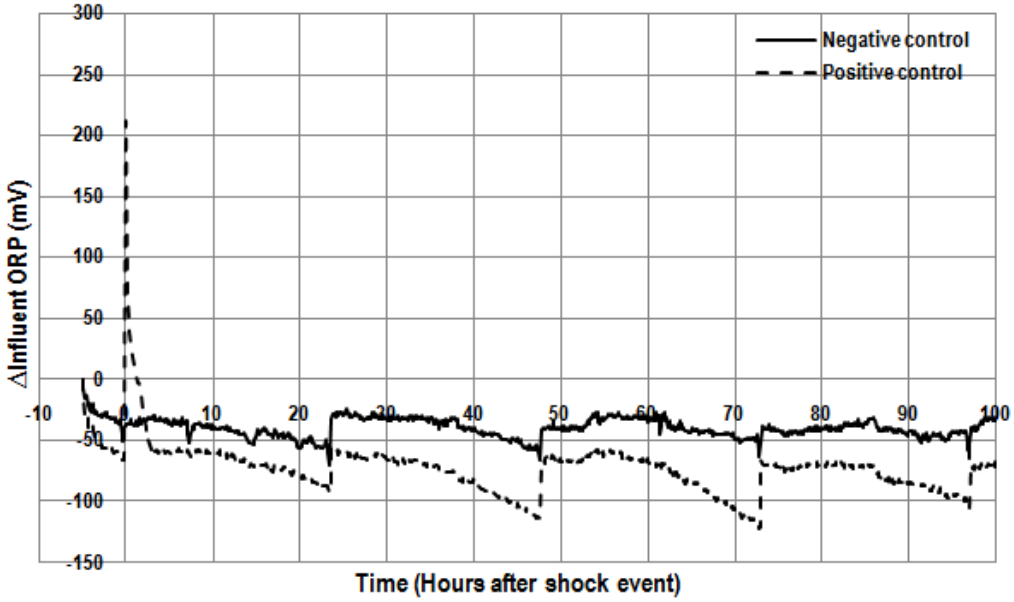

Figure B.50: Change in influent ORP for C-CEPT$_2$

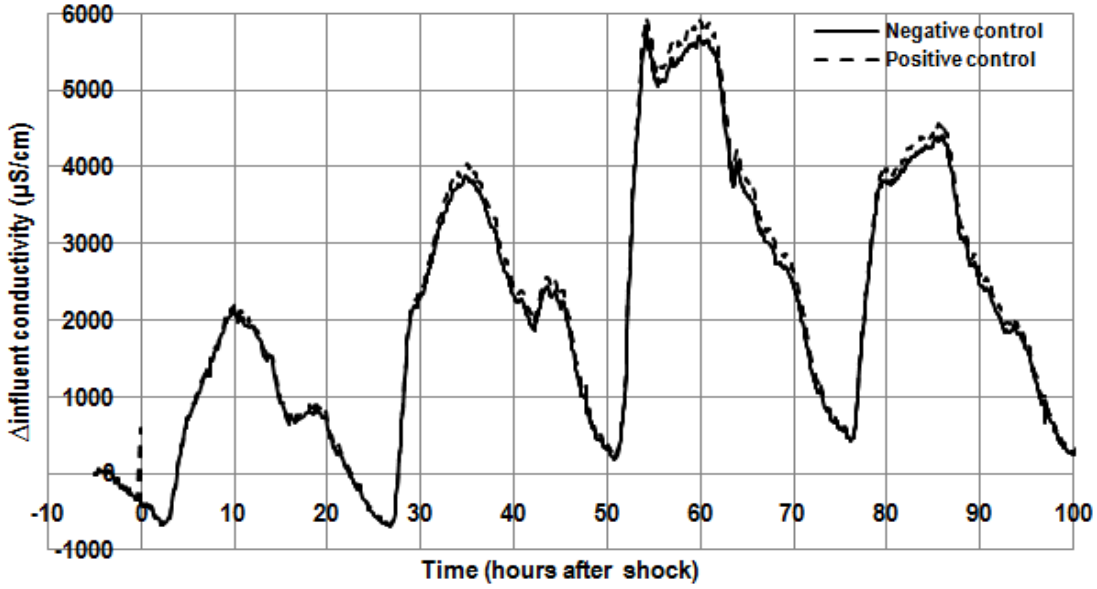

Figure B.51: Change in influent conductivity for C-CEPT$_2$

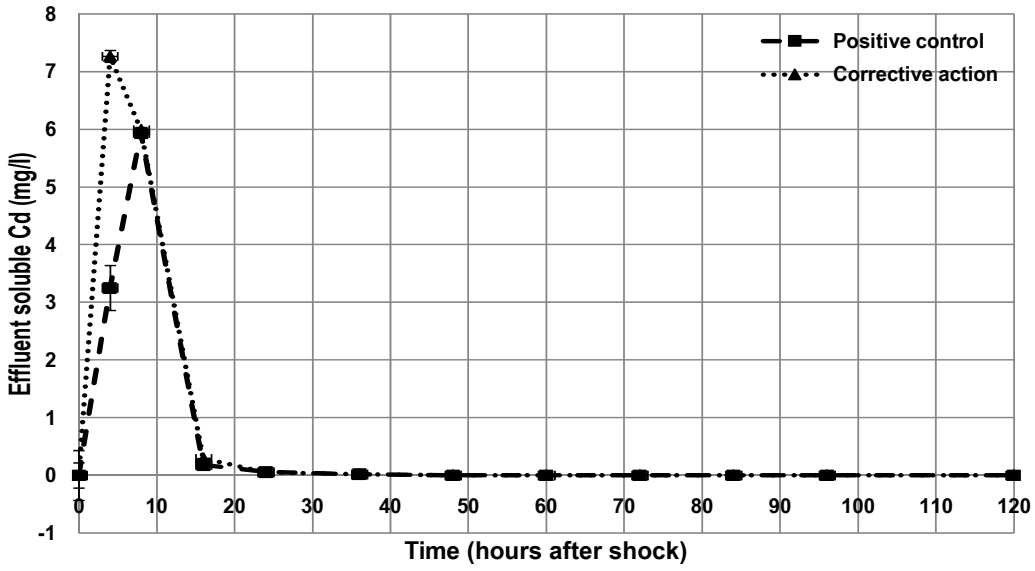

Figure B.52: Effluent soluble cadmium levels for C-CEPT$_2$

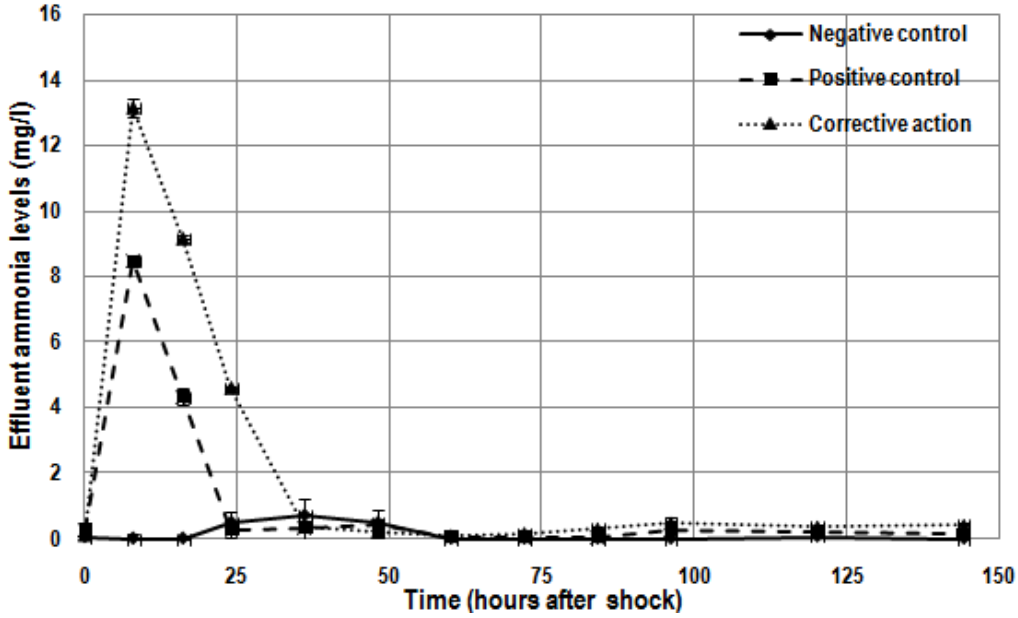

Figure B.53: Effluent ammonium-N levels for C-CEPT$_2$

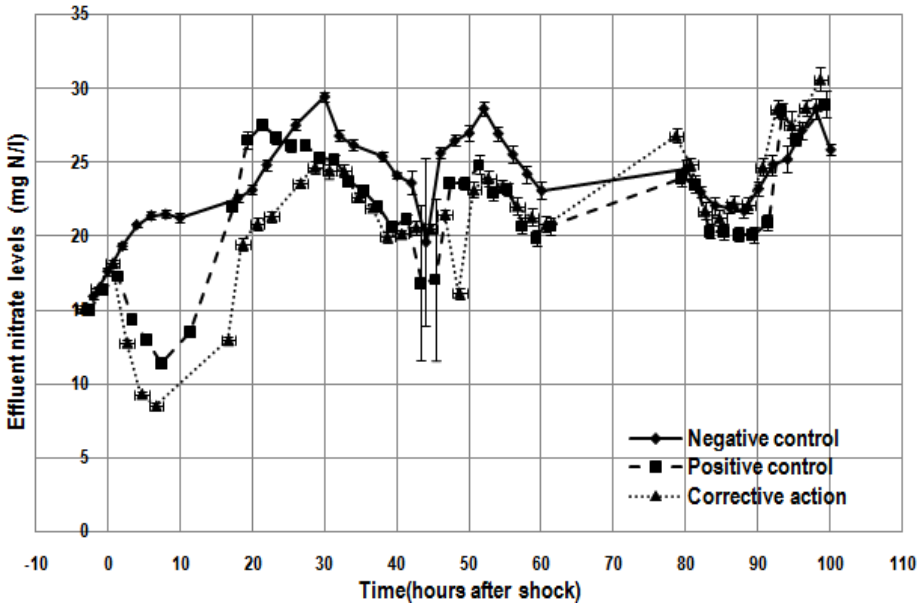

Figure B.54: Effluent nitrate-N levels for C-CEPT$_2$

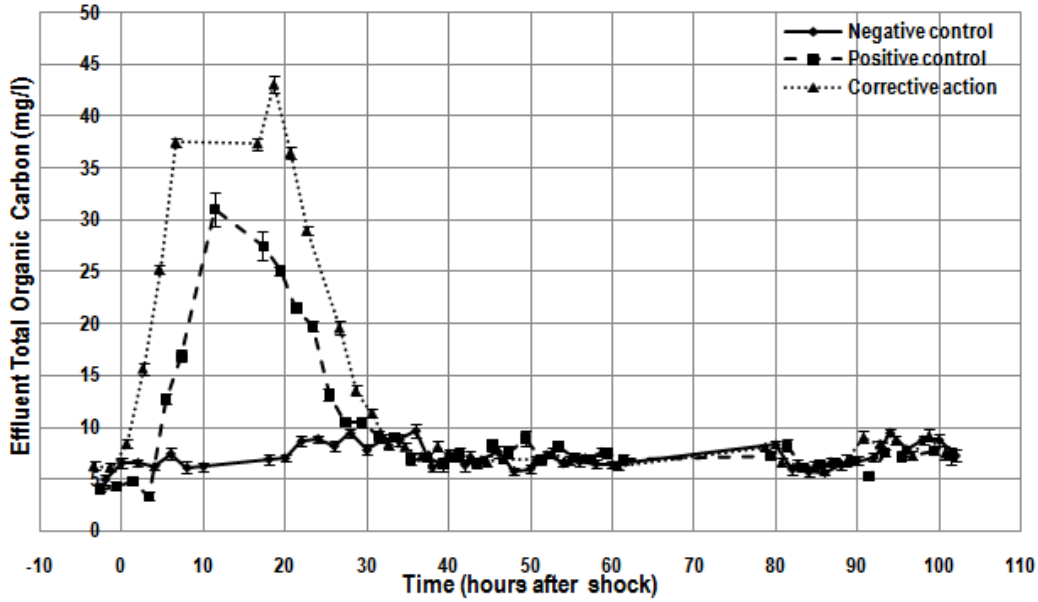

Figure B.55: Effluent TOC levels for C-CEPT$_2$

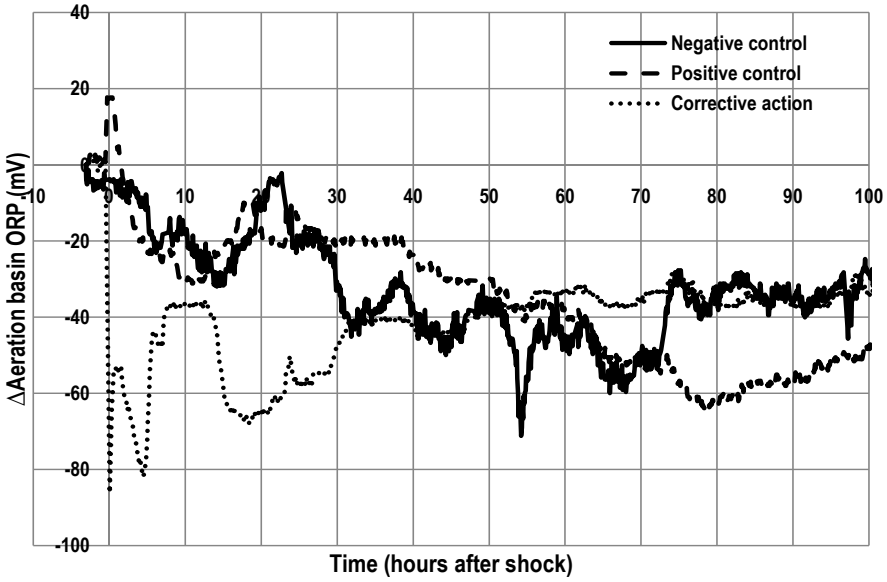

Figure B.56: Aeration basin ORP levels for C-CEPT$_2$

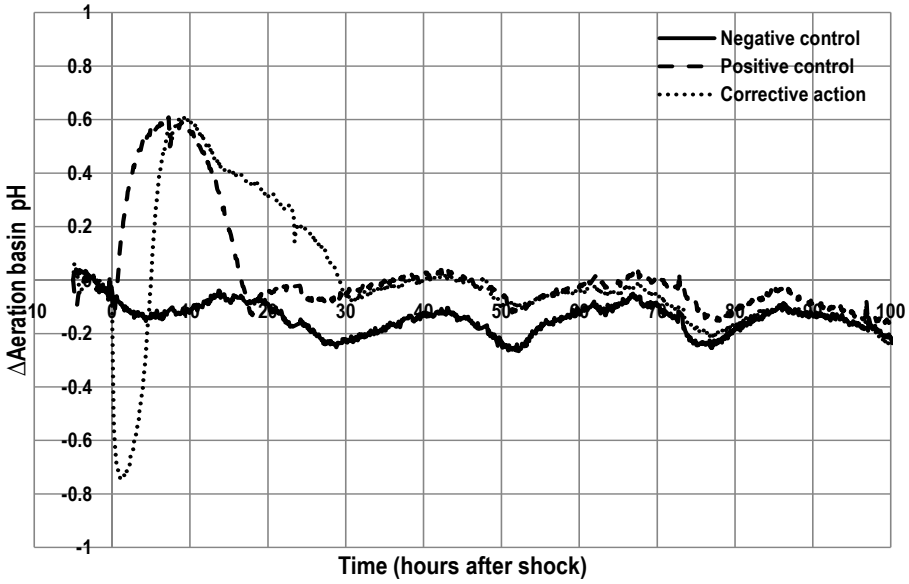

Figure B.57: Aeration basin pH levels for C-CEPT$_2$

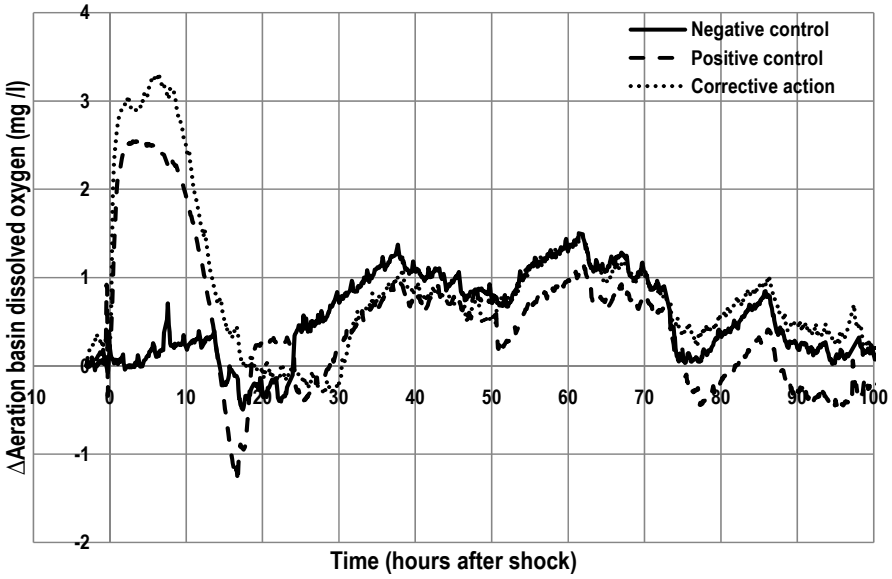

Figure B.58: Aeration basin DO levels for C-CEPT$_2$

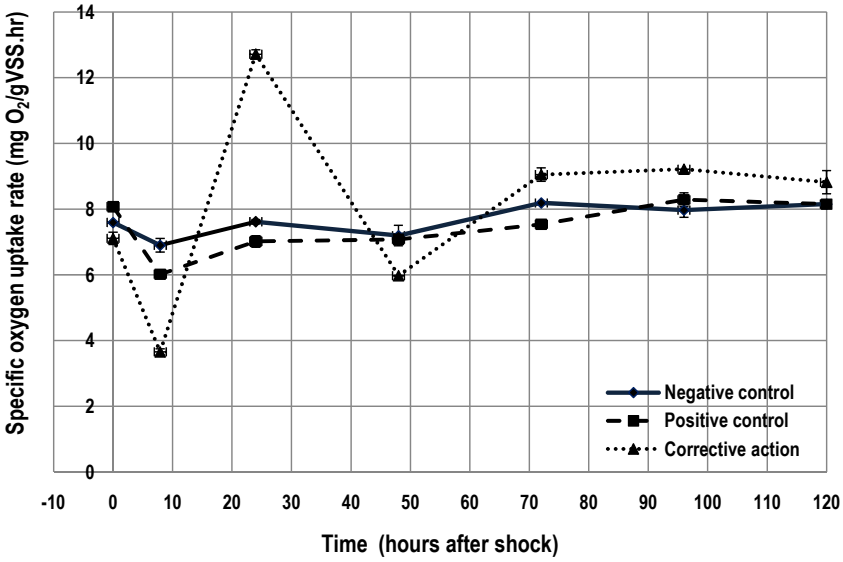

Figure B.59: Mixed liquor sOUR levels for C-CEPT$_2$

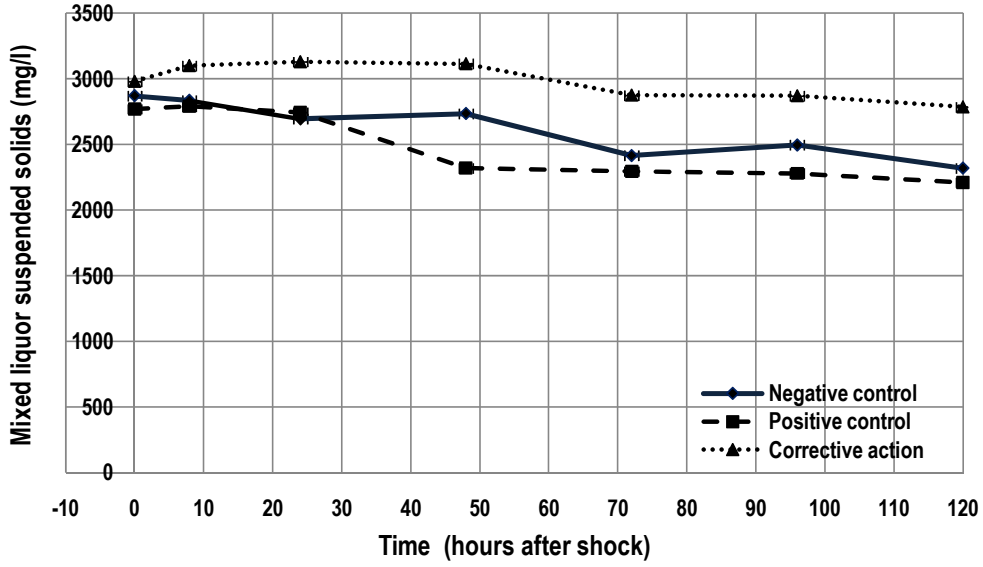

Figure B.60: Mixed liquor suspended solid levels for C-CEPT$_2$

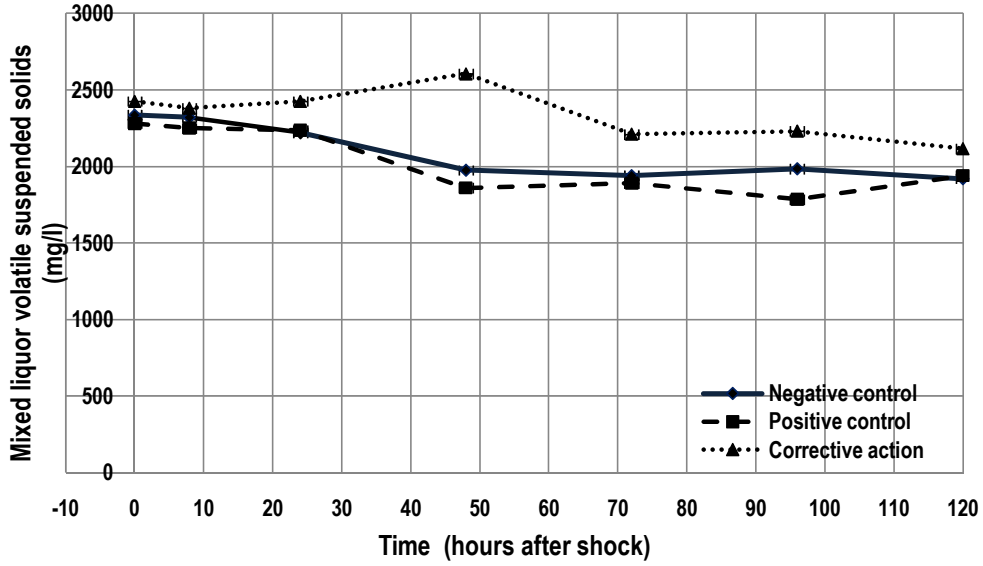

Figure B.61: Mixed liquor volatile suspended solid levels for C-CEPT$_2$

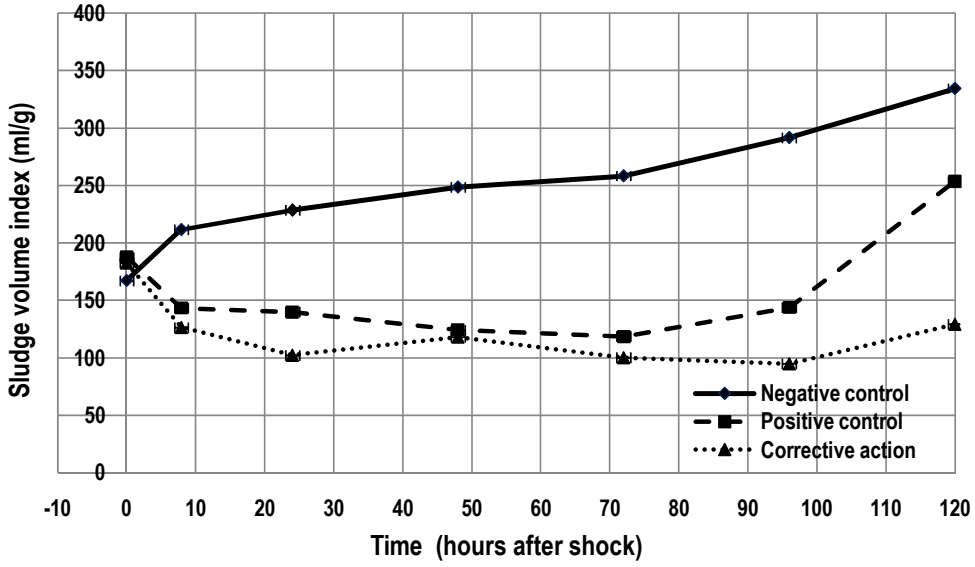

Figure B.62: Sludge volume index levels for C-CEPT$_2$

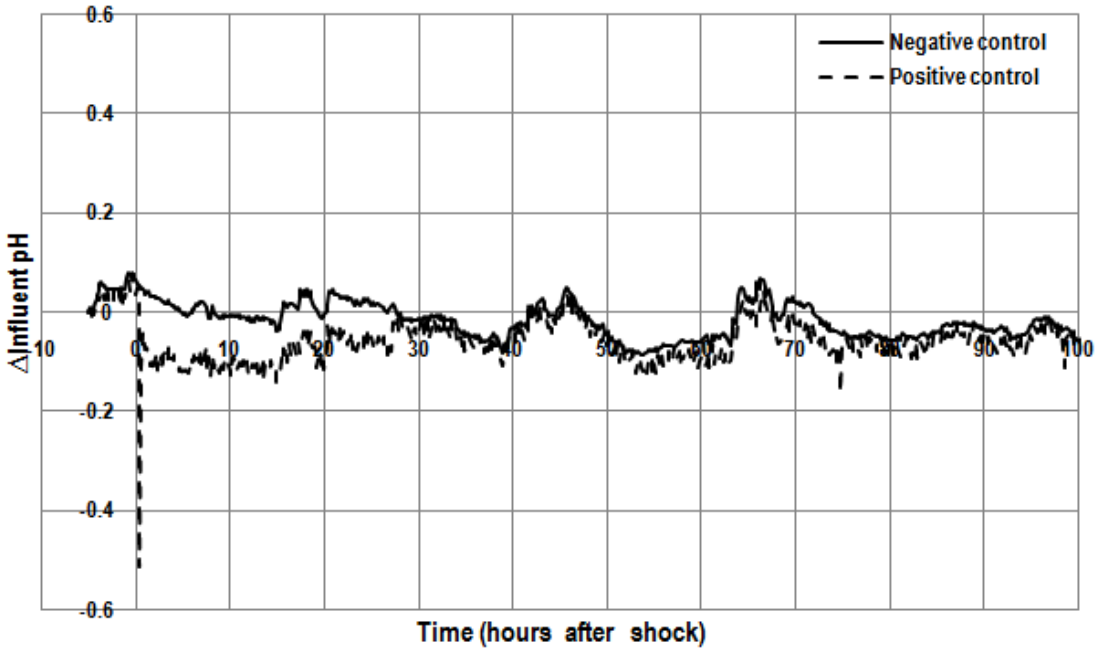

Figure B.63: Influent pH levels for C-SDS$_1$

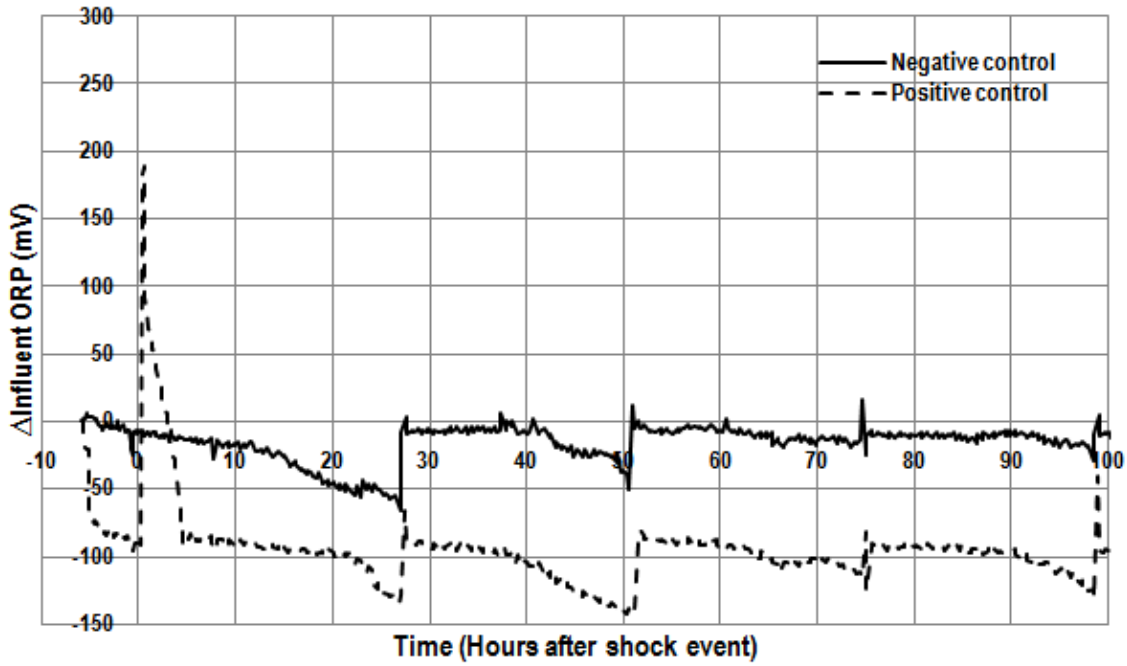

Figure B.64: Influent ORP levels for C-SDS$_1$

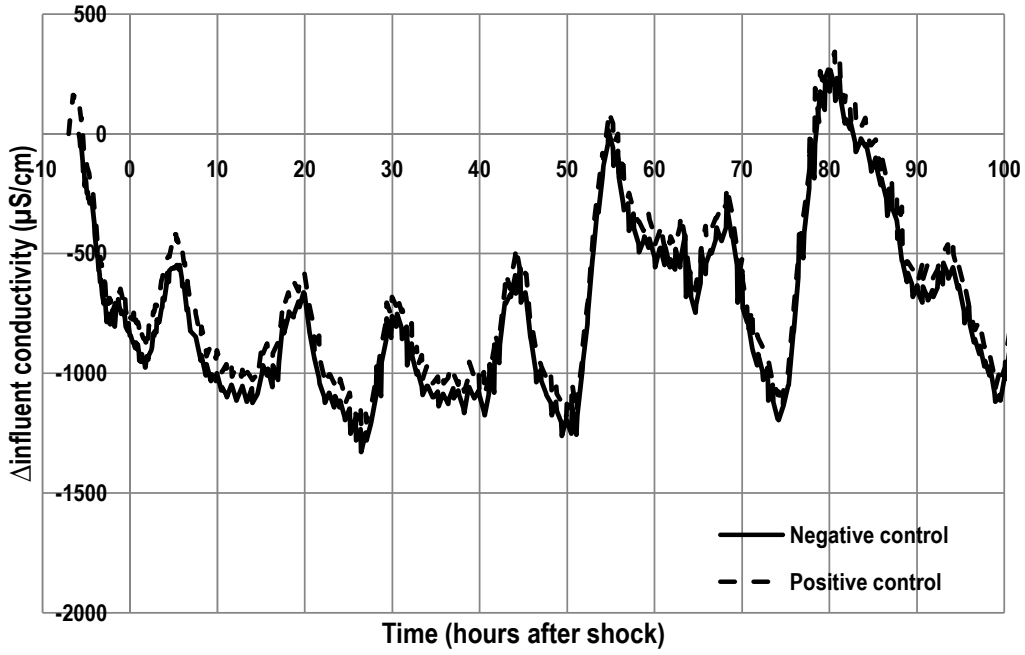

Figure B.65: Influent conductivity levels for C-SDS$_1$

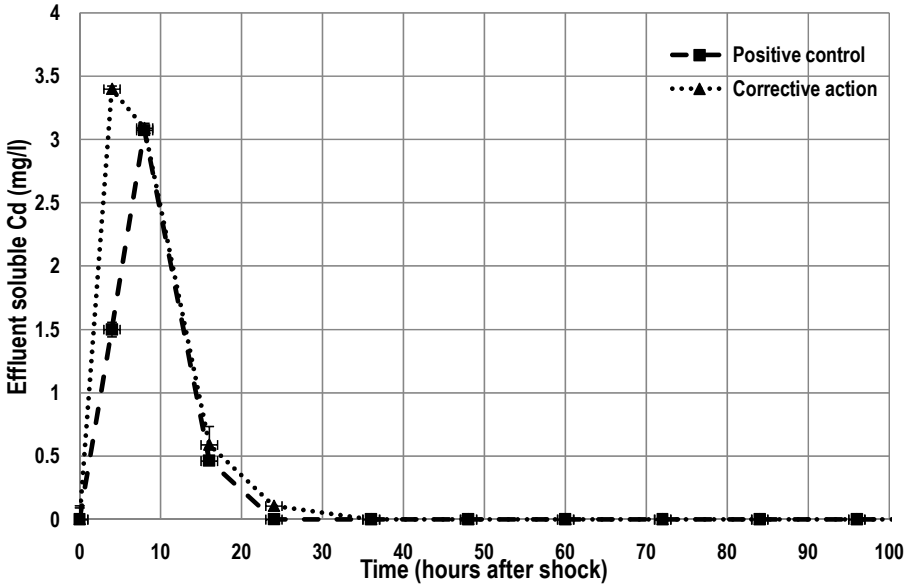

Figure B.66: Soluble cadmium levels for C-SDS$_1$

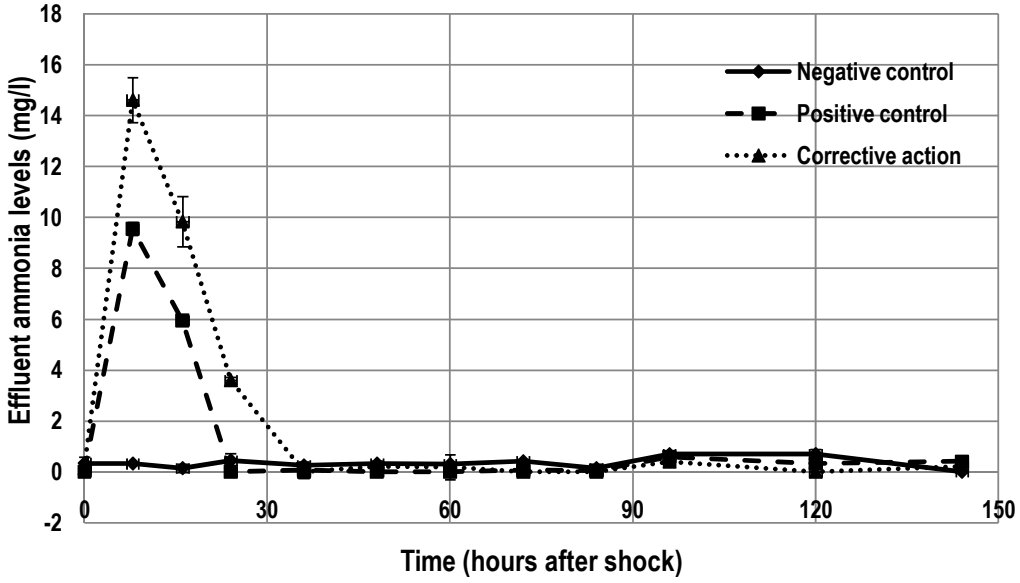

Figure B.67: Effluent ammonium-N levels for C-SDS$_1$

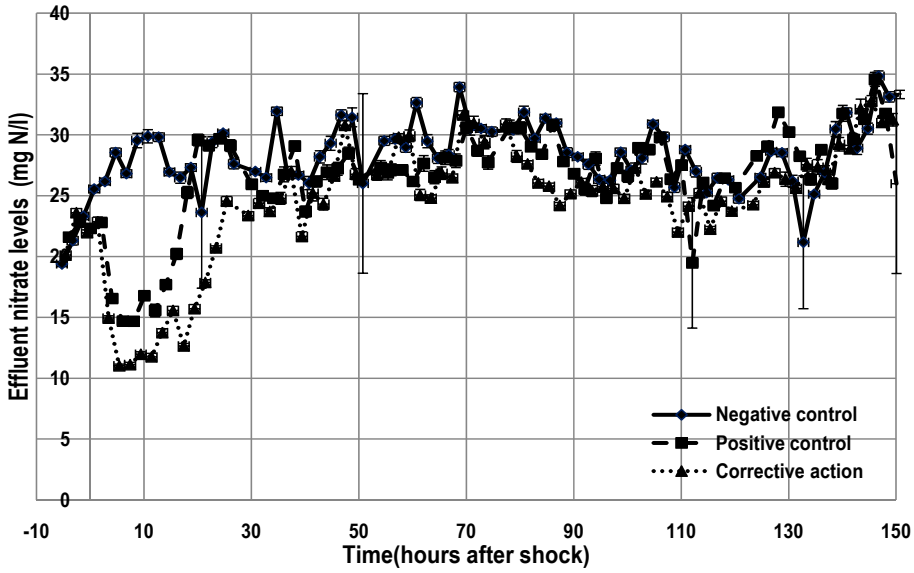

Figure B.68: Effluent nitrate-N levels for C-SDS$_1$

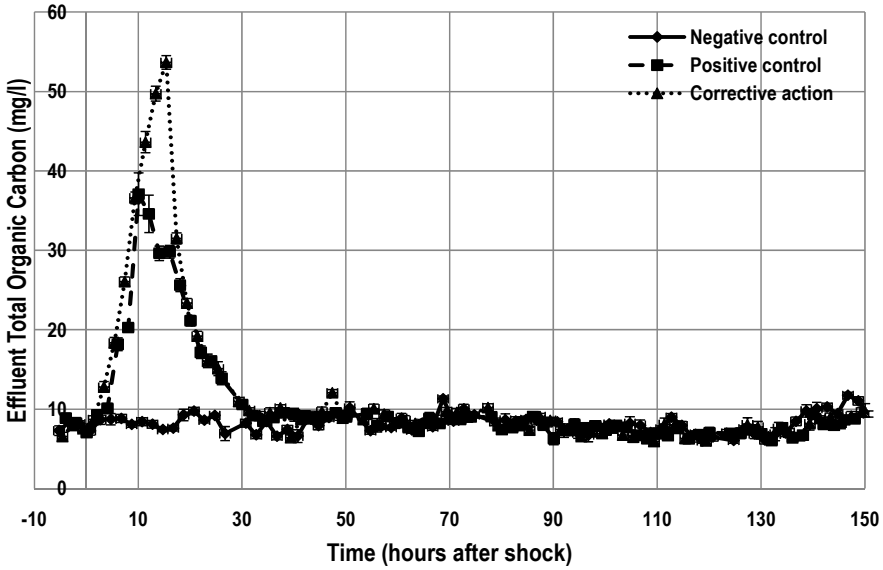

Figure B.69: Effluent TOC levels for C-SDS$_1$

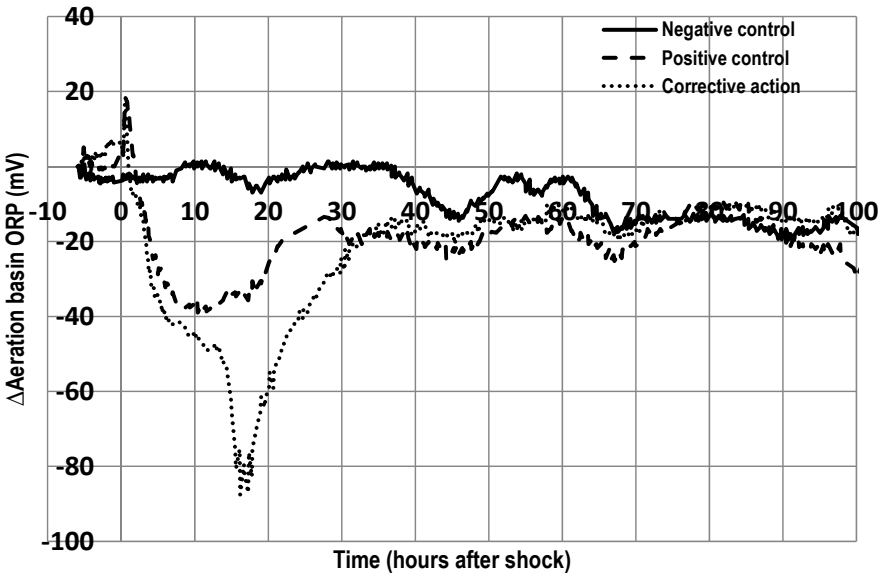

Figure B.70: Aeration basin ORP levels for C-SDS$_1$

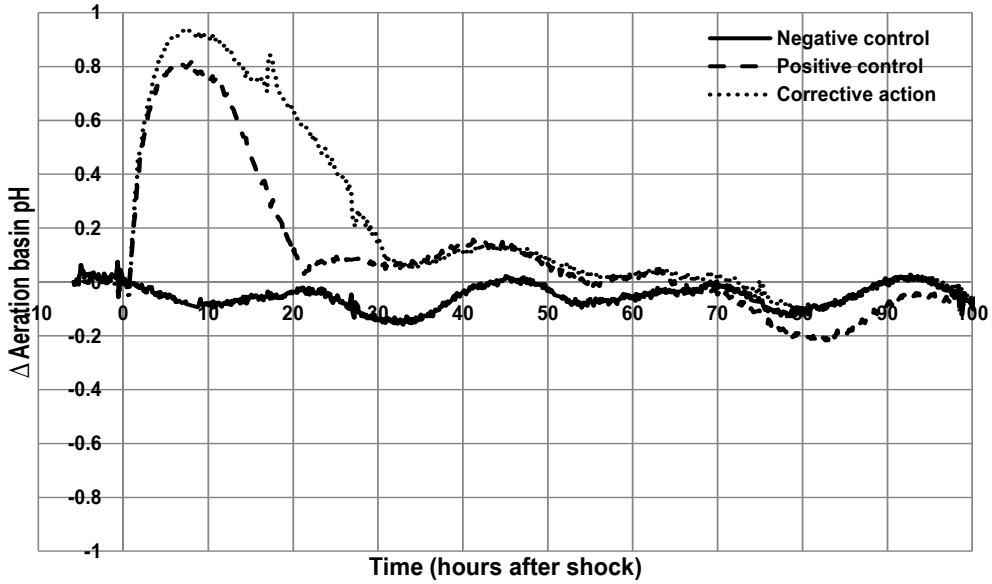

Figure B.71: Aeration basin pH levels for C-SDS$_1$

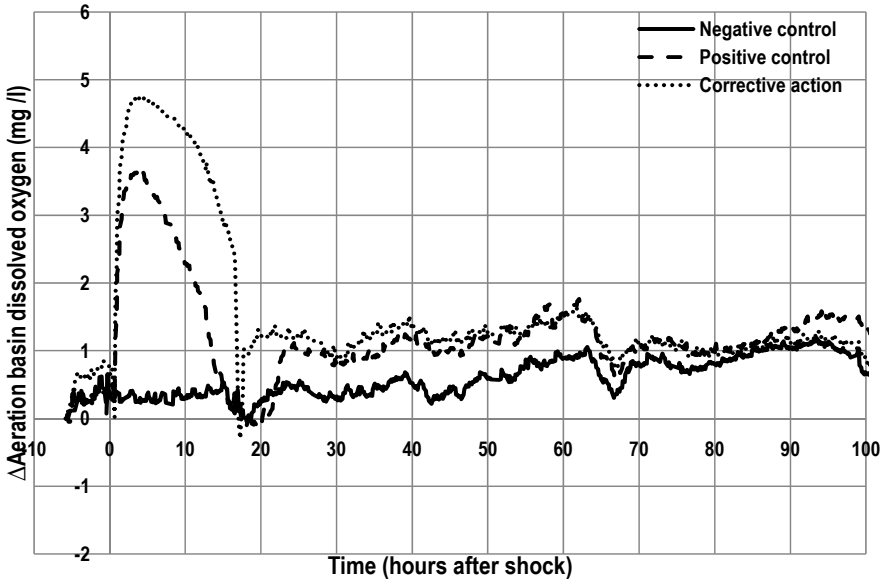

Figure B.72: Aeration basin DO levels for C-SDS$_1$

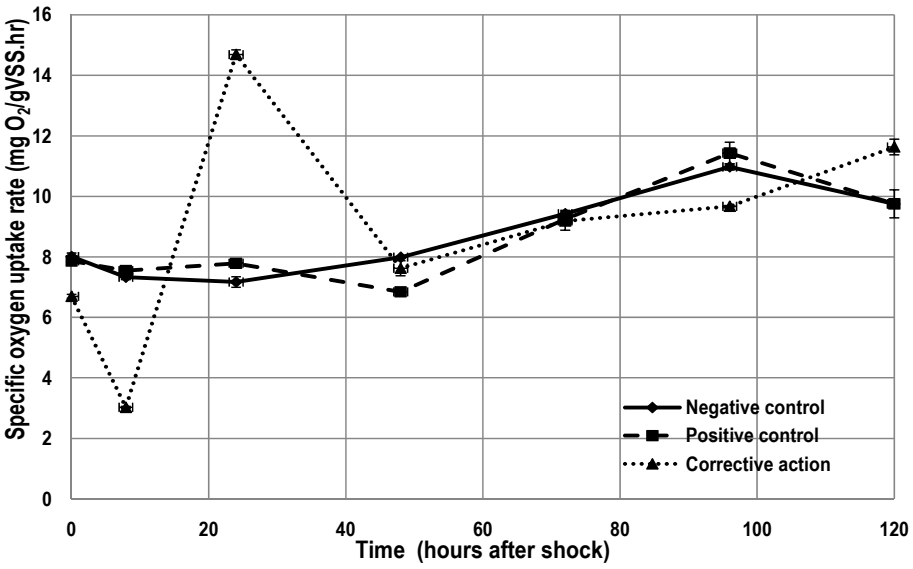

Figure B.73: Mixed liquor sOUR levels for C-SDS$_1$

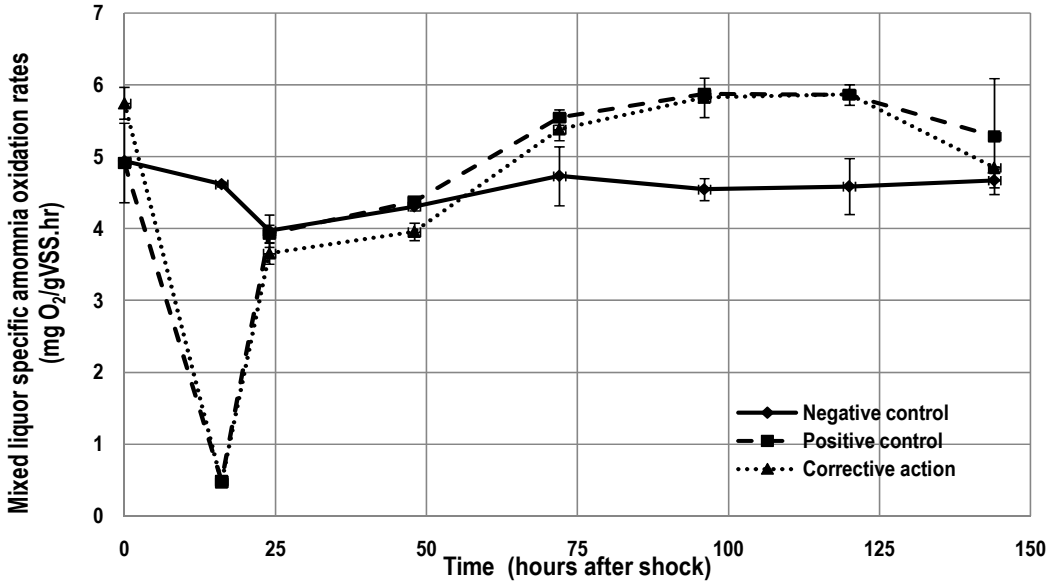

Figure B.74: Mixed liquor sAOR levels for C-SDS$_1$

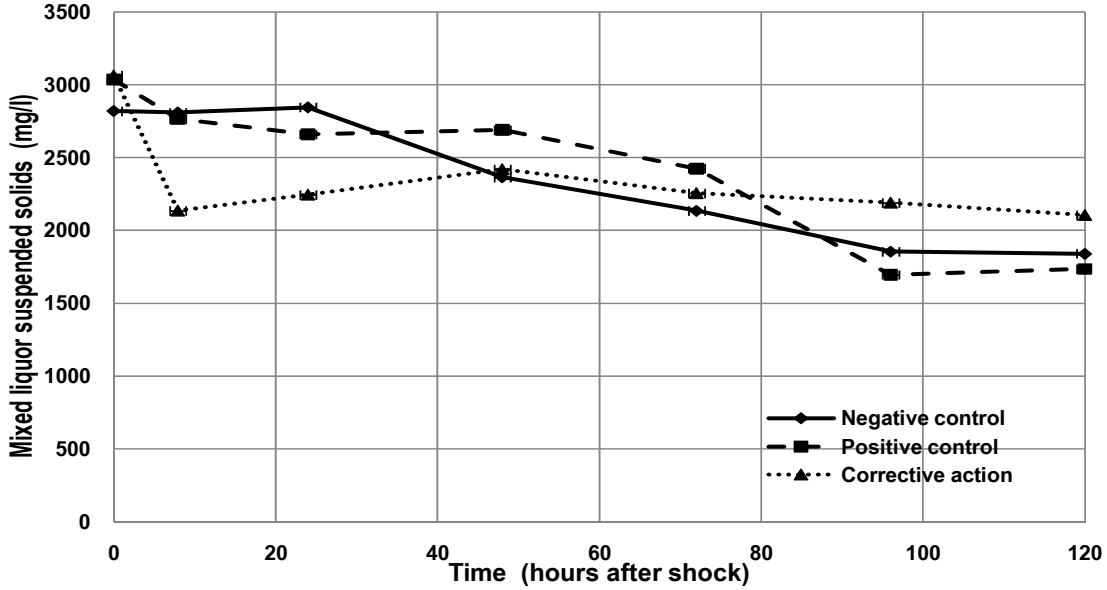

Figure B.75: Mixed liquor suspended solid concentrations for C-SDS$_1$

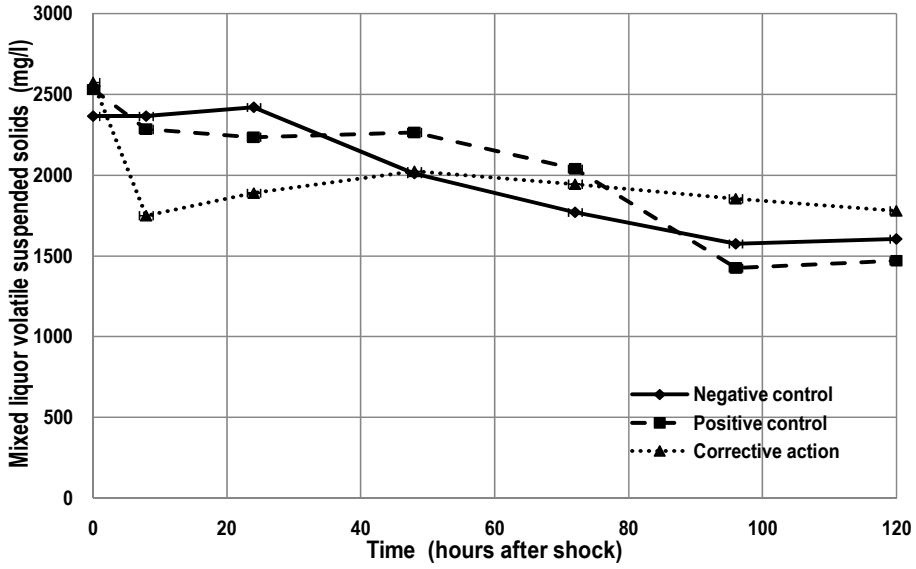

Figure B.76: Mixed liquor volatile suspended solid concentrations for C-SDS$_1$

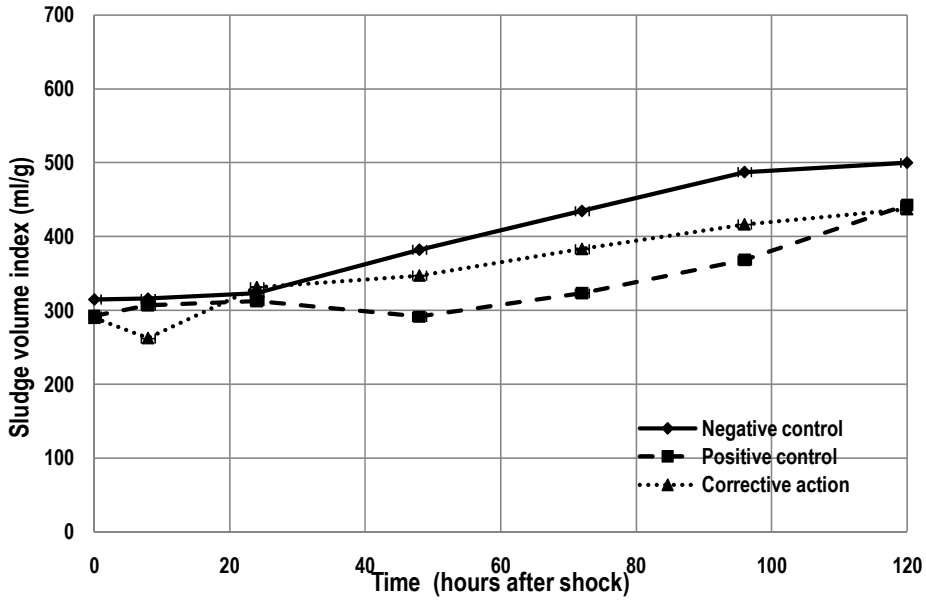

Figure B.77: Sludge volume index levels for C-SDS$_1$

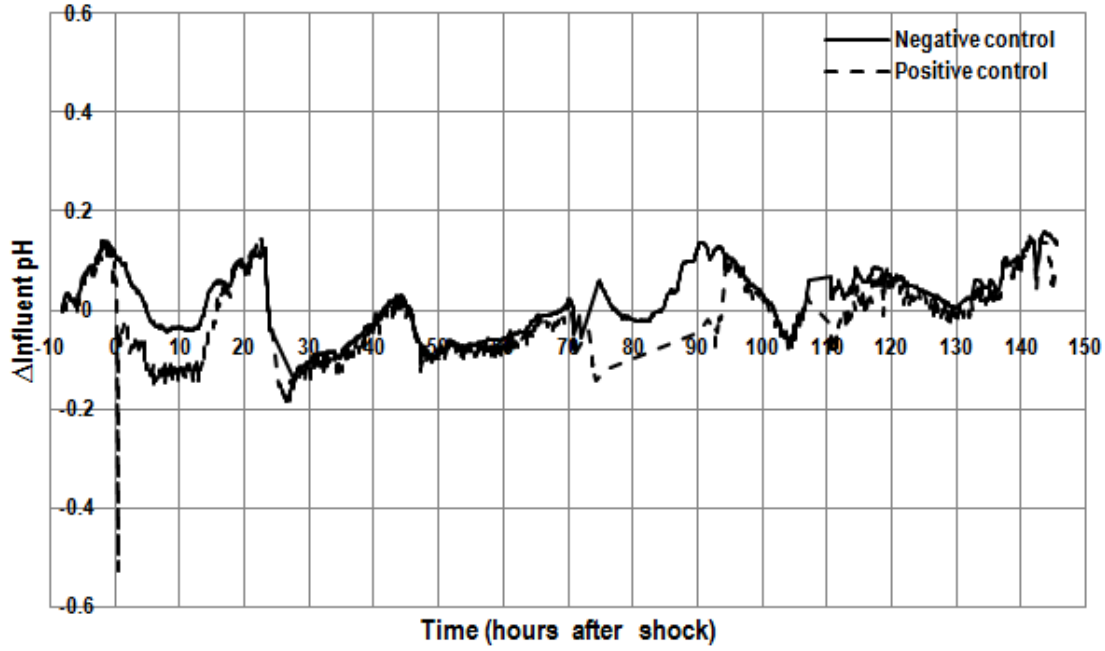

Figure B.78: Influent pH levels for C-SDS$_2$

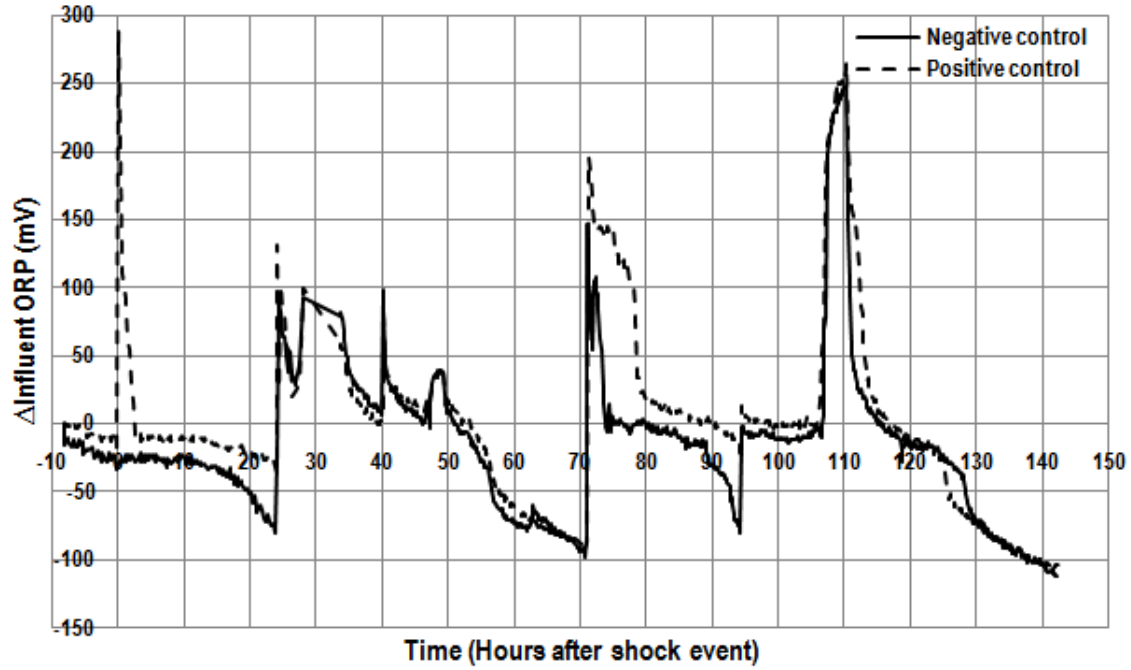

Figure B.79: Influent ORP levels for C-SDS$_2$

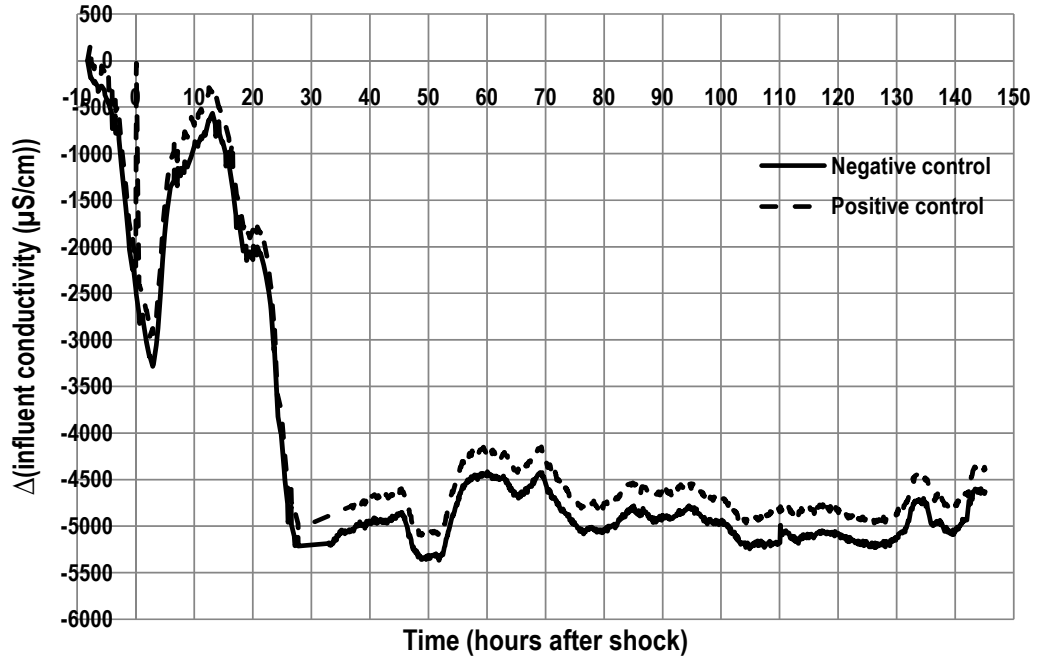

Figure B.80: Influent conductivity levels for C-SDS$_2$

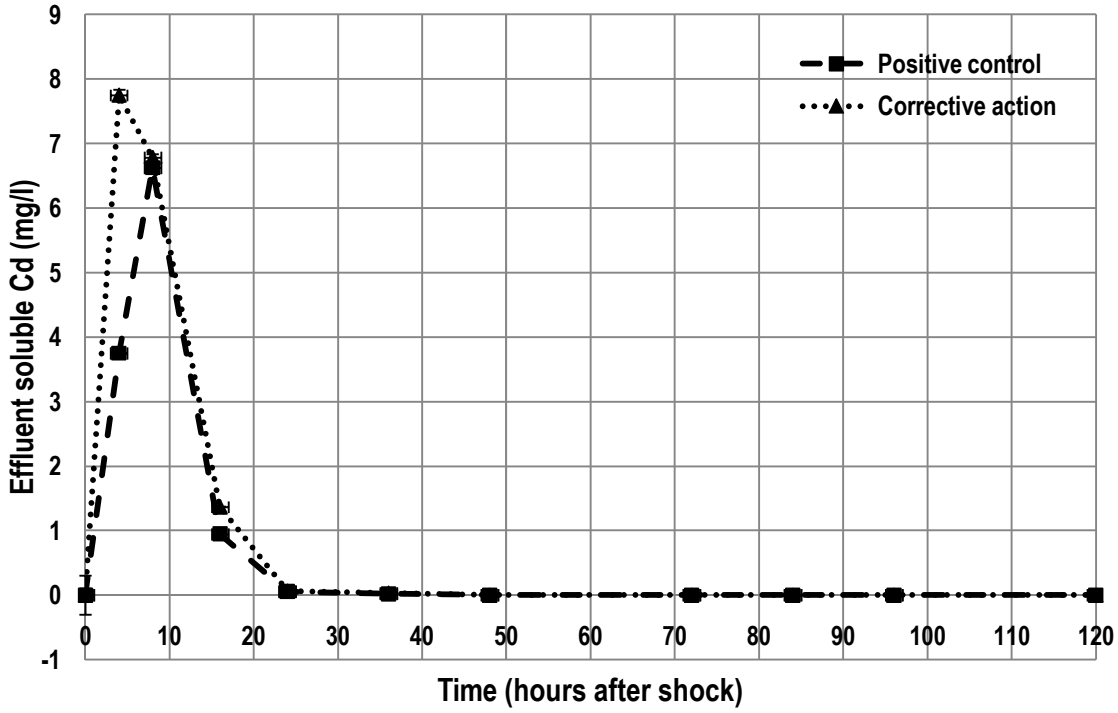

Figure B.81: Soluble cadmium levels for C-SDS$_2$

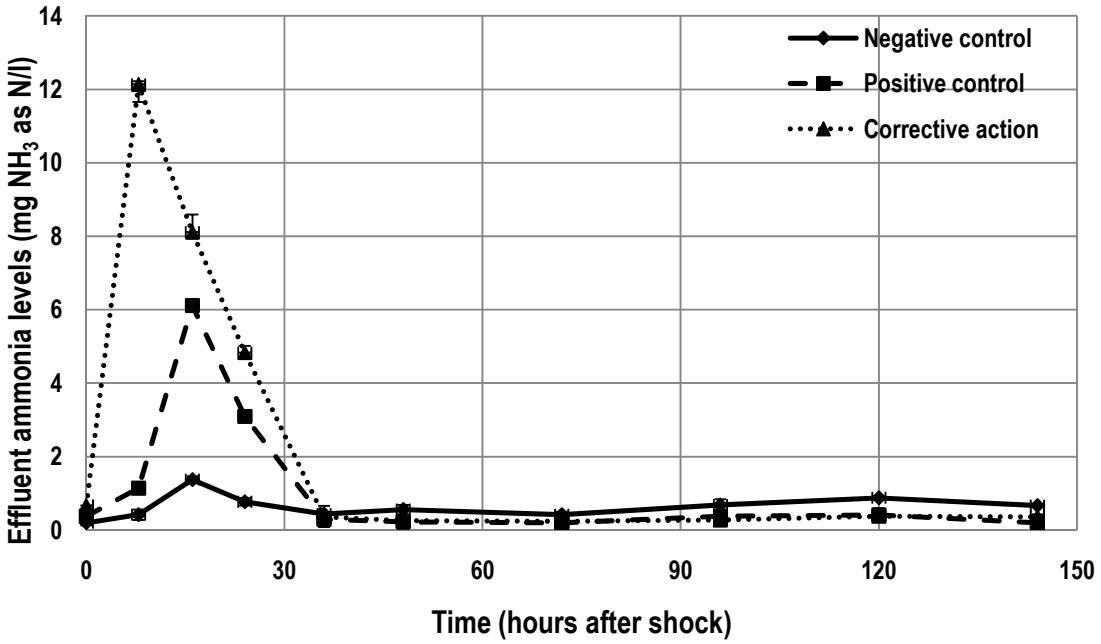

Figure B.82: Effluent ammonium-N levels for C-SDS_2

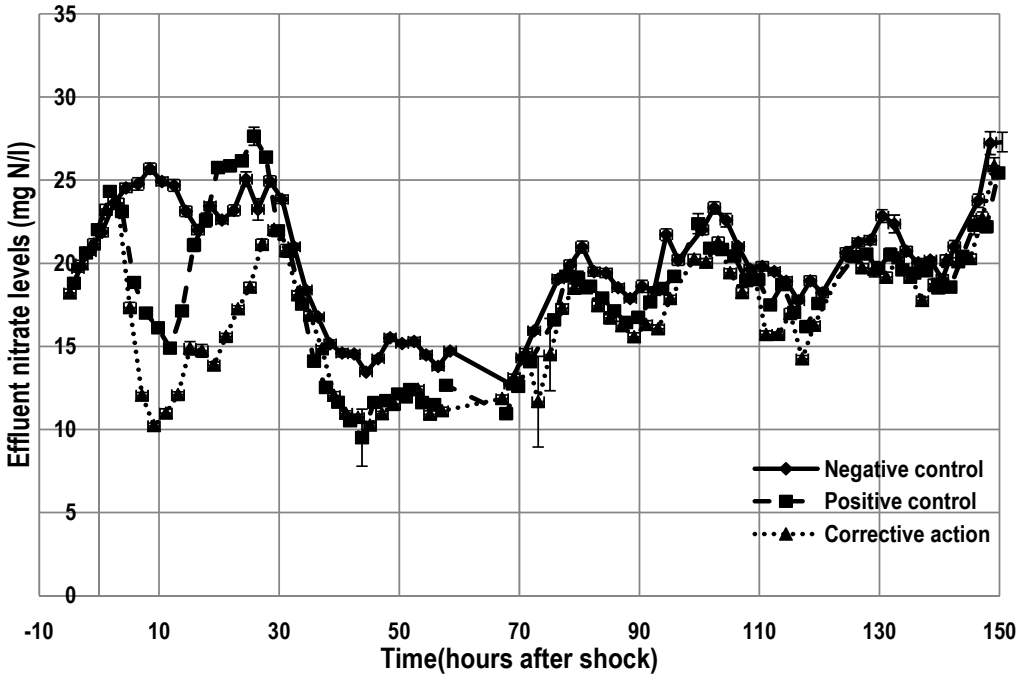

Figure B.83: Effluent nitrate-N levels for C-SDS_2

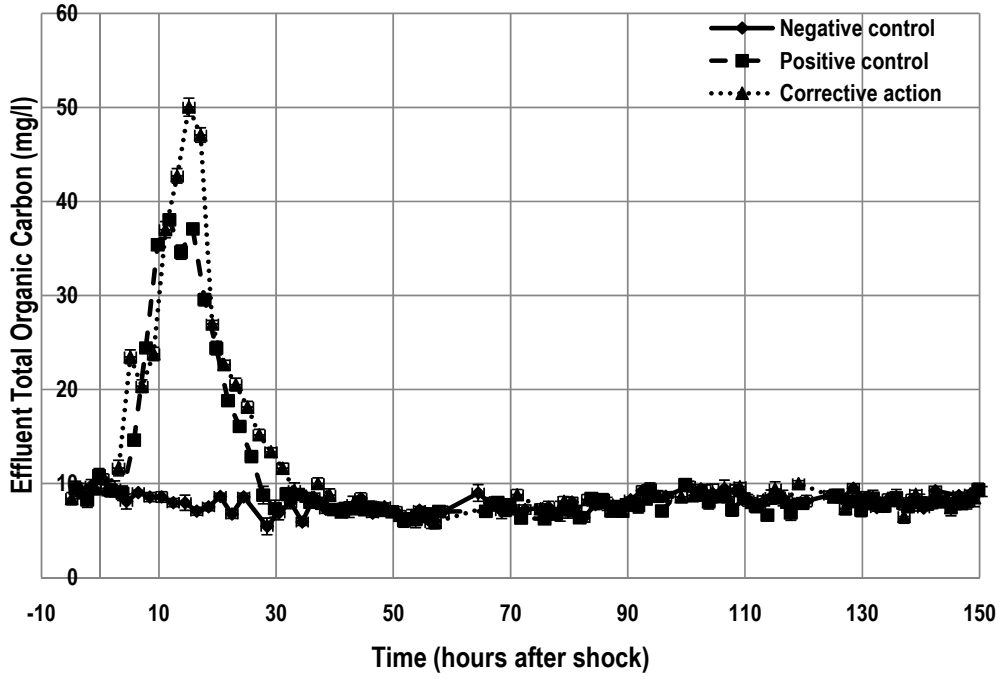

Figure B.84: Effluent TOC levels for C-SDS$_2$

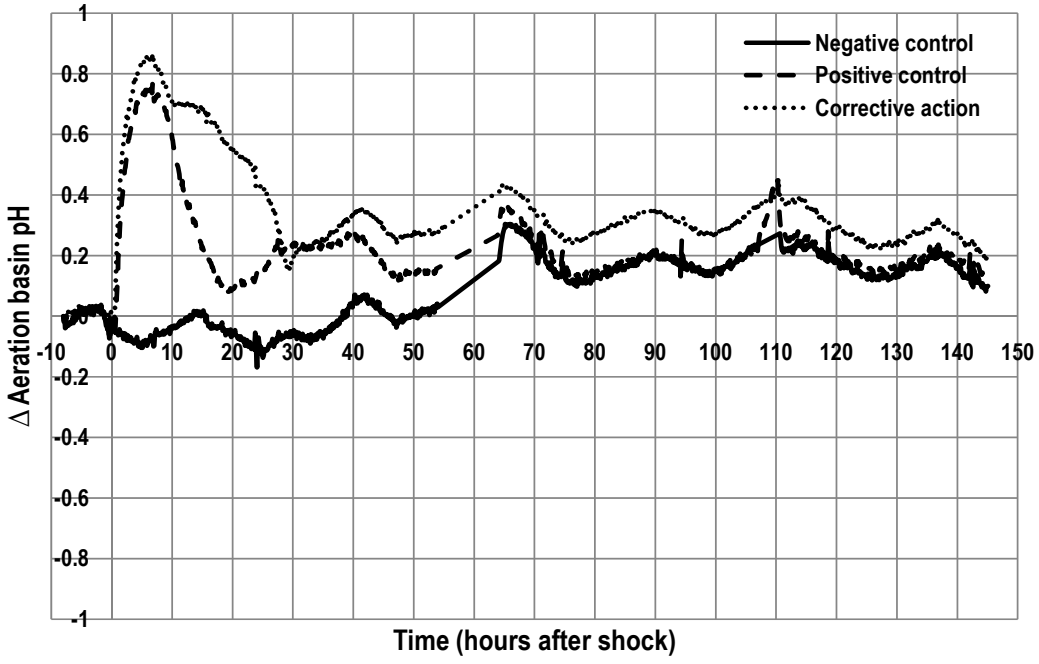

Figure B.85: Aeration basin pH levels for C-SDS$_2$

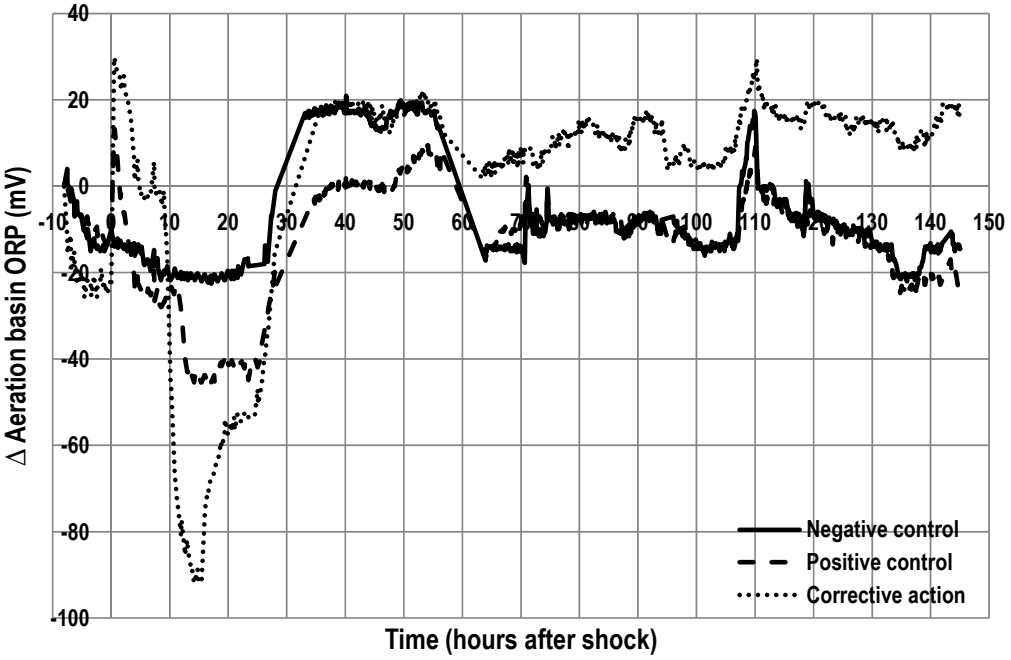

Figure B.86: Aeration basin ORP levels for C-SDS$_2$

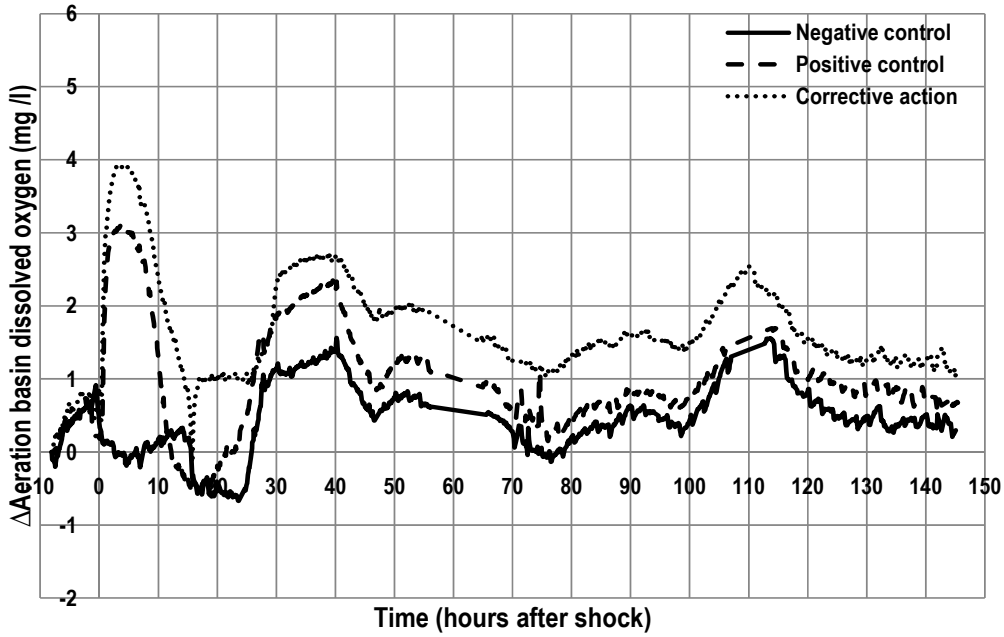

Figure B.87: Aeration basin DO levels for C-SDS$_2$

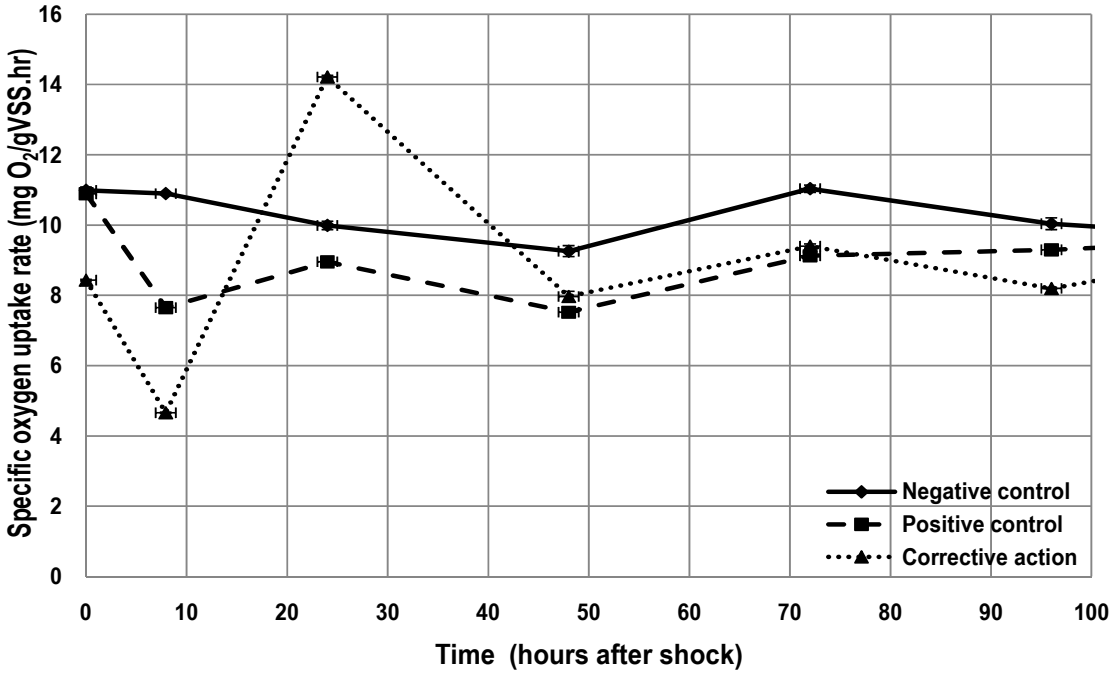

Figure B.88: Mixed liquor sOUR levels for C-SDS$_2$

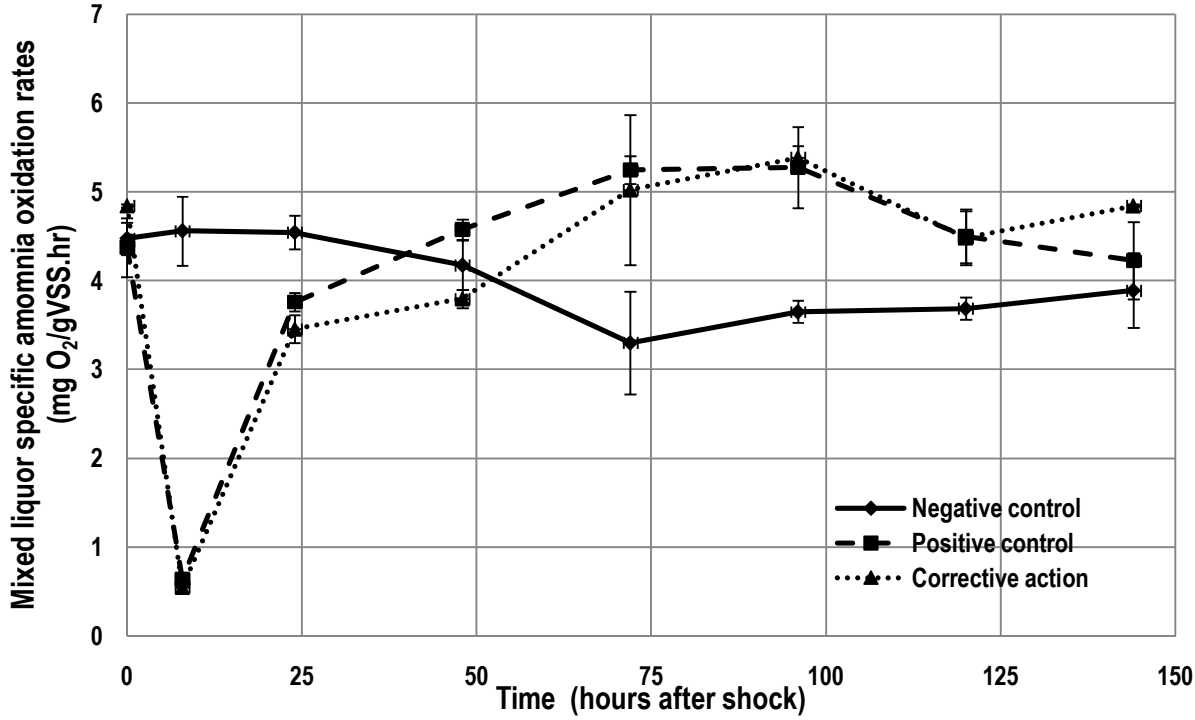

Figure B.89: Mixed liquor sAOR levels for C-SDS$_2$

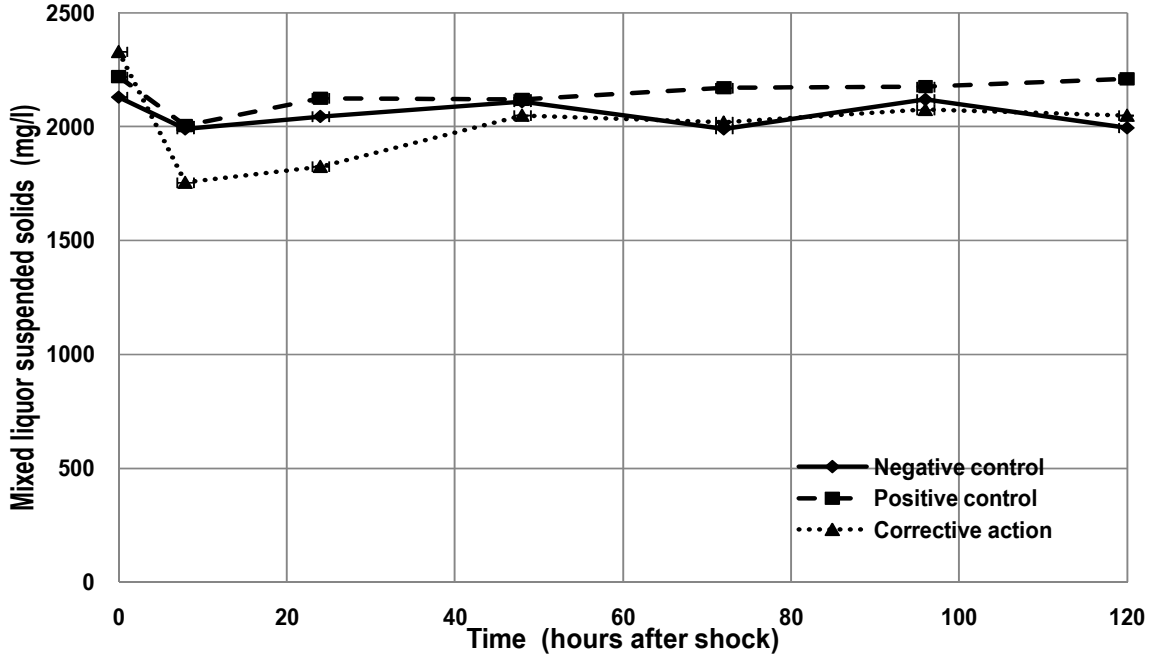

Figure B.90: Mixed liquor suspended solid concentrations for C-SDS$_2$

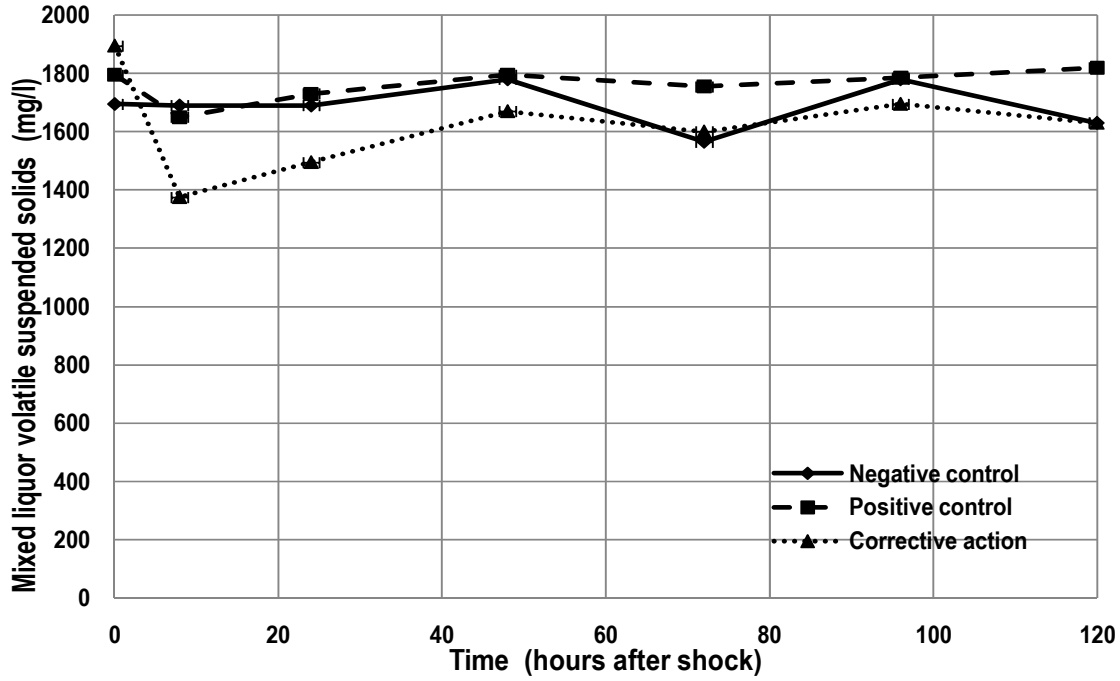

Figure B.91: Mixed liquor volatile suspended solid concentrations for C-SDS$_2$

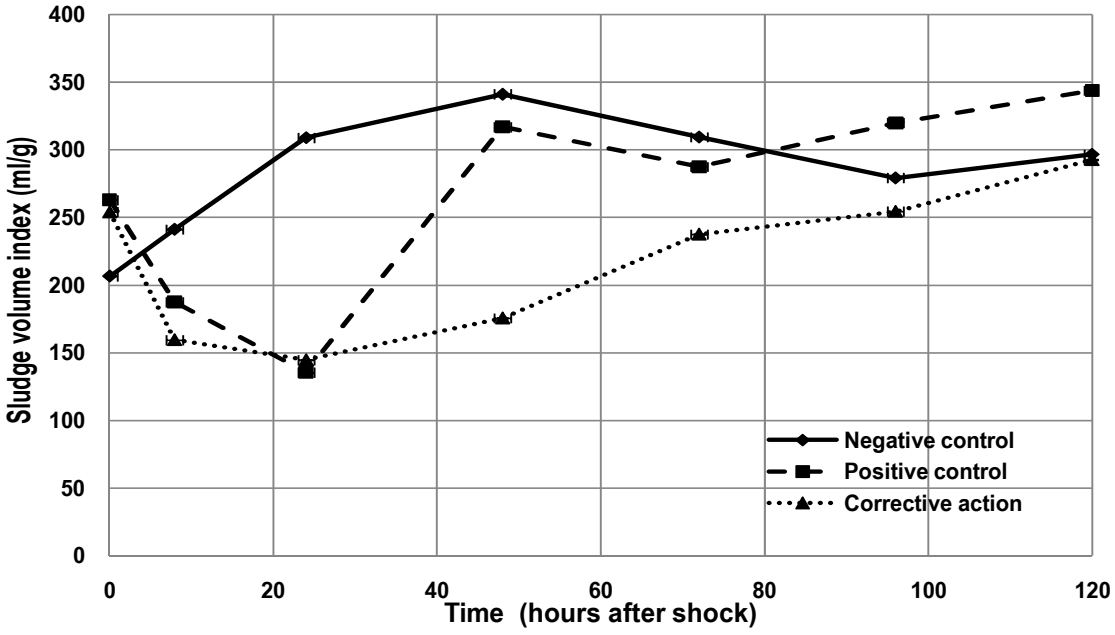

Figure B.92: Sludge volume index levels for C-SDS_2

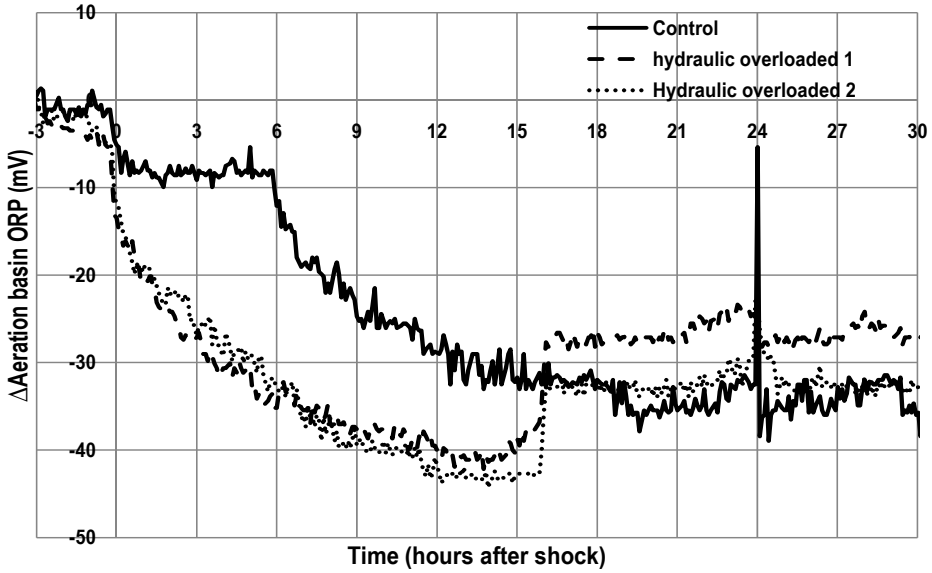

Figure B.93: Change in aeration basin ORP subject to hydraulic overloading

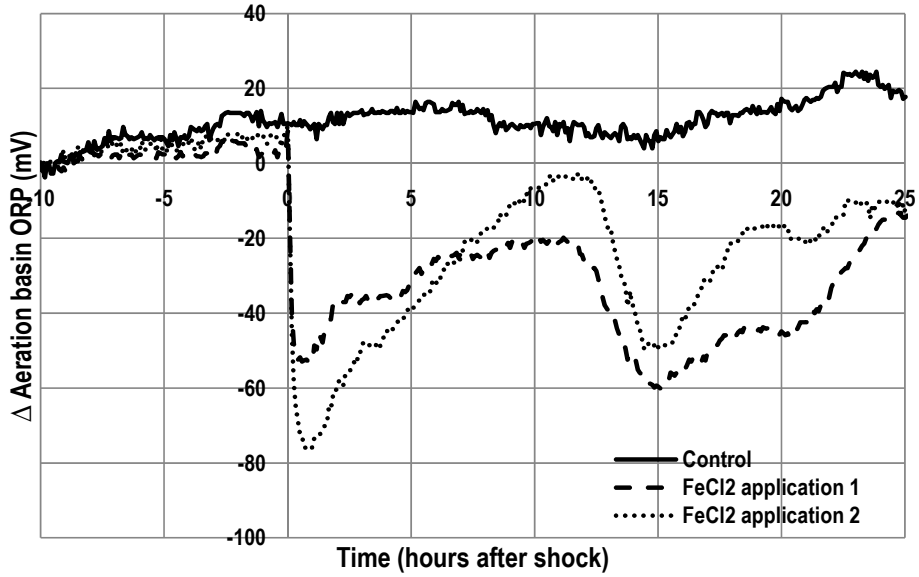

Figure B.94: Change in aeration basin ORP in response to $FeCl_2$ addition

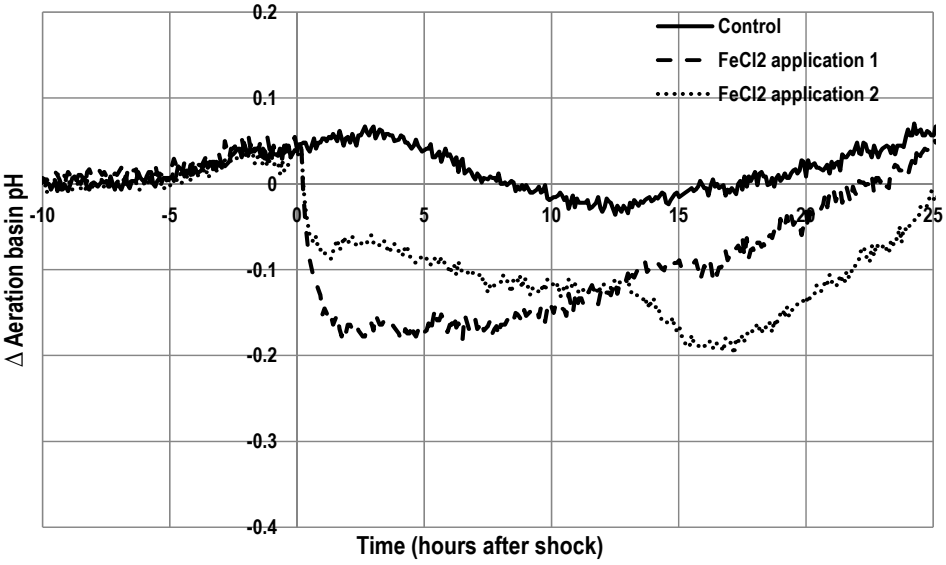

Figure B.95: Change in aeration basin pH in response to $FeCl_2$ addition

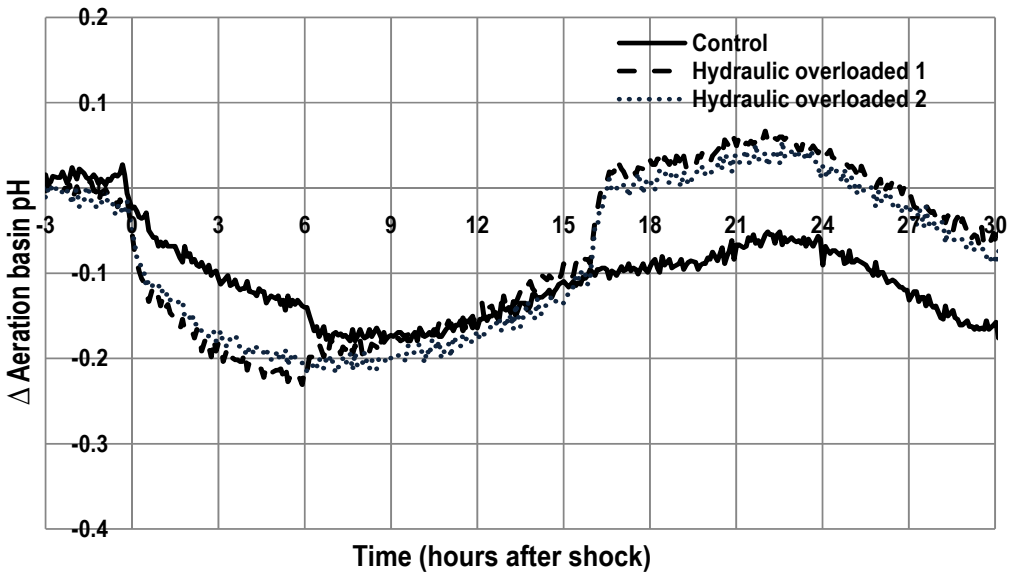

