Fate of Select Pharmaceutically Active Compounds in the Integrated Fixed Film Activated Sludge Process

by

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A thesis
presented to University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Applied Science
in
Civil Engineering

Waterloo, Ontario, Canada, 2014

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Based on a diverse consortia of research completed within the last 15 years, it has been found that Pharmaceutical Compounds (PCs) are present in detectable levels within a variety of environmental matrices, including tap water. This is largely attributed to anthropogenic activities as humans are the majority consumer of PCs. As a result, the primary method of disposal is via wastewater pathways resulting from human excretion of ingested PCs. Based on past research into PC fate via the wastewater treatment process, only limited biotic and abiotic transformations are achieved – most PC's are detected in the effluents of WWTP's. This suggests that improving the removal of PCs during the wastewater treatment process provides a promising strategy for limiting the conveyance of PCs to the environment.

Historically, studies regarding PC fate in WWTPs have predominantly focused on the activated sludge process. However, fixed film (biofilm) wastewater treatment technologies continue to gain popularity at full scale wastewater treatment facilities. The limited studies which investigated fixed film wastewater treatment processes have reported that improved transformation efficiencies were observed relative to activated sludge systems. Based on these previous studies, it was postulated that the more diverse bacterial consortium present within the Integrated Fixed Film Activated Sludge (IFAS) process, a novel treatment process which has recently gained popularity in North America, may lead to improved transformation efficiencies ("removals") of these very complex compounds. Only one previous study which investigated the transformation efficiencies of the IFAS process compared to a control was found. It was therefore considered that an additional investigation into the IFAS process warrants further investigation.

Four IFAS Sequencing Batch Biofilm Reactors (SBBRs) and four control Sequencing Batch Reactors (SBRs) were operated with varied experimental conditions in a 2^2 factorial design to investigate whether an observable difference in the level of PC transformations would result via the IFAS process when compared to a control. Experimental conditions were characterized by varying the operating Solids Retention Time (SRT) and mixed liquor temperature. For all other operational parameters, best efforts were made to ensure both reactors were operated under equivalent conditions. This permitted a true assessment of the effects of the inclusion of IFAS media.

Reactors were investigated through three phases of sampling, under which the performance of the reactors was investigated through the measurement of the following parameters:

- Conventional parameters (tCOD, sCOD, TAN, NO_3-N) within the initial and final samples;
- Operational parameters (MLSS, MLVSS, ESS); and
- The transformation efficiencies achieved for 5 PC (Carbamazepine, Sulfamethoxazole, Trimethoprim, Atenolol and Acetaminophen).

During all three phases of PC sampling, the pilot reactors were found to have been performing as anticipated with respect to conventional contaminant removals. Organic removals were found to be statistically similar between the

IFAS and control reactors across all four experimental conditions. Full nitrification was observed for all reactors with the exception of the control SBR operated under the low SRT, low temperature condition. The IFAS SBBRs were found to demonstrate improved nitrification kinetics when compared to their respective controls operated under the same experimental conditions. This was believed to be related to the more diverse bacterial consortia present as a result of the IFAS biofilms. All reactors were generally believed to be operating at steady state and were within an acceptable range of the target operating conditions.

Due to complications associated with the analysis of samples, only CBZ, TRIM, ATEN and ACE could be successfully quantitated. CBZ was found to not have been transformed to any appreciable level across all conditions investigated through either the IFAS SBBRs or control SBRs. ACE was transformed at efficiencies greater than 99% under all conditions and in both IFAS and control reactors and therefore no comparison could be made. TRIM and ATEN demonstrated improved transformation efficiencies under all conditions within the IFAS reactors. The presence of IFAS media, SRT and temperature were all found to be statistically significant effects through ANOVA using a confidence limit of 95%.

ACKNOWLEDGEMENTS

This thesis document is the product of the advice, assistance, suggestions and support of many people. Without these individuals, this thesis would not have been completed.

First I would love to express my sincere appreciation for the contributions of Dr. Wayne Parker. His advice, knowledge and willingness to act as a “sounding board” resulted in input that made the most significant influence on this thesis. I would also like to express my gratitude for his patience during my study at the University of Waterloo.

I would like to thank Dr. Mark Servos and Leslie Bragg for their assistance in completing this thesis. The world of LC-MS/MS is not well suited to engineers and it was very helpful to have such experienced guides. They are also amazing, compassionate people who made time for my questions and concerns. They made my problems their own and their support was very appreciated.

I would like to give special thanks to Shirley Anne Smyth and Dr. Lori Lishman who provided much needed support in terms of trace contaminant analysis and troubleshooting. Their knowledge of analytical techniques, trace contaminant sampling and behaviour and overall study management suggestions were invaluable.

Special thanks go to the support staff at the Canadian Centre for Inland Waters – Wastewater Technology Centre, particularly Scott Dunlop. Their knowledge and support were key to maintaining a successfully operating pilot reactor. Scott was an excellent resource and patiently donated his time to ensuring that all required analytical equipment and operation supplies were available and in good working order.

I would like to also express my gratitude to Headworks Bio Canada Inc. for supplying the IFAS media and for their technical assistance during this study. Particularly, I would like to thank Siva Angappan for sharing his experience and knowledge.

Lastly, I am very grateful to my wife, Rachel Gander, as well as my parents. Their support (both financial and other) helped me to keep it together over this very protracted thesis period.

DEDICATION

Dedicated to my mother, who instilled in me at a very young age the importance of academics. I hope you don't find this thesis too boring.

TABLE OF CONTENTS

Page No.

Declaration .... *ii.*
Abstract .... *iii.*
Acknowledgements .... *v.*
Dedication .... *vi.*
Table of Contents .... *vii.*
List of Figures .... *ix.*
List of Tables .... *xi.*

1.0 Introduction .... *1.*

2. Literature Review .... *3.*

2.1 Wastewater Treatment .... *3.*
2.1.1 The Activated Sludge Process .... *3.*
2.1.2 The Activated Sludge Process .... *5.*
2.1.3 Process Control Parameters .... *8.*
2.1.4 The IFAS Process .... *10.*
2.2 Pharmaceutical Compounds .... *12.*
2.3 Pharmaceutical Compounds .... *13.*
2.3.1 Removal Mechanisms .... *14.*
2.3.1.1 Volatilization .... *14.*
2.3.1.2 Sorption to Solids .... *14.*
2.3.1.3 Transformation Processes .... *16.*
2.3.2 Pharmaceutical Analysis in Wastewater .... *20.*
2.4. Prior Studies of Pharmaceutical Removal in Biofilm Systems .... *14.*

3. Materials and Methods .... *28.*

3.1 Experimental Procedure .... *28.*
3.1.1 Experimental Theory .... *28.*
3.1.2 Experimental Design .... *30.*
3.1.3 Selection of PC Compounds for Investigation .... *30.*
3.2 Pilot Reactor Configuration .... *32.*
3.3 Pilot Reactor Operation .... *35.*

3.3.1 Reactor Feed .... *39.*
3.4 Sampling Procedure .... *40.*

3.5 Conventional Analysis ....41.
3.5.1 Conventional Analysis Methods .... 42.
3.5.2 Batch Specific Nitrification Rate Testing .... 43.
3.6 Analysis of Pharmaceutical Compounds .... 45.
3.6.1 Sample Preparation .... 45.
3.6.2 Sample Analysis ....46.
3.6.3 Preliminary Investigations ....47.
3.6.4 Phase 1 Investigations ....46.
3.6.5 Phase 2 Investigations ....46.
3.6.6 Phase 3 Investigations ....46.

4. Results and Discussion .... 54.

4.1 Phase 1 Investigations .... 54.
4.2 Phase 2 Investigations .... 72.
4.3 Phase 3 Investigations .... 89.
4.4 Summary and Statistical Analysis of Results .... 112.
4.4.1 Conventional Parameters .... 112.
4.4.2 PC Analysis .... 118.

5. Conclusions and Recommendations... 129.

6. References .... 131.

LIST OF FIGURES

Figure 2.1: Typical Activated Sludge floc composition .... 4.

Figure 2.2: Biofilm development stages ……………….. 5.

Figure 2.3: 3D modeling of a bacterial biofilm based on in-situ molecular analysis........ 6.

Figure 2.4: HDPE media used in IFAS process with biofilm colonization.......................10.

Figure 2.5: Number of academic studies related to PCs as well as matrix investigated ………………………………………………………………………………...... 24.

Figure 3.1: Experimental treatment levels ……………... 31.

Figure 4.1: MLVSS concentrations within reactor K20 and B.......................................105.

Figure 4.2: MLVSS concentrations within reactor A20 and C.......................................105.

Figure 4.3: MLVSS concentrations within reactor K7 and E ....105.

Figure 4.4: MLVSS concentrations within reactor A7 and D ....105.

Figure 4.5: CBZ transformation efficiency for IFAS and Control at 20 d SRT and 18 ℃ ………………………………………………………………………………...................111.

Figure 4.6: CBZ transformation efficiency for IFAS and Control at 20 d SRT and 12 ℃ ………………………………………………………………………………...................111.

Figure 4.7: CBZ transformation efficiency for IFAS and Control at 7 d SRT and 18 ℃ ………………………………………………………………………………...................111.

Figure 4.8: CBZ transformation efficiency for IFAS and Control at 7 d SRT and 12 ℃ ………………………………………………………………………………...................111.

Figure 4.9: TRIM transformation efficiency for IFAS and Control at 20 d SRT and 18 ℃ ………………………………………………………………………………...................113.

Figure 4.10: TRIM transformation efficiency for IFAS and Control at 20 d SRT and 12 ℃ ………………………………………………………………………………...................113.

Figure 4.11: TRIM transformation efficiency for IFAS and Control at 7 d SRT and 18 ℃ ………………………………………………………………………………...................113.

Figure 4.12: TRIM transformation efficiency for IFAS and Control at 7 d SRT and 12 ℃ ………………………………………………………………………………...................113.

Figure 4.13: ATEN transformation efficiency for IFAS and Control at 20 d SRT and 18 ℃ ……………………………………………………………………………............. .....116.

Figure 4.14: ATEN transformation efficiency for IFAS and Control at 20 d SRT and 12 °C ………………………………………………………………………………............... ...116.

Figure 4.15: ATEN transformation efficiency for IFAS and Control at 7 d SRT and 18 °C ……………………………………………………………………………………...................116.

Figure 4.16: ATEN transformation efficiency for IFAS and Control at 7 d SRT and 12 °C ……………………………………………………………………………………...................116.

LIST OF TABLES

Table 2.1: Past literature related to PC fate investigations concerning biofilm Processes .... 27.

Table 3.1: Configuration of SBR stages utilized .... 33.

Table 3.2: Operating conditions for the six pilot reactors .... 36.

Table 3.3: Calculated minimum SRT required for nitrification in a conventional SBR .... 38.

Table 3.4: Average primary effluent contaminant concentrations, 2011 .... 39.

Table 3.5: Experimental conditions, reactors and sampling dates used for Phase 1, 2 and 3 .... 47.

Table 3.6: Statistical determination of minimum sample size .... 50.

Table 4.1: Conventional performance during PC sampling – Phase 1 .... 55.

Table 4.2: Results of solids monitoring during PC sampling – Phase 1 .... 58.

Table 4.3: Nitrification rate testing results – Phase 1 .... 60.

Table 4.4: Calculated and reported MQL's for Phase 1 analysis .... 63.

Table 4.5: Reported PC concentrations for Phase 1 investigation .... 65.

Table 4.6: Observed transformation efficiencies for Phase 1 Investigation .... 67.

Table 4.7: Conventional performance during Phase 2 sampling .... 69.

Table 4.8: Results of solids monitoring during Phase 2 sampling .... 72.

Table 4.9: Nitrification rate testing results – Phase 2 .... 73.

Table 4.10: Calculated and reported MQL's for Phase 2/Re-run analysis .... 75.

Table 4.11: Reported PC concentrations for Reactor A20 initial and final samples ..... 78.

Table 4.12: Reported PC concentrations for Reactor C initial and final samples .... 79.

Table 4.13: Observed transformation efficiencies for Phase 2 Investigation .... 81.

Table 4.14: Conventional performance during PC sampling - Phase 3 .... 84.

Table 4.15: Results of solids monitoring during PC sampling - Phase 3 .... 86.

Table 4.16: Nitrification rate testing results – Reactors K7, E, A7 and D ……................. *88.*

Table 4.17: Calculated and reported MQL's for Phase 3 ………………................... *91.*

Table 4.18: Reported PC concentrations for Reactor K7 initial and final samples ..... *93.*

Table 4.19: Reported PC concentrations for Reactor E initial and final samples .... *94.*

Table 4.20: Observed transformation efficiencies for Phase 3 Investigation – K7 and E …………………………………………………………………………………..... *96.*

Table 4.21: Reported PC concentrations for Reactor A7 initial and final samples ..... *98.*

Table 4.22: Reported PC concentrations for Reactor D initial and final samples .... *99.*

Table 4.23: Observed transformation efficiencies for Phase 3 Investigation – A7 and D …………………………………………………………………………………......*101.*

Table 4.24: Nitrification Rate Testing Summary……………………………………....... *107.*

1.0 INTRODUCTION

As populations continue to increase, concerns over the effects of anthropogenic stressors on our environment are continually gaining attention from both the academic community and society as a whole (WHO, 2012). One emerging area of concern which is attracting significant attention is the exposure of environmental waters and soils to emerging contaminants (ECs). EC's may include prescription or over the counter pharmaceuticals, drugs of abuse (or illegal substances), personal care products found in consumer products, such as household cleaning products, over the counter medicines or antimicrobials, veterinary medicines and industrial chemicals, such as pesticides (Metcalf and Eddy, 2003).

Pharmaceutical compounds (PCs) are predominantly excreted in either un-metabolized or metabolized forms, and are conveyed via wastewater streams to wastewater treatment facilities (WWTFs) where they undergo varying levels of biotic and abiotic transformations before being disposed of via effluent to a receiver (Daughton and Ternes, 1999). Some veterinary PCs are also excreted and are directly disposed of into rivers and streams (Shao et al., 2009). In addition to conveyance through sewage networks, stormwater can also transport PCs throughout various environmental matrices (Osenbrück et al., 2007). The application of biosolids to land as fertilizers also results in PCs being conveyed to rivers and streams via overland flow (Edwards et al., 2009). However, the concentrations observed in wastewater received at treatment facilities indicate that the majority of PCs are disposed of via wastewater streams (Ternes and Joss, 2006).

As wastewater effluents represents the most significant source of PC related environmental contamination, their removal in conventional activated sludge processes has been given considerable attention. However, the Integrated Fixed Film Activated Sludge (IFAS) process, which is an emerging technology, utilized for bioreactor retrofitting to meet increasingly stringent effluent requirements, has received very limited focus regarding the transformation potential of PCs (Kim et al., 2009). The various process advantages of the biofilm environment suggests, on superficial analysis, that many characteristics of the biofilm environment appear to be conducive to improved removal efficiencies based on the mechanisms for biotic and abiotic transformation of PCs reported in the literature. Past PC invesitgations related to biofilm systems have been limited, however the majority of these studies indicate that these processes provide enhanced PC transformation capabilities in comparison to activated sludge systems. In response to this absence of past experimental investigations, the study which forms the subject of this thesis was conducted.