Figure B.96: Change in aeration basin pH subject to hydraulic overloading

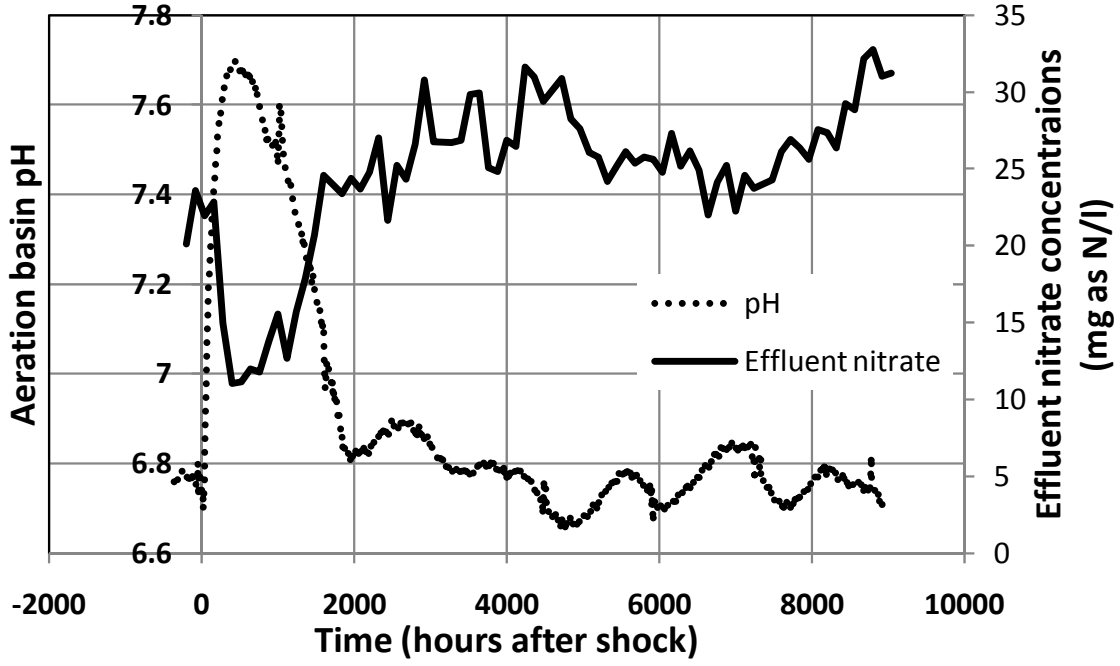

Figure B.97: Comparing aeration basin pH and effluent nitrate concentrations for cadmium stressed reactor

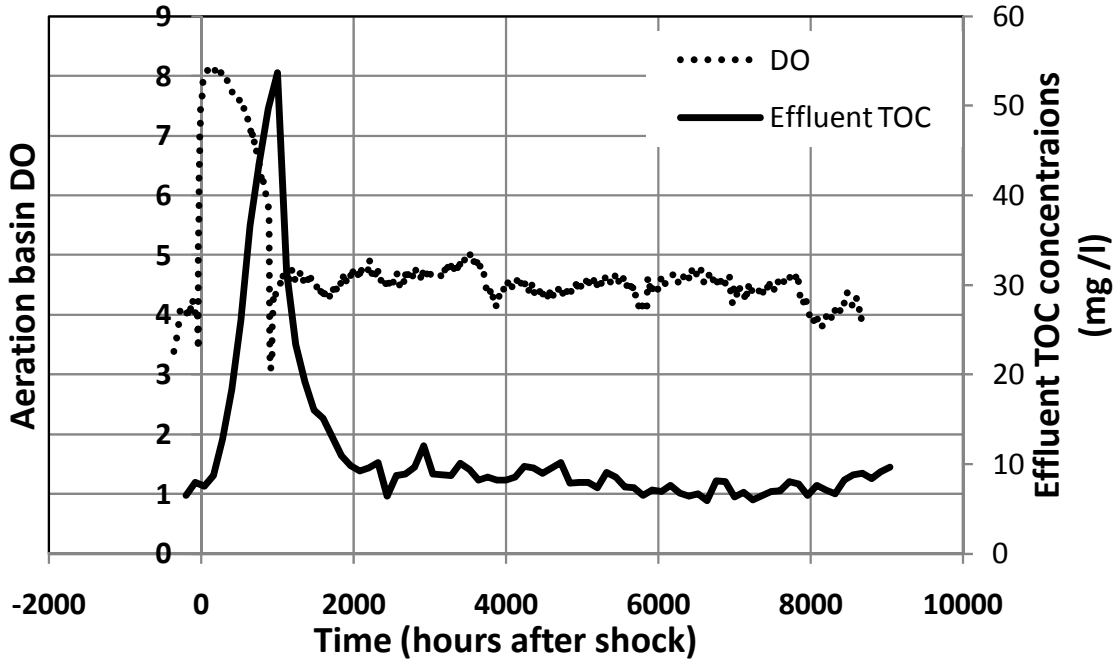

Figure B.98: Comparing aeration basin DO and effluent TOC concentrations for cadmium stressed reactor

Appendix C

Data in support of Chapter 5.0

Table C.1: Mixed liquor sOUR levels for laboratory scale cadmium stress 1

Mixed liquor specific oxygen uptake rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
-3	36.02	0.62	36.02	0.62	36.02	0.62
-2	29.19	1.16	29.19	1.16	29.19	1.16
-1	34.58	0.50	34.58	0.50	34.58	0.50
0	25.47	0.81	25.47	0.81	25.47	0.81
0.33	31.15	0.02	2.62	0.07	1.95	1.48
1	27.85	0.87	9.94	0.04	8.85	0.28
3	35.59	1.88	28.96	0.43	23.00	0.36
4	33.17	0.75	32.14	1.26	27.18	0.38
5	27.99	1.13	39.67	0.02	36.81	0.71
6	26.84	0.80	42.75	0.04	34.10	0.02
8	24.28	1.13	39.47	0.22	39.49	1.98
9	22.45	0.02	33.32	1.61	34.73	1.50
12	35.49	0.83	37.83	0.02	41.36	1.63
14	-	-	34.97	0.59	40.69	1.53

Table C.2: Mixed liquor sNGR levels for laboratory scale cadmium stress 1

Mixed liquor specific nitrite generation rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr
-2	1.39	0	1.39	0	1.39	0
-1	1.1	0.01	1.1	0.01	1.1	0.01
0	1.03	0	1.03	0	1.03	0
0.33	1.05	0.02	0	0.01	0.01	0
1	1.21	0.1	0.2	0.02	0.23	0.02
2	1.07	0.05	0.55	0.02	0.38	0.01
3	1.11	0.04	0.52	0.03	0.48	0.03
4	1.3	0.03	1.08	0.04	0.78	0.01
5	1.02	0.03	1.21	0.1	1.13	0.06
6	1.06	0.04	1.41	0.12	1.6	0.02
8	0.85	0.03	1.83	0.06	1.91	0.05

11	1.03	0.03	1.47	0	1.77	0
12	1	0	1.37	0.04	1.58	0.03
13	0	0	1.51	0.02	1.48	0.01

Table C.3: Mixed liquor sOUR levels for laboratory scale cadmium stress 2

Mixed liquor specific oxygen uptake rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
-2.0	36.75	0.93	36.75	0.93	36.75	0.93
-1.0	33.96	1.70	33.96	1.70	33.96	1.70
0.0	22.19	0.35	22.19	0.35	22.19	0.35
0.3	30.83	0.35	2.06	0.10	0.25	0.15
1	30.82	1.13	9.32	0.26	4.77	0.84
2	32.00	0.78	21.00	0.14	16.58	0.71
3	31.35	1.81	28.73	2.44	27.37	1.34
5	29.65	0.17	40.91	0.80	38.98	1.02
6	32.01	0.36	44.93	0.66	42.17	1.26
7	21.36	1.09	28.98	0.63	46.77	3.52
9			38.57	2.62	33.09	2.95
11	30.82	1.53	32.42	0.59	30.77	0.00
13	22.40	0.27	33.60	0.36	42.64	2.25

Table C.4: Mixed liquor sNGR levels for laboratory scale cadmium stress 2

Mixed liquor specific nitrite generation rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr
-1	1.84	0.01	1.84	0.01	1.84	0.01
0	1.3	0	1.3	0	1.3	0
0.33	1.39	0.02	0	0	0	0
1	1.24	0.03	0.27	0	0.24	0.05
2	1.12	0.03	0.62	0.04	0.39	0
3	1.21	0.02	0.86	0.02	0.58	0.06
4	1.37	0.01	1.57	0	0.87	0
5	1.44	0.03	2.4	0	1.37	0
6	1.56	0	3.61	0.21	3.2	0.14
7	1.43	0.1	2.82	0.07	3.17	0.13
9	1.07	0.03	1.84	0.01	1.83	0
11	1.35	0.02	1.38	0	1.32	0.05
13	1.31	0.06	1.19	0.02	2	0.04

Table C.5: Mixed liquor sOUR levels for pilot scale cadmium stress 1

Mixed liquor specific oxygen uptake rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
0	8.01	0.12	7.87	0.12	6.69	0.06
0.33	7.33	0.08	7.55	0	3.03	0.03
1	7.17	0.18	7.79	0.02	14.69	0.16
2	7.99	0.04	6.85	0.05	7.62	0.24
3	9.43	0.11	9.25	0.18	9.19	0.31
4	10.98	0.1	11.43	0.36	9.67	0.03
5	9.75	0.09	9.75	0.46	11.63	0.26
6	13.17	0.3	12.6	0.23	9.74	0.12

Table C.6: Mixed liquor sNGR levels for pilot scale cadmium stress 1

Mixed liquor specific nitrite generation rate						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr
0	1.44	0	1.43	0.16	1.67	0.06
0.33	1.35	0.01	0.14	0.02	0.14	0.01
1	1.16	0.06	1.14	0.04	1.07	0.04
2	1.26	0.02	1.28	0.01	1.15	0.04
3	1.38	0.12	1.62	0.03	1.57	0.05
4	1.33	0.04	1.71	0.01	1.7	0.08
5	1.34	0.11	1.71	0.04	1.71	0.02
6	1.36	0.03	1.54	0.24	1.41	0.01

Table C.7: Mixed liquor sOUR levels for pilot scale cadmium stress 2

Mixed liquor specific oxygen uptake rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr	mgO_2/gss.hr
0	10.99	0.1	10.89	0.09	8.43	0.02
0.33	10.9	0	7.65	0	4.66	0.02
1	9.99	0.12	8.95	0.05	14.22	0.05
2	9.26	0.16	7.52	0.1	7.97	0.14
3	11.03	0.11	9.12	0.03	9.4	0.06
4	10.04	0.17	9.29	0.13	8.2	0.02
5	9.6	0.06	9.6	0.16	9.4	0.06
6	9.8	0	9.97	0.17	9.21	0.25

Table C.8: Mixed liquor sNGR levels for pilot scale cadmium stress 2

Mixed liquor specific nitrite generation rate						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr
0	1.3	0.05	1.28	0.1	1.41	0
0.33	1.33	0.11	0.19	0	0.16	0.02
1	1.32	0.06	1.1	0.03	1.01	0.05
2	1.22	0.08	1.33	0.03	1.11	0.03
3	0.96	0.17	1.53	0.05	1.46	0.25
4	1.06	0.04	1.54	0.13	1.57	0.04
5	1.07	0.04	1.31	0.09	1.31	0.09
6	1.13	0.12	1.23	0.13	1.41	0

Table C.9: Effluent TSS for laboratory cadmium stress 3

Effluent total suspended solids						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	40	0	40	0	40	0