The objective of this study was to determine if the IFAS process, a novel treatment process which utilizes a suspended growth and biofilm phase for biological treatment, demonstrates significantly different PC transformation potential relative to the conventional activated sludge (CAS) process, which is the predominant process used for municipal wastewater treatment. This objective was supporting through the following sub-objectives:

- Pilot reactors configured as both IFAS and CAS processes were operated in parallel under identical process conditions, at different combinations of solids retention times (SRTs) and temperatures. By comparing the net transformation efficiencies of the compounds of interest through both a sequencing batch reactor (SBR)

and an IFAS equipped Sequencing Batch Biofilm Reactor (SBBR), while operated under identical conditions, the net effect of the biofilm was estimated;

- Pilot reactor performance was evaluated through the measurement of conventional pollutants as well as operational parameters to confirm that all reactors were operating under steady-state conditions prior to further investigations; and
- PC transformation efficiencies were assessed through a 2^2 factorial design by analyzing PC concentrations in municipal wastewater subjected to treatment through the IFAS and CAS processes. A thorough sampling and analysis procedure was developed, based on methods reported in prior PC fate investigations, to ensure that data obtained was significant.

The experiment was carried out using 6 bench scale 30 L sequencing batch reactors. To ensure that observations made using the bench scale systems were as conducive to real world conditions as possible, municipal wastewater was utilized. To ensure reactors were operating under steady state conditions conventional pollutants (total chemical oxygen demand (COD), filtered COD, Total Ammonia Nitrogen, Nitrate-N and Nitrite-N) were monitored as a means of assessing reactor performance. Batch nitrification testing was also conducted to assess the performance of the nitrifers within each reactor at each experimental condition. Effluent total suspended solids, Mixed Liquor suspended solids and SRT were monitored to ensure experimental conditions were met. Temperatures were maintained at experimental conditions through the usage of water jackets and water recirculation.

This investigation focused on the fate of five pharmaceutical compounds (PC), namely:

- the anti-epileptic Carbamazepine (CBZ);
- the non-steroidal anti-inflamatory drug Acetaminophen (ACE);
- the beta blocker Atenolol (ATEN); and,
- the antibiotics Trimethoprim (TRIM) and Sulfamethoxazole (SMX).

The 5 PCs investigated were selected on the basis of: prevalence in Canadian wastewaters and surface waters; encompassing a wide range of reported transformation potentials; suitability to available analytical techniques; and the availability of PC fate investigation data for the purposes of comparing the results of this study to those obtained previously.

PC concentrations were assessed by means of Liquid Chromatography coupled with high performance mass spectrometry (LC-MS/MS). Due to the difficulties associated with the measurement of PCs within wastewater, the isotope dilution method was utilized for all samples with the exception of one experimental condition. Preliminary investigations were completed using a commercial laboratory to assess the analytical precision and accuracy associated with the development of a proprietary analytical method. This method was found to produce high variability and poor accuracy and was ultimately abandoned. The remainder of analysis was conducted at a University of Waterloo lab, utilizing a previously developed methodology with minor modifications (Rahman et al., 2010). This method was found to produce excellent results for CBZ, ACE, ATEN and TRIM both in terms of accuracy and precision. However, due to poor performance and suspected contamination of the LC column, SMX transformation efficiencies could not be estimated.

2. LITERATURE REVIEW

To provide a context for the current investigation, a review of literature related to the wastewater treatment processes and pharmaceutical compounds was conducted. Pharmaceutical compounds have emerged as a significant area of study as a result of their presence in many environmental matrices and uncertainty regarding their effects to aquatic flora, fauna and humans through unintentional uptake through drinking water. The following section provides a background to the current investigation through a summary and critical review of past academic studies and other literature sources. The following topics are discussed in chapter 2:

- a general overview of suspended and fixed film wastewater treatment processes;
- factors that affect process performance;
- detailed review of IFAS/similar processes as well as design information;
- PCs within the wastewater treatment processes including:
  - Environmental significance
  - Fate mechanisms
  - Analytical methods in w/w matrices; and
- Prior studies of PC fate in biofilm processes

2.1. WASTEWATER TREATMENT

Wastewater treatment typically employs physical, chemical and biological processes to remove contaminants that are deleterious to aquatic habitat. Biological wastewater treatment relies on maintaining an environment that contains a concentrated bacterial population capable of utilizing organic and inorganic contaminants for cellular synthesis while producing a treated effluent which is low in oxidizable organic material, typically measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD_5). In addition, the requirement for nitrification, the oxidation of ammonia (NH_3) + ammonium (NH_4^+) (referred to in combination as total ammonia nitrogen, or TAN), has become a standard performance requirement for all new or upgraded wastewater treatment facilities (WWTF) in order to meet environmental regulations.
The activated sludge process is the predominant wastewater treatment process used to meet these treatment requirements (MOE, 2008). However, there is a growing interest in bioreactors that employ bacteria that are associated with surfaces (i.e., biofilms). Brief descriptions of activated sludge and biofilm processes are provided in the following sections.

2.1.1. THE ACTIVATED SLUDGE PROCESS

The activated sludge (AS) process employs biomass that is present as suspended solids and can be arranged in a number of process configurations that may utilize aerobic, anoxic or anaerobic conditions for the removal of organic contaminants, ammonia, phosphorus and nitrate. The activated sludge process was developed around 1910 for the biological treatment of wastewater and is still the most common process in usage today (Metcalf and Eddy, 2003).

Aerobic suspended growth processes utilize a bioreactor that provides a well mixed and aerated environment conducive to cellular growth requirements. Treated effluent is then discharged to a solids separation process to remove and retain biological solids and produce an effluent for further treatment or discharge. The activated sludge process relies on a bacterial population that is mostly found in biological floc, a clustering of bacteria bound by extracellular polymeric substances (EPS). The combination of influent wastewater and AS flocs is termed mixed liquor. Figure 2.1 demonstrates the typical composition of an activated sludge floc.

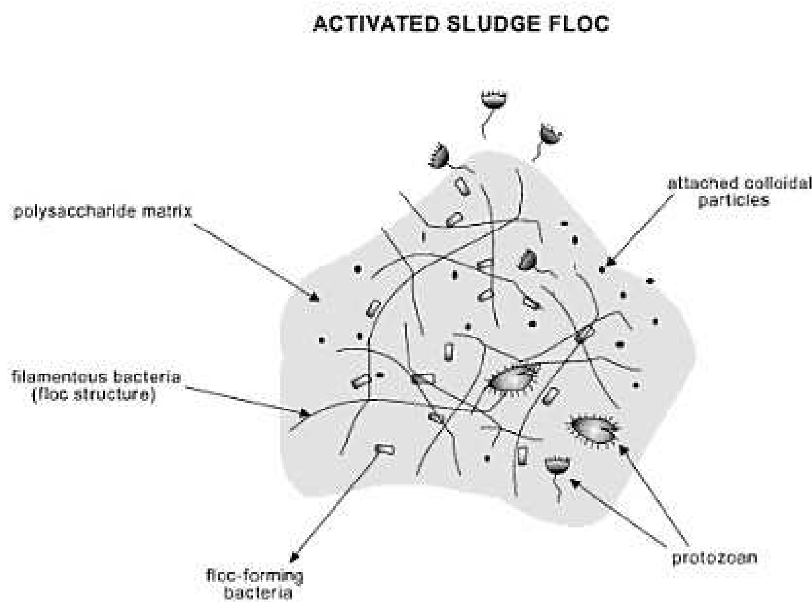


Figure 2.1 - Typical Activated Sludge Floc composition (Von Sperling, 2007)

The treatment capability of an activated sludge system relies on the ability to maintain a sufficient biomass population. Solids retention in suspended growth systems are typically achieved by secondary clarification: a quiescent environment which encourages the settlement of AS flocs. Settled flocs are then returned to the bioreactor to maintain a stable bacterial population, or biomass. As additional contaminants are introduced to the bioreactor via the influent, bacterial growth will continually increase the level of biomass. Excessive biomass concentrations can lead to operational issues and must periodically be removed from the system for additional treatment and disposal. Biomass removed from the bioreactor is termed waste activated sludge (WAS).

Poor settling sludge is cited as one of the most frequent operational issues in AS plants globally (Martins et al., 2004). Sludge settlement issues can lead to high effluent solids concentrations, reduced efficiency of disinfection and unpredictable biological treatment performance. The susceptibility of the suspended growth process to biomass loss as a result of process upset is one operational weakness of suspended growth systems. Additionally, secondary clarifiers require large land area relative to the total WWTP footprint to provide suitably low hydraulic and solids loadings to ensure efficient settling. While the activated sludge process is considered to be more straightforward

operationally, the performance and design constraints identified above may make an attached growth process a more attractive option to the designing engineer.

2.1.2. FIXED FILM PROCESSES

Under suitable conditions bacterial cells within wastewater treatment processes will excrete extracellular compounds, comprised of proteins and polysaccharides and these are used to attach and colonize a suitable surface. As cellular growth proceeds, a biofilm that consists of immobilized cells located within a porous matrix will develop. Attached growth systems exploit this process to develop a biomass, contained within the biofilm, which does not rely on solids separation processes for biomass retention, although separation is required to ensure a low solids effluent is produced.

Historically, biofilms have been used in the wastewater treatment processes since the 1890s when trickling filters, consisting of biofilms grown on a media surface, typically stone, were brought into use. As early as the 1920's the use of supplementary materials providing increased surface area for biofilm development into bioreactors was reported (Doman, 1929). In addition to trickling filters, several biofilm processes in common usage include the rotating biological contactor (RBC), the moving bed biofilm reactor (MBBR) and the biological aerated filter (BAF) (WEF, 2010).

Bacteria that compose the biofilm are not fecal in origin; they predominantly originate from soil. Infiltration of groundwater into WWTFs is therefore a pathway for colonizing bacteria introduction into the bioreactor and may have an effect on the time required for biomass growth. In a general sense, there are 3 phases of biofilm growth, as described below (WEF, 2010) and depicted in Figure 2.2:

- Organic conditioning layer coats the media
- Bacteria come into contact w/ "organically tempered" surface. Sorption and de-sorption is controlled by van der Waals forces
- Permanent, irreversible adhesion due to cells secreting glucocalyx that binds it to surface.

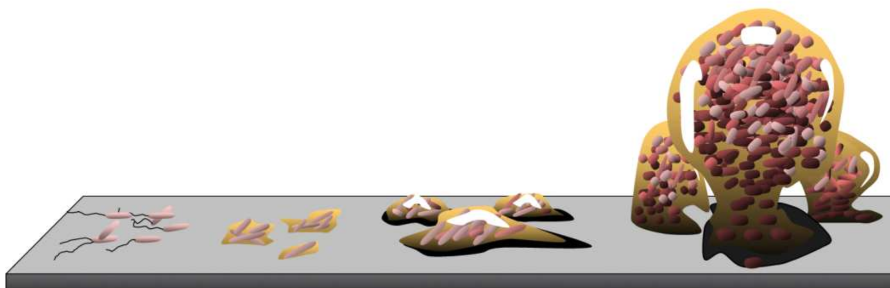


Figure 2.2 – Biofilm development stages, adopted from Monroe, 2007

Biofilms are theorized to develop in thin dense clusters of cells, which form roundly shaped colonies of bacterial consortia held together by extrapolymeric substances. These colonies are seperated by voids, which allow the passage of liquid and provide the biofilm a porous structure (Lewandowski et al., 1999). Recent in-situ modeling of a bacterial biofilm further elucidated the structural composition of biofilms (Berk et al., 2012). The results of this analysis are demonstrated in Figure 2.3 where bacterial cells are shown in blue and EPS components consisting of polysaccharides and proteins are shown in green, grey and red. Components shown in grey and green are noted to contribute to surface adhesion of the biofilm and components shown in red were found to provide a protective encasement, increasing the resistance of biofilms to antimicrobial attack.

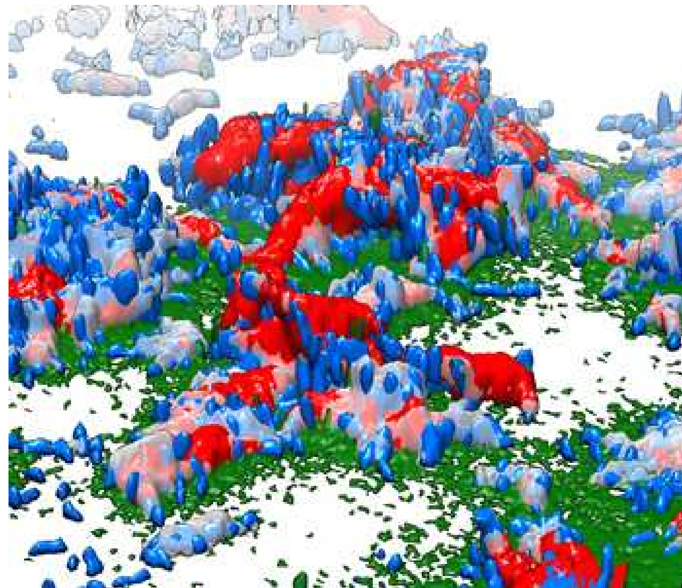


Figure 2.3 - 3D modelling of a bacterial biofilm based on in-situ molecular analysis (Berk et al., 2012).

The fixed film environment provides some advantages to the bacterial population. Bacteria contained within the biofilm are held in place through adhesion and are less susceptible to washout. Washout occurs when the bacteria are not provided a sufficient growth period and cannot sustain a population. However, the structure of the biofilm imposes diffusional limitations on substrates, such as organic compounds, nutrients and dissolved oxygen, resulting from resistance imposed by the liquid boundary layer and the biofilm matrix itself. This can lead to slower growing organisms being outcompeted for space by those that can uptake substrates and grow quicker. In wastewater bioreactors, the surface area provided is limited and therefore the process capabilities are limited as a function of the surface area available.

Varying process conditions will result in modifications of biofilm composition, leading to a constantly changing biofilm environment. As the biofilm thickens due to bacterial growth, diffusional limitations result in

deeper biofilm layers becoming anoxic or anerobic. However, the outer layers of the biofilm closest to the bulk liquid are more susceptible to sloughing by hydrodynamic forces. This results in biofilms being a somewhat self-regulating process in which diffusional resistance and biomass structure variability allows for constantly evolving environmental niches resulting in a highly diverse bacterial population. Daims et al., (2001) investigated the microbial diversity of biofilms treating sludge dewatering reject waters, characterized by high ammonia and salt content. Diverse species of ammonia and nitrite oxidizers were observed to co-exist within close proximity suggesting a mutualistic relationship was occurring. This result was contrasted with other studies that observed a primarily monoculture bacterial consortia in activated sludge reactors as a result of the extreme process conditions. As the transformation of complex and recalcitrant compounds is often attributed to co-metabolism and enzymatic activity with a broad specificity, the increased diversity provided by biofilms may result in improved biodegradation capabilities.

Depending on the depth of biofilm, diffusional limitations can lead to co-existing environmental niches comprised of aerobic, anoxic and anaerobic process conditions (Lewandowski and Beyenal, 2003). These environmental niches provide conditions favourable for a wider variety of co-existing species than otherwise may not occur in suspended growth systems. Observations made on trickling filter biomass indicated that heterotrophic bacteria and fungi resided in the upper aerobic layers. The presence of fungi is of interest when considering biotransformation of pharmaceuticals as some fungi can secrete enzymes with broad specificity and have been used for the enhanced transformation of PCs (Rodarte-Morales et al., 2011).

In the presence of both abundant ammonia and soluble organic substances, bacteria compete for available substrates and space within the biofilm. Investigations into the distribution of bacteria within biofilms typically indicate that heterotrophs reside on the outer surface of the biofilm where the higher presence of dissolved oxygen and organic substrates provides conditions allowing them to out-compete slower growing heterotrophs (WEF, 2010). Under high soluble organic loadings, nitrification performance is significantly reduced and may be entirely eliminated. The function of the biofilm will thus be tailored to specific environmental conditions. It is therefore possible that bacteria within the biofilm will become adapted to the presence of recalcitrant compounds, as a result of the extended retention times, and provide a greater potential for their utilization as substrates.

Diffusional limitations can be beneficial for biofilm organism and result in an increased resilience against perturbation. Wilderer and McSwain (2004) postulated that microorganisms growing in a biofilm environment are provided protection from bulk substrate conditions that may be inhibitory, such as pH, temperature and the presence of toxic compounds. Further, highly variable influent conditions, both in terms of physical and chemical composition, can lead to destabilization of AS performance (Metcalf and Eddy, 2003). Biofilm processes provide a protected environment in which process upset is minimized and slow growing bacteria can flourish. The biofilm also provides an environment that is resistant to starvation. Freeman and Lock (1995) postulated that the polysaccharide matrix traps nutrients and enzymes in close proximity to bacterial cells, providing a type of substrate “rationing”. This would also provide an environment that encourages the

development of bacterial species which may otherwise be inhibited or washed out in suspended growth systems.

The improved stability of the biofilm environment provides the ideal environment for the growth of slow growing micro-organisms, and hence may increase the ability for degradation of recalcitrant compounds. Wolfaardt et al., (1998) conducted an experiment in which a biofilm community was exposed to chlorinated aromatic molecules. These molecules were found within the EPS and the author observed utilization of these molecules as a carbon source during starvation of the biomass. The ability to degrade complex substrates may suggest that biofilms will demonstrate an enhanced ability to transform pharmaceutical compounds, which possess a similar chemical structure.

In summary, the biofilm environment, in comparison to a suspended growth environment, provides potential for high retention times of bacteria, entrapment of enzymes which may promote co-metabolism, and a more diverse bacterial consortium due in large part to the presence of multiple redox conditions. All of these factors result in a more stable biomass population, capable of improved treatment efficiencies of conventional compounds. It is possible that biofilms will provide improved opportunity for PC transformation as well. Despite these identified process advantages, very limited attention has been provided to fixed film processes in regards to PC fate. The current study was therefore conducted to elucidate whether these factors result in improve PC transformation relative to a suspended growth system.

2.1.3. PROCESS CONTROL PARAMETERS

As will be subsequently demonstrated biological reactors can be operated over a range of conditions that will affect their performance. This section reviews selected conditions that were deemed to be relevant to the current study. The key design and operational parameter governing oxidation processes in a suspended growth bioreactor is the solids retention time (SRT, θ). The SRT provides an estimate of the average time a bacterial cell spends in the bioreactor and is defined in Equation 2.1 (Metcalf and Eddy, 2003), below:

$$\theta = \frac{\forall_{bioreactor} \times X_{MLSS}}{[Q_{WAS} \times X_{WAS}] + [Q_{EFF} \times X_{EFF}]} \qquad (2.1)$$

where:

Θ = SRT (d)
$\forall$ = Volume (m^3)
X = Solids Concentration (g/m^3)
Q = Flow (m^3/d)

If bacteria are not retained in the system for a sufficient period of time to achieve cellular growth, functionality of that bacterial group will be lost. In suspended growth systems the rate of growth of organisms is inversely proportional to the SRT required, and hence long SRTs are required to retain slow growing organisms. In suspended growth systems, the SRT directly affects the ability of a WWTF to sustain nitrification, a process whereby ammonium (NH_4^+) is converted to nitrite (NO_2^-) by ammonia oxidizing bacteria (AOBs), and further from NO_2^- to Nitrate (NO_3) by nitrite oxidizing bacteria (NOBs) (Metcalf and Eddy, 2003). These processes are termed nitrification and nitritation, respectively. Both AOBs and NOBs are slow growing organisms that require extended SRTs to encourage a stable population achieving consistent performance. Nitrification performance is highly dependent on temperature as a result of substrate utilization and growth kinetics.

In biofilm systems complex mechanisms including biomass growth, sloughing and the spatial distribution of heterotrophs and autotrophs within the biomass structure make the retention times for the attached growth phase difficult to measure or control. Patel et al., (2005) postulated that detachment of porous, heterotrophic growth at the surface of the biofilm is higher than in the more dense autotrophic growth near the substratum. This implies that SRT varies through the depth of the biofilm, thus making an SRT estimation of little use for process control. Rather, the ability to achieve stable nitrification performance is a function of the surface area provided as well as the ability to create an environment low in soluble BOD_5 (WEF, 2010).

In addition to the SRT, the biodegradation of contaminants in both suspended growth and fixed film processes is governed by the hydraulic retention time (HRT, τ). This parameter is used to provide an average estimate of the period process wastewater is retained in the bioreactor prior to discharge. HRT is calculated based on the assumption that reactors are perfectly mixed and no short circuiting occurs. The HRT is defined mathematically by equation 2.2.

$$\tau = \frac{\forall_{Bioreactor}}{Q_{Influent}} \qquad (2.2)$$

where:

τ = HRT (d)

In addition to providing a sufficient SRT and HRT, operational parameters such as pH, alkalinity and dissolved oxygen must be controlled to create an environment that is suitable for bacterial growth. The nitrification reaction consumes alkalinity and if the wastewater does not possess sufficient alkalinity, the operating pH will be suppressed leading to the inhibition and eventually complete elimination of nitrification abilities. At a pH less than 6.5, substantial nitrification does not occur (Paredes et al., 2007) and therefore if the nitrification requirements exceed the background level of alkalinity within the bioreactor alkalinity must be supplemented by an additional source, such as sodium carbonate, sodium bicarbonate, or a base must be added, such as sodium hydroxide.

2.1.4. *THE IFAS PROCESS*

The IFAS is a hybrid process that utilizes a suspended growth phase as well as a fixed film phase to achieve low levels of COD and TAN. In order to encourage the development of an attached growth phase, artificial media are introduced into the bioreactor to provide surface area for biomass development. The IFAS process can be categorized by media type: those utilizing fixed media (Muller, 1998) and those utilizing free floating media in a moving bed biofilm configuration (Odegaard et al., 1994). The usage of free floating HDPE media to create a moving bed configuration is believed to provide a better configuration for biofilm growth as it prevents excessive biofilm thickness and the development of higher organisms that prey of bacteria due to media collisions (McQuarrie and Boltz, 2011). The media was specifically designed to provide a protected surface area for biofilm without excessive hydrodynamic shear or mechanical scouring resulting from media collisions. An illustrative example of moving bed media with biofilm growth is provided in Figure 2.4.

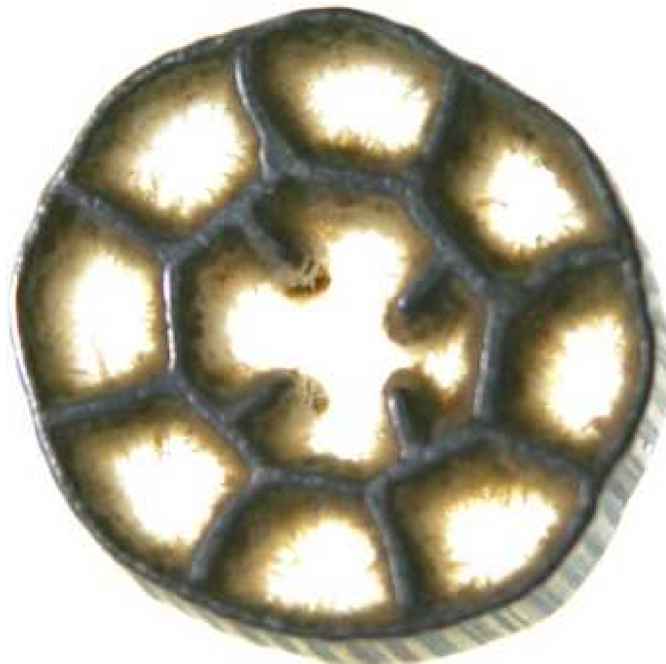


Figure 2.4 – HDPE media used in IFAS Process with biofilm colonization (300% magnification, Headworks International)

In the case of a moving bed biofilm configuration, media is added to the bioreactor at a fill fraction (v/v) between 30 and 65%. The IFAS media are retained within the bioreactor, typically by mesh screens, which permit the MLSS to flow through to a secondary clarifier. The ability to operate at a reduced MLSS results in lower solids loading rates applied to the downstream clarifiers, significantly reducing land area requirements. Settled biomass is returned to the reactor in the same fashion as an AS process. Most IFAS designs utilize a mixed liquor concentration between 2000 and 3000 mg/L, HRTs of 4-6 hours and operating SRTs between 2 and 8 days (WEF, 2010).

Early studies have reported that full nitrification under low mixed liquor SRT and thermally challenging winter conditions could be achieved by the addition of HDPE free floating IFAS media (Jones et al., 1999) to the bioreactor. Subsequent research has demonstrated that IFAS systems achieve consistent nitrification performance despite temperature variance (Bjornberg et al., 2010). Under similar operating conditions, AS has been observed to undergo settling issues (Morgan-Sagastume and Allen, 2003) and nitrification capacity reduction (Hwang and Oleszkiewicz, 2007). Some studies have reported that the incorporation of IFAS media resulted in a system more resistant to colonization by filamentous organisms (Sriwiriyarat et al., 2008).

Despite the literature demonstrating its benefits, IFAS has not evolved as a mainstream treatment process. Boltz et al., (2012) reported that as of 2011, only 20 IFAS systems were in usage, 15 of which were located in the US. This is likely due to the lack of design reference materials; the information currently available is mostly fragmented and is not well documented. In Canada, IFAS has not experienced wide implementation and is typically restricted to retro-fit applications (Nutt et al., 2011). As better design information and full scale process data becomes available, upgrades to aging WWTF infrastructure required to address capacity demands may result in a wider implementation of IFAS technology, particularly where full nitrification has become a standard requirement.

Moving Bed Biofilm Reactor (MBBR) wastewater treatment processes are a similar treatment process to IFAS. However, MBBRs rely solely on fixed film biofilms for treatment; no mixed liquor is maintained within the reactor. An inverse relationship between nitrification capacity and the organic loading applied to MBBR systems has been demonstrating, allowing MBBRs to be designed based on empirical data (Hem et al., 1994). For IFAS systems, this relationship is more complicated due to the interactions between the suspended growth and fixed film phases. The level of nitrification in IFAS systems has been demonstrated to be directly influenced by the mixed liquor SRT and the concentrations of substrates present. Maas et al., (2008) observed that the operating food to microorganism ratio (F:M, gBOD/gMLSS·d) is noted to be correlated directly with the attached growth total solids (AGTS) and inversely correlated to nitrification capacity.

Recognizing the inherent difficulty in assessing the SRT of IFAS biofilms, Maas et al., (2008) devised a novel technique to directly assess the nitrification capacity of an IFAS system. The authors studied the biofilm developments of a plug flow 4 cell, IFAS system operated at full scale. As the systems developed and the biofilm thickness reached steady state, AGTS was found to be inversely correlated to nitrification capacity and was thus not considered a reliable measurement to assess performance. This observation has been noted by others (Jones et al., 1999). Maas et al., utilized a modified oxygen uptake rate test, with IFAS media pieces withdrawn from the full scale bioreactor, to estimate nitrification capacity. This testing method was compared to batch nitrification testing and in-basin grab sampling from the full scale WWTP. The results of all three testing methods were found to be in good agreement. Nitrification rates, depending on the operating F:M, were found to vary between 0.3 to to 1.2 gN/m^2/d.

2.2. *PHARMACEUTICAL COMPOUNDS*

Pharmaceutical compounds (PCs) are drugs that are used in veterinary and human medicine (Ternes and Joss, 2006). Although PCs have been recognized as an environmental contaminant for many decades (Richardson and Bowron, 1985), studies into the fate and environmental effects of PCs in natural matrices were rare, mainly due to the difficulty in quantification at the extremely low concentrations present. The development of new enrichment methods and the advancement of analytical techniques within the last 20 years have resulted in a significant increase in PC investigations (Richardson and Ternes, 2005).
It is estimated that there are more than 3000 pharmaceuticals in common usage (Richardson and Ternes, 2011). Research has primarily focused on PCs with the highest prescription rates, or those that are believed to pose the greatest risk to the environment at large, with a particular focus on unintentional human consumption through water re-use. Pharmaceuticals can generally be classified by their intended use, as identified by Ternes and Joss (2006):

- Antiphlogistics
- Antiepileptics
- Calcium antagonists
- Diuretics
- Synthetic and natural sex hormones
- Antibiotics
- Beta blockers
- Psychotropics
- Decongestants
- Drugs of abuse
- Antidiabetics
- Antihistimines
- Muscle relaxants
- Antigout

Pharmaceuticals are of particular concern as an environment contaminant because of their resistance to biodegradation and their polar nature, resulting in persistence within environmental matrices (Halling-Sorensen, 1998). Lam et al., (2004) utilized several 12 m^3 microcosm aquatic environments containing fish, aquatic plants, zooplankton, phytoplankton, macrophytes and bacteria to study the fate of selected PCs in environmental systems. The results demonstrated that biodegradation was not a significant transformation pathway, with PC half-lives ranging from approximately 1 day for Acetaminophen, to 5 to 20 days for the antibiotics Trimethoprim and Sulfamethoxazole, respectively. The highly persistent compound Carbamazepine was found to have a half-life as high as 93 days. This suggests that PCs conveyed to surface waters undergo slow and limited transformation processes, potentially exposing aquatic organisms to continuous low levels of PCs.

The persistence of PCs and the potential for human exposure has been demonstrated through characterization of source and treated drinking waters. Benotti et al., (2008) performed an investigation of US drinking water and found detectable levels of several PCs in source, treated and ‘at tap’ drinking waters indicating that unintentional uptake of PCs through potable water is occurring. A Canadian study investigating the occurrence of pharmaceutical compounds in treated drinking water did not find detectable concentrations, however this study was restricted to 9 pharmaceutical compounds (Servos et al., 2007) and therefore is far from conclusive in proving that unintentional uptake of PCs through potable water is not occurring in Canada.

PCs at levels found in environmental waters are currently not considered a proven environmental hazard to humans as a result of unintentional uptake through potable water reuse. However, antibiotic resistance bacteria arising due to: wastewater treatment (Da Silva et al., 2006); increased agricultural usage of antibiotics (Smith et al., 2005); and, assimilation by agricultural crops of antibiotics resulting from land application of manure or wastewater biosolids all have been cited as requiring further investigation and assessment of risk (Grote et al., 2007). Despite the lack of conclusive evidence that PCs are a threat to human health, it is widely acknowledged that the potential long term effects of PC discharges are not well understood and therefore cannot been ruled out as a concern.

PCs within the aquatic and terrestrial environment have been shown to produce a multitude of detrimental effects. Investigations related to the feminization of fish resulting from the exposure to wastewater effluents have demonstrated the estrogenic nature of wastewater effluents (Harries et al., 1997). The feminization of fish has been cited as popularizing the study of PCs as it captured the attention of the public as well as researchers (Ternes and Joss, 2006). Further, diclofenac, an anti-inflammatory, was found to cause a significant decline in the vulture population in Pakistan. The vultures were found to be consuming cattle that were given diclofenac for veterinary purposes, demonstrating that unintended exposure could have effects via the food chain. The loss was estimated to be as high as 40 million vultures (Oaks et al., 2004). In addition, it has been suggested that the release of antibiotics in low concentrations may affect the biodegradation of leaf and other plant materials, that serve as primary food source for aquatic life in rivers and streams (Richardson and Ternes, 2011). This demonstrates that the potential for negative effects as a result of PC discharges into environmental waters is a complex and poorly understood problem. The uncertainty regarding aquatic environmental effects, is made more complex by the ongoing discovery of transformation products of PCs which are generally more polar, may be more toxic and hence warrant continued attention. As the wastewater treatment process has been identified as the predominant source of PC loadings to environmental matrices, the investigation of PC fate throughout the wastewater treatment process is considered to be a topic deserving of additional focus.