-2	36.5	6.36	36.5	6.36	36.5	6.36
-1	34	1.41	34	1.41	34	1.41
0	5	0	3	2.83	8.5	0.71
1	24	1.41	71.5	2.12	95	0
2	39	1.41	72	0	22.5	0.71
3	18	4.24	68	4.24	79.5	0.71
4	41.5	7.78	68.5	2.12	58	0
5	72	7.07	49	4.24	19	5.66
6	21	5.66	14.5	2.12	17.5	3.54
7	20.5	0.71	18.5	0.71	53.5	0.71
8	45.5	3.54	26.5	4.95	19	2.83
9	27.5	2.12	28	1.41	18.5	2.12
10	25.5	2.12	18	0	15	7.07
11	34	0	29	1.41	37.5	7.78
12	36	4.24	29	0	49.5	0.71
13	21	2.83	18.5	0.71	26.5	2.12
14	26	2.83	36	4.24	34	1.41
15	47	1.41	31.5	0.71	27.5	2.12
16	30.5	4.95	19	1.41	28	1.41
17	40.5	0.71	17	0	42	2.83
18	15	1.41	12	0	20.5	2.12
19	19.5	0.71	18.5	0.71	18	4.24
20	23.5	0.71	12.5	3.54	20.5	0.71

21	30.5	7.78	15	0	24.5	2.12
22	28	1.41	17	2.83	19	1.41
23	26	4.24	16	0	12.5	2.12
24	34.5	0.71	18	4.24	49	1.41
25	35	0	15	0	26.5	0.71
26	15	0	15	1.41	55	0
27	14	0	16	0	19.5	0.71
28	13	5.66	14.5	0.71	17	2.83

Table C.10: MLSS for laboratory cadmium stress 3

Mixed liquor suspended solids						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	2170	57	2170	57	2170	57
-2	2175	50	2175	50	2175	50
-1	2565	21	2565	21	2565	21
0	2565	0	2565	0	2565	0
0.33	2590	28	2535	92	1940	42
1	2350	42	2355	191	2150	99
2	2430	57	2600	99	2385	64
3	2405	92	2755	106	2185	247
4	2395	106	2735	148	2500	28
5	2425	120	2625	120	2460	71
6	2235	35	2250	28	2025	35
7	2060	14	2360	28	2105	50
8	2185	21	2525	35	2400	14
9	2195	7	2445	21	2340	28
10	2000	57	2265	163	2125	92
11	2090	28	2415	134	2660	665
12	2180	113	2440	71	2115	35
13	2005	35	2370	113	2170	0
13	2075	50	2230	57	2010	28
14	1795	7	2040	99	2050	28
15	2060	28	2055	64	2035	35
16	2100	28	2095	7	2175	21
17	1860	42	2025	7	2025	64
18	1865	7	2000	28	2060	14
19	1770	42	1975	92	1995	21
20	1805	7	2100	14	2115	7
21	1695	21	2120	0	2145	7
22	1500	28	1975	78	2000	28
23	1560	28	1995	35	2110	42
24	1585	21	2040	14	2025	7
25	1540	0	1925	21	1935	7
26	1525	21	1880	0	1720	42

27	1360	57	1880	0	1645	21
28	1485	7	1755	7	1685	21

Table C.11: MLVSS for laboratory cadmium stress 3

Mixed liquor volatile suspended solids						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	1760	40	1760	40	1760	40
-2	1670	10	1670	10	1670	10
-1	2085	5	2085	5	2085	5
0	2085	0	2085	0	2085	0
0.33	2160	40	2125	65	1595	25
1	1940	20	1985	65	1775	55
2	2105	15	2120	30	1995	35
3	1995	45	2225	65	1800	170
4	2025	55	2230	50	1980	50
5	1975	25	2160	40	1995	45
6	1925	5	1840	10	1715	15
7	1690	10	1960	30	1785	45
8	1865	35	2060	30	1925	15
9	1800	30	1965	15	1950	30
10	1760	0	1875	25	1745	65
11	1815	35	1945	75	2265	535
12	1860	20	1995	5	1750	20
13	1705	15	1990	40	1815	15
13	1775	35	1865	15	1675	15
14	1555	5	1695	45	1700	20
15	1765	25	1725	15	1715	15
16	1845	45	1770	0	1800	10
17	1590	0	1705	25	1685	15
18	1655	25	1700	20	1735	15
19	1570	40	1665	65	1670	10
20	1595	15	1795	25	1805	5
21	1490	10	1805	15	1830	10
22	1300	20	1690	30	1710	20
23	1385	15	1735	5	1835	25
24	1395	25	1720	20	1710	0
25	1315	5	1685	15	1660	20
26	1340	10	1625	5	1470	20
27	1210	30	1615	15	1430	0
28	1295	15	1520	10	1450	10

Table C.12: SVI for laboratory scale cadmium shock 3

Sludge volume index			
	UC	SR1	SR2
Days	Average	Average	Average
	ml/g	ml/g	ml/g
-3	88.48	88.48	88.48
-2	88.28	88.28	88.28
-1	81.09	81.09	81.09
0	81.09	81.09	81.09
0.33	67.95	88.36	90.72
1	88.51	98.51	96.74
2	88.89	92.31	90.57
3	86.49	90.02	95.19
4	93.53	106.76	89.6
5	92.37	112.76	91.06
6	103.8	124.44	114.57
7	120.39	152.54	117.81
8	131.81	120.4	120
9	105.69	124.34	99.15
10	120	123.62	112.94
11	107.18	125.88	84.21
12	99.08	127.87	102.13
13	109.73	165.4	101.38
14	120.33	137.25	105.37
15	100.97	128.47	102.21
16	102.86	126.01	99.31
17	86.02	122.47	79.01
18	102.95	128	93.2
19	112.99	176.2	100.25
20	124.1	148.57	105.91
21	117.99	113.21	93.24
22	154.67	141.77	116
23	133.33	148.37	98.58
24	121.14	117.65	94.81
25	129.87	108.05	103.36
26	110.16	140.43	97.67
27	129.41	127.66	106.99
28	129.29	145.87	113.95

Table C.13: Effluent alkalinity for laboratory scale cadmium shock 3

Effluent alkalinity						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3.00	301.62	1.22	301.62	1.22	301.62	1.22
0.00	141.28	1.42	141.28	1.42	141.28	1.42
0.33	163.98	0.20	237.97	0.81	308.91	0.41
0.67	175.34	1.01	326.35	0.41	416.14	1.42
1.00	208.98	0.20	323.71	0.20	378.04	1.01
1.50	184.46	2.03	307.90	0.20	314.39	0.20
2.00	174.52	0.20	258.65	2.43	239.59	1.62
2.50	162.36	0.20	190.13	0.00	196.82	0.20
3.00	167.84	0.41	183.65	0.81	212.63	0.61
4.00	174.73	0.41	179.19	0.41	175.54	0.81
5.00	210.61	0.20	217.09	0.20	220.74	0.61
6.00	228.85	0.20	249.73	0.41	251.55	2.23
7.00	163.58	1.82	168.85	0.20	169.46	0.00
8.00	196.62	0.00	202.50	0.61	201.89	0.81
9.00	251.15	0.20	253.17	0.20	256.01	0.20
10.00	186.28	0.61	185.07	1.01	190.13	0.41
11.00	173.31	0.61	172.30	0.00	178.58	0.20
12.00	181.01	1.01	184.25	1.42	187.29	0.81
13.00	214.05	0.81	212.43	0.81	211.21	0.41
13.30	253.38	0.00	250.54	0.81	260.47	1.01
13.70	171.08	0.41	257.43	0.00	279.93	0.20
14.00	164.80	0.20	185.67	0.81	234.12	0.20
14.50	165.20	0.61	170.47	0.20	168.04	1.82
15.00	164.19	0.00	166.42	0.20	167.43	0.81
15.50	131.96	0.20	134.80	1.01	141.89	0.41
16.00	131.55	0.20	135.00	0.00	135.20	0.61
17.00	159.12	0.20	152.03	0.00	160.94	0.41
18.00	152.23	0.20	151.82	0.20	152.63	0.61
19.00	203.11	0.00	204.12	0.20	205.54	0.81
20.00	190.94	0.00	194.39	0.20	203.92	1.62
21.00	157.30	0.00	155.88	0.20	158.31	0.20
22.00	166.21	0.00	167.02	1.22	165.40	0.41
23.00	163.98	0.20	163.38	0.00	161.55	0.20
24.00	173.92	0.41	168.04	0.20	168.44	0.20
25.00	176.75	0.00	171.89	0.41	171.69	0.20
26.00	233.20	0.40	230.40	0.80	231.00	0.20

Table C.14: Mixed liquor pH for laboratory scale cadmium shock 3

Mixed liquor pH			
	UC	SR1	SR2
Days	Average	Average	Average
-3	7.90	7.90	7.88
-2	7.90	7.89	7.90
-1	7.85	7.84	7.84
0	7.73	7.73	7.73
0	7.48	7.96	8.15
1	7.52	8.09	8.25
1	7.57	7.83	8.00
2	7.55	7.99	7.98
2	7.39	7.67	7.70
3	7.48	7.50	7.60
3	7.42	7.49	7.69
4	7.47	7.46	7.52
5	7.66	7.66	7.72
6	7.60	7.64	7.72
7	7.36	7.36	7.41
8	7.59	7.57	7.66
9	7.65	7.66	7.96
10	7.50	7.47	7.57
11	7.51	7.48	7.57
12	7.52	7.49	7.57
13	7.64	7.59	7.67
14	7.58	7.52	7.75
15	7.63	7.48	7.60
16	7.27	7.27	7.35
17	7.53	7.46	7.56
18	7.49	7.45	7.56
19	7.63	7.63	7.74
20	7.61	7.61	7.70
21	7.52	7.50	7.61
22	7.53	7.51	7.62
23	7.51	7.50	7.60
24	7.57	7.53	7.67
25	7.55	7.51	7.64
26	7.75	7.74	7.85
27	7.61	7.57	7.71
28	7.46	7.43	7.56

Table C.15: Mixed liquor DO for laboratory scale cadmium shock 3

Mixed liquor DO			
	UC	UC	UC
Days	Average	Average	Average
	mg/l	mg/l	mg/l
-3	5.90	5.40	5.50
-2	5.80	5.30	5.60
-1	5.20	5.10	5.00
0	5.60	5.70	5.90
0	5.20	6.50	6.50
1	5.60	6.40	6.70
1	5.10	5.10	5.50
2	4.90	5.90	5.80
2	4.80	5.00	5.30
3	4.50	4.50	4.80
3	5.00	5.20	5.60
4	5.30	4.80	5.40
5	5.90	5.40	6.00
6	4.90	4.60	5.20
7	5.20	4.70	5.30
8	5.20	4.70	5.50
9	4.80	4.40	6.40
10	4.90	4.30	5.00
11	5.50	4.40	5.10
12	6.10	4.50	5.20
13	5.00	4.50	5.20
14	5.20	4.60	5.50
15	4.90	5.50	5.40
16	4.40	4.70	5.30
17	5.20	4.70	5.60
18	4.90	4.40	5.10
19	5.20	4.80	5.60
20	5.20	4.60	5.30
21	5.10	4.30	5.20
22	5.00	4.20	5.10
23	4.70	4.30	5.30
24	4.60	4.10	5.10
25	4.30	4.00	4.00
26	5.40	5.00	5.70
27	5.10	4.80	5.60
28	4.20	3.90	5.10

Table C.16: Effluent pH for laboratory scale cadmium shock 3

Effluent pH			
	UC	SR1	SR2
Days	Average	Average	Average
-3	7.78	7.77	7.77
-2	7.79	7.80	7.72
-1	7.83	7.84	7.80
0	7.65	7.63	7.58
0	7.43	7.87	8.09
1	7.46	8.02	8.21
1	7.52	7.74	7.87
2	7.50	7.89	7.82
2	7.34	7.56	7.53
3	7.39	7.39	7.47
3	7.37	7.43	7.49
4	7.40	7.39	7.36
5	7.59	7.62	7.65
6	7.56	7.64	7.68
7	7.30	7.33	7.35
8	7.51	7.53	7.59
9	7.56	7.60	7.74
10	7.47	7.42	7.48
11	7.47	7.43	7.47
12	7.46	7.44	7.46
13	7.60	7.55	7.60
14	7.52	7.32	7.59
15	7.48	7.41	7.52
16	7.20	7.18	7.28
17	7.39	7.42	7.44
18			
19	7.55	7.59	7.69
20	7.51	7.55	7.63
21	7.43	7.44	7.54
22	7.44	7.46	7.55
23	7.42	7.43	7.54
24	7.47	7.46	7.59
25	7.46	7.45	7.56
26	7.66	7.68	7.80
27	7.52	7.51	7.60
28	7.38	7.39	7.47

Table C.17: Effluent ammonium-N for laboratory scale cadmium shock 3

Effluent ammonia						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	0	0.04	0	0.04	0	0.04
-2	0	0.02	0	0.02	0	0.02
-1	0.62	0.07	0.62	0.07	0.62	0.07
0	0	0.04	0	0.04	0	0.04
0.33	0.09	0.05	14.22	0.95	25.89	0.52
0.67	0.09	0.01	23.63	0.31	37.31	1.33
1	0.11	0.03	17.01	0.18	23.63	0.16
1.5	0.17	0.01	19.59	0.78	18.23	0.77
2	0.15	0.05	12.23	0.36	9.45	0.03
2.5	0.09	0.01	1.64	0.03	3.88	0.12
3	0.11	0.01	0.15	0.02	1.46	0.06
3	0.09	0.04	0.16	0.01	0.27	0.06
5	0.13	0.01	0.17	0.02	0.02	0.01
6	0.13	0.02	0.12	0.01	0.1	0.1
7	0.11	0.02	0.13	0.01	0.04	0.01
8	0.19	0.12	0.27	0.04	0.23	0.01
9	0.23	0.02	0.34	0.12	0.32	0.04
10	0.34	0.04	0.33	0.08	0.32	0.01
11.00	0.24	0.14	0.25	0.01	0.11	0.01
12.00	0.41	0.03	0.28	0.01	0.08	0.01
13.00	0.25	0.04	0.35	0.07	0.1	0.02
13.30	0.07	0.25	11.44	0.85	12.12	0.38
13.67	0.21	0	14.17	0.44	15.48	0.42
14.00	0.19	0.04	4.29	0.19	9.66	0.41
14.50	0	0.27	0.45	0.58	0.4	0.15
15.00	0.33	0.02	0.22	0.01	0.09	0.07
15.50	0.26	0.32	0.36	0.21	0.1	0.37
16.00	0.24	0.04	0.21	0.02	0.17	0.06
17.00	0.36	0.1	0.25	0.02	0.12	0.06
18.00	0.49	0.04	0.4	0.05	0.22	0.08
19.00	0.24	0.01	0.2	0.05	0.17	0.07
20.00	0.23	0.12	-0.11	0.03	0.07	0.08
21.00	0.32	0.01	0.33	0.02	0.25	0.07
22.00	0.31	0.04	0.33	0.04	0.17	0.05
23.00	0.3	0.06	0.97	0.01	0.11	0.02
24.00	0.37	0.08	0.42	0.04	0.48	0.24
25.00	0.48	0.19	0.31	0.01	0.24	0.17
26.00	0.48	0.16	0.32	0.21	0.38	0.39
27.00	0.29	0.28	0.37	0.15	0.42	0.43
28.00	0.32	0.22	0.24	0.27	0.19	0.26