2.3. Pharmaceutical Fate Through the Wastewater Treatment Process

Pharmaceutical compounds (PCs) are excreted, either in un-metabolized or metabolized forms, and are conveyed via wastewater streams to wastewater treatment facilities (WWTFs) where they undergo varying levels of biotic and abiotic transformations before being disposed of via effluent to a receiver (Daughton and Ternes, 1999). The application of biosolids to land as fertilizers has been postulated to result in PCs being conveyed to rivers and streams via overland flow (Edwards et al., 2009). The concentrations observed in wastewater received at treatment facilities suggest that the majority of PCs are disposed of via wastewater, making WWTFs the primary source of PC discharges into the environment (Ternes and Joss, 2006). As a result of their environmental significance, the wastewater treatment process has been the subject of extensive research focused on methods to better engineer treatment processes for maximal PC eliminations (Joss et al., 2008).

Early investigation methods lead to the conclusion that PCs were non-biodegradable (Richardson and Bowron, 1985). The methods used in many of these early studies were not well informed regarding biological treatment mechanisms, and resulted in experimental conditions which were not reflective of full scale wastewater systems (Ingerslev and Halling-Sorensen, 2000). As analytical techniques developed, PC fate investigations of full scale treatment systems were contradictory with these findings, demonstrating that process configuration and other operation factors warrant consideration.

A review of observations published for a variety of batch scale systems and full scale systems have demonstrated inconsistent trends regarding PC eliminations citing the HRT, SRT, temperature, influent characteristics and redox conditions as having influence on biological transformation processes. However, many of these factors have been found to influence the transformations of PCs on a compound specific basis, demonstrating that no single process variable can be solely attributed to improved elimination efficiency.

2.3.1. REMOVAL MECHANISMS

The removal of PC's through wastewater treatment can be attributed to three main fate mechanisms, namely: volatilization, sorption and transformation (Schwarzenbach, 2003). To quantify the fate of the PC's through the pilot bioreactors used for the current study, an understanding of these mechanisms is required.

2.3.1.1. VOLATILIZATION

Compounds that are easily volatilized may experience removal in the wastewater treatment process due to the vigorous aeration provided. This is typically referred to as air stripping. A PCs affinity to volatilize is determined by the Henry's law constant for a target compound, which provides a partitioning relationship between air and water. Struijs et al., (1991) estimated that for compounds with a Henry's law constant less than 1×10^{-4} ($atm \cdot m^3 \cdot mol^{-1}$), volatilization is expected to account for approximately 5% or less of the total removal from a wastewater treatment process. Similarly, Ternes and Joss (2006) report that a Henry's Law constant greater than 3×10^{-3} ($Pa \cdot m^3 \cdot mol^{-1}$) is required to observe any losses due to air stripping in a bioreactor with fine bubble aeration.

2.3.1.2. SORPTION TO SOLIDS

Historically, mechanistic models were used to describe the removal of PCs as a result of contact with activated sludge. These models implemented sorption and desorption rates through the use of a single K_D value. The determination of K_D can only occur when the concentration of the compound sorbed to solids is in equilibrium with the liquid phase concentration (and by extension the sorption and desorption rates of the compound to and from solids). As full-scale wastewater treatement processes experience a high degree of change due in large part to influent conditions, determinations of sorption values for wastewater sludge has largely been conducted in batch scale experiments under relatively steady-state conditions.

This process can be described mathematically by equation 2.1, below (Joss and Ternes, 2006):

$$K_d = \frac{X}{X_{SS} \cdot S} \quad (2.1)$$

Where:

- K_D is solid-water distribution coefficient [L gss^{-1}]
- X is the concentration sorbed onto sludge, per unit reactor volume. [ng L^{-1}]
- X_{SS} is the suspended solids concentration in raw wastewater or production of suspended solids in primary and/or secondary treatment per L of wastewater. [gSS L^{-1}]
- S is dissolved concentration of substance [ng L^{-1}]

If the total concentration, C (ng·L^{-1}), is considered to be equal to the sum of the soluble and sorbed concentrations, the total concentration is characterized by equation 2.2:

$$C = S \cdot (1 + X_{SS} \cdot K_d) \quad (2.2)$$

The ratio of pharmaceutical compounds can also be projected as a function of the losses due to sorption to waste activated sludge based on equation 2.3, below:

$$\frac{X}{C} = \frac{K_d \times Y}{1 + K_d \times Y} \quad (2.3)$$

Where:

- Y = Sludge Yield = gMLSS produced/$gBOD_5$ removed·d

The level of sorption of PCs largely depends upon the ionic state of the compound when exposed to typical WWTP pH conditions. The compounds analyzed in this analysis are largely non-ionic (neutral) within the WWTP. As a result, these compounds may be absorbed in the lipid fractions or sorbed onto organic matter via van der Waals interactions (Golet et al., 2003).

Solid-liquid partitioning coefficients were estimated by Ternes et al., (2004) using batch experiments in which high concentrations of pharmaceutical compound standards were added to a sample of primary and secondary sludge under conditions which suppressed aerobic biomass growth. It was not clear if this testing was done under truly anaerobic conditions or anoxic conditions, the latter of which may have led to some

level of bio-transformation (Nödler et al., 2012). However, mass balances on the sorbed and dissolved concentrations were found to be in good agreement with the spiked amounts. At a solids concentration of 4 gss·L^{-1}, equilibrium was found to be reached 0.5h after the addition of the pharmaceuticals to the batch reactors (Joss and Ternes, 2006). It should be noted that in the context of secondary sludge, reported K_D values were related to the quantity of sludge generated per unit of wastewater treated as opposed to the mixed liquor concentration.

Horsing et al., (2011) conducted sorption experiments using municipal sludge that had been biologically inhibited. This method measured only the dissolved fraction and thus may have overestimated the sorbed fraction if abiotic transformation processes were occurring. However, a good agreement with previous measurements was reported.

Ternes and Joss (2006) postulate that compounds with a K_D value less than 0.3 L·gSS^{-1} can be expected to experience removal of <10 % in a typical municipal sewage treatment plant. It was assumed for the purposes of this experiment that sorption coefficients determined for activated sludge reflect the sorption capabilities of biofilm. It is acknowledged that the presence of EPS or other structural differences between biofilms and activated sludge flocs may have an impact on sorption as biofilms are noted to be hydrophilic and negatively charged under neutral pH (Bryers, 2000**)**. Only one study was encountered in which sorption rates to biofilm were studied (Wunder et al., 2011) however this study achieved poor precision and repeatability. Two batch tests were conducted using SMX standards spiked at 0.33 μg/L and 3.33 μg/L which resulted in estimated K_{oc} values ranging from -2000 to 7000 L/kg. The final average estimate obtained for SMX was 4000 $\pm$ 1000 L/kg, which is approximately an order of magnitude higher than previously reported values for soil and activated sludge. The results presented within this study were considered to be too inaccurate for practical use.

2.3.1.3. TRANSFORMATION PROCESSES

Historically, investigations into contaminant fate within the wastewater treatment process have focused primarily on biotic removals (biodegradation). Richardson and Bowron (1985) appears to be one of the earliest PC fate investigations and predicted that sorption and volatilization can be largely ignored, and therefore the observed change in PCs through a WWTP must be due to biological activity. However, a number of studies have demonstrated that observed “removals” of PCs can be attributed to chemical transformations (Celiz et al., 2009; Nödler et al., 2012). Biotransformation may include full biodegradation to innocuous products or partial degradation, which may result in the creation of daughter products with unknown toxicity.

The majority of PC fate studies calculate elimination by comparison of concentrations of PCs measured in the soluble phase of influents and effluents. However, the disappearance of the parent compound cannot be considered to imply complete biodegradation (i.e., mineralization or loss of toxicity). Usage of the term

biotransformation, as opposed to biodegradation, has been recommended by some researchers to describe the perceived eliminations detected to avoid confusion with mineralization and the associated loss of toxicity (Onesios et al., 2009; Plosz et al., 2012).

Biodegradation rates (referred to in this document as biotransformation) are difficult to characterize with any significant level of certainty, largely due to the unknown degradation pathways and associated products that may be generated/cleaved to form the parent compound (Ternes et al., 2004). Early tests used to assess biodegradability of PCs failed to acknowledge the importance of the sludge used for testing and the presence or absence of key bacterial populations, such as autotrophs (Eichhorn et al., 2005). In many early studies it was assumed that all wastewater bacteria possess a homogenous ability to degrade PCs (Richardson and Bowron, 1985). Typically, sludges were provided the PC being investigated as the sole source of substrate without providing other nutrients required for cellular synthesis (Ingerslev and Halling-Sorensen, 2000). This likely led to the conclusion that many substrates were "not biodegradable" or that bacteria required a significant adaptation period before biodegradation would occur. However, this effect may have been masked by co-metabolism resulting from endogenous decay which would increasingly occur in an aerobic environment with an absence of growth substrates.

Based on published methods, biodegradation rates have been estimated using a pseudo first order kinetic equation, generally based on differences between influent and effluent concentrations after losses attributed to sorption have been taken into consideration. Biodegradation rates are characterized by k_{bio} coefficients (Joss et al., 2006). The k_{bio} value may be approximated by equation 2.4, below (Schwarzenbach et al., 2003):

$$\frac{dC}{dt} = \frac{C_{t+dt} - C_t}{dt} = -k_{bio} \cdot X_{SS} \cdot S \qquad (2.4)$$

Where:

- C is total compound concentration ($ng{\cdot}L^{-1}$)
- t is time (d)
- k_{bio} is reaction rate constant ($L{\cdot}gSS^{-1}{\cdot}d^{-1}$)

Substituting the sorption equation (2.2) into equation 2.4 yields equation 2.5, which provides a general model for contaminant fate within a bioreactor:

$$\frac{dS}{dt} = \frac{-k_{bio}}{1 + K_d{\cdot}X_{SS}} \cdot X_{SS} \cdot S \qquad (2.5)$$

This method of expressing removal of contaminants via assumed biological degradation is a generalization and does not consider the biological pathway or the products which may be formed and which may also be reversible based on different process conditions. It is noted that for the majority of studies, k_{bio} is expressed per sludge dry matter concentration (MLSS). This would appear to be counterintuitive given that PC removal is largely attributed to co-metabolic processes and therefore MLVSS would be a more accurate method of normalization (Majewsky et al., 2011). Depending on the age of sludge samples used in biodegradation studies, and the amount of time samples have been operated without substrate, the VSS/TSS ratio may demonstrate considerable variance, resulting in a skewing of rates being reported. The majority of recent studies appear to simply state observed removal rates (in percentage) based on comparisons of influent vs. effluent concentrations.

The theory of co-metabolism being the responsible mechanism for the removal of poorly degradable organic compounds is long standing and has been cited as a likely removal mechanism for PCs by a number of researchers. Namkung et al., (1983) postulated that trace organics do not contribute to bacterial growth and thus a primary substrate is required to sustain a microbial population. Co-metabolism can be theoretically described by the multisubstrate monod growth relationship, utilizing monod growth coefficients, which suggests that the total growth rate is the sum of the growth occurring for a variety of compounds utilized via co-metabolism. Equation 2.6, below, describes this relationship (Schwarzenbach et al., 2003):

$$\mu_{tot} = \sum_{i=1}^{n} \mu i, \quad \mu_i = \frac{\mu_{max,i} \cdot S_i}{K_{i,S} + \sum_{j=1}^{n} \left(\frac{k_{i,S}}{k_{j,S}}\right) \cdot S_j} \qquad (2.6)$$

Where:

- μ_{tot} is the specific biomass growth rate (d^{-1})
- μ_i is the specific biomass growth rate on substance i
- $\mu_{max,i}$ is the maximum specific biomass growth rate on substance i (d^{-1})
- S_i is dissolved concentration of substance i [ng L^{-1}]
- S_j is the dissolved concentration of substance j (mg/L)

$K_{i,S}$ is the half-saturation constant associated with substance i

- $K_{j,s}$ is the half saturation constant associated with substance j

Janke and Fritsche (1985) suggested that co-metabolism of xenobiotic compounds in natural eco-systems is likely a slow process as the specific micro-ogranisms responsible are likely to be low in population, and the introduction of recalcitrant xenobiotic compounds will not result in significant growth. However, the authors suggest that in engineered systems, such as the wastewater treatment process, high concentrations of

biomass and an abundance of co-metabolic substrate mixtures could lead to significant attenuations of xenobiotics. This can be somewhat confirmed by the rather slow transformation process observed by Lam et al., (2004) using replicated aquatic environments. This would suggest that improved elimination of PCs through the wastewater treatment process would provide protection to the aquatic environment and is a process requiring better understanding. Additionally, the wastewater treatment process is likely to possess a diverse bacterial consortium that will be more successful in degrading pharmaceuticals as a result of complementary transformation processes resulting from multiple microorganisms participating in degradation.

In environmental systems such as WWTPs, mixed bacterial consortia contribute to PC degradation present at trace concentrations relative to high co-metabolic substrate concentrations. Therefore, the biotransformation rates and products produced are expected to be determined by the microbial diversity of the system and the available pool of enzymes. However, Larcher and Yargeau (2011) found that the transformation of SMX in pure culture systems, utilizing species of rodococcus and pseudomonas, was greater than a mixed culture containing the same bacteria, demonstrating anti-synergistic effects.

As identified by Clara et al., (2005) if a pharmaceutical is biodegradable and degradation via co-metabolism can be described by the above relationship, even in low concentrations, a specific SRT required to grow organisms responsible for co-metabolism of that compound can be determined. In addition to SRT, temperature influences the maximum growth rate of wastewater bacteria. This has a direct effect on the required minimum SRT, particularly for nitrifying organisms which are very susceptible to wash-out under challenging growth conditions.

TRANSFORMATION PRODUCTS

Namkung et al., (1983) postulated that co-metabolism may result in partial degradation of PCs, resulting in the formation of intermediates, or transformation products (TPs). Despite acknowledgement of TPs as likely occurring (Richardson and Bowron, 1985, Daughton and Ternes, 1999), early investigations into PC fate through the wastewater treatment processes focused primarily on parent compound "removals". Usage of the term "removals" appears to be a misnomer as this could be interpreted as elimination of the risk to the aquatic environment. Some authors, but not all, acknowledge that the parent compound may have undergone biotic or abiotic transformations to form a TP, of which the structure, persistence and toxicological effect is unknown.

TP's occuring in environment due to biological induced transformations can be classified into 3 categories (Escher and Fenner, 2011):

- Metabolites of organic compounds formed during Ph. I and II metabolism (mammalian and human metabolites);
- TP's formed during advanced treatment processes, such as advanced oxidation processes used in drinking water treatment; and

- TP's formed from transformation reactions occuring in environmental and engineered systems such as microbial degradation, hydrolysis and photolysis.

Transformation products have recently been given increased focus, largely due to the historical difficulties in obtaining standards to facilitate their measurement using common analytical techniques. Studies which attempt to elucidate the degradation pathways either require the synthesis of transformation products to allow for analysis using analytical techniques or the use of time of flight mass spectrometry which allows TP structures to be estimated based on detected masses (Ternes and Joss, 2006). Helbling et al., (2010) suggested that the amide functional group is a common structure in many PCs and reactions occurring in relation to this group may be a primary mechanism for the formation of TPs. In this study, various bench scale experiments were performed using seed sludge from an MBR reactor to detect TPs associated with 30 amine containing PCs. The degradation pathways observed included: amide hydrolysis and N-dealkylation, hydroxylation, oxidation, ester hydrolysis, dehalogenation, nitro reduction, and gluthatione conjugation.

Transformation products should be considered during fate investigations or those concerned with determining the toxicological effects of PC releases on the environment. Several studies have been conducted in which TPs were found to be more toxic than the parent compound. Bedner and MacCrehan, (2006) found that two of the 11 TPs of Acetaminophen detected under chlorination of wastewater effluents, 1,4-benzoquinone and N-acetyl-p-benzoquinone imine, have toxicities higher than their parent compound. A TP of CBZ, CBZ-10,11-epoxide, which has been found in wastewater effluents (Miao and Metcalfe, 2003) and surface water (Kern et al., 2009), was found to result in malformations of fetal mice. A Toxicology study using D. magna completed by Trovo et al., (2009) found that SMX byproducts of photodegradation were more toxic than the parent compound. When micropollutants degrade in environmental matrices they may form persistent and potentially toxic TP's which should be included in risk assessment of parent TPs (Daughton and Ternes, 1999).