Table C.18: Effluent nitrate-N for laboratory scale cadmium shock 3

Effluent nitrate						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	24.06	0.04	24.06	0.04	24.06	0.04
-2	32.28	0.00	32.28	1.36	32.28	1.36
-1	26.70	0.69	26.70	0.69	26.70	0.69
0	41.15	1.32	41.15	1.32	41.15	1.32
0.33	40.09	0.00	23.98	0.05	13.63	0.53
0.67	40.29	0.17	14.50	0.02	4.86	0.02
1	33.87	0.05	16.35	0.02	9.53	0.01
1.5	37.35	0.00	14.71	0.00	15.09	0.00
2	40.12	0.00	24.46	0.00	26.61	0.62
2.5	40.45	0.02	34.95	0.07	32.47	0.19
3	39.72	0.02	35.85	0.02	29.75	0.06
4	36.45	0.00	34.99	0.04	35.23	0.26
5	29.65	0.10	30.20	0.00	30.65	0.10
6	30.70	0.00	26.70	0.00	26.80	0.00
7	37.77	0.09	37.47	0.02	36.72	0.07
8	31.16	0.06	31.69	0.03	30.97	0.81
9	23.04	0.04	22.27	0.02	21.15	0.06
11	40.36	0.00	39.73	0.00	38.99	0.21
12	39.95	0.06	39.38	0.08	39.21	0.15
13	37.68	0.08	37.58	0.05	36.89	0.09
13.3	41.84	0.01	29.40	0.00	26.88	0.26
13.67	45.69	0.03	28.65	0.18	25.26	0.00
14	41.47	0.28	37.12	0.59	29.40	0.26
14.5	38.94	0.00	36.76	0.00	35.88	0.00
15	40.07	0.00	36.76	0.00	38.05	0.00
15.5	41.15	0.00	37.29	0.00	37.16	0.00
16	38.64	0.00	40.64	0.00	37.14	0.11
17	40.42	0.00	42.32	0.00	40.40	0.00
18	42.02	0.00	42.58	0.00	42.80	0.00
19	34.80	0.00	35.26	0.00	34.79	0.00
20	38.92	0.00	38.92	0.00	37.28	0.00
21	39.61	0.01	40.23	0.11	40.47	0.03
22	39.35	0.02	39.82	0.08	40.22	0.08
23	42.43	0.04	43.47	0.07	43.79	0.15
24	38.57	0.08	39.79	0.12	40.81	0.19
25	36.48	0.04	38.16	0.04	38.70	0.04
26	27.77	0.00	29.51	0.02	30.16	0.00
27	29.53	0.02	32.09	0.02	31.49	0.00
28	41.83	0.05	44.47	0.04	44.55	0.00

Table C.19: Effluent nitrite for laboratory scale cadmium shock 3

Effluent nitrite						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	0.48	0	0.48	0	0.48	0
-2	0.04	0.01	0.04	0.01	0.04	0
-1	0.01	0	0.01	0	0.01	0
0	0.04	0	0.04	0	0.04	0
0.33	0.06	0	0.04	0	0	0
1	0.09	0.02	0.06	0	0.01	0
2	0.12	0.01	0.17	0	0.23	0
3	0.24	0	0.4	0.01	0.26	0
4	0.12	0	0.38	0	0.5	0
6	0.1	0	0.22	0	0.32	0
7	0.09	0	0.9	0.01	0.33	0
9	0.11	0.01	0.01	0	0.03	0
10	0.31	0	0.14	0	0.11	0
13	0.21	0.02	0.29	0.01	0.12	0.01
13.67	0.15	0.01	0.02	0	0.05	0.05
14	0	0.01	0.05	0	0.18	0.07
15	0.01	0.01	0.02	0.03	0.04	0.06
16	0.15	0.03	0.03	0.01	0.04	0.05
17	0.17	0.03	0.01	0	0	0
19	0.14	0.03	0.01	0	0.02	0
20	0.16	0.08	0.01	0	0.01	0.01
22	0.26	0.07	0.01	0	0.01	0.01
24	0.28	0.08	0.09	0	0.01	0
26	0.67	0.08	0.48	0	0.47	0
28	0.33	0.08	0.09	0.01	0.06	0

Table C.20: Effluent soluble COD for laboratory scale cadmium shock 3

Effluent chemical oxygen demand						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
-3	23.92	0	23.92	0	23.92	0
-2	36.29	2.47	36.29	2.47	36.29	2.47
-1	35.06	1.62	35.06	1.62	35.06	1.62
0	26.16	2.43	26.16	2.43	26.16	2.43
0.333	29.39	2.43	43.96	2.43	88.45	1.62
0.67	45.56	9.43	81.7	9.43	135.91	3.93
1	22.11	1.62	52.85	0	65.8	1.62
1.5	29.8	0.79	58.29	0.79	53.54	0.79

2	25.35	1.62	53.66	5.66	34.25	0.81
2.5	36.13	2.37	49.58	3.16	53.54	0.79
3	35.34	3.16	39.3	0.79	41.67	1.58
4	43.21	0.79	56.56	0	62.85	3.14
5	22.11	1.62	35.87	4.04	31.82	0
6	35.35	0.79	43.99	0	59.71	3.14
7	23.73	4.85	35.87	2.43	54.47	9.71
8	25.85	3.16	37.71	2.37	46.42	0
9	42.46	3.96	46.42	7.91	31.38	0.79
10	36.92	0.79	46.35	13.36	36.14	0
11	33.78	0.79	36.14	0	39.28	1.57
12	42.42	1.57	36.14	0	36.92	0.79
13	30.73	0.79	39.47	1.59	33.11	1.59
13.33	32.32	0.79	31.52	1.59	29.93	0
13.67	18.01	3.97	21.19	0.79	41.06	3.18
14	37.09	7.15	31.52	0	35.5	2.38
14.5	44.24	4.77	38.68	0.79	30.73	0.79
15	21.99	0	25.96	0.79	38.68	2.38
15.5	30.73	0.79	25.17	1.59	23.58	1.59
16	22.78	0.79	21.99	0	25.96	0.79
17	30.59	0	30.59	1.58	31.38	0.79
18	29.8	0.79	29.8	3.96	38.51	4.75
19	35.34	1.58	33.76	3.16	31.38	0.79
20	30.59	0	42.46	3.96	36.92	1.58
21	16.42	0.79	28.34	0	27.55	2.38
22	28.7	0	27.11	3.19	29.5	0.8
24	25.51	1.59	24.72	0.8	23.92	3.19
26	24.72	0.8	19.93	0.8	23.92	0
28	23.92	3.19	18.34	2.39	20.73	0

Table C.21: Mixed liquor sOUR levels for laboratory scale cadmium shock 3

Mixed liquor specific oxygen uptake rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$	$mgO_2/gss.hr$
-3.0	43.07	0.46	43.07	0.46	43.07	0.46
-2.0	41.60	0.29	41.60	0.29	41.60	0.29
-1.0	39.80	1.04	39.80	1.04	39.80	1.04
0.0	30.85	0.55	30.85	0.55	30.85	0.55
0.3	39.01	2.76	37.82	0.41	3.91	0.08
1	39.99	0.28	25.44	0.44	17.07	0.14
2	35.13	0.27	34.88	0.95	32.83	0.44
3	33.94	0.11	27.26	0.13	36.43	0.60
4	31.45	1.88	34.49	0.91	34.92	2.83
6	30.94	1.26	42.36	1.30	38.92	2.92

7	40.26	1.53	42.57	1.15	30.12	0.54
9	34.00	0.80	52.87	0.60	43.71	1.95
10	30.99	1.91	46.05	0.70	45.01	2.23
13	27.68	1.32	36.86	0.86	34.69	1.44
13.33	32.92	0.78	23.94	0.06	20.94	0.88
14	27.43	0.77	26.81	1.12	26.10	0.86
15	24.63	1.65	24.33	0.68	24.56	0.42
16	28.36	0.23	26.07	0.10	27.33	0.73
17	25.66	0.15	26.15	0.04	28.13	0.04
19	0.00	0.00	37.33	1.48	29.96	0.97
21	25.83	0.99	26.76	0.30	30.30	0.00
23	23.18	1.04	22.25	0.36	24.87	0.11
25	31.05	0.43	23.23	0.02	23.06	0.25
28	0.00	0.00	0.00	0.00	0.00	0.00

Table C.22: Mixed liquor sNGR for laboratory scale cadmium shock 3

Mixed liquor specific nitrite generation rates						
	UC		SR1		SR2	
Days	Average	+/-	Average	+/-	Average	+/-
	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr	$mgNO_2^-$ as N/gss.hr
-3	2.31	0.01	2.31	0.01	2.31	0.01
-2	1.81	0	1.81	0	1.81	0
-1	1.34	0.01	1.34	0.01	1.34	0.01
0	1.58	0.01	1.58	0.01	1.58	0.01
0.33	1.56	0.03	1.54	0	0.01	0.01
1	1.64	0.04	0.63	0.01	0.71	0.03
2	1.53	0.06	1	0.02	1.32	0.03
3	1.59	0.03	1.78	0	1.64	0
4	1.52	0.05	2.05	0	2.17	0.1
6	1.94	0.06	2.6	0.01	2.46	0.02
7	2.01	0.01	2.35	0.1	2.57	0.13
9	1.49	0.02	2.17	0.06	2.73	0.01
10	1.27	0.08	1.56	0.06	1.95	0.04
13	1.53	0.02	1.47	0.05	1.48	0.05
13.33	1.47	0.01	0.12	0.01	0.09	0
14	1.52	0.01	1.3	0.03	1.27	0.05
15	1.36	0.03	1.73	0.01	1.6	0.1
16	1.51	0.06	2.23	0.01	2.08	0.03
17	1.4	0.16	2.22	0.1	2.01	0.08
19	1.63	0.04	2.49	0.03	0	0
20	1.37	0.1	2.15	0.02	2.05	0.07
22	1.51	0	2.05	0.09	1.98	0
24	1.78	0.01	1.6	0.02	1.61	0.23
26	1.28	0.04	1.1	0.04	1.07	0.04
28	1.94	0.03	1.31	0	1.45	0.04

Table C.23: Total cadmium levels for laboratory scale cadmium shock 3

Total cadmium levels				
	SR1		SR2	
Days	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l
0	0	0	0	0
0.33	1.96	0	3.22	0.01
0.67	2.36	0.01	2.69	0.01
1	1.91	0	1.8	0
1.5	3.72	0.01	1.67	0
2	2.73	0.01	1.41	0
2.5	1.82	0	1.48	0
3	1.26	0	1.91	0
4	0.72	0	1.18	0
5	0.26	0	0.59	0
6	0.06	0	0.1	0
13.33	3.15	0.01	3.23	0.01
13.67	2.33	0.01	2.28	0.01
14	1.74	0	1.77	0
14.5	1.34	0	1.51	0
15	1.03	0	0.99	0
15.5	0.82	0	0.98	0
16	0.69	0	0.83	0
17	0.55	0	0.61	0
19	0.09	0	0.1	0
21	0.06	0	0.05	0

Table C.24: Soluble cadmium levels for laboratory scale cadmium shock 3

Soluble cadmium levels				
	SR1		SR2	
Days	Average	+/-	Average	+/-
	mg/l	mg/l	mg/l	mg/l
0	0	0	0	0
0.33	37.93	0.3	59.18	0
0.67	45.08	0.19	57.61	0
1	44.26	0.08	57	0.36
2	70.05	1.02	47.68	0.56
3	60.67	0.22	63.39	0.18
6	37.22	0.13	45.06	0.16
13	24.78	0.08	27.97	0.16
13.33	65.7	0.29	73.73	0.22
13.67	63.12	0.22	71.14	0.45
14	49.24	0.1	65.16	0.22
16	38.87	0.06	47.72	0.18

21	31.93	0.08	35.55	0.06
24	25.75	0.06	28.61	0.06
28	19.47	0.08	19.75	0.06

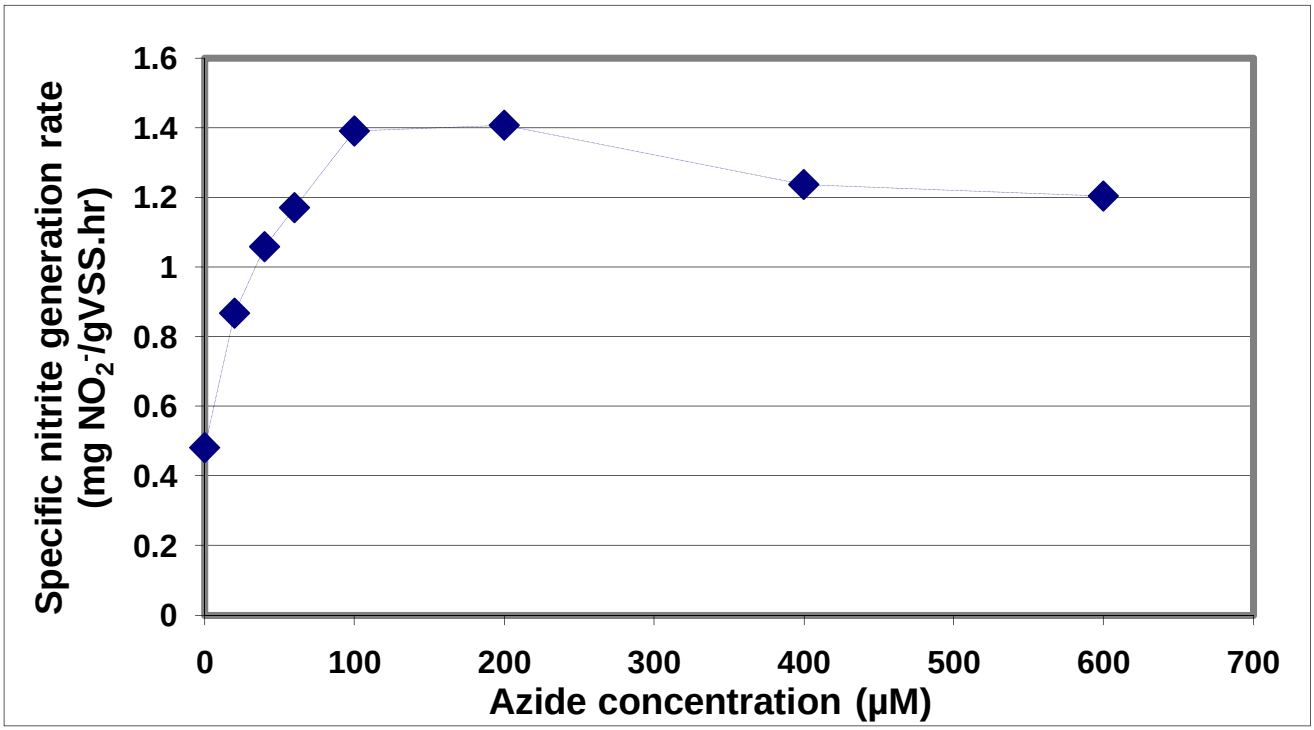

Figure C.1: Optimization of azide concentration for complete inhibition of nitrite oxidizing bacteria: Trial 1

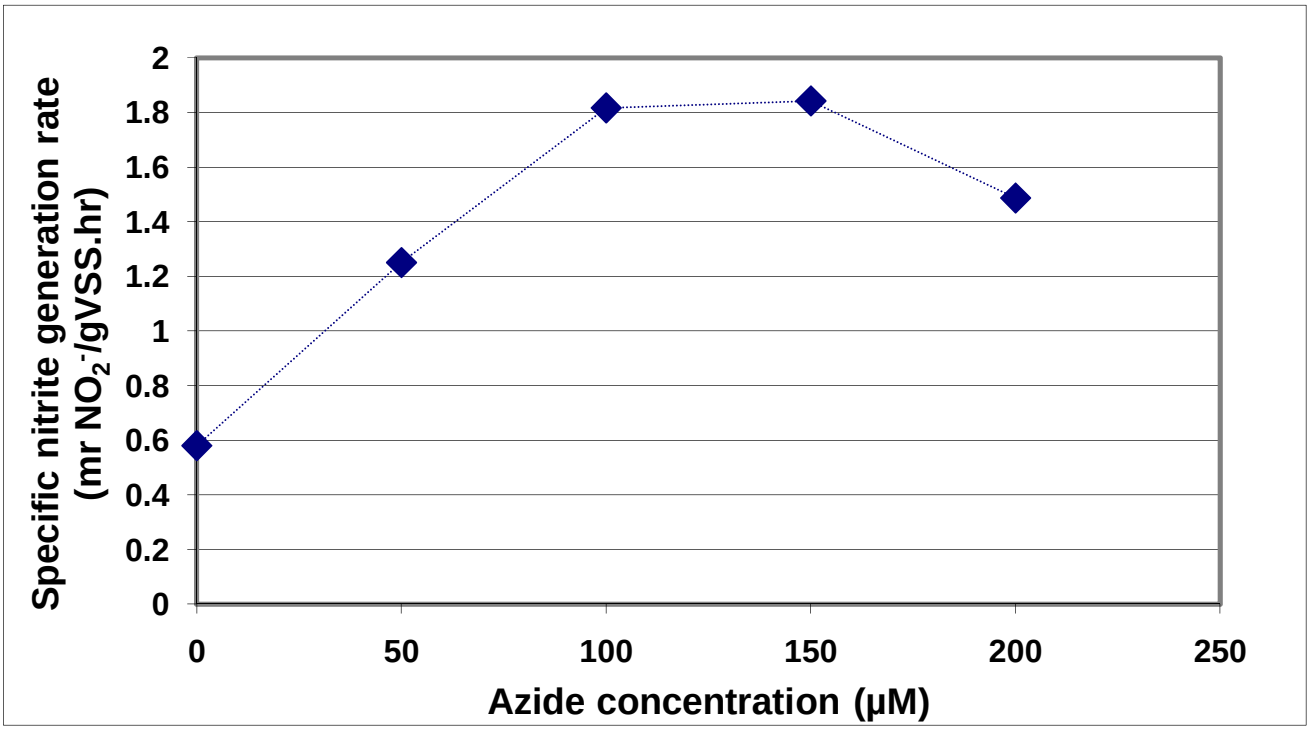

Figure C.2: Optimization of azide concentration for complete inhibition of nitrite oxidizing bacteria: Trial 2

Table C.25: Primer sequences used for qPCR, T-RFLP, and clone library generation

Primer	Target	Orientation	Sequence
For qPCR			
CTO 189(A/B)F	AOB 16SrRNA	Forward	GGA GRA AAG CAG GGG ATC G
CTO 189CF	AOB 16SrRNA	Forward	GGA GGA AAG TAG GGG ATC G
RT1r	AOB 16S rRNA	Reverse	CGT CCT CTC AGA CCA RCT ACTG
Eub338F	General bacteria 16S rRNA	Forward	ACT CCT ACG GGA GGC AGC AG
Univ518r	General bacteria 16S rRNA	Reverse	ATT ACC GCG GCT GCT GG
For T-RFLP			
Eub338F	General bacteria 16S rRNA	Forward	6FAM-ACT CCT ACG GGA GGC AGC AG
Univ1492r	General bacteria 16S rRNA	Reverse	GGT TAC CTT GTT ACG ACT T
Nso1225r	AOB specific 16S rRNA	Reverse	CGC CAT TGT ATT ACG TGT GA
For cloning			
Eub338F	General bacteria 16S rRNA	Forward	6FAM-ACT CCT ACG GGA GGC AGC AG
Nso1225r	AOB specific 16S rRNA	Reverse	CGC CAT TGT ATT ACG TGT GA
amoA-1F	amoA	Forward	GGG GTT TCT ACT GGT GGT
amoA-2R	amoA	Reverse	CCC CTC KGS AAA GCC TTC TTC

Table C.26: Enzyme ranking based on Enzyme Resolving Power Analysis (ERPA). Top 10 enyzymes were further analyzed based on Restriction Enzyme Picker (REPK) analysis of clone library and Taq1 (rank 4) was chosen for further analysis.

Enzyme rank	Enzyme name	Restrict Site	Total good sequence hits	Full Length	Forward fragments		
					Number of fragments	Average length	Range of fragment length
1	HpyCH4V	TG/CA	429	429	45	354.224	169.838
2	Hpy188III	TC/NNGA	429	428	41	425.702	305.815
3	Sau96I	G/GNCC	429	429	39	553.016	85.068
4	TaqI	T/CGA	429	425	39	500.788	175.102
5	BstDEI	C/TNAG	429	296	37	590.196	220.389
6	RseI	CAYNN/NNRTG	429	390	35	802.065	58.09
7	BaeGI	GKGCM/C	429	421	35	690.033	92.235
8	MaeI	C/TAG	429	344	35	437.636	240.743
9	AflIII	A/CRYGT	429	394	28	488.73	148.692
10	FaeI	CATG/	429	429	28	217.601	198.748
12	AspLEI	GCG/C	429	429	27	205.396	120.849
13	HinP1I	G/CGC	429	429	27	203.396	120.849
14	SpeI	A/CTAGT	429	270	26	643.261	199.932
15	MspR9I	CC/NGG	429	429	23	264.914	48.497

Table C.27: Quantitative PCR data for laboratory scale cadmium stress 1

Reactor	Time	DNA	AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
	Days		Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
ALL	-1	106.00	5.56E+05	2.24E+04	5.24E+03	2.11E+02	2.91E+07	4.89E+05	2.74E+05	4.61E+03
SR2	0	104.00	9.04E+05	4.46E+04	8.69E+03	4.29E+02	5.24E+07	2.18E+06	5.04E+05	2.10E+04
SR2	0.33	130.50	9.19E+05	1.25E+05	7.04E+03	9.60E+02	3.00E+07	2.69E+06	2.30E+05	2.06E+04
SR2	1	125.50	1.49E+06	8.21E+04	1.19E+04	6.54E+02	8.78E+07	5.49E+06	6.99E+05	4.37E+04
SR2	2	163.50	4.11E+06	7.89E+04	2.51E+04	4.83E+02	1.38E+08	4.24E+06	8.45E+05	2.60E+04

SR2	3	125.50	2.96E+06	3.24E+05	2.36E+04	2.58E+03	1.03E+08	5.42E+06	8.24E+05	4.32E+04
SR2	4	181.00	4.39E+06	5.86E+05	2.43E+04	3.24E+03	1.50E+08	6.94E+06	8.27E+05	3.83E+04
SR2	5	129.50	3.09E+06	7.24E+04	2.39E+04	5.59E+02	1.04E+08	6.13E+06	8.06E+05	4.73E+04
SR2	6	235.00	4.79E+06	2.71E+05	2.04E+04	1.15E+03	1.50E+08	2.28E+07	6.40E+05	9.70E+04
SR2	8	195.50	4.88E+06	5.32E+05	2.49E+04	2.72E+03	1.62E+08	6.33E+06	8.31E+05	3.24E+04
SR2	10	196.00	2.49E+06	2.63E+05	1.27E+04	1.34E+03	1.06E+08	3.93E+06	5.41E+05	2.01E+04
SR2	12	189.50	3.31E+06	3.39E+05	1.75E+04	1.79E+03	1.42E+08	4.66E+06	7.50E+05	2.46E+04
SR1	0.33	193.00	1.95E+06	1.27E+05	1.01E+04	6.55E+02	9.93E+07	3.05E+06	5.14E+05	1.58E+04
SR1	1	191.00	1.98E+06	9.57E+04	1.04E+04	5.01E+02	1.00E+08	3.50E+06	5.26E+05	1.83E+04
SR1	2	166.00	4.22E+06	1.27E+05	2.54E+04	7.65E+02	1.65E+08	6.11E+06	9.94E+05	3.68E+04
SR1	3	192.00	4.18E+06	5.63E+05	2.18E+04	2.93E+03	1.64E+08	1.60E+07	8.56E+05	8.36E+04
SR1	4	155.00	3.97E+06	1.73E+05	2.56E+04	1.12E+03	1.29E+08	8.44E+06	8.30E+05	5.44E+04
SR1	5	161.50	4.55E+06	1.89E+05	2.81E+04	1.17E+03	1.48E+08	4.88E+06	9.18E+05	3.02E+04
SR1	6	177.50	8.84E+06	4.91E+05	4.98E+04	2.77E+03	2.51E+08	1.30E+07	1.42E+06	7.33E+04
SR1	8	188.50	3.43E+06	1.36E+05	1.82E+04	7.20E+02	1.48E+08	1.23E+07	7.86E+05	6.53E+04
SR1	10	130.50	2.35E+06	6.55E+04	1.80E+04	5.02E+02	1.16E+08	7.08E+06	8.89E+05	5.43E+04
SR1	12	148.00	2.38E+06	2.27E+05	1.61E+04	1.53E+03	1.20E+08	3.49E+06	8.13E+05	2.36E+04
UC	0.33	151.50	1.73E+06	7.76E+04	1.14E+04	5.13E+02	8.88E+07	6.56E+06	5.86E+05	4.33E+04
UC	1	142.50	1.43E+06	6.37E+04	1.00E+04	4.47E+02	7.47E+07	1.55E+06	5.24E+05	1.09E+04
UC	2	190.00	1.15E+06	1.25E+05	6.06E+03	6.57E+02	6.36E+07	6.39E+06	3.35E+05	3.37E+04
UC	3	211.67	1.03E+06	7.15E+04	4.87E+03	3.38E+02	5.53E+07	2.77E+06	2.61E+05	1.31E+04
UC	4	200.50	1.17E+06	8.81E+04	5.85E+03	4.39E+02	5.71E+07	4.18E+06	2.85E+05	2.08E+04
UC	5	226.50	9.24E+05	1.09E+05	4.08E+03	4.82E+02	4.15E+07	9.12E+05	1.83E+05	4.02E+03
UC	6	239.50	1.74E+06	2.04E+05	7.28E+03	8.52E+02	6.73E+07	1.59E+06	2.81E+05	6.64E+03
UC	8	218.50	9.61E+05	4.05E+04	4.40E+03	1.85E+02	3.67E+07	9.63E+05	1.68E+05	4.41E+03
UC	10	157.00	8.59E+05	8.94E+04	5.47E+03	5.69E+02	3.19E+07	3.94E+05	2.03E+05	2.51E+03
UC	12	182.00	7.12E+05	8.06E+04	3.91E+03	4.43E+02	2.48E+07	1.87E+06	1.36E+05	1.03E+04

Table C.28: Quantitative PCR data for laboratory scale cadmium stress 2

Reactor	Time	DNA	AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
	Days		Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
ALL	-2	281.50	1.08E+06	6.58E+04	3.84E+03	2.34E+02	1.33E+08	2.00E+07	4.72E+05	7.10E+04
ALL	-1	263.00	1.08E+06	1.01E+05	4.12E+03	3.86E+02	9.42E+07	7.24E+06	3.58E+05	2.75E+04
ALL	0	255.50	1.04E+06	1.02E+05	4.08E+03	3.99E+02	8.72E+07	9.67E+06	3.41E+05	3.78E+04
SR2	0.33	263.00	1.16E+06	1.11E+05	4.39E+03	4.22E+02	7.34E+07	1.39E+07	2.79E+05	5.30E+04
SR2	1	211.50	9.04E+05	7.96E+04	4.27E+03	3.76E+02	6.31E+07	6.53E+06	2.99E+05	3.09E+04
SR2	2	217.00	2.03E+06	2.09E+05	9.35E+03	9.64E+02	8.41E+07	6.82E+06	3.88E+05	3.14E+04
SR2	3	265.00	5.01E+06	6.31E+05	1.89E+04	2.38E+03	1.70E+08	1.13E+07	6.41E+05	4.27E+04
SR2	4	227.00	5.52E+06	2.56E+05	2.43E+04	1.13E+03	1.38E+08	1.20E+07	6.06E+05	5.31E+04
SR2	5	231.50	6.34E+06	3.75E+05	2.74E+04	1.62E+03	1.84E+08	1.21E+07	7.93E+05	5.25E+04
SR2	6	251.00	7.05E+06	6.84E+05	2.81E+04	2.73E+03	1.64E+08	1.90E+07	6.52E+05	7.55E+04
SR2	7	329.00	7.96E+06	5.04E+05	2.42E+04	1.53E+03	2.27E+08	2.14E+07	6.90E+05	6.50E+04
SR2	9	214.00	6.73E+06	6.43E+05	3.15E+04	3.01E+03	2.49E+08	2.76E+07	1.16E+06	1.29E+05
SR2	11	184.50	3.58E+06	5.06E+05	1.94E+04	2.74E+03	1.75E+08	1.61E+07	9.48E+05	8.73E+04
SR2	13	192.00	1.81E+06	1.20E+05	9.44E+03	6.27E+02	1.03E+08	1.39E+07	5.38E+05	7.26E+04
SR1	0.33	204.50	1.52E+06	2.30E+04	7.41E+03	1.12E+02	1.44E+08	1.77E+07	7.06E+05	8.64E+04
SR1	1	204.00	1.27E+06	1.09E+05	6.22E+03	5.36E+02	9.08E+07	2.94E+06	4.45E+05	1.44E+04
SR1	2	157.33	2.20E+06	1.22E+05	1.40E+04	7.78E+02	1.00E+08	8.32E+06	6.37E+05	5.29E+04
SR1	3	182.00	2.57E+06	2.15E+05	1.41E+04	1.18E+03	1.32E+08	9.13E+06	7.23E+05	5.02E+04
SR1	4	193.67	3.26E+06	2.10E+05	1.68E+04	1.08E+03	1.44E+08	6.11E+06	7.46E+05	3.15E+04
SR1	5	174.33	3.57E+06	1.37E+05	2.05E+04	7.88E+02	1.66E+08	1.28E+07	9.50E+05	7.33E+04
SR1	6	219.67	3.61E+06	2.88E+05	1.64E+04	1.31E+03	1.60E+08	1.95E+07	7.30E+05	8.86E+04
SR1	7	195.67	4.22E+06	5.50E+05	2.16E+04	2.81E+03	1.86E+08	1.04E+07	9.51E+05	5.29E+04
SR1	9	161.00	2.53E+06	1.74E+05	1.57E+04	1.08E+03	1.52E+08	1.53E+07	9.43E+05	9.50E+04
SR1	11	164.67	1.40E+06	1.47E+05	8.53E+03	8.90E+02	9.06E+07	8.24E+06	5.50E+05	5.00E+04
SR1	13	112.00	8.34E+05	1.18E+05	7.44E+03	1.05E+03	6.39E+07	2.32E+06	5.71E+05	2.07E+04
UC	0.33	155.00	1.43E+06	1.94E+05	9.24E+03	1.25E+03	1.16E+08	8.21E+06	7.49E+05	5.30E+04