2.3.2. PHARMACEUTICAL ANALYSIS IN WASTEWATER

Most PC's found in environmental waters are in the ng/L range, or in the case of wastewater, the low ug/L range. In order to reliably quantitate PCs at these levels, advanced analytical methods and equipment are required. The predominant analytical techniques used to quantitate PCs in aqueous matrices are either liquid chromatography (LC) or gas chromatography (GC) coupled to some form of mass spectrometry (MS). Due to the ability to analyze polar compounds without derivatization, LC coupled with MS has emerged as the most popular analytical technique for PC investigations, however GC is still used in some instances with good analytical performance reported (Kimura et al., 2007; Togola and Budzinski, 2008; Kosma et al., 2010; Bisceglia et al., 2010). A 2010 review of pharmaceutical detection in environmental matrices completed by Petrovic et al., suggested that LC with tandem MS (MS/MS) was the most widely used analytical method. However, LC-MS/MS, particularly when utilizing electrospray ionization (ESI), is noted to be highly

susceptible to matrix effects, which can represent a significant source of uncertainty in data if not quantitated.

The mechanisms of matrix effects are not conclusively understood, however they are believed to be the result of competition between the analyte of interest and co-eluting compounds present in the sample matrix which react with the primary ions formed in the LC interface, resulting in reduced or increased detection of analyte signals (ion suppression or enhancement), interfering with the reproducibility and accuracy of measurements (Matuszewski et al., 2003). The usage of HPLC for chromatographic separation results does not provide for explicit detection of these impurities, leading to the misconception of high selectivity and accuracy. Due to the high sensitivity of HPLC analysis some researchers develop methods without sample concentration/clean up procedure (referred to as Direct Injection), however the limitations of these methods are evident by the high limits of quantification (Busetti et al., 2008). In particular, the presence of humic substances has been demonstrated to produce matrix effects in surface waters (Steen et al., 1999). Similarly, Renew and Huang (2004) reported that increasing concentrations of organic compounds were found to result in a similar increase in the limits of quantitation achievable during the analysis of wastewater.

To quantitate the presence of PCs in environmental samples, samples are injected into the LC under high pressure along with a mobile phase, typically a polar organic solvent, where separation of chemicals occurs via a stationary phase present within the LC column. Samples are then conveyed to the MS whereby precursor and product ions are detected based on their respective mass to charge ratio (m/z). This results in the production of a chromatogram which demonstrates signal intensities detected based on the retention time (Ramanathan, 2011). In order to quantitate samples with unknown concentrations, reference standards must be measured first. Reference standards at various concentrations are measured using the instrument, producing a calibration curve which relates the signal detected by the MS (peak areas on the chromatogram) to known values. Calibration curves should span the range of concentrations expected within the sample and should display a high degree of linearity ($r^2 \geq 0.99$). If samples are measured and found to be outside the linear range of the calibration curve, dilution of samples may be required; results obtained from sample measurements taken outside of the linear range of the calibration curve have a significantly reduced certainty and cannot be considered accurate. Calibration curves should consist of at least 5 points and may include a blank to assess the background noise associated with the equipment (Ternes and Joss, 2006). The obtained calibration curve can then be used with linear regression methods, allowing sample concentrations to be determined by inference.

Calibration curves can consist of known concentrations of the analytes of interest prepared in solvent, or matrix matched standards such as surface waters which are known to be free of detectable concentrations of PCs. The usage of internal standards, such as deuterated or isotopically labelled standards (ILS) are often used to correct for systematic errors and matrix effects, as these standards are expected to behave in an identical fashion to the native non-labelled analytes. However, historically, due to limited availability and high cost, these standards were not always included in research methods (Ternes and Joss, 2006). The

standard addition method can also be used, in which small (known) volumes of analyte reference standards are spiked into a sample at various levels and measured. By subtracting the spiked (known) amount, an estimation of the native concentration of the analyte can be determined. This method effectively eliminates the errors associated with matrix effects, but is quite time consuming (approximately 5 times the analytical effort of calibration curve methods (Hao et al., 2008) and is predicated on the assumption that the response at various concentrations will be linear. Due to the required laboratory effort, this method is rarely used.

In order to facilitate quantitation of most PCs at environmentally relevant concentrations, multi-step sample preparation has been nearly universally adopted. To achieve accurate analysis at the low levels found in environmental samples, as well as to reduce the amount of co-eluting compounds contained with the sample, solid phase extraction (SPE) is almost exclusive utilized as a sample preparation measure (Petrovic et al., 2010). Oasis HLB SPE cartridges, used for samples extraction under neutral pH, is the most common SPE method used in multi-residue (multi analyte) methods due to the HLB's ability to retain a broad spectrum of PCs (Vanderford and Snyder, 2006; Gros et al., 2006; Hao et al., 2008; Van Nuijs et al., 2010; Gomez et al., 2010). Further sample manipulation steps including additional SPE steps (Shao et al., 2009) and dilution of sample extracts (Hernando et al., 2004, Gomez et al., 2006) has been practiced to limit matrix effects, although both of these methods are expected to introduce additional errors due to the additional preparation steps.

To assess the lower limits of sample detection, limits of detection (LOD) and limits of quantitation (LOQ) are typically determined by the researcher/analyst. Relative standard deviations are also provided to determine the precision of the method (Ternes, 2001). In early studies, LODs or LOQs were estimated based on the second lowest point on the calibration curve (Ternes et al., 1998). LOQ and LOD in more recent studies are expressed based on a selected ratio of the signal for a given analyte to the noise measured on the chromatogram (typically 3 and 10 times, respectively) (Gros et al., 2006), or are determined by statistical means (Lishman et al., 2006). Most of these methods utilize reference solutions prepared in solvents or laboratory grade water to determine the LOQ/LOD and do not account for the matrix effects and lack of sensitivity associated with actual sample matrices. Extreme caution should be applied to data obtained through these methods as the level of accuracy and precision are likely grossly overstated.

With the improved sensitivity and selectivity of high performance liquid chromatography (HPLC), multi-residue methods have been developed which allow for quanitification of a large number of analytes, within different chemical classes and possessing different physic-chemical properties. According to research published by Gomez et al., (2010) Multi-residue methods are useful for environmental screening purposes, and prior methods using LC-MS/MS have been used to quantitate as many as 150-200 compounds in a single analytical run. In an attempt to expand on the limits of quantitation, Gomez et al., (2010) used time of flight mass spectrometry for the simultaneous detection of almost 400 compounds in a single analytical run. However, these methods lead to higher potential for analytical error as a result of compromised

chromatographic separation and mass spectrometry detection, as well as increased potential for "cross talk" amongst MS channels and analyte carryover.

As the fate of PC's through the wastewater treatment process is reliant on consistent measurements for comparison, researchers must consider methods to ensure that data obtained is reliable. As a result, quality assurance (QA) and quality control (QC) methods are considered to be of the utmost importance. QA and QC measures are typically employed which may include, as a minimum, quantification of matrix spikes to determine recoveries as well as blanks to detect for contamination within the preparation and analytical equipment (Ternes and Joss, 2006). Matrix spikes are prepared using an aqueous matrix that is free of PC's; typically lab grade water, tap water, or surface waters are used, although some methods used spiked aliquots of the sample matrix in a similar method to standard addition. The analysis of matrix spikes allows for the quantitation of standards at a known concentration, allowing the recovery of the method to be determined, typically expressed as a percentage of the spiked concentration. The recovery can be considered a gross measure of the errors associated with sample preparation and analysis. Generally, most studies aim to achieve a recovery within 20% of the spiked amount as a determination of good accuracy. However, as many studies utilize laboratory grade water with very minimal co-eluting matrix components, the recovery determined through matrix spikes may be vastly different from the recovery achieved with the actual sample matrix. The analysis of blank samples should produce no detected peaks; otherwise contamination of the equipment used in sample preparation or the analytical instrument is present and must be accounted for in the analysis.

Since the 1980's, the study of PC's in environmental matrices has attracted significant academic interest, resulting in a highly active field focused on quantitation, toxicological effects and the fate of PCs. However, limitations of technology made these early investigations technically challenging as they were characterized by a high level of analytical uncertainty, particularly in the case of difficult matrices such as wastewater. Recently, significant advances in analytical technology and methods have facilitated investigations with a much higher degree of sensitivity, accuracy and precision while significantly reducing laboratory labour. This has caused a significant increase in the number of studies completed, with academic interest increasing every year. Fatta-Kassinos et al., (2011) graphically demonstrated the increasing popularity of PC investigations, as well as the investigated sample matrix, during 2000 to 2010. These results are displayed below as Figure 2.5.

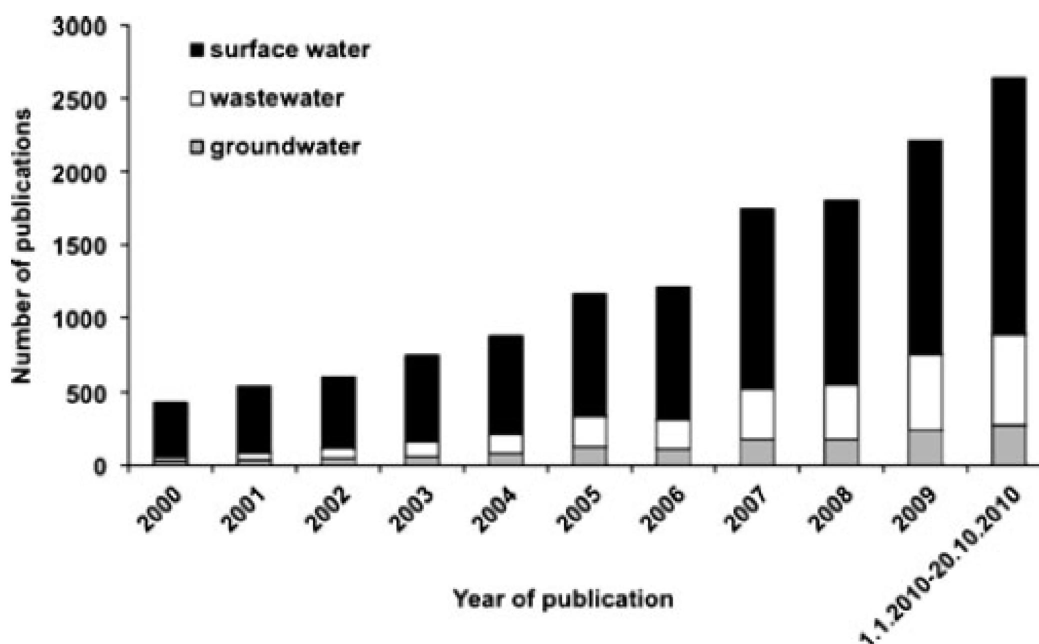


Figure 2.5 – Number of academic studies related to PCs as well as matrix investigated - 2000 to 2010 (Fatta-Kassinos et al., 2011)

As can be observed, despite the wastewater treatment process representing the most significant pathway for PC's into the environment, investigations which focus on wastewater are much less prevalent. Fatta-Kassinos et al., (2011) postulated that the lower number of studies related to wastewater can be attributed to the complexity of the matrix and to the fact that only a small number of laboratories have the capability to perform these analyses. However, in relative terms, the number of studies related to wastewater analysis, and the development of advanced methods with additional quality assurance considerations, has increased dramatically since 2006.

A study completed by Zhang et al., (2011) attempted to quantitate the difficulties and pitfalls associated with the analysis of certain sample matrices using LC-MS/MS, including fish muscle and brain tissue, blood, bile, tap water, surface water, and influent and effluent wastewater. This study concluded that the significant matrix effects present in wastewater can lead to reduced accuracy, precision, poorer detection limits and significantly limit the linear range of quantitation. In a comparison between biological samples, wastewater was found to produce the highest variability. Due to the matrix effects present in wastewater, LOQs were observed to increase by 2 to 10 times for wastewater samples relative to LOQs obtained for standards in neat solvents. This is in good agreement with other articles focused on critically investigating wastewater analysis methods which demonstrate significant increases in LOQ values for wastewater samples relative to neat solvents (Laven et al., 2009), lab grade water (Rodil et al., 2009) and uncontaminated surface water

samples (Tarcomnicu et al., 2010). These studies demonstrate the importance of quantitating matrix effects and ensuring that their impacts regarding the data obtained are understood. On a practical basis, wastewater influents have been noted to increase LOQs by 2 to 10 times relative to effluents (Laven et al., 2009). These factors make wastewater analysis particularly challenging and require that methods make best attempts to minimize, or at a minimum, understand the uncertainties inherent in the analytical process utilized.

The analysis of wastewater influents and effluents requires that samples be concentrated by as much as 400 times to facilitate quantitation using LC/MS-MS. However, this concentration step leads to a higher probability of matrix effects impacting analysis as well as the magnification of errors inherent in sample preparation and analysis. The usage of surrogate compounds as internal standards has become a consistently used analytical practice, as these compounds allow samples to be corrected for laboratory and analytical errors and, in particular, sample matrix effects. Deuterated (H_3) or isotopically labelled (C_{13}) versions of the analytes of interest provide the best results as these compounds will theoretically behave in an identical fashion to the non-labelled analyte.

The isotope dilution method, as described by Vanderford and Snyder (2006) has become widely used and has been proven to be a robust process which is compatible with multi-residue investigations providing a high degree of accuracy and repeatability. Some researchers have modified this method to use a single labelled standard to represent an entire class of PC's, although due to the wide physic-chemical parameters between PC analyte groups, and even within the same class of PCs, this method is considered somewhat of a compromise (Van Nuijs et al., 2010, Nurmi and Pellenin, 2011). Many recent studies which have focused on the difficulties in the analysis of wastewaters have identified that the use of ILS is compulsory to ensure valid data is obtained (Gros et al., 2006; Hao et al., 2008; Shao et al., 2009; Tarcomnicu et al., 2010)

Early studies into PC fate were conducted with methods which did not account for matrix effects and therefore the data presented must be interpreted with caution. Ternes (1998) observed that during a rainfall event, the removal rates of several antiphlogistics and lipid regulating agents were significantly reduced during a wet weather event. The author suggested that this effect may have been caused by biological or sorption related processes which were impacted by increased flows and reduced retention times. While the exact cause of this reduction is unclear, it should be dilution is known to significantly reduce signal suppression issues associated with LC-MS/MS analysis. It is therefore possible that the concentration differences may have been the product of wastewater dilution, resulting in a significant reduction in ion suppression and producing a more representative peak. These uncertainties highlight the importance of accurate analytical methods as well as the requirement for matrix effect investigations.

2.4. Prior Studies Of Pharmaceutical Removal in Biofilm Systems

The majority of past WWTP contaminant fate investigations have focused on activated sludge processes (Onesios et al., 2009). Based on a review of the literature, the investigations listed in Table 2.1 represent the limited number of PC fate investigations that have involved biofilm processes. It is noted that data regarding

IFAS and MBBR processes are limited to only several studies. Other studies exist, however these are primarily focused on very novel process situations, which include: MBBR utilized as a tertiary treatment process (Lundström et al., 2010) and the MBBR removing very specific contaminants, such as iodinated contrast media (Hapeshi et al., 2013).

From Table 2.1 it was concluded that the research conducted to date focusing on the ability of biofilm technologies to transform PCs has been significantly limited and that no transformation data exists for several commonly prescribed PCs. It was also noted that all studies investigating biofilm processes which utilize a moving bed configuration reported that these processes generally demonstrated increased PC elimination in comparison to activated sludge. This is likely partially due to MBBR and IFAS being relatively 'new' technologies that have experienced limited implementation at full scale. Given that it has been shown that these technologies provide improved process efficiencies, it is considered likely that their implementation in future will increase.

When it is considered that:

- environmental exposure to PCs has been identified as a major uncertainty warranting continued academic attention;
- the wastewater treatment process has been identified as the most significant source of PC loadings to the environment;
- optimization of wastewater treatment processes may provide a means of source control of PC loads discharged to the environment; and
- biofilm processes have been identified as potentially providing enhanced means of PC eliminations from wastewater streams,

It can be concluded that further research into biofilm processes and PC transformation is highly warranted.

Table 2.1 – Past Literature Related to PC Fate Investigation Concerning Biofilm Processes

Author	Process Investigated	PC's Quantitated	Comments
Stumpf et al., (1999)	TF/AS	DCF, IBP, GEM, KET, FEN, BZF, IDM, NAX	For all compounds eliminations appeared to be worse for TF
McAvoy et al., (2002)	TF/AS	TCS	Eliminations through TF were notably worse and less consistent than AS
Simonich et al., (2002)	AS/OX/TF/RBC/LAG	16 Fragrances	Eliminations through TF appeared to be worse. RBC had better removals but worse than AS, OX and LAG.
Joss et al., (2005)	FB/AS	CBZ, DCF, AHTN, N_4AC-SMX, SMX, IBP, IPM, NAX, HHCB, ROX	Fixed bed showed very similar performance to AS despite 1/10th the HRT of AS.
Thompson et al., (2005)	RBC/TF	TCS	RBC had worse elimination performance than TF and AS. AS was slightly improved over TF.
Batt et al., (2007)	RBC/AS/EA	CIP, SMX, TC, TRIM	With the exception of CIP, RBC has better eliminations for SMX and TC, similar for TRIM.
Kim et al., (2009)	IFAS/CAS (both operated as A_2O)	DEET, BPA, TCS, MTCL, CBZ, ATZ, NOPA, NNPA, E3, E2, E1, EE2, EEQ	IFAS demonstrated improved eliminations for DEET, BPA, TCS, NOPA, NNPA, E3, EE2 and EEQ, similar removals for CBZ, MTCL, E2, E1 and worse removals for ATZ only.
Falas et al., (2012)	MBBR/AS	IBP, KET, NAX, DCF, CLO, MFNA, GEM	MBBR demonstrated greater removal potential per unit biomass than AS for DCF, KET, GEM, CLO and MFNA. IBP and NAX were eliminated to a similar degree in both MBBR and AS.
Zupanc et al., (2013)	MBBR/AS	IBP, KET, NAX, CBZ, DCF, CLO	IBP, DCF and CLO noted to undergo significant increased elimination under the MBBR process when compared with CAS.
Margot et al., (2013)	MBBR/AS	BPA, NOR, ATEN, OFX, BEZ, m-BZT, MTN, TRIM, SMV, GEM, KET, IBP, MFNA, NAX, AZM, SOL, IMP	Data suggests that all compounds investigated, with the exception of IBP and NAX, were eliminated to a higher degree in MBBR compared to CAS.

Notes

TF – Trickling Filter

AS – Activated Sludge

OX – Oxidation Ditch

RBC – Rotating Biological Contactor

LAG – Lagoon

FB – Fixed Bed Reactor (Biostyr®)

EA – Extended Aeration

IFAS – Integrated Fixed Film Activated Sludge

PCs: DCF – Diclofenac, IBP – Ibuprofen; GEM – Gemfibrozil; KET – Ketoprofen; FEN – Fenofibric Acid; BZF – Bezafibrate; IDM – Indometacine; NAX – Naproxen; TCS – Triclosan; CBZ – Carbamazepine; AHTN – Tonalide; N_4AC-SMX N_4-Acetyl-Sulfamethoxazole; SMX – Sulfamethoxazole; IPM – Iopromide; HHCB – Galaxolide; ROX – Roxithromycin; CIP – Ciprofloxacin, TC- Tetracycline; TRIM – Trimethoprim; DEET- Deet; BPA – Bisphenol-A; MTCL – Metaclor; ATZ – Atrazine; NOPA – n-octylphenol; NNPA – n-nonylphenol; E3 – Estriol; E2 – Estradiol; E1 – Estrone; EE2 – Ethinyl estradiol; EEQ – Estrogen Equivalent Concentration; CLO – Clofibric Acid; MFNA – Mefenamic Acid; NOR – Norfloxacin; ATEN – Atenolol; OFX – Ofloxacin; BEZ – Bezafibrate; m-BZT – Methylbenzotriazole; MTN – Metronidazole; SMV – Simvastatin; AZM – Azithromycin; SOL – Sotalol; IMP – Iomeprol.

3. MATERIALS AND METHODS

As the urban wastewater system has been identified as the most significant source of PC loadings to the environment, methods of improving the transformation potential achievable through wastewater treatment facilities has become a thoroughly investigated topic. In the past 20 years, the number of wastewater treatment facilities utilizing biofilm processes for treatment has increased substantially. However, after an extensive review of prior studies, it is evident that the fate of PCs through biofilm processes is not well understood and has been provided very limited attention. To address this gap in the literature, the fate of several PCs frequently detected in Canadian wastewaters was assessed in a wastewater treatment process incorporating a biofilm and a suspended growth reactor that was used as a control. The following section describes the materials and methods used to conduct the investigation.

The experimental set up was located at the Canada Centre for Inland Waters-Wastewater Technology Centre (WTC) in Burlington, Ontario. The experimental reactors, process control and monitoring equipment, consumable materials used for pilot reactor operation, and conventional analytical reagents and supplies were provided by the Government of Canada.

3.1 EXPERIMENTAL PROCEDURE

3.1.1 EXPERIMENTAL THEORY

As environmental regulations in Canada become increasingly stringent, the requirement for full nitrification is becoming a key design objective for WWTFs. To ensure full nitrification is achieved during all seasons, the key parameter utilized in the design and operation of the activated sludge (AS) bioreactor is the solids retention time (SRT) (MOE Design Guidelines, 2008). In addition to SRT, temperature is a key parameter affecting the performance of the wastewater treatment process as it directly affects growth kinetics and thereby the capabilities of the biomass involved in biological treatment. The most notable effect of temperature can be observed with slow growing organisms such as those responsible for nitrification. Under the colder temperatures experienced at most Canadian WWTFs, nitrification can be difficult to achieve; minimum SRT requirements can be doubled as a result of a 10 ℃ difference in bioreactor operating temperatures.

Fixed Film systems, which utilize biofilm processes, are noted to provide ideal growth conditions for slow growing organisms (Wilderer 1995), such as the autotrophic bacteria responsible for nitrification. Under stable hydraulic conditions biofilms provide an extended SRT as a result of the enmeshment of bacteria. Biofilms also reduce the impacts of shock loads (process upsets) due to diffusional limitations and improve the performance of slow growing organisms, particularly under cold process conditions which can make their sustained performance a challenge in AS systems (Daims et al., 2001). This has been demonstrated by fixed film processes, such as the rotating biological contactor, the integrated fixed film activated sludge process and the moving bed biofilm reactor in which nitrification occurs even under very low temperature conditions (WEF, 2010). Gieske et al., (2001) demonstrated that nitrifying biofilms contained a diversity of Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) species

which co-exist despite being in direct competition for the same substrates. The enrichment of bacterial consortia was postulated as the reason for the ability of fixed film systems to maintain high stability and resistance to perturbation under inclement conditions.

Past studies investigating the fate of certain pharmaceutical compounds (PCs) have identified a linkage between treatment processes which operate under extended SRTs and an increased ability to “remove” PCs (Clara et al., 2005, Leu et al., 2012). The majority of these studies have used parallel set ups to investigate differences between low SRT activated sludge (AS) processes, typically used primarily for organic reduction, and processes which incorporate a higher operating SRT, such as nitrifying CAS (Kreuzinger et al., 2004), nutrient removal processes (Rosal et al., 2010) and MBRs (Radjenovic et al., 2009). The results from a majority of studies investigating PC transformations via the AS process have led to the general conclusion that many PCs experience higher rates of transformation under extended SRT conditions. It was therefore postulated that autotrophic bacteria, or those requiring similar process conditions, may be primarily responsible for these removals.

A long term study conducted by Santos et al., (2009) which characterized the removal of several PCs through four WWTP’s utilizing AS processes operated under varying process conditions over a period of 1 year. The author identified statistically significant correlations between the TKN content of influent wastewater, the removal rates of conventional organic and nutrient contaminants, the removal of most of the pharmaceutical compounds monitored, and the operating HRT. The author noted that only a weak correlation was found between SRT and that the removals achieved for various PCs investigated were inconsistent in their linkage to these identified factors. However, a prior study conducted by Joss et al., (2004) compared the transformation rates of 9 in an AS process and a fixed bed reactor. The fixed bed reactor, which utilizes a biofilm process for treatment, demonstrated comparable transformation rates despite operation under very short HRTs (approximately 1/10th that of the AS reactor used as control). These observations suggest that the removal mechanisms associated with PCs are diverse and no individual process variable can be attributed to improved PC removals.

Despite many studies reporting improved performance of systems operated at extended SRTs, recent researchers have postulated that this relationship does not prove true for certain PCs. Majewsky et al., (2011) performed batch tests using sludges obtained from a short SRT and an extended SRT process and found that the reactor which had a higher heterotrophic population and low SRT exhibited improved PC transformation rates. This study also noted that transformation rates improved under extended HRTs. Falas et al., (2012) attributed increased removal rates of seven pharmaceutical compounds to heterotrophic activity and postulated that improved PC removals is not associated with nitrification capacity. These conflicting observations have resulted in uncertainty regarding the true mechanisms responsible for PC transformations and the operating conditions required to maximize their removal.

As identified in Section 2.4, historically, PC fate investigations have almost exclusively been based on the AS process and its many permutations. Despite their significant implementation internationally, fixed film processes have received relatively little attention with regards to the fate of PCs. Additional investigation into the effects of fixed film processes and their ability for PC transformations is therefore considered necessary. To address these research

gaps, an investigation regarding the PC transformation capabilities of a fixed film process relative to an activated sludge process has been conducted.

The current investigation was conducted to investigate whether the inclusion of a biofilm provides improved capabilities for PC transformation. The theory of co-metabolism suggests that specific organisms capable of generating enzymes with broad specificity may be responsible for the removal of PCs. Past research has suggested that nitrifying conditions lead to improved removals of certain PCs, leading to the postulation by many researchers that operation under increased SRT increases PC transformation rates. SRT and temperature were therefore selected as independent variables for this investigation to determine how these process variables affect PC fate and whether the biofilm environment provides an observable advantage relative to activated sludge under varying operational conditions.

3.1.2. EXPERIMENTAL DESIGN

To investigate the effects of the inclusion of a biofilm process, the integrated fixed film activated sludge (IFAS) process was used. As previously discussed, the two key process variables used for the current investigation were the operating SRT and temperature. In order to provide experimental conditions which were conducive to direct assessment of the influence of the biofilm process, a comparative investigation was conducted in which the ability of both an AS and biofilm process to transform select PCs was investigated. This was achieved by operating two bench scale wastewater treatment pilot reactors under similar process conditions. One reactor was operated with only an activated sludge biomass (control) and the other operated with both a biofilm biomass and an activated sludge biomass (IFAS). By operating the two reactors under similar experimental conditions, and at various SRT and temperatures, the net effect of the biofilm as well as the SRT and temperature was examined.

In practice the IFAS process allows the bioreactor to be operated under significantly reduced suspended growth SRTs while still achieving nitrification. However, in this study both the control and the IFAS were operated under SRT conditions typically required to achieve nitrification with AS bioreactors. While these operating conditions are likely to be exaggerated for the IFAS reactor in comparison to typical full-scale operation, this selection was made to allow for a direct comparison of the effects of the biofilm without confounding the effects of non-equivalent SRT.

To provide an efficient method of investigating the effects of the target variables, a 2^2 factorial design was used. A factorial design was selected to facilitate statistical analysis of results using the ANOVA method. The conventional performance and the PC fate associated with both the control and IFAS reactors was investigated at 4 distinct treatment conditions, characterized by varying the SRT and temperature as independent variables. Figure 3.1 summarizes the treatment levels for SRT and temperature. Four of the six pilot SBR reactors used as experimental controls in this study were shared with another researcher. Hence, the experimental conditions and reactor operation were based on a compromise between the target parameters of each experiment to accommodate both sets of research goals.

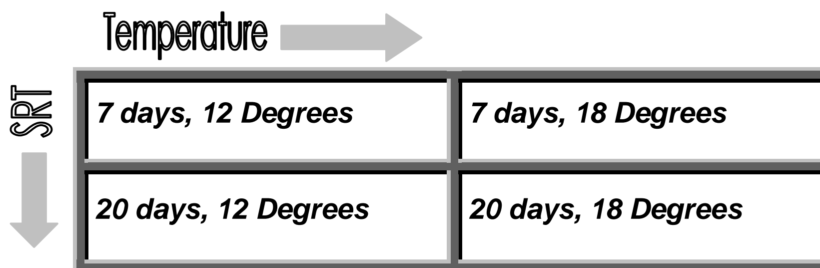


Figure 3.1 Experimental treatment levels

PC transformations in this investigation were assessed by measuring concentrations in the reactor effluent relative to the concentrations present at the beginning of a treatment cycle. Previous studies have attempted to normalize the observed losses of PC's relative to biomass concentrations, or active populations of specific bacterial species or classes. The results obtained by these methods may be site specific with limited applicability in predicting transformation rates at other locations. The current investigation was concerned only with the difference observed between the two parallel treatment processes and thus the mean transformation rate, expressed as a percentage, was used to assess transformational capabilities.

In addition to monitoring PC concentrations, organic and nutrient removals were measured to assess the performance of each process and ensure the reactors were operating under steady state conditions at the time of PC sample collection. As the IFAS is primarily utilized to provide nitrification, the ability of the various pilot reactors to achieve nitrification was given considerable focus. The following conventional parameters were monitored throughout the experiment:

- mixed liquor suspended solids;
- mixed liquor volatile suspended solids;
- soluble chemical oxygen demand removal;
- total chemical oxygen demand removal;
- total ammonia nitrogen removal; and
- nitrate and nitrite generation.

When operating conditions of the reactors require modification, samples were not taken until steady state operation was reached. Typically, a period equivalent to 3 SRTs is allowed to reach steady state operating conditions (Sobeck and Higgins, 2002). In the current study steady state operation was assessed based on the on-going measurement of conventional parameters as well as meeting the target SRT requirements.

3.1.3. SELECTION OF PC COMPOUNDS FOR INVESTIGATION

The primary goal of this experiment was to determine if there was a difference between the transformation of PCs observed in the IFAS process and AS processes. In order to provide a broad yet relevant assessment of transformational capabilities, compounds representing multiple classes and a range of physico-chemical properties were selected.

The compounds investigated for this experiment were chosen based on the following criteria:

- a range of biotransformation potential previously observed in other investigations;
- a variety of pharmacokinetic properties;
- prevalence in Canadian waters;
- limited potential for volatilization under aerobic conditions within the pilot bioreactors;
- limited potential for sorption to biomass solids;
- prevalence in prior studies to permit comparisons with published data;
- ability to be quantitated using available analytical equipment utilizing multi-residue methods; and
- availability of labelled standards.

The following 5 pharmaceutical compounds that met the above criteria were selected for analysis:

- Acetaminophen - Non-steroidal Anti-inflammatory Drug (antiphlogistic)
- Atenolol - Beta Blocker
- Carbamazepine - Antiepileptic
- Sulfamethoxazole - Antibiotic
- Trimethoprim – Antibiotic

A further profile of each PC selected is included in **Appendix A.**

3.2 PILOT REACTOR CONFIGURATION

The experiment was conducted using 6 bench-scale pilot reactors, each providing a total volume of approximately 30L. The 6 cylindrical glass tanks were operated as sequencing batch reactors (SBRs). The SBR process is a complete mix, activated sludge process which operates on a fill and draw basis, wherein the aeration and clarification occur in the same tankage. The SBR represents an ideal reactor configuration for contaminant fate investigations as the batch nature of operation permits a simplified investigation of contaminant fate. Studies involving continuous feed reactors have an unavoidable uncertainty as a result of hydraulic variations which make a direct comparison of influent versus effluent impossible.