UC	1	183.00	1.77E+06	2.21E+05	9.68E+03	1.21E+03	1.06E+08	6.04E+06	5.77E+05	3.30E+04
UC	2	251.33	1.54E+06	1.53E+05	6.13E+03	6.08E+02	1.00E+08	4.72E+06	3.99E+05	1.88E+04
UC	3	228.00	1.65E+06	1.46E+05	7.22E+03	6.41E+02	9.75E+07	6.26E+06	4.28E+05	2.75E+04
UC	4	213.33	1.89E+06	1.68E+05	8.87E+03	7.87E+02	1.13E+08	5.43E+06	5.31E+05	2.55E+04
UC	5	229.57	1.74E+06	2.63E+05	7.58E+03	1.14E+03	9.84E+07	3.43E+06	4.28E+05	1.50E+04
UC	6	222.00	2.04E+06	2.59E+05	9.18E+03	1.17E+03	1.21E+08	3.64E+06	5.47E+05	1.64E+04
UC	7	202.00	1.58E+06	1.74E+05	7.84E+03	8.63E+02	8.29E+07	2.55E+06	4.10E+05	1.26E+04
UC	9	149.67	1.17E+06	3.76E+04	7.79E+03	2.51E+02	6.18E+07	4.15E+06	4.13E+05	2.77E+04
UC	11	161.00	9.51E+05	5.15E+04	5.91E+03	3.20E+02	3.64E+07	1.55E+06	2.26E+05	9.66E+03
UC	13	179.67	1.01E+06	8.99E+04	5.62E+03	5.00E+02	4.83E+07	2.20E+06	2.69E+05	1.23E+04

Table C.29: Quantitative PCR data for laboratory scale cadmium stress 3

Reactor	Time	DNA (ng/ µl)	AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
	Days		Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
ALL	-3	405.00	1.20E+06	1.15E+05	2.96E+03	2.84E+02	1.02E+08	1.37E+07	2.52E+05	3.39E+04
ALL	-2	231.33	1.35E+06	9.45E+04	5.83E+03	4.09E+02	1.06E+08	1.01E+07	4.56E+05	4.36E+04
ALL	-1	257.67	1.38E+06	3.64E+04	5.36E+03	1.41E+02	9.65E+07	8.83E+06	3.75E+05	3.43E+04
ALL	0	221.30	8.65E+05	6.28E+04	3.91E+03	2.84E+02	6.28E+07	6.85E+06	2.84E+05	3.09E+04
SR2	0.33	141.00	2.98E+06	6.54E+04	2.11E+04	4.64E+02	2.42E+08	7.67E+06	1.72E+06	5.44E+04
SR2	1	99.00	3.72E+06	3.45E+05	3.75E+04	3.49E+03	3.07E+08	2.36E+07	3.10E+06	2.38E+05
SR2	2	131.50	5.96E+06	5.47E+05	4.54E+04	4.16E+03	4.26E+08	2.26E+07	3.24E+06	1.72E+05
SR2	3	122.00	7.85E+06	5.37E+05	6.43E+04	4.40E+03	5.37E+08	1.70E+07	4.40E+06	1.40E+05
SR2	4	109.00	8.20E+06	1.11E+05	7.53E+04	1.02E+03	4.83E+08	3.43E+07	4.43E+06	3.14E+05
SR2	6	81.50	5.83E+06	3.87E+05	7.15E+04	4.74E+03	3.36E+08	4.23E+07	4.13E+06	5.19E+05
SR2	7	156.00	4.80E+06	6.00E+05	3.08E+04	3.85E+03	5.87E+08	7.51E+07	3.76E+06	4.81E+05
SR2	9	181.50	8.10E+06	7.69E+05	4.46E+04	4.24E+03	6.77E+08	5.47E+07	3.73E+06	3.02E+05
SR2	10	143.00	6.34E+06	4.62E+05	4.43E+04	3.23E+03	4.82E+08	3.58E+07	3.37E+06	2.51E+05
SR2	13	153.50	4.47E+06	3.26E+05	2.91E+04	2.12E+03	3.35E+08	1.11E+07	2.19E+06	7.20E+04
SR2	13.33	123.00	5.51E+06	2.70E+05	4.48E+04	2.19E+03	3.06E+08	2.88E+07	2.49E+06	2.34E+05

SR2	14	126.50	5.64E+06	5.45E+05	4.45E+04	4.31E+03	4.47E+08	5.22E+07	3.54E+06	4.12E+05
SR2	15	135.50	5.31E+06	3.09E+05	3.92E+04	2.28E+03	4.41E+08	4.45E+07	3.25E+06	3.28E+05
SR2	16	125.50	5.36E+06	3.50E+05	4.27E+04	2.79E+03	4.36E+08	5.28E+07	3.48E+06	4.21E+05
SR2	17	134.00	4.80E+06	5.32E+05	3.58E+04	3.97E+03	3.79E+08	2.75E+07	2.83E+06	2.05E+05
SR2	19	151.50	3.68E+06	7.52E+04	2.43E+04	4.96E+02	4.52E+08	2.65E+07	2.98E+06	1.75E+05
SR2	20	175.50	8.87E+06	1.36E+06	5.06E+04	7.74E+03	8.51E+08	4.96E+07	4.85E+06	2.83E+05
SR2	22	183.00	5.44E+06	2.27E+05	2.97E+04	1.24E+03	4.01E+08	1.50E+07	2.19E+06	8.21E+04
SR2	25	131.50	2.62E+06	2.33E+05	1.99E+04	1.77E+03	2.29E+08	1.13E+07	1.74E+06	8.57E+04
SR2	26	161.00	2.86E+06	1.11E+05	1.78E+04	6.91E+02	2.44E+08	1.50E+07	1.51E+06	9.34E+04
SR2	28	148.00	4.99E+06	5.37E+05	3.37E+04	3.63E+03	2.22E+08	1.58E+07	1.50E+06	1.07E+05
SR1	0.33	142.50	1.86E+06	2.37E+05	1.30E+04	1.66E+03	2.35E+08	2.21E+07	1.65E+06	1.55E+05
SR1	1	203.00	6.26E+06	7.33E+05	3.08E+04	3.61E+03	5.24E+08	2.37E+07	2.58E+06	1.17E+05
SR1	2	147.50	8.60E+06	8.00E+05	5.83E+04	5.42E+03	5.14E+08	5.36E+07	3.48E+06	3.63E+05
SR1	3	207.00	9.65E+06	1.04E+06	4.66E+04	5.02E+03	2.74E+08	2.77E+07	1.33E+06	1.34E+05
SR1	4	262.50	1.14E+07	1.05E+06	4.33E+04	3.99E+03	3.60E+08	2.59E+07	1.37E+06	9.86E+04
SR1	6	178.00	6.33E+06	8.00E+05	3.55E+04	4.49E+03	2.47E+08	1.41E+07	1.39E+06	7.91E+04
SR1	7	193.50	6.63E+06	1.75E+05	3.43E+04	9.05E+02	2.71E+08	2.56E+07	1.40E+06	1.32E+05
SR1	9	241.00	6.96E+06	6.05E+05	2.89E+04	2.51E+03	3.35E+08	7.34E+06	1.39E+06	3.05E+04
SR1	10	237.50	6.74E+06	8.07E+05	2.84E+04	3.40E+03	2.98E+08	8.29E+06	1.25E+06	3.49E+04
SR1	13	230.00	4.04E+06	3.56E+05	1.76E+04	1.55E+03	1.69E+08	7.27E+06	7.35E+05	3.16E+04
SR1	13.33	125.50	6.92E+06	9.28E+05	5.52E+04	7.39E+03	2.70E+08	1.17E+07	2.15E+06	9.31E+04
SR1	14	115.00	7.30E+06	8.19E+05	6.35E+04	7.12E+03	2.49E+08	1.22E+07	2.17E+06	1.06E+05
SR1	15	123.00	5.01E+06	4.51E+05	4.07E+04	3.67E+03	2.52E+08	3.06E+06	2.05E+06	2.49E+04
SR1	16	138.50	4.92E+06	5.83E+05	3.55E+04	4.21E+03	1.94E+08	1.55E+07	1.40E+06	1.12E+05
SR1	17	176.00	4.97E+06	5.27E+05	2.82E+04	2.99E+03	1.66E+08	1.50E+07	9.46E+05	8.52E+04
SR1	19	127.00	5.20E+06	5.32E+05	4.10E+04	4.19E+03	2.10E+08	4.98E+06	1.65E+06	3.92E+04
SR1	20	97.00	4.22E+06	4.42E+05	4.35E+04	4.56E+03	1.26E+08	1.54E+06	1.29E+06	1.59E+04
SR1	22	97.00	7.30E+06	2.39E+05	7.53E+04	2.46E+03	1.84E+08	8.82E+06	1.90E+06	9.09E+04
SR1	25	88.00	6.07E+06	3.51E+05	6.90E+04	3.98E+03	1.39E+08	1.04E+07	1.58E+06	1.19E+05
SR1	26	106.00	3.74E+06	4.85E+05	3.53E+04	4.57E+03	9.90E+07	2.87E+06	9.34E+05	2.71E+04

SR1	28	135.00	5.81E+06	3.42E+05	4.31E+04	2.54E+03	1.18E+08	8.78E+06	8.72E+05	6.50E+04
UC	0.33	192.50	2.46E+06	2.75E+04	1.28E+04	1.43E+02	1.40E+08	6.25E+06	7.29E+05	3.25E+04
UC	1	133.50	2.65E+06	8.10E+04	1.98E+04	6.07E+02	1.58E+08	6.38E+06	1.18E+06	4.78E+04
UC	2	124.00	2.49E+06	3.78E+05	2.01E+04	3.05E+03	1.43E+08	4.24E+06	1.15E+06	3.42E+04
UC	3	119.50	2.04E+06	2.30E+05	1.71E+04	1.92E+03	1.06E+08	1.01E+07	8.91E+05	8.45E+04
UC	4	115.00	1.88E+06	1.30E+05	1.63E+04	1.13E+03	8.80E+07	3.72E+06	7.65E+05	3.23E+04
UC	6	131.00	2.87E+06	1.13E+05	2.19E+04	8.64E+02	1.28E+08	4.91E+06	9.79E+05	3.75E+04
UC	7	153.00	2.19E+06	2.13E+05	1.43E+04	1.39E+03	2.13E+08	8.31E+05	1.39E+06	5.43E+03
UC	9	105.50	7.53E+05	9.16E+04	7.14E+03	8.69E+02	2.27E+08	2.57E+07	2.16E+06	2.44E+05
UC	10	130.50	7.18E+05	8.96E+04	5.50E+03	6.86E+02	1.81E+08	2.43E+07	1.38E+06	1.86E+05
UC	13	152.00	7.08E+05	8.11E+04	4.66E+03	5.34E+02	1.82E+08	2.51E+07	1.20E+06	1.65E+05
UC	13.33	134.50	8.01E+05	9.09E+04	5.96E+03	6.76E+02	1.63E+08	1.85E+07	1.21E+06	1.38E+05
UC	14	73.50	5.63E+05	6.62E+04	7.65E+03	9.01E+02	1.48E+08	2.14E+07	2.01E+06	2.91E+05
UC	15	102.00	6.19E+05	7.33E+04	6.07E+03	7.19E+02	1.37E+08	5.27E+06	1.34E+06	5.17E+04
UC	16	111.50	6.12E+05	4.05E+04	5.49E+03	3.63E+02	1.19E+08	5.35E+06	1.07E+06	4.80E+04
UC	17	122.50	6.33E+05	5.70E+04	5.17E+03	4.65E+02	1.30E+08	1.54E+07	1.06E+06	1.25E+05
UC	19	113.50	7.15E+05	2.87E+04	6.30E+03	2.53E+02	9.62E+07	4.98E+06	8.47E+05	4.39E+04
UC	20	170.00	5.35E+05	6.83E+04	3.15E+03	4.02E+02	1.26E+08	9.85E+06	7.44E+05	5.80E+04
UC	22	100.50	4.86E+05	3.08E+04	4.83E+03	3.07E+02	1.22E+08	1.49E+07	1.22E+06	1.49E+05
UC	24	151.00	7.39E+05	4.45E+04	4.89E+03	2.95E+02	1.59E+08	6.12E+06	1.05E+06	4.05E+04
UC	25	121.00	1.55E+06	1.58E+05	1.28E+04	1.30E+03	2.66E+08	2.65E+07	2.20E+06	2.19E+05
UC	26	90.50	5.53E+05	3.67E+04	6.12E+03	4.06E+02	1.13E+08	8.97E+05	1.24E+06	9.91E+03

Table C.30: Quantitative PCR data for pilot scale cadmium stress 1

Reactor	Time	DNA	AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
			Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
ALL	0	138.00	1.14E+06	1.41E+05	8.26E+03	1.02E+03	1.16E+08	4.99E+06	8.44E+05	3.62E+04
SR2	0.33	147.00	1.27E+06	1.63E+05	8.65E+03	1.11E+03	1.14E+08	3.69E+06	7.77E+05	2.51E+04
SR2	1	106.00	1.95E+06	1.60E+05	1.84E+04	1.51E+03	1.72E+08	8.43E+06	1.62E+06	7.95E+04

SR2	2	148.50	2.51E+06	1.28E+05	1.69E+04	8.60E+02	2.23E+08	7.71E+06	1.50E+06	5.19E+04
SR2	3	128.50	2.52E+06	2.49E+05	1.96E+04	1.94E+03	2.17E+08	7.95E+06	1.69E+06	6.19E+04
SR2	4	150.00	2.10E+06	7.28E+04	1.40E+04	4.85E+02	1.82E+08	1.34E+07	1.21E+06	8.96E+04
SR2	5	123.00	2.12E+06	1.43E+05	1.73E+04	1.16E+03	1.85E+08	1.55E+07	1.50E+06	1.26E+05
SR2	6	180.00	2.52E+06	1.35E+05	1.40E+04	7.53E+02	1.70E+08	1.53E+07	9.43E+05	8.51E+04
SR1	0.33	136.50	2.48E+06	2.65E+05	1.82E+04	1.94E+03	2.19E+08	1.06E+07	1.60E+06	7.78E+04
SR1	1	82.50	1.45E+06	1.29E+05	1.76E+04	1.56E+03	1.36E+08	1.59E+06	1.65E+06	1.93E+04
SR1	2	110.00	2.01E+06	7.24E+04	1.83E+04	6.58E+02	1.88E+08	3.14E+06	1.71E+06	2.85E+04
SR1	3	126.00	2.51E+06	2.80E+05	1.99E+04	2.22E+03	2.07E+08	8.04E+06	1.65E+06	6.38E+04
SR1	4	110.50	2.22E+06	1.03E+05	2.00E+04	9.36E+02	1.55E+08	6.53E+06	1.40E+06	5.91E+04
SR1	5	180.00	2.16E+06	1.13E+05	1.20E+04	6.28E+02	1.70E+08	5.80E+06	9.42E+05	3.22E+04
SR1	6	272.50	2.44E+06	1.63E+05	8.97E+03	5.98E+02	1.39E+08	9.12E+06	5.11E+05	3.35E+04
UC	0.33	249.00	2.80E+06	2.26E+05	1.13E+04	9.06E+02	1.62E+08	1.31E+07	6.50E+05	5.26E+04
UC	1	248.00	1.23E+06	1.81E+05	4.95E+03	7.30E+02	1.06E+08	1.46E+06	4.27E+05	5.91E+03
UC	2	250.00	1.25E+06	1.20E+05	5.01E+03	4.81E+02	1.14E+08	8.66E+05	4.56E+05	3.46E+03
UC	3	243.50	1.78E+06	9.53E+04	7.32E+03	3.92E+02	1.34E+08	1.24E+06	5.49E+05	5.08E+03
UC	4	180.50	1.93E+06	1.62E+05	1.07E+04	8.99E+02	1.35E+08	4.30E+06	7.48E+05	2.38E+04
UC	5	213.50	1.72E+06	1.51E+05	8.06E+03	7.09E+02	1.31E+08	3.97E+06	6.15E+05	1.86E+04
UC	6	101.00	1.14E+06	9.37E+04	1.13E+04	9.28E+02	8.73E+07	4.08E+06	8.65E+05	4.04E+04

Table C.31: Quantitative PCR data for pilot scale cadmium stress 2

Reactor	Time	DNA	AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
			Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
ALL	0	72.00	7.85E+05	1.17E+05	1.09E+04	1.63E+03	7.99E+07	6.05E+06	1.11E+06	8.40E+04
SR2	0.33	108.00	1.70E+06	2.54E+05	1.58E+04	2.35E+03	1.05E+08	4.61E+06	9.68E+05	4.27E+04
SR2	1	52.50	1.10E+06	6.46E+04	2.09E+04	1.23E+03	9.99E+07	8.23E+06	1.90E+06	1.57E+05
SR2	2	98.50	1.78E+06	1.54E+05	1.81E+04	1.57E+03	1.94E+08	2.03E+07	1.97E+06	2.06E+05
SR2	3	54.00	1.60E+06	1.16E+05	2.95E+04	2.15E+03	1.44E+08	4.01E+06	2.67E+06	7.43E+04

SR2	4	73.00	2.15E+06	2.20E+05	2.95E+04	3.01E+03	1.62E+08	7.99E+06	2.22E+06	1.09E+05
SR2	5	122.00	2.02E+06	1.54E+05	1.65E+04	1.26E+03	1.54E+08	2.32E+06	1.27E+06	1.90E+04
SR2	6	92.50	2.10E+06	2.13E+05	2.27E+04	2.30E+03	1.36E+08	1.35E+06	1.47E+06	1.46E+04
SR1	0.33	82.00	2.28E+06	2.72E+05	2.78E+04	3.31E+03	1.12E+08	2.95E+06	1.36E+06	3.60E+04
SR1	1	84.50	1.61E+06	1.67E+05	1.91E+04	1.97E+03	9.82E+07	1.26E+07	1.16E+06	1.50E+05
SR1	2	105.00	2.61E+06	3.27E+05	2.48E+04	3.12E+03	2.19E+08	8.28E+06	2.09E+06	7.89E+04
SR1	3	88.50	1.64E+06	2.14E+05	1.85E+04	2.42E+03	1.38E+08	3.52E+06	1.56E+06	3.97E+04
SR1	4	136.00	1.48E+06	1.23E+05	1.08E+04	9.04E+02	1.18E+08	8.28E+05	8.70E+05	6.09E+03
SR1	5	138.00	1.89E+06	1.22E+05	1.37E+04	8.83E+02	1.38E+08	1.10E+06	1.00E+06	7.98E+03
SR1	6	88.50	1.94E+06	1.24E+05	2.20E+04	1.41E+03	1.15E+08	4.90E+06	1.30E+06	5.53E+04
UC	0.33	109.00	1.12E+06	5.26E+04	1.03E+04	4.82E+02	7.86E+07	6.00E+05	7.21E+05	5.50E+03
UC	1	143.50	1.84E+06	1.38E+05	1.28E+04	9.59E+02	9.70E+07	5.29E+06	6.76E+05	3.69E+04
UC	2	166.00	1.52E+06	1.91E+05	9.13E+03	1.15E+03	1.08E+08	8.39E+05	6.48E+05	5.05E+03
UC	3	103.00	1.13E+06	5.16E+04	1.10E+04	5.01E+02	8.41E+07	3.79E+05	8.17E+05	3.68E+03
UC	4	100.00	1.23E+06	8.35E+04	1.23E+04	8.35E+02	8.75E+07	3.52E+06	8.75E+05	3.52E+04
UC	5	78.00	1.37E+06	4.03E+04	1.76E+04	5.17E+02	8.71E+07	4.98E+06	1.12E+06	6.39E+04
UC	6	113.00	1.24E+06	1.02E+05	1.10E+04	9.00E+02	7.14E+07	1.20E+06	6.31E+05	1.06E+04

Table C.32: AOB specific terminal restriction fragments and their relative distribution for laboratory scale cadmium stress 1

	AOB specific Terminal Restriction Fragments and their relative distribution											
	SR2				SR1				UC			
T-RF length (bp)	106	382	485	679	106	382	485	679	106	382	485	679
Day												
-1	63.31	0	19.46	17.23	63.31	0	19.46	17.23	63.31	0	19.46	17.23
0	67.67	0	15.95	16.38	67.67	0	15.95	16.38	67.67	0	15.95	16.38
0.33	73.82	0	14.31	11.86	73.39	0	12.94	13.67	78.51	0	10.32	11.17
1	77.78	0	12.05	10.17	82.34	0	7.63	10.03	76.12	0	13.8	10.07
2	76.46	0	9.58	13.96	81.26	0	7.74	11	84.12	0	15.88	0

3	81.43	0	0	18.57	86.02	4.64	0	9.35	74.83	3.45	13.15	8.58
4	86.55	0	0	13.45	85.92	0	0	14.08	75.76	3.07	13.95	7.22
5	82.9	6.59	0	10.5	86.19	0	0	13.81	67.2	7.17	13.41	12.21
6	88.47	0	0	11.53	80.64	7.58	0	11.78	77.43	3.59	11.22	7.75
8	83.61	7	0	9.39	79.46	10.82	0	9.73	73.84	4.19	13.86	8.11
10	76.77	0	9.65	13.58	73.03	7.59	11.17	8.21	73.56	8.84	9.63	7.96
12	88.83	0	7.75	3.42	72.76	11.42	8.46	7.36	85.22	0	8.65	6.13

Table C.33: AOB specific terminal restriction fragments and their relative distribution for laboratory scale cadmium stress 2

	AOB specific Terminal Restriction fragments and their relative distribution														
	SR2					SR1					UC				
T-RF length (bp)	106	382	485	625	679	106	382	485	625	679	106	382	485	625	679
Day															
-2	76.4	16.15	0	0	7.45	76.4	16.15	0	0	7.45	76.4	16.15	0	0	7.45
-1	76.87	13.5	3.59	0	6.03	76.87	13.5	3.59	0	6.03	76.87	13.5	3.59	0	6.03
0	79.62	7.49	4.42	0	8.47	79.62	7.49	4.42	0	8.47	79.62	7.49	4.42	0	8.47
0.33	82.7	0	6.3	0	11	83.89	6.36	3.52	0	6.23	83.84	0	0	0	16.16
1	77.61	10.11	5.99	0	6.29	89.28	0	0	0	10.72	80.8	0	0	0	19.2
2	80.58	10.27	3.87	0	5.28	85.62	0	0	0	14.38	73.58	0	8.38	0	18.04
3	83.62	10.65	0	0	5.73	83.01	0	0	0	16.99	78	0	8.05	0	13.95
4	78.45	16.01	0	0	5.54	83.16	0	0	0	16.84	85.44	0	0	0	14.56
5	73.83	19.8	0	0	6.37	81.37	0	0	0	18.63	88.22	0	0	0	11.78
6	68.39	20.72	0	0	10.89	69.97	0	0	0	30.03	85.64	0	0	0	14.36
7	65.49	19.25	5	4.21	6.04	80.52	0	0	0	19.48	84.18	0	0	0	15.82
9	63.86	17.07	9.63	4.15	5.29	82.94	0	0	0	17.06	73.93	0	0	0	26.07
11	53.3	15.55	19.33	6.19	5.64	81.86	0	0	0	18.14	74.18	0	10.92	0	14.9

Table C.34: AOB specific terminal restriction fragments and their relative distribution for laboratory scale cadmium stress 3

	AOB specific Terminal Restriction Fragments and their relative distributions											
	SR2				SR1				UC			
T-RF length (bp)	106	382	485	679	106	382	485	679	106	382	485	679
Day												
-3	69.95	0	0	30.05	69.95	0	0	30.05	69.95	0	0	30.05
-2	65.12	0	14.72	20.16	65.12	0	14.72	20.16	65.12	0	14.72	20.16
-1	59.92	0	19.44	20.64	59.92	0	19.44	20.64	59.92	0	19.44	20.64
0	54.03	0	17.42	28.55	54.03	0	17.42	28.55	54.03	0	17.42	28.55
0.33	79.49	0	7.16	13.35	58.75	0	24.96	16.29	62.62	7.19	19.97	10.22
1	81.27	0	4.09	14.63	71.68	7.93	9.51	10.87	69.31	5.86	14.81	10.02
2	73.86	0	6.32	19.82	83.43	0	0	16.57	65.47	5.13	18.18	11.22
3	65.45	11.69	8.37	14.5	83.28	0	0	16.72	77.44	0	13.49	9.07
4	68.27	7.85	9.77	14.1	74.72	0	10.36	9.46	74.94	0	11.28	13.78
6	70.34	9.65	8.73	11.29	66.5	0	23.26	10.24	82.26	0	6.82	10.92
7	73.6	0	14.13	12.28	67.68	0	22.71	9.61	71.75	0	8.54	19.71
9	73.94	0	14.42	11.64	61.94	0	27.78	10.28	78.06	0	8.19	13.75
10	69.14	0	18.67	12.19	67.85	0	21.88	10.26	74.23	0	8.61	17.17
13	73.35	0	14.77	11.88	67.34	0	22.57	10.1	79.33	0	6.49	14.18
13.33	79.22	0	7.47	13.31	81.24	0	11.34	7.42	86.52	0	0	13.48
14	80.47	0	3.57	15.96	85.12	0	8.79	6.09	84.52	0	0	15.48
15	81.92	0	7.47	10.62	84.27	0	7.52	8.21	79.14	0	0	20.86
16	76.79	0	12	11.21	81.76	0	10.04	8.21	85.79	0	0	14.21
17	77.62	0	9.44	12.93	81.19	0	8.49	10.32	80.65	0	0	19.35
19	79.49	0	10.53	9.97	84.28	0	7.17	8.55	79.81	0	0	20.19
20	82.4	0	9.49	8.11	83.25	0	7.58	9.16	78.21	0	0	21.79
22	73.41	0	10.05	16.55	88.08	0	5.04	6.89	78.98	0	0	21.02
25	65.52	0	21.87	12.61	86.09	0	6.17	7.74	89.63	0	0	10.37

26	66.8	0	21.01	12.19	82.49	0	9.69	7.83	72.03	0	12.41	15.57

Table C.35: AOB specific terminal restriction fragments and their relative distribution for pilot scale cadmium stress 1

	AOB specific Terminal Restriction fragments and their relative distribution											
	SR2				SR1				UC			
T-RF length (bp)	106	127	625	679	106	127	625	679	106	127	625	679
Day												
0	75.12	0	0	24.88	75.12	0	0	24.88	75.12	0	0	24.88
0.3	75.12	0	0	24.88	75.9	0	11.27	12.83	86.98	0	0	13.02
1	78.01	0	0	21.99	76.46	0	8.45	15.09	83.21	0	0	16.79
2	80.44	0	0	19.56	75.95	0	0	24.05	74.62	0	0	25.38
3	68.37	11.18	0	20.45	67.31	7	8.64	17.04	83.26	0	0	16.74
4	82.9	0	0	17.1	86.23	0	0	13.77	81.1	0	7.21	11.69
5	84.83	0	0	15.17	83.94	0	0	16.06	86.46	0	0	13.54
6	89.85	0	0	10.15	81.99	0	0	18.01	88.28	0	0	11.72

Table C.36: AOB specific terminal restriction fragments and their relative distribution for pilot scale cadmium stress 2

	AOB specific Terminal Restriction fragments and their relative distribution											
	SR2				SR1				UC			
T-RF length (bp)	106	127	625	679	106	127	625	679	106	127	625	679
Day												
0	83.9	0	0	16.1	83.9	0	0	16.1	83.9	0	0	16.1
0.3	79.18	0	7.6	13.22	87	0	0	13	86.48	0	0	13.52
1	71.57	0	0	28.43	86.16	0	0	13.84	87.8	0	0	12.2
2	73.81	13.48	0	12.71	75.57	6.2	8.93	9.3	86.48	0	0	13.52
3	80.78	9.5	0	9.72	80.96	6.95	0	12.09	83.44	5.5	0	11.06
4	78.7	7.45	0	13.85	78.76	0	0	21.24	89.03	0	0	10.97

5	90.34	0	0	9.66	85.94	0	0	14.06	88.1	0	0	11.9
6	89.47	0	0	10.53	87.84	0	0	12.16	88.68	0	0	11.32

Table C.37: Non dimensionalized matrix scaling using Bray Curtis Similarity Index for T-RFLP profiles for laboratory scale cadmium stress 1

	Non dimensionalized matrix scaling using Bray Curtis Similarity Index					
Time (days)	SR2		SR1		UC	
	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2
-1	-0.26209	-0.08484	-0.26331	-0.08548	-0.26287	-0.08463
0	-0.19497	-0.04867	-0.19665	-0.0495	-0.19603	-0.04907
0.33	-0.08954	-0.00374	-0.08741	-0.02125	-0.00826	-0.01087
1	-0.02788	0.002987	0.056698	-0.02615	-0.04872	0.006269
2	-0.03265	-0.03702	0.042112	-0.02843	-0.05314	0.019019
3	0.1206	-0.13008	0.18968	0.014067	-0.05464	0.073636
4	0.16698	-0.07256	0.16245	-0.07702	-0.05701	0.076181
5	0.17414	0.045185	0.16494	-0.0754	-0.17009	0.092644
6	0.18319	-0.06714	0.16385	0.06526	-0.01672	0.073611
8	0.18087	0.050511	0.1632	0.11362	-0.01997	0.078461
10	-0.02897	-0.03365	-0.04548	0.12995	-0.01903	0.14592
12	0.30999	-0.1239	-0.00886	0.18897	0.065569	-0.06689

Table C.38: Non dimensionalized matrix scaling using Bray Curtis Similarity Index for T-RFLP profiles for laboratory scale cadmium stress 2

	Non dimensionalized matrix scaling using Bray Curtis Similarity Index					
Time (days)	SR2		SR1		UC	
	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2
-2	-0.1198	-0.04674	-0.12054	-0.04701	-0.11921	-0.0465
-1	-0.12124	0.007416	-0.11952	0.007367	-0.12543	0.008052
0	-0.04064	0.018095	-0.03991	0.018189	-0.04131	0.018112
0.33	0.052058	0.01598	-0.02483	-0.04581	0.11247	-0.02198
1	-0.09081	0.032308	0.097266	-0.08327	0.11919	0.013122
2	-0.07535	-0.00394	0.11169	-0.04288	0.10369	0.1203
3	-0.0417	-0.07092	0.11309	-0.01312	0.065845	0.077327
4	-0.10596	-0.05398	0.11252	-0.01515	0.11201	-0.03996
5	-0.17399	-0.06629	0.11541	0.00793	0.09579	-0.0734

6	0.10793	-0.05193	0.26131	0.092494	0.109	-0.04302
7	-0.27896	0.012669	0.11954	0.016066	0.11187	-0.02514
9	-0.30271	0.060741	0.11521	-0.01295	0.20467	0.073441
10	-0.49577	0.073857	0.11583	0.001781	0.0813	0.12873
11	-0.1198	-0.04674	-0.12054	-0.04701	-0.11921	-0.0465
12	-0.12124	0.007416	-0.11952	0.007367	-0.12543	0.008052

Table C.39: Non dimensionalized matrix scaling using Bray Curtis Similarity Index for T-RFLP profiles for laboratory scale cadmium stress 3