Within the many configurations of the activated sludge process, the SBR provides an ideal configuration for the culturing of slow growing organisms. Due to the batch mode of operation, which results in a gradual introduction of feed diluted by the existing mixed liquor, the perturbations caused by inhibitory substances (Wobus and Roske,

2000) or varying influent conditions (Daims et al., 2001) are minimized. The quiescent conditions provided during the settle phase, as well as the feast and famine conditions imposed due to intermittent feeding, allows the SBR to selectively culture microorganisms that may be otherwise washed out in a continuous flow bioreactor (Wilderer, 1995). In the context of the current experiment, an environment that facilitates the proliferation of slow growing organisms, resulting in increased diversity of bacterial species, makes the SBR the ideal process to promote the development of a diverse bacterial consortium, increasing the opportunity for co-metabolism.

The SBRs used for the current research were operated in 5 phases, namely: (1) Fill; (2) React (aeration); (3) Waste; (4) Settle (clarification); and, (5) Decant. These phases were operated on a timed basis by means of a programmable logic controller (PLC). The 5 phases used in the operation of the reactor formed one cycle, each 6 hours in duration, for a total of 4 cycles per day. The duration of each phase was determined based on the constraints of the peristaltic pumps controlling the feed, waste and decant cycles as well as to provide a sufficient period for settling. The periods used for each of the 5 phases are provided below in Table 3.1.

Table 3.1 – Configuration of SBR Stages Utilized

Stage	Duration (mins)
Feed	30
React	200
Waste	5
Settle	55
Decant	70

To provide sufficient freeboard, the conventional SBRs were operated with a total fill volume of 20L. The volume decanted during each cycle was 13.3 L, with an equivalent volume added to the SBRs during the feed cycle, resulting in a volume of 6.7 L remaining after decant representing the settled and compacted biomass. The conventional SBRs were therefore operated under an HRT of 9 hours.

The IFAS process is typically used for retrofitting existing AS reactors. As such, IFAS bioreactors are typically operated as a plug flow configuration. However, as the SBR was identified as ideal in regards to the current investigation, the IFAS pilot reactors were operated as sequencing batch biofilm reactors (SBBRs). Wilderer (1992,1995, and 2004) found that the SBBR is an ideal treatment process for the biodegradation of 'difficult wastewaters' due to the ability to maintain a culture of slow growing organisms that may be otherwise washed out by another process. The SBBR was therefore considered to be well suited to the current investigation.

WEF (2010) suggests that IFAS reactors are typically operated with a media fill fraction between 40 and 60 percent (volume/volume). Initially, a fill fraction of 50 percent was selected for the IFAS reactors. However, after 10 L of media was added to the pilot reactors, media bulking as well as displacement due to the dissolved oxygen and pH probes caused some of the media to be exposed to atmospheric oxygen for extended periods. To mitigate this issue,

the volume remaining after decant was increased to 12 L in an attempt to maintain all IFAS pieces within the mixed liquor. To provide a consistent contaminant loading between all pilot reactors, the operating volume of the IFAS reactors was increased to 25 L to permit a feed volume of 13L. It is reported that IFAS media displaces approximately 171 mL per L of media. This results in a displacement of approximately 1.7 L in the IFAS reactors, achieving an effective operating volume of 23.3 L and a slightly increased HRT of 10.8 hours.

All reactors were equipped with a water jacket that continuously received recirculated water from either a chilled (approximate volume: 400L) or heated sump (approximate volume: 50L) maintained at temperatures of 11ºC and 20ºC respectively. Intermittent temperature depression occurred when the pilots received feed during the winter months as a result of low influent temperature, however the target operating temperatures were generally reached within an hour of the initiation of the feed phase.

To maintain a pH conducive to nitrification, each reactor was equipped with a HACH pH probe to continuously monitor in-situ pH. pH conditions were maintained between 7.0 and 7.5 by alkalinity (Sodium Bicarbonate) supplementation conveyed to the pilot reactors by Masterflex peristaltic pumps controlled by PLC. Operating pH conditions within the reactors was verified monthly by means of a secondary portable pH probe. pH probes observed to be out of calibration were either recalibrated, or if re-calibration was not successful, replaced.

Dissolved oxygen was controlled by means of HACH LDO probes and the PLC. The PLC operated solenoid valves independently controlled the flow of compressed air to all reactors to maintain the target D.O. during the react phase. The conventional SBRs were operated with a target D.O. level of 2 mg/L as recommended by MOE Design Guidelines for activated sludge bioreactors (MOE Design Guidelines, 2008). The IFAS SBBRs were operated at 4 mg/L based on typical operation conditions suggested by WEF (2010). The IFAS units require a higher D.O. concentration to overcome oxygen transfer limitations occurring within the biofilm.

The conventional SBRs were equipped with mechanical mixers to ensure that the bioreactors were well mixed during periods when aeration was not active. However, mechanical mixers were not used within the SBBRs as even when operated under very low rotational speeds, the mixers were observed to rapidly destroy the IFAS media. As a result, the IFAS reactors were mixed by aeration only. This resulted in intermittent periods without mixing when dissolved oxygen levels exceeded 4 mg/L. These periods were generally of limited duration (several minutes) and this operating strategy was not observed to inhibit performance as demonstrated through nitrification testing and conventional removals. In order to ensure that waste volumes had equivalent solids concentration to the reactor mixed liquor, aeration was turned on for the entire duration of the waste phase for all pilot reactors.

Diffusers for the conventional reactors consisted of 4 porous aquarium ceramic stone diffusers which were believed to provide a similar sized bubble as fine bubble diffusers used in full scale systems. Maas et al., (2008) suggested that IFAS carriers should be mixed in a double-roll pattern, with upwelling in the centre and downwelling at the sides. Optimal mixing promotes detachment of excessive biomass (self-cleaning function) resulting in improved mass transfer between liquid and the biofilm. Typically, IFAS reactors are equipped with medium bubble aeration systems

consisting of small pores drilled into stainless steel pipe. The IFAS reactors were equipped with stainless steel tubing, in a semi-circle arrangement, drilled with 3 mm holes. Best efforts were made to minimize 'dead zones', such as in areas proximal to pH and LDO probes in which media can became trapped and may have become exposed to atmospheric conditions during decant.

Authentic municipal wastewater was received at the WTC from the Skyway WWTP by means of forcemain conveyance. The Skyway WWTP, located approximately 1.5 km from the WTC, serves the residents of the City of Burlington (population 175,779, 2011 Canada Census). Flows received at the WTC undergo primary clarification prior to entering the feed tank for the 6 bench-scale pilots. Primary effluent within the feed tank was conveyed to the pilot reactors by submersible pump and feed lines. Primary effluent was continuously circulated through the feed tubing to prevent septic conditions. The feed, decant and waste volumes were all conveyed to and from the reactors by means of Masterflex peristaltic pumps operated using Teflon tubing. The volumetric throughputs of the pumps were monitored on a biweekly or monthly basis and adjusted to maintain the target operating conditions as needed. As the wasted volume has a significant effect on the SRT, the waste pumps were monitored with a higher frequency.

Gabb et al., (1989) reported that *S. Natans*, a filamentous organism that can cause sludge bulking and foaming, is common in laboratory-scale reactors as it grows preferentially on tubing, reactor walls and equipment surfaces. To inhibit the formation of *S. Natans* and other filamentous organisms, probes and tank walls were regularly cleaned by means of pressurized water jets and scrubbing with a coarse brush. Feed tanks were drained, sprayed with hot water and scrubbed to inhibit biofilm formation. Piping associated with the feed and recirculation lines was also regularly flushed with hot water.

The PLC used to control the process was subject to a reset in the event of power loss, causing the internal timer of the PLC to reset to t=0. Early in the experimental phase it was observed that PLC resets could result in a "double feed" if decanting had not occurred prior to the reset, resulting in overflow and a significant loss of reactor biomass. In order to control this condition, the pilot reactors were outfitted with level sensors which were used to automatically shut off the feed pumps in the event of overfilling. Additionally, the PLC was provided with a surge protector and uninterrupted power supply (UPS) in an attempt to maintain a consistent time schedule. Despite the usage of the UPS, the PLC was found to regularly be 'behind schedule' as a result of frequent power losses. Occasionally, the WTC underwent planned power outages. In the event of an extended power loss, supplementary synthetic feed was dosed into the reactors to achieve an initial feed concentration of 350 mg/L COD and 30 mg/L TAN to delay endogenous conditions when feed pumps were out of service for extended periods of time. Sampling for PCs was not attempted until sufficient time had passed following these upsets and reactor performance was confirmed to be at relative steady state.

3.3 PILOT REACTOR OPERATION

Of the 6 pilot reactors available, four were operated as SBRs and two were employed for IFAS operation. Hence, a conventional SBR was employed for each of the 4 treatment levels and the IFAS reactor conditions were changed temporally. The process conditions and labelling used for each of the 6 reactors are provided in Table 3.2.

Table 3.2 – Operating Conditions for the Six Pilot Reactors

Reactor Label	Process Configuration	Operating Temperature	Operating SRT
A20/A7	IFAS	12 °C	20d → 7d
B	Conventional	12 °C	20d
C	Conventional	18 °C	20d
K20/K7	IFAS	18 °C	20d → 7d
D	Conventional	18 °C	7d
E	Conventional	12 °C	7d

The IFAS reactors were initially operated at the high SRT condition to minimize the steady state waiting period required after process modifications were made. Media and seed biomass was added to IFAS reactors ‘A’ and ‘K’ on June 21, 2011 and August 15, 2011, respectively. Both reactors were initially operated at 20 °C for a period greater than 1 year before process conditions were altered to ensure that each IFAS reactor contained a fully developed steady state biomass. During this time period, several minor process adjustments were required to reach the desired process performance. Hwang and Oleszkiewicz, 2007 demonstrated that sharp decreases in temperature (10°C) can inhibit nitrification to a greater degree than if the temperature is varied gradually (2°C/day). To avoid temperature shocks which could lead to washout of slow growing organisms, operating temperatures were adjusted at a rate of 1 °C per day.

Wastewater bioreactors have been shown to be highly dynamic in terms of bacterial population composition (Kaewpipat and Grady Jr., 2002), even when operated under steady state conditions. Despite operating under identical conditions and from the same bacterial seed sludge, pilot bioreactor mixed liquor compositions have been shown to significantly diverge in terms of bacterial populations over relatively short periods of time (Ayarza et al., 2010). However, despite the differences in bacterial community, similar performance in terms of removals of conventional pollutants can be achieved due to the presence of functionally redundant species, which may all contribute to the overall removal performance observed (McMahon et al., 2007). Bacterial populations within lab scale pilot reactors maintain similarity if the biomass is mixed after an acclimation period within the reactors. In an attempt to maintain the highest degree of similarity between reactors, biomass cultures from SBRs operating at the same SRT were periodically intermixed through the addition of collected WAS. In addition, the IFAS reactors were initially seeded with WAS collected from reactor B.

The pilot reactors were operated at the target SRT based on measurements of the effluent TSS and the mixed liquor suspended solids (MLSS). This procedure establishes the SRT of the suspended growth biomass only. Estimation of the biofilm SRT was not attempted for the IFAS biofilm in this experiment as this would require measurements of sloughed biomass, under process conditions, as well as investigations into the bacterial species present to determine species loss. Similarly, the presence of grazing organisms and the impacts on the populations of heterotrophs and

autotrophs would need to be considered. Due to the complexity of this procedure biofilm SRT measurements were considered beyond the scope of this investigation.

The SBR configuration allowed for a simplified method of controlling the operating SRT; the solids concentration of the wasted volume was equivalent to the MLSS, allowing the SRT to be controlled on a strictly volumetric basis. The Conventional SRT relationship, which was previously presented as equation 2.1, is provided below:

$$\theta = \frac{\forall_{bioreactor} \times X_{MLSS}}{[Q_{WAS} \times X_{WAS}] + [Q_{EFF} \times X_{EFF}]} \quad (2.1)$$

where:

$\theta = SRT\ (d)$

$\forall$ = Volume (m^3)

X = Solids Concentration $\left(\frac{g}{m^3}\right)$

Q = Flow $\left(\frac{m^3}{d}\right)$

Under normal operation, the effluent TSS concentration for all reactors was found to be generally below 15 mg/L, resulting in a minor contribution to the SRT. However, it is noted that the SRT calculation is sensitive to the effluent TSS. Process upsets, which led to high effluent TSS concentrations, resulted in significant impacts to the operating SRT. As a result, effluent TSS was monitored frequently to ensure that the operating SRT was maintained within range of the target SRT. Under typical SBR operation, the SRT can be determined based on equation 3.1:

$$\theta = SRT\ (d) = \frac{\forall_{bioreactor} \times X_{MLSS}}{[Q_{WAS} \times X_{MLSS}]} \quad (3.1)$$

Equation 3.1 can be further simplified to reflect the equivalence of the solids concentrations within the WAS and the bioreactor, as demonstrated by equation 3.2:

$$\theta = SRT\ (d) = \frac{\forall_{bioreactor}}{Q_{WAS}} \quad (3.2)$$

It should be noted that the SRT calculation method used in these tests represents the total solids retention time within the reactor. The conditions within the SBR were not consistently aerobic and became anoxic, and periodically anaerobic, during the settle and decant phases. As a result, the SRT during which the reactor maintains aerobic conditions was reduced. The aerobic SRT was described by equation 3.3 (Artan & Orhon, 2005):

$$\theta_{Aerobic} = \theta \frac{T_{Aerobic}}{T_{Cycle}} \quad (3.3)$$

where:
$T_{Aerobic}$ = duration of aerated operation
T_{Cycle} = total time of each cycle

Aerobic conditions are generally defined as operation under a dissolved oxygen concentration greater than 0.5 mg/L (Metcalf and Eddy, 2003). Under the SBR operation cycle defined above, the fill, react and waste phases all occurred under aerated conditions and therefore the duration of the aerobic period was 235 minutes, or approximately 65 percent of each cycle. Under the target SRT conditions of 7 and 18 days, the aerobic SRT was therefore equivalent to approximately 4.6 and 11.7 days, respectively.

Calculation of minimum SRT using equation 3.4 (Metcalf and Eddy, 2003) can be used to estimate whether a sufficient autotroph population will be established under given process conditions and achieve nitrification:

$$\frac{1}{\theta_{Aerobic,Crit}} = \left(\frac{\mu_N \cdot N}{K_N + N}\right)\left(\frac{D.O.}{K_O + D.O.}\right) - K_{dn} \qquad (3.4)$$

where:
$\Theta_{Aerobic,\mathrm{Crit}}$ = the critical aerobic SRT to maintain autotrophs [d]
μ_n = maximum autotrophic growth rate [1/d]
N = mixed liquor ammonia concentration [mg/L]
K_N = ammonia nitrogen half saturation coefficient (mgN/L)
D.O. = operating dissolved oxygen concentration (mg/L)
K_O =Half saturation coefficient of dissolved oxygen (mg/L)
K_{dn} = autotrophic endogenous decay coefficient (1/day)

Using a typical influent TAN concentration of 12 mg/L, the target operating D.O. concentrations, as well as default kinetic parameters provided in Wastewater Engineering: Treatment and Reuse (Metcalf and Eddy, 2003), minimum SRTs for a conventional SBR were estimated. The results of these calculations are provided in Table 3.3.