	Non dimensionalized matrix scaling using Bray Curtis Similarity Index					
Time (days)	SR2		SR1		UC	
	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2
-3	-0.00634	-0.15382	-0.00635	-0.15363	-0.00631	-0.15405
-2	0.09502	-0.04014	0.094751	-0.04	0.094488	-0.03986
-1	0.15288	-0.04624	0.15271	-0.04615	0.15253	-0.04606
0	0.18469	-0.12135	0.18488	-0.1215	0.18477	-0.12143
0.33	-0.04238	0.007704	0.18563	-0.00615	0.15021	0.08382
1	-0.10675	-0.02995	0.020889	0.099747	0.069146	0.08147
2	-0.06722	-0.07701	-0.09748	-0.04094	0.11646	0.069603
3	0.059336	0.1386	-0.09694	-0.04183	-0.00467	0.044562
4	0.047849	0.096724	-0.02498	0.037279	0.006215	0.013499
6	0.025465	0.11546	0.1234	0.047088	-0.06384	0.027999
7	0.029595	0.023304	0.11369	0.051304	-0.06508	-0.08028
9	0.028977	0.027268	0.1757	0.052071	-0.02807	0.007871
10	0.081692	0.027508	0.10706	0.04585	0.001168	-0.01804
13	0.03559	0.025821	0.11403	0.048238	-0.04433	-0.00082
13.33	-0.11223	-0.02361	-0.04695	0.060282	-0.11854	-0.01735
14	-0.09794	-0.0408	-0.08652	0.067149	-0.10489	-0.03207
15	-0.05968	0.031111	-0.08116	0.047751	-0.06909	-0.07468
16	-0.00335	0.030547	-0.05362	0.051772	-0.11335	-0.0224
17	-0.0198	0.016357	-0.04948	0.035725	-0.07951	-0.06261
19	-0.02995	0.037914	-0.15179	0.012313	-0.07311	-0.06926
20	-0.06042	0.052536	-0.07114	0.041563	-0.06283	-0.08246
22	0.011621	-0.01021	-0.12137	0.05406	-0.0678	-0.07607
25	0.12052	0.027091	-0.16116	0.018376	-0.09735	-0.04146
26	0.10898	0.030699	-0.0609	0.054608	-0.14169	0.00583
28	-0.10496	0.038212	-0.10029	0.047252	0.031662	-0.00173

Table C.40: Non dimensionalized matrix scaling using Bray Curtis Similarity Index for T-RFLP profiles for pilot scale cadmium stress 1

Time (days)	Non dimensionalized matrix scaling using Bray Curtis Similarity Index					
	SR2		SR1		UC	
	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2
0	-0.22091	-0.08024	-0.22005	-0.07985	-0.21855	-0.07908
0.33	-0.21945	-0.07953	-0.03816	0.27104	0.2896	-0.05886
1	0.063611	-0.19271	-0.03753	0.26866	-0.07208	0.018104
2	0.12307	-0.15975	-0.25967	-0.08464	-0.16231	-0.05801
3	-0.00758	-0.05311	-0.14304	0.17469	0.053721	0.001382
4	0.22963	-0.09436	0.084071	0.037743	0.081841	0.19132
5	0.12549	-0.00673	-0.02854	0.009593	0.082515	0.03732
6	0.34458	-0.03825	-0.0278	0.009621	0.17754	0.045645

Table C.41: Non dimensionalized matrix scaling using Bray Curtis Similarity Index for T-RFLP profiles for pilot scale cadmium stress 2

Time (days)	Non dimensionalized matrix scaling using Bray Curtis Similarity Index					
	SR2		SR1		UC	
	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2	Ordination axis 1	Ordination axis 2
0	-0.1543	-0.13767	-0.15372	-0.13716	-0.15471	-0.13804
0.33	0.15142	-0.19624	0.076377	0.079159	-0.11823	-0.11554
1	-0.44912	0.28148	0.050042	0.074721	-0.0729	-0.10069
2	0.022587	0.070917	0.09445	-0.3366	-0.0693	-0.1073
3	0.089768	-0.01246	0.078363	0.079874	0.10763	0.10693
4	-0.1115	-0.1127	-0.21339	0.12829	0.13028	0.1175
5	0.15292	0.029968	0.006443	-0.03494	0.16445	0.12515
6	0.15762	0.12284	0.096773	0.099528	0.11806	0.11297

Table C.42: Microscopically quantified crawling ciliates for SBR experiment

Crawling ciliates (organisms/ml)									
	Day	0		1		2		3	
Reactor		Avg	+/-	Avg	+/-	Avg	+/-	Avg	+/-
Control 1		2.72E+06	2.80E+05	3.07E+06	5.00E+05	3.11E+06	3.00E+05	4.32E+06	5.40E+05
Control 2		2.72E+06	2.80E+05	2.81E+06	3.00E+04	3.23E+06	2.50E+05	3.92E+06	2.70E+05
IC10-R1		2.72E+06	2.80E+05	7.25E+05	1.90E+05	1.75E+06	2.60E+05	3.88E+06	2.10E+05
IC10-R2		2.72E+06	2.80E+05	7.90E+05	6.00E+04	1.95E+06	1.10E+05	3.96E+06	4.00E+04
IC25-R1		2.72E+06	2.80E+05	3.45E+05	5.00E+04	1.04E+06	1.60E+05	9.50E+05	6.00E+04
IC25-R2		2.72E+06	2.80E+05	3.50E+05	1.40E+05	8.10E+05	1.20E+05	8.70E+05	3.50E+05
Salt spike-R1		2.72E+06	2.80E+05	1.73E+06	1.80E+05	2.75E+06	1.13E+06	3.98E+06	8.20E+05
Salt spike-R2		2.72E+06	2.80E+05	2.11E+06	2.00E+05	2.90E+06	4.50E+05	4.12E+06	3.30E+05

Table C.43: Microscopically quantified free swimming ciliates for SBR experiment

Free swimming ciliates (organisms/ml)									
	Day	0		1		2		3	
Reactor		Avg	+/-	Avg	+/-	Avg	+/-	Avg	+/-
Control 1		5.30E+05	4.40E+05	3.20E+05	8.00E+04	6.15E+05	2.10E+05	1.72E+06	4.00E+05
Control 2		5.30E+05	4.40E+05	5.55E+05	1.10E+05	7.40E+05	1.60E+05	1.65E+06	2.10E+05
IC10-R1		5.30E+05	4.40E+05	2.75E+05	1.90E+05	1.90E+05	8.00E+04	3.70E+05	2.50E+05
IC10-R2		5.30E+05	4.40E+05	1.40E+05	1.60E+05	2.20E+05	1.20E+05	5.50E+05	1.20E+05
IC25-R1		5.30E+05	4.40E+05	6.50E+04	3.00E+04	1.10E+05	2.00E+04	3.50E+05	6.00E+04
IC25-R2		5.30E+05	4.40E+05	1.80E+05	6.00E+04	1.45E+05	9.00E+04	2.80E+05	1.00E+05
Salt spike-R1		5.30E+05	4.40E+05	2.30E+05	6.00E+04	5.45E+05	4.10E+05	2.16E+06	2.40E+05
Salt spike-R2		5.30E+05	4.40E+05	2.55E+05	1.20E+05	4.45E+05	3.30E+05	1.82E+06	1.40E+05

Table C.44: Microscopically quantified sessile ciliates for SBR experiment

Sessile ciliates (organisms/ml)									
	Day	0		1		2		3	
Reactor		Avg	+/-	Avg	+/-	Avg	+/-	Avg	+/-
Control 1		5.52E+06	6.90E+05	5.75E+06	7.10E+05	4.56E+06	1.90E+05	3.96E+06	6.20E+05
Control 2		5.52E+06	6.90E+05	4.77E+06	1.10E+06	4.56E+06	6.70E+05	4.02E+06	3.50E+05
IC10-R1		5.52E+06	6.90E+05	2.25E+06	2.90E+05	2.71E+06	3.50E+05	2.35E+06	4.50E+05
IC10-R2		5.52E+06	6.90E+05	2.94E+06	4.70E+05	2.49E+06	3.40E+05	3.28E+06	3.90E+05
IC25-R1		5.52E+06	6.90E+05	1.38E+06	3.10E+05	1.76E+06	2.10E+05	1.75E+06	7.00E+04
IC25-R2		5.52E+06	6.90E+05	1.42E+06	1.80E+05	1.84E+06	1.80E+05	1.86E+06	1.50E+05
Salt spike-R1		5.52E+06	6.90E+05	3.71E+06	7.80E+05	3.99E+06	2.10E+05	4.32E+06	6.90E+05
Salt spike-R2		5.52E+06	6.90E+05	3.21E+06	4.00E+05	3.55E+06	6.00E+05	4.02E+06	2.00E+05

Table C.45: Microscopically quantified total ciliates for SBR experiment

Total ciliates (organisms/ml)									
	Day	0		1		2		3	
Reactor		Avg	+/-	Avg	+/-	Avg	+/-	Avg	+/-
Control 1		8.77E+06	7.41E+06	9.14E+06	2.95E+06	8.28E+06	2.96E+06	1.00E+07	3.07E+06
Control 2		8.77E+06	7.41E+06	8.13E+06	2.47E+06	8.52E+06	2.32E+06	9.59E+06	1.62E+06
IC10-R1		8.77E+06	7.41E+06	3.25E+06	2.43E+06	4.65E+06	2.16E+06	6.60E+06	4.65E+06
IC10-R2		8.77E+06	7.41E+06	3.87E+06	4.47E+06	4.66E+06	2.63E+06	7.79E+06	1.94E+06
IC25-R1		8.77E+06	7.41E+06	1.79E+06	9.53E+05	2.91E+06	7.74E+05	3.05E+06	5.70E+05
IC25-R2		8.77E+06	7.41E+06	1.95E+06	1.04E+06	2.80E+06	1.80E+06	3.01E+06	1.64E+06
Salt spike-R1		8.77E+06	7.41E+06	5.67E+06	1.99E+06	7.28E+06	6.25E+06	1.05E+07	2.96E+06
Salt spike-R2		8.77E+06	7.41E+06	5.58E+06	2.76E+06	6.89E+06	5.35E+06	9.96E+06	1.21E+06

Table C.46: MLSS levels for SBR experiment

Mixed liquor suspended solids (mg/l)									
	Day	**0**		**1**		**2**		**3**	
Reactor		**Avg**	**+/-**	**Avg**	**+/-**	**Avg**	**+/-**	**Avg**	**+/-**
Control 1		1990	40	2075	30	1990	160	1890	0
Control 2		1990	40	2090	20	1865	70	1890	80
IC10-R1		1990	40	2145	70	2005	190	1890	120
IC10-R2		1990	40	2050	40	2125	170	1885	70
IC25-R1		1990	40	2155	370	1875	150	1825	30
IC25-R2		1990	40	2040	100	1905	130	1475	90
Salt spike-R1		1990	40	2125	50	1955	310	1995	170
Salt spike-R2		1990	40	2190	220	1945	290	1970	40

Table C.47: MLVSS levels for SBR experiment

Mixed liquor volatile suspended solids (mg/l)									
	Day	0		1		2		3	
Reactor		Avg	+/-	Avg	+/-	Avg	+/-	Avg	+/-
Control 1		1505	50	1650	40	1555	30	1490	20
Control 2		1505	50	1660	40	1465	30	1485	30
IC10-R1		1505	50	1695	50	1565	130	1475	130
IC10-R2		1505	50	1610	40	1685	150	1480	40
IC25-R1		1505	50	1700	300	1435	130	1440	60
IC25-R2		1505	50	1580	60	1460	120	1150	60
Salt spike-R1		1505	50	1685	30	1520	240	1585	130
Salt spike-R2		1505	50	1725	170	1480	260	1515	30

Table C.48: Effluent TSS for SBR experiment

Effluent total suspended solids									
	Day	0		1		2		3	
Reactor		Avg	+/-	Avg	+/-	Avg	+/-	Avg	+/-
Control 1		16.5	0.73	24	0.75	26	1	21	0
Control 2		16.5	0.73	29	0.79	23.23	2.18	18.56	0.29
IC10-R1		16.5	0.73	44	0.81	44	4.47	27.12	3.76
IC10-R2		16.5	0.73	66	0.8	48.33	3	33.33	0.06
IC25-R1		16.5	0.73	84	0.78	62.3	5.19	36.67	4.33
IC25-R2		16.5	0.73	100	0.78	68.63	7.06	33.93	0.49
Salt spike-R1		16.5	0.73	22.22	0.8	30.61	0.55	19	1.35
Salt spike-R2		16.5	0.73	23.23	0.77	24.49	0.43	23	0.59

Table C.49: Sludge volume index for SBR experiment

	Sludge volume index (ml/g)			
Day	**0**	**1**	**2**	**3**
Reactor				
Control 1	75.38	72.29	70.35	68.78
Control 2	75.38	66.99	75.07	63.49
IC10-R1	75.38	65.27	69.83	79.37
IC10-R2	75.38	68.29	61.18	79.58
IC25-R1	75.38	55.68	64	82.19
IC25-R2	75.38	63.73	62.99	88.14
Salt spike-R1	75.38	80	81.84	85.21
Salt spike-R2	75.38	86.76	92.54	86.29

Table C.50: Quantitative PCR analyses on DNA extracts from MLSS for SBR experiment

Reactor	Time Days	DNA (ng/ µl)		AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
		Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
Control 1	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	132.45	8.5	2.83E+07	2.09E+06	2.13E+05	2.09E+04	2.83E+07	2.09E+06	2.13E+05	2.09E+04
	2	162.4	3	7.50E+07	7.70E+06	4.62E+05	4.82E+04	7.50E+07	7.70E+06	4.62E+05	4.82E+04
	3	144.65	3.1	8.32E+07	1.15E+07	5.75E+05	8.05E+04	8.32E+07	1.15E+07	5.75E+05	8.05E+04
Control 2	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	136	19.6	2.09E+07	2.50E+06	1.53E+05	2.88E+04	2.09E+07	2.50E+06	1.53E+05	2.88E+04
	2	136	19.6	2.09E+07	2.50E+06	1.53E+05	2.88E+04	2.09E+07	2.50E+06	1.53E+05	2.88E+04
	3	141.4	8.8	8.04E+07	8.01E+06	5.68E+05	6.68E+04	8.04E+07	8.01E+06	5.68E+05	6.68E+04
IC_{10}- R1	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	150.1	11.8	6.06E+07	7.15E+06	4.04E+05	5.72E+04	6.06E+07	7.15E+06	4.04E+05	5.72E+04
	2	125.3	4.6	6.27E+07	6.55E+06	5.01E+05	5.54E+04	6.27E+07	6.55E+06	5.01E+05	5.54E+04
	3	148.6	1	1.10E+08	1.30E+07	7.37E+05	8.77E+04	1.10E+08	1.30E+07	7.37E+05	8.77E+04
IC_{10}- R2	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	128.05	14.9	4.51E+07	4.11E+06	3.52E+05	5.21E+04	4.51E+07	4.11E+06	3.52E+05	5.21E+04
	2	149.95	2.5	7.66E+07	9.12E+06	5.11E+05	6.14E+04	7.66E+07	9.12E+06	5.11E+05	6.14E+04
	3	144.95	3.7	9.73E+07	9.02E+06	6.71E+05	6.45E+04	9.73E+07	9.02E+06	6.71E+05	6.45E+04
IC_{25}- R1	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	120.65	8.5	5.50E+07	6.87E+06	4.56E+05	6.54E+04	5.50E+07	6.87E+06	4.56E+05	6.54E+04
	2	114.5	2.6	5.51E+07	4.04E+06	4.82E+05	3.70E+04	5.51E+07	4.04E+06	4.82E+05	3.70E+04
	3	129.05	2.1	8.19E+07	1.10E+07	6.35E+05	8.60E+04	8.19E+07	1.10E+07	6.35E+05	8.60E+04
IC_{25}- R2	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	153.15	26.3	5.86E+07	4.21E+06	3.83E+05	7.12E+04	5.86E+07	4.21E+06	3.83E+05	7.12E+04
	2	128	3	6.83E+07	9.50E+06	5.33E+05	7.53E+04	6.83E+07	9.50E+06	5.33E+05	7.53E+04
	3	129.4	2.4	7.88E+07	7.88E+06	6.09E+05	6.19E+04	7.88E+07	7.88E+06	6.09E+05	6.19E+04
Salt spk 1	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	109.05	0.9	4.58E+07	5.17E+06	4.20E+05	4.76E+04	4.58E+07	5.17E+06	4.20E+05	4.76E+04

	2	141.15	2.5	6.48E+07	7.74E+06	4.59E+05	5.55E+04	6.48E+07	7.74E+06	4.59E+05	5.55E+04
	3	125.15	5.3	7.91E+07	6.87E+06	6.32E+05	6.11E+04	7.91E+07	6.87E+06	6.32E+05	6.11E+04
Salt spk 2	0	143.65	8.5	3.20E+07	3.59E+06	2.23E+05	2.82E+04	3.20E+07	3.59E+06	2.23E+05	2.82E+04
	1	142.15	16.3	3.09E+07	2.39E+06	2.18E+05	3.01E+04	3.09E+07	2.39E+06	2.18E+05	3.01E+04
	2	163.4	3.4	8.46E+07	1.11E+07	5.18E+05	6.87E+04	8.46E+07	1.11E+07	5.18E+05	6.87E+04
	3	144.55	3.1	6.57E+07	7.22E+06	4.54E+05	5.09E+04	6.57E+07	7.22E+06	4.54E+05	5.09E+04

Table C.51: Quantitative PCR analyses on DNA extracts from effluent biomass for SBR experiment

Reactor	Time Days	DNA (ng/ µl)		AOB 16S copies per µl		AOB 16S copies/ng DNA		Bacterial 16S copies per µl		Bacterial 16S copies/ng DNA	
		Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
Control 1	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	2	124.4	3.6	2.44E+07	8.95E+05	1.96E+05	9.17E+03	4.37E+08	3.43E+07	3.51E+06	2.94E+05
	3	104.35	4.1	1.98E+07	1.26E+06	1.90E+05	1.42E+04	1.37E+09	1.03E+08	1.31E+07	1.11E+06
Control 2	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	115.2	2	1.86E+07	2.64E+06	1.61E+05	2.31E+04	4.24E+08	3.10E+07	3.68E+06	2.77E+05
	2	115.2	2	1.86E+07	2.64E+06	1.61E+05	2.31E+04	4.24E+08	3.10E+07	3.68E+06	2.77E+05
	3	162.9	6.8	3.16E+07	5.89E+05	1.94E+05	8.86E+03	1.06E+09	8.40E+07	6.53E+06	5.83E+05
IC_{10}- R1	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	112.55	11.1	1.23E+07	1.66E+06	1.10E+05	1.83E+04	2.92E+08	1.87E+07	2.59E+06	3.05E+05
	2	136.2	3.4	2.31E+07	2.50E+06	1.69E+05	1.89E+04	4.17E+08	2.91E+07	3.06E+06	2.27E+05
	3	216.35	2.5	3.83E+07	4.75E+06	1.77E+05	2.20E+04	9.60E+08	9.19E+07	4.44E+06	4.28E+05
IC_{10}- R2	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	322.3	379	9.55E+06	1.05E+06	2.96E+04	3.50E+04	7.08E+08	3.00E+06	2.20E+06	2.58E+06
	2	139.15	8.7	1.24E+07	1.50E+06	8.91E+04	1.22E+04	7.82E+08	3.67E+07	5.62E+06	4.39E+05
	3	204.05	6.3	2.93E+07	4.47E+06	1.44E+05	2.24E+04	1.54E+09	1.85E+07	7.53E+06	2.49E+05
IC_{25}- R1	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	114.35	8.7	1.94E+07	3.28E+06	1.70E+05	3.14E+04	5.31E+08	1.25E+07	4.64E+06	3.70E+05
	2	110.1	0	1.61E+07	6.16E+05	1.46E+05	5.60E+03	1.17E+09	3.10E+07	1.07E+07	2.82E+05

	3	182.1	7.4	2.48E+07	3.80E+06	1.36E+05	2.16E+04	1.53E+09	2.58E+08	8.38E+06	1.46E+06
IC_{25}- R2	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	122.7	0.8	2.89E+07	3.01E+06	2.36E+05	2.46E+04	7.43E+08	4.76E+07	6.05E+06	3.90E+05
	2	118.55	1.1	2.03E+07	2.17E+06	1.71E+05	1.83E+04	1.09E+09	7.12E+07	9.22E+06	6.06E+05
	3	161.7	5.8	2.43E+07	3.27E+06	1.50E+05	2.09E+04	1.65E+09	9.05E+07	1.02E+07	6.68E+05
Salt spk 1	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	100	1	1.99E+07	2.09E+05	1.99E+05	2.89E+03	8.45E+08	4.74E+07	8.45E+06	4.81E+05
	2	158.6	3.6	3.01E+07	4.17E+06	1.89E+05	2.67E+04	1.44E+09	1.70E+07	9.11E+06	2.33E+05
	3	139	1.6	2.59E+07	2.21E+06	1.86E+05	1.61E+04	1.48E+09	6.41E+07	1.06E+07	4.77E+05
Salt spk 2	0	138.8	4.2	2.19E+07	2.21E+06	1.57E+05	1.66E+04	4.97E+08	4.19E+07	3.58E+06	3.21E+05
	1	124	9.6	1.84E+07	1.50E+06	1.48E+05	1.67E+04	5.88E+08	4.15E+07	4.74E+06	4.97E+05
	2	148.85	5.1	1.78E+07	2.06E+06	1.19E+05	1.45E+04	7.95E+08	1.50E+07	5.34E+06	2.09E+05
	3	173.3	0	2.36E+07	1.37E+06	1.36E+05	7.88E+03	9.31E+08	5.85E+07	5.37E+06	3.38E+05

Table C.52: General bacteria Taq1 terminal restriction fragments and their relative distribution for control reactors for SBR experiment

	MLSS						Effluent					
	Control 1			Control 2			Control 1			Control 1		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)												
12.44	0	0	0	0	1.97	1.89	0	0	0	0	1.47	0
13.61	0	0	0	0	0	0	0	0	0	0	0	2.57
14.31	0	0	5.85	0	0	0	0	0	0	0	0	0
15.48	0	0	0	0	0	0	0	0	0	0	0	1.32
35.02	0	0	0	0	0	0	0	0	0	0	0	0
35.97	0	0	0	0	0	0	0	0	0	0	0	0
61.58	7.91	8.22	9.24	9.67	7.93	7.98	9.24	4.85	5.58	9.05	6.71	10.75
72.95	0	0	0	0	0	0	0	0	0	0	0	1.2
97.79	0	0	0	0	0	0	0	0	0	0	0	0.87
101.21	2.99	3.79	0	0	3.23	1.27	3.65	2.2	2.21	3.52	3.72	3.29
102.51	0	0	0	0	0	0	10.38	5.69	6.93	7.96	2.82	1.14
117.8	0	0	0	0	0	0	0	0	0	0	0	1.16

235.64	0	0	0	0	0	0	0	0	0	0	0	0
270.82	0	0	0	0	0	0	0	0	0	0	0	0
280.6	4.16	4.65	5.2	3.47	3.97	3.8	5.63	5.25	5.98	4.91	5.66	5.88
287.61	0	0	0	0	0	0	0	0	0	0	0	0
291.23	0	0	0	0	0	0	0	1.22	0.87	1.42	1.72	1.17
296.99	0	0	0	0	1.43	1.37	0	2.2	3.08	0	1.71	2.13
297.85	0	2.66	0	0	1.88	1.8	0	1.22	1.16	0	1.25	0
311.82	0	0	0	0	0	0	0	0	0	0	0	1.46
312.73	0	0	0	0	0	0	0	0	0	0	0	0
316.21	0	0	0	0	0	0	0	0	0	0	0	0
365.57	0	0	0	0	0	0	0	0	0	0	0	0
397.63	0	0	0	0	0	0	0	0	0	0	0	0
398.6	0	0	0	0	0	0	0	0	0	0	0	0
416.66	0	0	0	0	0	0	0	0	0	0	0	0
418.44	0	0	0	0	0	0	0	0	0	0	0	0
419.06	0	0	0	0	0	0	0	0	0	0	0	0
422.46	0	0	0	0	0	0	0	0	0	0	0	0
432.42	0	0	0	0	0	0	0	0	0	0	0	0
476.57	0	0	0	0	1.52	1.45	0	0	0	0	0	0
477.47	0	0	0	0	1.76	1.68	0	2.39	2.28	0	0	0
478.46	0	0	0	0	0	0	0	0	0	0	0	0
480.94	0	0	0	0	4.2	0	0	0	0	0	0	0
482.47	37.36	29.82	33.17	32.2	23.99	32.72	33.78	36.78	36.38	34.11	31.85	26.05
484.57	0	0	0	0	0	0	0	0	0	0	0	0
517.29	9.66	6.95	8.02	11.76	6.41	6.13	8.13	6.96	5.68	7.28	7.35	6.54
517.41	0	0	0	0	0	0	0	0	0	0	0	0
546.56	0	0	0	0	0	0	0	0	0	0	0	0
593.37	0	0	0	0	0	0	0	0	0	0	0	0
595.04	0	0	0	0	0	0	0	0	0	0	2.66	0
596.32	0	8.24	8.53	0	10.13	9.69	0	0	0	0	0	0
605.68	5.38	4.83	0	3.96	4.24	4.06	0	2.86	2.73	3.26	2.92	2.35
611	0	0	0	0	0	0	0	0	0	0	0	0
613.93	0	0	0	0	0	0	0	0	0	0	0	0
619.18	15.15	12.45	13.5	19.27	11.99	11.47	13.55	15.16	14.48	13.19	14.35	14.46
620.91	17.38	18.38	16.5	19.68	15.35	14.68	15.64	13.22	12.63	15.32	15.82	17.68

621.55	0	0	0	0	0	0	0	0	0	0	0	0

Table C.53: General bacteria Taq1 terminal restriction fragments and their relative distribution for IC_{10} reactors for SBR experiment

	MLSS						Effluent					
	IC_{10}-R1			IC_{10}-R2			IC_{10}-R1			IC_{10}-R2		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)												
12.44	0	1.32	0	0	3.72	0.82	0	0	0	0	0	0
13.61	0	0	0	0	0	0	0	0	13.11	0	2.43	0
14.31	0	0	0	0	0	0	0	0	0	0	0	0
15.48	0	0	0	0	0	0	0	0	0	0	0	0
35.02	0	0	0	0	0	0	0	0	0	0	0	0
35.97	0	0	0	0	0	0	0	0	0	0	0	0
61.58	7.68	6.12	5.94	6.97	4.48	6.5	4.87	5.45	4	5.27	4.59	0
72.95	0	0	0	0	0	0	0	0	0	0	0	0
97.79	0	0	0	0	0	0	0	0	0	0	0	0
101.21	2.83	3.58	3.42	2.98	3.85	3.61	0	2.62	2.91	3.73	2.1	2.14
102.51	0	0	0	0	0	0	5.09	2.37	0	0	1.52	0
117.8	0	0	0	0	0	0	0	0	0	0	0	0
235.64	0	1.1	0	0	1.47	0.81	0	0	0	0.84	0	0
270.82	0	0	0	0	0	0.93	0	0	0	0	0	0
280.6	4.01	6.01	7.68	4.33	6.65	8.56	6.71	8.19	9.04	10.86	9.9	11.25
287.61	0	0	0	0	0	0.92	0	0	0	0.95	0	0
291.23	0	0	0	0	0	0.82	0	0	0	0	0	0
296.99	0	0	0	0	0	0	0	3.07	0	0	1.73	1.86
297.85	0	3.41	0	0	5.22	2.44	0	2.49	0	2.52	3.57	0
311.82	0	1.71	0	0	0	2.13	0	2.14	0	2.2	2.11	2.76
312.73	0	0	0	0	0	0	0	0	0	0	0	0
316.21	0	0	0	0	0	0.79	0	0	0	0.82	0	0
365.57	0	0	0	0	0	0.91	0	0	0	0.95	0	0
397.63	0	0	0	0	0	0	0	0	0	0	0	0
398.6	0	0	0	0	0	0	0	0	0	0	0	0
416.66	0	0	0	0	0	0	0	0	0	0	0	0

418.44	0	0	0	0	0	1.73	0	0	0	0.31	0	0
419.06	0	0	0	0	0	0	0	0	0	0	0	0
422.46	0	0	0	0	0	0	0	0	0	0	0	0
432.42	0	1.51	0	0	0	1.27	0	0	0	0.52	1.6	2.07
476.57	1.62	4.1	4.56	2.27	3.63	1.6	5.08	2.06	0	4.34	2.93	0
477.47	0	0	0	0	0	1.65	0	0	0	0	1.69	0
478.46	0	1.82	0	0	1.85	0	0	0	0	0	0	0
480.94	0	0	0	0	0	0	0	0	0	0	0	0
482.47	32.94	24.68	31.13	34.54	25.14	26.45	37.4	29.99	33.52	27.35	29.5	34.46
484.57	0	0	0	0	0	0	0	0	0	0	0	0
517.29	6.38	5.49	5.76	6.27	6.03	5.57	6.76	5.82	7.29	5.76	5.8	8.86
517.41	0	0	0	0	0	0	0	0	0	0	0	0
546.56	0	0	0	0	0	0	0	0	0	0	0	0
593.37	0	0	0	0	0	0	0	0	0	0	0	0
595.04	3.78	4.04	4.87	3.42	4.03	4.04	0	0	0	4.18	2.54	4.27
596.32	5.17	7.29	8.84	4.9	7.26	6.52	0	3.13	0	6.74	2.39	0
605.68	5.6	3.99	4.2	5.5	5.17	3.64	0	2.68	0	3.76	2.41	2.64
611	0	0	0	0	0	0	0	0	0	0	0	0
613.93	0	0	0	0	0	0	0	0	0	0	0	0
619.18	13.2	11.94	10.53	13.13	12.37	8.93	18.96	16.16	13.63	9.23	13.59	16.51
620.91	16.78	11.9	13.07	15.7	9.13	9.34	15.13	13.83	16.51	9.66	9.62	13.16
621.55	0	0	0	0	0	0	0	0	0	0	0	0

Table C.54: General bacteria Taq1 terminal restriction fragments and their relative distribution for IC_{25} reactors for SBR experiment

	MLSS						Effluent					
	IC_{25}-R1			IC_{25}-R2			IC_{25}-R1			IC_{25}-R2		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)												
12.44	1.3	4.84	0	0	0	0	0	0	0	0	5.41	4.04
13.61	0	0	0	15.48	0	0	0	0	0	0	1.63	0
14.31	0	0	0	0	0	0	0	0	0	0	0	0
15.48	0	0	0	22.29	0	0	0	0	0	0	0	0
35.02	0	0	0	44.72	0	0	0	0	0	0	0	0

35.97	0	0	0	17.51	0	0	0	0	0	0	0	0
61.58	13.45	8.21	4.71	0	6.66	0	3.78	8.09	5.16	6.44	6.64	4.26
72.95	0	0	0	0	0	0	0	0	0	0	0	0
97.79	0	0	0	0	0	0	0	0	0	0	0	0
101.21	2.02	3.59	2.25	0	2.55	2.1	3.36	1.65	1.2	1	1.83	1.09
102.51	0	0	0	0	0	0	0	1.37	0	1.31	1.34	0
117.8	0	0	0	0	0	0	0	0	0	0	0	0
235.64	1.2	0	1.48	0	0	1.5	0.26	1.21	1.38	0.86	1.32	1.18
270.82	0	0	0	0	0	0	0	0	0	0	0	0
280.6	2.51	6.5	6.55	0	6.49	6.97	8.72	3.71	7.75	0	3.06	6.79
287.61	0	0	0	0	0	0	0	0	0	0	0	0
291.23	1.15	0	0	0	0	0	0	0	0	0	0	0
296.99	0	0	0	0	0	0	0	0	0	0	0	0
297.85	3.06	0	10.49	0	7.78	8.61	3.26	9.74	8.32	3.67	8.32	7.77
311.82	0	0	0	0	0	0	0	0	0	0	0	0
312.73	0	0	0	0	0	0	0	0	0	0	0	0
316.21	0	0	0	0	0	0	0	0	0	0	0	0
365.57	0	0	0	0	0	0	0	0	0	0	0	0
397.63	0	0	0	0	0	0	0	0	0	1.44	0	0
398.6	0	0	0	0	0	0	0	0	0	0	1.48	0
416.66	0	0	0	0	0	0	0	0	0	0	0	0
418.44	0	0	0	0	0	0	0	0	0	0	0	0
419.06	0	0	0	0	0	0	0	0	0	2.11	0	0
422.46	0	0	0	0	0	0	0	0	0	0	1.81	0
432.42	0	0	0	0	0	0	0	0	0	0	0	0
476.57	3.51	0	9.15	0	7.57	9.01	6.83	7.07	6.34	3.71	6.88	6.43
477.47	2.7	0	0	0	0	0	0	0	0	0	0	0
478.46	0	0	0	0	0	0	0	0	0	0	0	0
480.94	0	0	0	0	0	0	0	3.51	0	4.15	3.34	0
482.47	23.96	28.99	14.83	0	31.74	16.91	14.83	12.64	15.65	8.46	9.69	14.44
484.57	0	0	0	0	0	0	0	0	0	0	0	0
517.29	5.04	7.23	2.92	0	0	3.89	5.06	5.81	5.8	6.15	4.92	4.12
517.41	0	0	3.69	0	0	3.38	3.69	0	0	0	0	3.27
546.56	0	0	0	0	0	0	0	0	0	0	0	0
593.37	0	0	0	0	0	0	0	1.92	0	0	1.59	1.79

595.04	0	0	0	0	0	3.79	0	0	3.24	0	0	2.59
596.32	8.26	0	11.81	0	0	9.76	14.36	10.48	2	20.06	12.47	1.73
605.68	4.43	4.34	3.91	0	0	4.57	3.91	0	1.7	0	0	1.78
611	0	0	2.07	0	0	2.62	2.07	1.97	3.03	0	1.33	2.28
613.93	0	0	0	0	0	0	0	4.25	0	0	3.5	2.27
619.18	11.23	19.51	16	0	22.13	16.66	16.01	26.57	29.3	40.63	23.45	26.41
620.91	16.18	16.8	0	0	15.07	0	0	0	9.12	0	0	7.75
621.55	0	0	10.15	0	0	10.22	13.87	0	0	0	0	0

Table C.55: General bacteria Taq1 terminal restriction fragments and their relative distribution for salt spike reactors for SBR experiment

	MLSS						Effluent					
	Salt spike-R1			Salt spike -R2			Salt spike -R1			Salt spike -R2		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)	1.14	0	0	0	0	1.5	0	1.45	8.08	0	0	6.54
12.44	5.88	0	0	6.51	0	0	0	0	0	0	0	0
13.61	0	0	0	0	0	0	0	0	0	0	0	0
14.31	3.19	0	0	3.5	1.78	0	0	0	1.68	0	0	1.75
15.48	0	0	0	0	0	0	0	0	0	0	0	0
35.02	0	0	0	0	0	0	0	0	0	0	0	0
35.97	7.02	5.41	5.63	8.04	5.18	4.57	7.66	6.79	7.16	4.74	6.29	5.34
61.58	0	0	0	0	0	0	0	0	0	0	0	3.27
72.95	0	0	0	0	0	0	0	0	0	0	0	0
97.79	2.38	3.28	3.1	2.18	3.1	2.86	3.52	3.51	3.88	2.32	2.84	2.27
101.21	0	0	0	0	0	0	9.8	2.18	1.1	5.78	8.63	0
102.51	0	0	0	0	0	0	0	1.36	0	0	0	1.15
117.8	0.9	0	0	0	1.14	0	0	0	0	0	0	0
235.64	0	0	0	0	0	0	0	0	0	0	0	0
270.82	2.55	6.52	5.6	4.58	7.33	9.73	4.64	5.93	7.45	5.42	6.6	7.89
280.6	0	0	0	0	0	0	0	0	0	0	0	0
287.61	0	0	0	0	0	0	0	1.44	0	0	0	0.9
291.23	0	0	0	0	0	0	2.29	2.96	2.76	2.38	2.23	4.1
296.99	2.34	0	2.13	0	2.01	0	0	1.33	0.98	0	0	0
297.85	0	0	0	0	0	0	0	0	2.01	0	0	0

311.82	0	0	0	0	0	1.13	0	1.2	0	0	0	1.34
312.73	0	0	0	0	0	0	0	0	0	0	0	0
316.21	0	0	0	0	0	0	0	0	0	0	0	0
365.57	0	0	0	0	0	0	0	0	0	0	0	0
397.63	0	0	0	0	0	0	0	0	0	0	0	0
398.6	0	0	0	0	0	0.69	0	0	0	0	0	0
416.66	0	0	0	0	0	0	0	0	0	0	0	0
418.44	0	0	0	0	0	0	0	0	0	0	0	0
419.06	0	0	0	0	0	0	0	0	0	0	0	0
422.46	0	0	0	0	0	1.57	0	0	0	0	0	0
432.42	2.28	0	0	0	1.89	0	0	0	0	0	0	0
476.57	2.66	0	0	0	1.65	1.52	0	1.85	0	0	0	0
477.47	0	0	0	0	2.31	0	0	0	0	0	0	0
478.46	0	0	0	0	0	0	0	0	0	0	0	0
480.94	25.71	34.76	35.08	36.61	35.17	41.42	31.76	28.82	28.37	38.2	34.32	29.19
482.47	1.96	0	0	3.13	0	0	0	0	0	0	0	0
484.57	5.71	8.51	6.8	7.19	7.37	6.69	6.91	6.59	5.62	7.26	6.41	5.59
517.29	0	0	0	0	0	0	0	0	0	0	0	0
517.41	0	0	0	0	0	0	0	0	0	0	0	1.31
546.56	0	0	0	0	0	0	0	0	0	0	0	0
593.37	0	0	0	0	3.19	2.75	0	3.36	3.09	0	0	2.33
595.04	6.94	9.79	10.2	0	5.82	5.21	0	0	0	0	0	0
596.32	3.58	4.92	5.36	3.62	3.46	3.29	3.68	3.55	2.57	2.86	3.25	2.23
605.68	0	0	0	0	0	0	0	0	0	0	0	0
611	0	0	0	0	0	0	0	0	0	0	0	0
613.93	10.44	11.76	9.92	10.75	8.01	7.37	13.74	12.16	10.43	16.44	14.05	12.04
619.18	15.31	15.04	16.17	13.88	10.6	9.69	16	15.52	14.8	14.61	15.38	12.76
620.91	0	0	0	0	0	0	0	0	0	0	0	0
621.55	1.14	0	0	0	0	1.5	0	1.45	8.08	0	0	6.54

Table C.56: General bacteria Rsa1 terminal restriction fragments and their relative distribution for control reactors for SBR experiment

	MLSS						Effluent					
	Control 1			Control 2			Control 1			Control 1		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)												
12.52	0	0	0	0	0	0	0	0	0	0	0	0
13.65	0	0	0	0	0	0	0	0	0	0	0	0
15.42	0	0	0	0	0	0	0	0	0	0	0	0
35.17	0	0	0	0	0	0	0	0	0	0	0	0
36.16	0	0	0	0	0	0	0	0	0	0	0	0
61.57	9.63	7.96	12.94	12.41	8.13	9.31	7.53	5.76	8.47	7.39	6.1	7.8
100.89	0	0	0	0	0	0	3.26	2.16	7.57	3.97	2.48	8.12
102.34	0	0	0	0	0	0	0	0	0	0	0	0
120.28	0	2.7	0	2.29	3.28	2.59	4.25	4.65	2.92	4.77	4.62	2.64
128.82	0	0	0	0	0	0	0	0	0	0	0	0
134.78	2.29	2.23	0	1.74	1.91	2.63	0	1.86	0	0	2.05	0
139.78	14.09	12.18	17.43	12.61	12.05	13.37	6.29	9.31	4.36	7.46	9.42	4.25
140.57	0	0	0	0	0	0	2.56	0	0	0	0	0
143.47	1.8	0	0	2.2	1.33	0	0	0	0	0	0	0
144.9	10.1	10.15	12.54	12.17	10.2	10.9	8.04	6.91	7.44	7.62	6.24	6.86
145.96	6.59	6.24	7.19	7.02	5.49	5.97	3.52	6.26	3.13	3.19	5.93	3.28
235.99	0	0	0	0	0	0	0	0	0	0	0	0
280.65	0	0	0	0	0	0	0	0	0	0	0	0
316.16	5.39	5.26	7.96	5.71	4.86	5.61	4.95	3.85	3.91	3.47	3.51	3.46
406.84	0	0	0	0	0	0	0	0	0	0	0	0
423.75	0	0	0	0	0	0	3.53	3.74	0	3.09	3.32	0
457.36	0	3.03	0	0	2.6	3.07	7.03	4.89	9.82	6.95	4.67	10.67
482.51	0	0	0	0	0	0	0	0	0	0	0	0
484.63	0	0	0	0	0	0	0	0	0	0	0	0
512.3	0	0	0	0	0	0	0	0	0	0	0	1.08
517.47	0	0	0	0	0	0	0	0	0	0	0	0
524.01	0	0	0	0	1.91	1.77	0	1.67	0	0	1.98	0
525.75	0	0	0	0	2.55	2.6	4.75	4.94	4.5	5.08	4.69	3.64

527.08	5.56	8.62	0	5.17	6.15	5.76	7	5.5	6.96	6.15	6.01	7.16
536.1	0	0	0	0	0	0	0	1.19	0	0	0	0
543.29	8.92	6.27	9.89	6.99	6.1	6.24	0	5.12	3.94	5.35	5.63	3.04
544.96	6.03	3.22	0	5.35	3.08	2.9	0	1.63	1.47	0	1.77	1.52
549.25	10.48	11.13	15.26	9.6	10.02	9.51	13.95	8.71	10.32	11.85	9.45	13.33
550.7	6.75	9.52	10.74	6.55	6.96	7.26	0	0	5.62	0	0	0
551.97	2.52	0	0	3.15	2.44	2.06	11.74	4.68	7.06	7.28	4.43	7.09
552.33	0	0	0	0	0	0	0	6.29	0	0	6.52	0
605.69	0	0	0	0	0	0	0	0	0	0	0	0
619.32	0	0	0	0	0	0	0	0	0	0	0	0
621.07	0	0	0	0	0	0	0	0	0	0	0	0
905.39	0	0	0	0	0	0	0	0	0	0	0	0
905.99	0	0	0	0	0	0	0	0	0	0	0	0
909.52	0	0	0	0	0	0	0	0	1.64	0	0	1.56
911.53	0	0	0	0	0	0	0	0	0	0	0	0
1040.1	0	0	0	0	2.25	0	0	3.64	0	0	0	4.14
1040.9	0	0	0	0	0	0	0	0	4.73	4.04	0	0
1041.8	0	0	0	0	0	0	0	0	0	0	0	0
1055	0	0	0	0	0	3.46	0	0	0	0	4.64	2.51
1055.8	0	0	0	0	0	0	0	4.78	2.18	0	0	0
1056.7	7.43	0	0	0	0	0	0	0	0	0	0	0
1057.3	0	0	0	0	4.73	0	0	0	0	0	0	0
1058.6	0	0	0	7.04	0	0	0	0	0	0	0	0
1065.7	0	0	0	0	0	0	0	0	0	0	0	0
1067.6	0	0	0	0	0	0	0	0	3.96	0	0	0
1069.7	0	0	0	0	0	0	0	2.46	0	0	2.58	0

Table C.57: General bacteria Rsa1 terminal restriction fragments and their relative distribution for IC_{10} reactors for SBR experiment

	MLSS						Effluent					
	IC_{10}-R1			IC_{10}-R2			IC_{10}-R1			IC_{10}-R2		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)												
12.52	0	0	0	0	0	0	0	0	0	1.06	0	0
13.65	0	0	0	0	0	0	0	0	0	0	0	6.77
15.42	0	0	0	0	0	0	0	0	0	0	0	0
35.17	0	0	0	0	0	0	0	0	0	0	0	0
36.16	0	0	0	0	0	0	0	0	0	0	0	0
61.57	9.06	7.68	5.74	8.95	7.12	8.42	9.6	8.19	6.96	7.62	6.34	5.8
100.89	0	0	0	0	0	0	0.87	0	0	1.02	0	0
102.34	0	0	0	0	0	0	0	0	0	1.59	1.89	0
120.28	2.17	3.51	1.83	2.77	3.75	0	1.6	2.2	0	1.37	2.25	0
128.82	0	0	0	0	0	0	0	0	0	1.07	0	0
134.78	2.61	2.03	1.55	2.64	2.37	0	1.8	2.17	0	1.26	1.78	0
139.78	13.06	13.37	17.1	14.62	12.25	19.67	14.18	14.11	13.37	18.99	21.66	13.96
140.57	0	0	0	0	0	0	0	0	0	0	0	0
143.47	1.42	0	0	1.75	0	0	1.04	0	0	0.69	0	0
144.9	9.05	9.18	8.52	8.85	7.12	8.07	9.16	9.4	7.79	6.71	8.63	6.82
145.96	5.91	7.08	8.84	6.47	6.18	10.03	6.53	5.15	6.78	5.75	5.65	5.21
235.99	0	0	0	0	0	0	0	0	0	0.7	0	0
280.65	0	0	0	0	0	0	0	0	0	0	0	0
316.16	4.03	4.46	6.42	4.09	5.32	5.86	6.04	4.96	6.56	5.42	4.4	5.53
406.84	0	0	0	0	0	0	0	0	0	1.17	0	0
423.75	0	0	0	0	0	0	0	0	0	0	0	0
457.36	1.28	3.85	0	1.84	4.76	0	0	3.17	0	0	2.4	0
482.51	0	0	0	0	0	0	0	0	0	0	0	0
484.63	0	0	0	0	0	0	0	0	0	0	0	0
512.3	0	0	0	0	0	0	0	0	0	0	0	0
517.47	0	0	0	0	0	0	0	0	0	0	0	0
524.01	0	0	2.86	2.74	0	0	1.48	0	0	0	0	0
525.75	3.81	2.99	0	2.76	4.45	0	1.31	2.72	0	0	0	0

527.08	4.45	6.69	4.09	5.06	7.89	0	3.52	6.34	0	2.54	4.69	3.36
536.1	1.18	0	0	0	0	0	0.9	0	0	0	0	0
543.29	6.53	7.2	10.67	7.49	8.51	10.65	8.47	7.8	21.56	9.95	12.26	17.29
544.96	3.93	3.06	3.15	4.83	3.9	3.82	3.32	3.07	5.47	2.96	3	6.01
549.25	9.43	9.89	8.87	9.6	13.03	9.53	9.2	9.37	11	7.86	6.61	10.08
550.7	5.35	6.31	7.57	5.45	5.18	4.92	10.17	7.8	11.01	9.87	5.49	8.84
551.97	2.56	3.49	3.07	3.45	5.62	3.52	0	0	0	0	1.77	0
552.33	0	0	0	0	0	0	0	0	0	0	0	0
605.69	0	0	0	0	0	0	0	0	0	0	0	0
619.32	0	0	0	0	0	0	0	0	0	0	0	0
621.07	0	0	0	0	0	0	0	0	0	0	0	0
905.39	1.95	0	0	0	0	0	0	0	0	0	2.46	0
905.99	0	0	0	0	0	0	0	0	0	1.73	0	0
909.52	0	0	0	0	0	0	0	0	0	0	0	0
911.53	0	0	0	0	0	0	0	0	0	1.37	0	0
1040.1	0	0	0	0	0	0	0	0	0	0	0	0
1040.9	0	0	0	0	0	0	0	0	0	0	0	0
1041.8	1.92	0	0	0	0	0	0	0	0	0	0	0
1055	0	0	0	0	0	0	0	0	0	0	0	0
1055.8	0	4.66	0	0	0	0	0	0	0	0	0	0
1056.7	0	0	0	0	0	0	0	0	0	5.51	0	0
1057.3	0	0	0	0	0	0	0	0	0	0	0	0
1058.6	0	0	0	0	0	0	0	0	0	0	0	0
1065.7	0	0	0	0	0	0	0	0	0	0	0	0
1067.6	0	0	0	0	0	0	0	0	0	0	0	0
1069.7	0	0	0	0	0	0	0	0	0	0	0	0

Table C.58: General bacteria Rsa1 terminal restriction fragments and their relative distribution for IC_{25} reactors for SBR experiment

	MLSS						Effluent					
	IC_{25}-R1			IC_{25}-R2			IC_{25}-R1			IC_{25}-R2		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)												
12.52	0	0	0	0	0	0	0	0	0	0	0	0
13.65	0	0	0	15.58	0	0	0	0	0	0	0	0
15.42	0	0	0	24.58	0	0	0	0	0	0	0	0
35.17	0	0	0	42.04	0	0	0	0	0	0	0	0
36.16	0	0	0	17.8	0	0	0	0	0	0	0	0
61.57	7.94	6.34	6.53	0	5.55	5.52	7.88	8.62	8.06	10.05	9.94	9.43
100.89	2.9	2.95	5.79	0	3.4	6.15	0	0	0	0	0	0
102.34	0	0	0	0	0	0	0	0	0	0	0	0
120.28	0	5.85	3.86	0	5.15	4	2.29	1.35	0	1.8	1.69	0
128.82	0	0	0	0	0	0	0	0	0	0	0	0
134.78	0	2.27	0	0	2.1	0	1.56	1.17	0	1.51	1.63	0
139.78	0	8.11	10.14	0	7.92	10.79	15.92	13.45	15.34	13.43	13.54	16.05
140.57	0	0	0	0	0	0	0	0	0	0	0	0
143.47	0	0	0	0	0	0	0	1.39	0	0	1.43	0
144.9	0	7.86	9.22	0	7.31	9.42	10.91	9.01	10.89	11.56	9.48	13.72
145.96	0	5.71	6.97	0	5.47	7.05	7.04	7.02	6.91	7.25	6.08	8.06
235.99	0	0	0	0	0	0	0	0	0	0	0	0
280.65	5.2	0	0	0	0	0	0	0	0	0	0	0
316.16	0	4.49	4.73	0	3.51	5.56	6.02	5.11	6.63	7.81	5.42	7.62
406.84	0	0	0	0	0	0	2	0	0	0	0	0
423.75	0	5.16	3.24	0	3.25	3.1	0	0	0	0	0	0
457.36	0	5.54	0	0	4.9	0	0	0	0	0	0	0
482.51	38.01	0	0	0	0	0	0	0	0	0	0	0
484.63	3.2	0	0	0	0	0	0	0	0	0	0	0
512.3	0	0	0	0	0	0	0	0	0	0	0	0
517.47	7.66	0	0	0	0	0	0	0	0	0	0	0
524.01	0	0	0	0	0	0	0	0	0	0	0	0
525.75	0	5.41	4.45	0	5.38	3.73	0	0	0	0	0	0

527.08	0	6.83	0	0	6.34	0	3.18	3.31	0	3.15	3.71	0
536.1	0	0	0	0	0	0	0	0	0	0	0	0
543.29	0	0	6.46	0	4.12	5.86	7.63	17.91	11.05	7.09	16.63	11.2
544.96	0	0	0	0	2.23	1.84	3.46	5.22	4.58	3.15	5.41	5.79
549.25	0	9.91	13.75	0	10.35	11.96	11.5	9	12.19	11.17	9.17	12.97
550.7	0	0	0	0	0	0	11.37	9.6	9.03	10.72	8.45	10.21
551.97	0	5.04	11.38	0	8.65	7.77	0	0	2.47	2.65	0	0
552.33	0	7.82	0	0	0	0	0	0	0	0	0	0
605.69	3.6	0	0	0	0	0	0	0	0	0	0	0
619.32	11.18	0	0	0	0	0	0	0	0	0	0	0
621.07	14.4	0	0	0	0	0	0	0	0	0	0	0
905.39	0	0	0	0	0	0	0	0	0	0	0	0
905.99	0	0	0	0	0	0	0	0	0	0	0	0
909.52	0	0	0	0	0	0	0	0	0	0	0	0
911.53	0	0	0	0	0	0	0	0	0	0	0	0
1040.1	0	0	0	0	0	0	0	0	0	0	0	0
1040.9	0	0	0	0	4.88	0	0	0	0	0	0	0
1041.8	0	0	0	0	0	0	0	0	0	0	0	0
1055	0	0	0	0	0	0	0	0	0	0	0	0
1055.8	0	0	0	0	0	5.29	0	0	0	0	0	0
1056.7	0	0	0	0	0	0	0	6.62	7.55	0	0	0
1057.3	0	0	0	0	0	0	0	0	0	0	0	0
1058.6	0	0	0	0	0	0	0	0	0	0	0	0
1065.7	0	0	0	0	0	0	1.97	0	0	0	0	0
1067.6	0	0	0	0	0	0	0	0	0	0	0	0
1069.7	0	0	0	0	2.23	0	0	0	0	0	0	0

Table C.59: General bacteria Rsa1 terminal restriction fragments and their relative distribution for salt spike reactors for SBR experiment

	MLSS						Effluent					
	Salt spike-R1			Salt spike -R2			Salt spike -R1			Salt spike -R2		
Time	1	2	3	1	2	3	1	2	3	1	2	3
TRF(bp)	0	0	0	0	0	0	0	0	0	0	0	0
12.52	0	0	0	0	0	0	0	0	0	0	0	0
13.65	0	0	0	0	0	0	0	0	0	0	0	0
15.42	0	0	0	0	0	0	0	0	0	0	0	0
35.17	0	0	0	0	0	0	0	0	0	0	0	0
36.16	8.46	6.92	8.48	8.98	6.42	6.3	7.96	7.09	7.59	7.19	8.2	6.66
61.57	1.55	0	0	1.64	0	0	0	0	0	0	0	0
100.89	0	0	0	0	1.36	0	0	0	0	0	0	0
102.34	2.43	2.99	1.7	2.76	2.72	0	3.23	2.92	2.42	2.57	3.44	2.48
120.28	0	0	0	0	0	0	0	0	0	0	0	0
128.82	2.18	2.46	1.71	2.18	1.93	1.41	2.04	1.24	1.45	2.18	0	0
134.78	11.2	11.86	16.97	10.08	16.77	16.91	15.55	14.43	17.03	16.88	12.69	18.31
139.78	0	0	0	0	0	0	0	0	0	0	0	0
140.57	0	0	0	0	0	0	0	1.26	0	0	1.39	0
143.47	8.26	10.26	8.89	7.72	7.84	7.97	7.55	8.37	8.31	6.57	8.23	6.62
144.9	6.39	6	7.14	5.9	5.5	6.32	7	5.68	7.44	8.16	4.54	6.36
145.96	0	0	0	0	0	2.18	0	0	0	0	0	0
235.99	0	0	0	0	0	0	0	0	0	0	0	0
280.65	4.17	5.7	7.39	4.03	3.88	8.96	4.87	5.55	7.17	4.48	5.31	5.8
316.16	0	0	0	0	0	0	0	0	0	0	0	0
406.84	0	0	0	0	0	0	0	0	0	0	0	0
423.75	3.07	2.85	0	3.8	2.45	0	4.12	0	0	3.63	2.67	0
457.36	0	0	0	0	0	0	0	0	0	0	0	0
482.51	0	0	0	0	0	0	0	0	0	0	0	0
484.63	0	0	0	0	0	0	0	0	0	0	0	0
512.3	0	0	0	0	0	0	0	0	0	0	0	0
517.47	1.92	0	2.19	2.38	0	0	0	0	0	0	0	0
524.01	3.21	0	0	3.72	3.52	0	4.15	2.97	2.27	3.29	3.24	0
525.75	5.22	6.79	3.48	6.13	5.53	3.46	6.61	3.46	3.42	5.83	4.48	4.28

527.08	0	0	0	1.12	0	0	0	0	0	0	0	0
536.1	5.92	6.97	9.35	5.89	9.49	11.89	10	15.35	10.8	10.13	12.52	14.35
543.29	3.87	3.45	3.21	3.89	2.87	3.06	2.89	5.1	3.41	3.42	4.9	5.4
544.96	8.39	11.7	10.08	7.5	7.66	10.64	9	9.22	9.15	9.3	11.53	9.44
549.25	6.91	7.87	10.37	6.62	5.77	13.34	5.77	6.34	7.22	0	6.42	6.31
550.7	0	3.27	0	2.81	2.64	0	2.92	2.47	2.92	4.84	0	4.42
551.97	0	0	0	0	0	0	0	0	0	3.62	0	0
552.33	0	0	0	0	0	0	0	0	0	0	0	0
605.69	0	0	0	0	0	0	0	0	0	0	0	0
619.32	0	0	0	0	0	0	0	0	0	0	0	0
621.07	0	0	0	0	0	0	0	0	0	0	0	0
905.39	0	0	0	0	0	0	0	0	0	0	0	0
905.99	0	0	0	0	0	0	0	0	0	0	0	0
909.52	0	0	0	0	0	0	0	0	0	0	0	0
911.53	0	0	0	3.56	0	0	0	0	0	0	0	0
1040.1	0	0	0	0	0	0	0	0	0	0	0	0
1040.9	0	0	0	0	0	0	0	0	0	0	0	0
1041.8	0	0	0	0	0	0	0	0	0	0	0	0
1055	0	0	0	0	0	0	0	0	0	0	0	0
1055.8	0	0	0	0	0	0	6.33	0	0	0	0	0
1056.7	0	0	0	0	0	0	0	0	0	0	0	0
1057.3	0	0	0	0	0	0	0	0	0	0	0	0
1058.6	0	0	0	0	0	0	0	0	0	0	0	0
1065.7	0	0	0	0	0	0	0	0	0	0	0	0
1067.6	0	0	0	0	0	0	0	0	0	0	0	0
1069.7	0	0	0	0	0	0	0	0	0	0	0	0

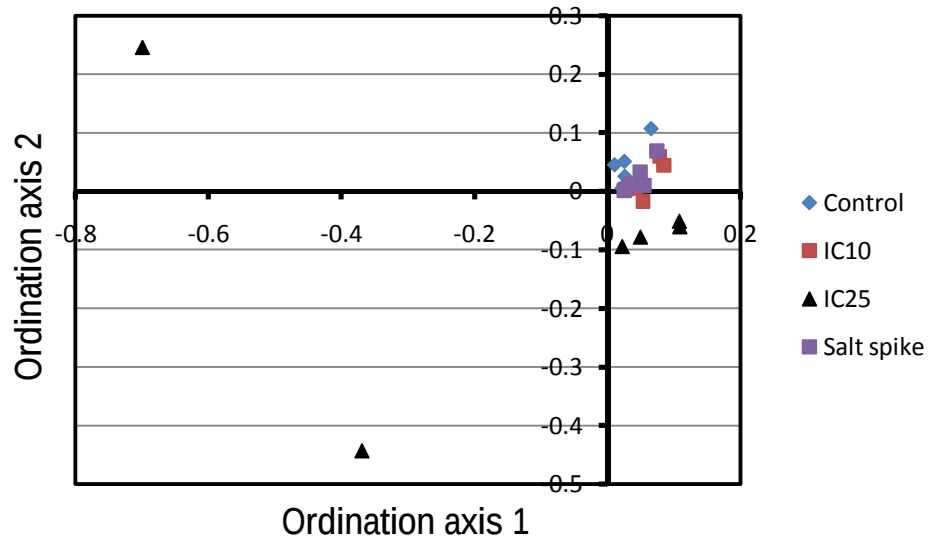

Figure C.3: Non dimensionalized scaling using Bray-Curtis similarity index on T-RFLP profiles for Rsa1 digest

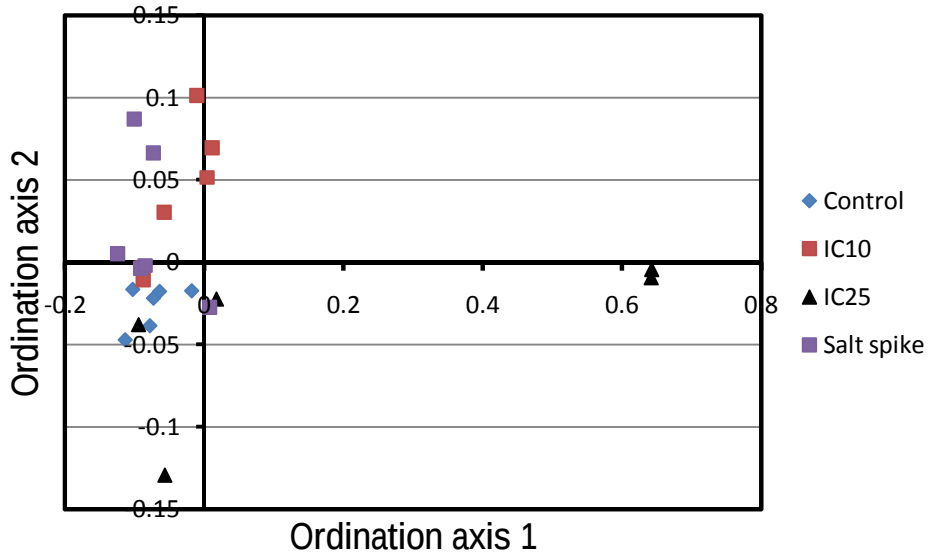

Figure C.4: Non dimensionalized scaling using Bray-Curtis similarity index on T-RFLP profiles for Taq1 digest

AOB specific 16S rRNA sequence alignment used to construct phylogenetic tree.

>AOB_16s_seq_1
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GGTTGTAAAGCTCTTTCAGTCGAGAAGAAAAGGCTGCAGTGAATAACTGTAGTTTATGACGGTATCGACAGAAGAAGCACCG
GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGC-
GAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTTTTGTAAGTCAGATGTGAAATCCCCGGGCTTAACCTG
GGAATTGCGTTTGAAACTACAAGACTAGAGTGTGGCAGAGGGAGGTGGAATTCCATGTGTAGCAGTGAAATGCGTAGAGAT
TTGGAAGAACATCGATGGCGAAGGCAGCCTCCTGGGTTAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGCCT--
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AATTTTCCAGAGATGGAT-TAGTGCCTTC--
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CCCTTGTCATTAATTGCCATCA-TTTA-
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GGGAACGCTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA
CCCTTGTCATTAATTGCCATCA-TTTA-
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ACTCCT-
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GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGC-
GAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTTTTGTAAGTCAGATGTGAAATCCCCGGGCTTAACCTG
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CCCTTGTCATTAATTGCCATCA-TTTA-
GTTGGGCACTTTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTA
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CTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGC-
GAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTTTTGTAAGTCAGATGTGAAATCCCCGGGCTTAACCTG
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