Table 3.3 – Calculated Minimum SRT required for Nitrification in a Conventional SBR

Operating Temperature	Minimum SRT for Conventional SBR
12 ℃	6.2 d
20 ℃	3.9 d

Based on the values calculated for the pilot reactors, the conventional SBR was not expected to provide full nitrification when operated at 12 degrees and an SRT of 7 days. Based on WEF MOP 35, the IFAS reactors were expected to nitrify at SRTs above 2 days due to the presence of the biofilm. However, this is subject to influent COD loadings, which, if sufficiently high, can cause excessive heterotroph growth which displaces autotrophics, and provided that sufficient media area exists to develop a sufficient biofilm. The operating temperature and SRT

determines whether the suspended growth phase will contribute to nitrification. It was therefore anticipated that the two pilot reactors operated at 12 °C and an SRT of 7 days would allow for an evaluation of the impacts of nitrification capabilities on the transformation achievable for the selected PCs.

3.3.1 REACTOR FEED

The WTC receives a constant flow of preliminary treated municipal sewage from the Burlington Skyway WWTP via a 500 mm forcemain at a rate of 7 L/s. Due to the low flow within the forcemain, the estimated HRT within the pipeline was approximately 11.7 h, which likely resulted in substantial conjugation/deconjugation reactions associated with PCs, as well as partial removal of organic and nutrient contaminants. Further, these conditions were also likely favourable for the generation of reduced sulphur compounds and the proliferation of filamentous organisms (Jenkins et al., 2004).

The Skyway WWTP, located approximately 1.5 km from the WTC, serves the residents of the City of Burlington (population 175,779, 2011 Canada Census) and has an average daily flow capacity of 118,000 m^3/d (Regional Municipality of Halton Wastewater Treatment Systems 2010 Performance Report). It should be noted that the Skyway WWTP receives sewage from the Joseph Brant Memorial Hospital, located approximately 1 km from the Skyway WWTP. A characterization of the primary effluent at the WTC during 2011 is provided in Table 3.4.

Table 3.4 – Average Primary Effluent Contaminant Concentrations, 2011

Parameter	Annual Average[1] (n= 15)
Alkalinity (mg/L as $CaCO_3$)	275
$cBOD_5$ (mg/L)	83
COD (mg/L)	248
TAN (mg/L)	20.9
TKN (mg/L)	26.8
TSS (mg/L)	88
TP (mg/L)	8.0

The primary effluent data indicate that the wastewater received reflects typical municipal sewage and contains sufficient substrates for both heterotrophic and autotrophic growth. Van Der Gast et al., (2008) observed that bacterial community diversity increased in the presence of municipal wastewater relative to industrial wastewater sources. Therefore utilization of municipal wastewater is anticipated to produce a more diverse microbial consortium which may result in a better opportunity to observe PC transformations. However, it was anticipated that the analysis of influent and effluent municipal wastewater for PCs would be challenging due to the matrix effects and low concentrations of the target PCs.

3.4 SAMPLING PROCEDURE

The method of sample collection for characterization of PC fate in wastewater processes can lead to significant variability and reduced statistical certainty if all potential factors influencing error are not properly considered or controlled. Ort et al., (2006) found that individual measurements of benzotriazole varied from -70% to +160% from the average concentration when sampling frequencies were varied between 30 seconds and several minutes. A follow up critical review conducted by Ort et al., (2010) demonstrated a chronic lack of consideration of temporal variation in most PC studies reviewed, particularly when certain PC loads may be attributed to a small fraction of the total population. However, the authors noted that variability could be largely reduced through sampling from the primary clarifier. The HRT provided in the primary clarifier used in this investigation was expected to attenuate any significant temporal variability.

The main route of PCs into sewage networks is via human excretion. Concentrations are therefore expected to display the typical diurnal variability observed for conventional contaminants. However, due to the ubiquitous nature of the PCs selected and the large population serviced by the Skyway WWTP, a relatively consistent concentration of PCs during the sampling periods proposed was expected. In consideration of the HRT provided in the forcemain conveying flows to the WTC (approximately 12 hours) and the primary clarifier HRT (estimated to be at least 2.5 hours based on typical design criteria), sampling was conducted commensurate with influent flow during afternoon and evening periods in an attempt to capture the diurnal effects associated with evening and morning sewage flows. Best efforts were made to collect samples during the same time over consecutive days, however due to minor equipment issues, this was not always achievable. Sampling campaigns were conducted between Monday and Friday to minimize temporal variability and capture typical conditions.

Due to the batch nature of the SBR configuration, samples required blending to provide a true estimate of the PCs and conventional contaminants present in the reactors at the beginning of each react phase (t = 30 min). As a fraction of the mixed liquor is retained after each cycle, effluent from the previous cycle was used to characterize the mass of PCs within the mixed liquor retained through decant. All effluent discharged from the previous cycle prior to sampling was collected in 20 L stainless steel containers. This effluent was then blended with primary effluent collected from the feed tank to create a representative sample of the pilot reactor contents at the beginning of the react phase. Samples collected from both the effluent and primary influent were added to each bottle at the volumetric fraction equivalent to their volume within the reactor. To further minimize variability associated with the influent, representative samples were collected from the feed tank at 5, 15 and 25 minute intervals during the feed cycle.

Effluent samples (t = 360 min) were collected by capturing the entire decanted volume in a 20 L stainless steel container. As effluent concentrations of TSS may vary throughout the decant cycle, it was considered necessary to create a composite to homogenize the effluent. This was accomplished by "swaying" the poured effluent stream in and out of a beaker to provide a composite volume representative of the total effluent volume. All 20 L stainless steel cylinders, graduated cylinders and beakers used for influent and effluent collection were rinsed with methanol and

thrice with DI water prior to the next sampling event. At the end of each sampling campaign, 20 L stainless steel cylinders were permitted to soak in a dilute solution of Contrad-70 (Decon Labs, PA. USA) to ensure no PC carryover occurred between campaigns.

All samples were stored in 500 mL amber silanized glass bottles (Systems Plus). Studies completed by Vanderford et al., (2011) and Baker and Kasprzyk-Hordern (2011) found that the use of silanized glass resulted in samples collected for PC analysis that were more stable during prolonged holding times. Silanized glass reduces the polarity of the container glass, reducing the likelihood of PC adsorption. EPA method 1694 (Englert, 2007) recommends that protracted holding periods be avoided to minimize potential for PC transformation prior to analysis. To ensure the maximum stability of analytes, EPA recommends samples should be extracted onto an SPE cartridge as soon as possible and stored at -20℃ if analysis is not practical within a period of 48 hours. This was noted to be a best practice approach with limited stability investigations conducted at the time the method was published. Due to the number of samples collected during the final phase of sampling, extraction within 48 hours was not achievable, although best attempts were made to perform sample preparation as quickly as was practical.

Vanderford et al., (2011) observed the stability of ATEN, CBZ, SMX, TRIM and several other common PCs held for a period of 28 d within surface water and treated waters. A suitable stability (concentration change <15%) was achieved for all PCs when samples were preserved using Ascorbic Acid and Sodium Azide and refrigerated at 4℃. Trenholm et al., (2009) also investigated contaminant stability of ATEN, CBZ and TRIM in samples of wastewater effluents preserved under the same methods and found little difference between samples analyzed 7 months apart. To prolong holding times, all samples collected during this investigation were dosed with premade solutions of sodium azide and ascorbic acid, achieving an in-bottle concentration of 1 g/L and 50 mg/L, respectively. Small aliquots were collected from the sample bottles prior to the addition of ascorbic acid and sodium azide for the analysis of conventional contaminants. All samples were kept refrigerated at 4℃ until samples could be processed.

3.5 CONVENTIONAL ANALYSIS

Prior to the PC fate investigations, grab samples of primary effluent (reactor feed) and secondary effluent were analyzed for conventional contaminants to determine the treatment performance of the reactor. Mixed liquor samples were also collected periodically from the reactors for the purposes of SRT adjustments and to determine whether reactors were operating at steady state. Samples collected for the purposes of PC fate investigations using the procedure described above were analyzed concurrently with the PCs for conventional contaminants to assess the reactor performance achieved during each treatment cycle.

The following physical parameters were analyzed to determine the operating SRT within the reactors as well as to determine the settling performance of sludge:

- mixed liquor suspended solids (MLSS)/ mixed liquor volatile suspended solids (MLVSS); and
- effluent total suspended solids (ESS).

In addition, the following chemical parameters were analyzed to assess the performance of the reactors on a temporal basis as well as relative to anticipated performance:

- influent and effluent total COD (tCOD);
- influent and effluent soluble COD (sCOD);
- influent and effluent NH_3-N (TAN);
- influent and effluent NO_3-N; and
- effluent NO_2-N.

To further assess the performance of the pilot reactors, batch nitrification testing was also performed periodically. The procedures utilized for the analysis of all conventional parameter testing is described below.

3.5.1 CONVENTIONAL ANALYSIS METHODS

The analysis of all conventional contaminants was performed using the methods described in Standard Methods for the Investigation of Water and Wastewater (Eaton and Franson, 2005). All chemical parameters were analyzed using HACH Test n' Tube methods and a HACH DR 2800 spectrophotometer.

Mixed liquor and Volatile Suspended Solids

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were examined through analysis of WAS collected during the waste cycle. These parameters were determined using method 2540D and 2540E in Standard Methods (Eaton and Franson, 2005). Briefly, 25 - 30 mL of WAS was stirred to ensure good mixing before being filtered using Whatman 0.45 µm filter paper, which was first dried at 105°C, desiccated and weighed (tare weight). The samples were then heated for the required periods and at the temperatures defined in the procedure identified above, cooled in a desiccator and weighed again.

Effluent Suspended Solids

Effluent suspended solids (ESS) were monitored by collection of the entire effluent volume decanted from the reactor at the end of each cycle. Samples were ensured to be well mixed through utilization of a mechanical mixer operated at 300 RPM for several minutes before 500 mL was extracted for ESS testing. ESS was determined based on the same procedure as Method 2540D. Briefly, Whatman 0.45 µm filter papers were first dried at 105°C, desiccated and weighed (tare weight). Depending on the effluent TSS concentration, assessed by sample appearance, 125 or 250 mL were filtered. Filtrates were then heated at 105 °C for a minimum 2 hours, desiccated and measured.

Soluble and Total Chemical Oxygen Demand

The closed reflux colorimetric method (method 5220D, Eaton and Franson, 2005) was used to determine the chemical oxygen demand (COD) of reactor influents and effluents. Samples used for sCOD measurements were first filtered through a 0.45 µm pore size Whatman filter paper prior to analysis. tCOD measurements were made without

filtering. Hach Test n' Tube method 8000 was utilized in which samples were digested at 150°C for 2 hours. Sample tubes were then quenched and analyzed using a HACH DR 2800. HACH reports that the accuracy of this method is between 3 and 4 percent of the maximum concentration listed in the method range.

Total Ammonia Nitrogen (NH_3-N), Nitrate nitrogen (NO_3-N) and Nitrite ($N0_2$-N)

All influent and effluent samples used for measurements of TAN, NO_3-N and NO_2-N were filtered through a 0.45 µm pore size Whatman filter paper prior to analysis. All three parameters were analyzed using HACH Test N Tube methods through the salicylate, chromotropic acid and diazotization methods, respectively. Samples were analyzed using either method 10031 (High Range – 0.4 to 50 mg/L) or 10023 (Low Range – 0.02 to 2.5 mg/L) depending on the anticipated TAN concentration. Samples were analyzed for NO_3-N using method 10020 and NO_2-N using method 10019. Samples which exceeded the limits of the NO_2-N method range were serially diluted to achieve a result within the specified limits. HACH reports that these methods typically achieve an accuracy of 5 percent of the maximum concentration listed within the method range.

3.5.2 BATCH SPECIFIC NITRIFICATION RATE TESTING

Batch specific nitrification rate (SNR) tests were used to assess the nitrification rate. The batch tests assessed the reduction of TAN or specific ammonia removal rate (SARR), and the nitrogen production rate, measured as the creation of NO_3-N and NO_2-N or specific NO_x-N production rate (SNPR). As autotrophic bacteria are more susceptible to reactor washout and reduced kinetics under process upset conditions, the nitrification capacity was used to assess whether steady state operation was established in the pilot reactors. Typically, this testing is conducted on the basis of normalizing the observed rates based on the measured VSS concentration within the reactor. This procedure was utilized for both control SBR and the IFAS SBBR pilots, however, it is acknowledged that the biofilm associated within the SBBR IFAS pilots leads to a skewed estimate of specific nitrification rates.

In order to perform a true assessment of the IFAS SNR, measurement of the volatile solids within the biofilm would be required. The methods for determining this value were considered beyond the scope of the current study. Therefore, SNRs were calculated as well as net removal rates to facilitate a direct process performance comparison. As the IFAS process is widely used as an upgrade technology, the usage of specific removal rates demonstrates the ability of the IFAS process to achieve improved nitrification without increased solids loading on downstream clarification. It should be acknowledged that the SNR and the SNPR will differ slightly as heterotrophic organisms utilize ammonia as a source for metabolic growth. As a result, SNPR was utilized for an assessment of reactor nitrification capacity.

The SBRs were employed for the batch tests. In the tests the entire volume of feed for each reactor was retained within a 20 L HDPE container and analyzed to determine the initial TAN concentration. Anhydrous ammonium chloride (NH_4Cl) was then added to the feed volume to bring the starting reactor TAN concentration to approximately 30 mg/L. During the testing, sufficient $NaHCO_3$ was provided to the reactors to maintain a pH within the experimental target of 7.0 to 7.5. Reactor temperatures were maintained at their target values during the testing procedure.

Following the completion of the feed cycle, the collected feed was added to the reactors with a funnel and 38 mm tubing to avoid excessive hydraulic shear. After the entire volume of the feed had been added to the pilot reactors, a sample representing t=0 was collected. In both the control and IFAS pilot reactors, a 50 mL MLSS sample was also collected at the beginning of the test (t=0) for the determination of the MLVSS concentration within the suspended growth stage using the methodology outlined in previous sections. SNR testing was typically conducted over a period of 2.5 to 3 hours with samples collected at intervals of 30 minutes to 1 hour. All collected samples were analyzed for TAN, NO_3-N and, at a reduced frequency, NO_2-N using the methodologies identified previously. Under conditions in which the nitrifying bacteria are not inhibited, the response in terms of TAN reduction and NOx creation should be approximately linear. Linear regression methods were used to determine the nitrification rate, measured in units of mg TAN/day as well as the nitrogen generation rate, measured as mg NO_x-N/day. These values were then normalized based on MLVSS observations and reactor volumes.

The expected nitrification performance for each SBR was estimated using equation 3.5 and 3.6. This provided a coarse estimation of the reactors performance relative to typical full scale systems as means of further assessing whether steady state conditions were achieved. Equation 3.5 (Metcalf and Eddy, 2003) provides an estimate of the nitrifiers existing in the bioreactor at the time of batch nitrification testing.

$$X_n = \frac{Q \cdot Y_n \cdot NO_x \cdot \theta}{[1 + k_{dn} \cdot \theta]\forall} \qquad (3.5)$$

where:
X_n = Concentration of nitrifiers present in mixed liquor (mg/L)
Y_n = biomass yield (gVSS/gNH_4-N)
NO_x = Amount of TAN nitrified (mg/L)

The concentration of nitrifiers within each reactor was estimated based on process monitoring conducted during PC sampling, as well as default kinetic parameters presented in Wastewater Engineering: Treatment and Reuse (Metcalf and Eddy, 2003). Equation 3.6 can be used to estimate the react time required to achieve the level of nitrification observed in the batch tests.

$$t = \frac{K_n \cdot \ln\left(\frac{S_0}{S_f}\right) + \left(S_0 - S_f\right)}{X_n \cdot \frac{\mu_n}{Y_n} \cdot \frac{D.O.}{K_O + D.O.}} \qquad (3.6)$$

where:
t = Reaction time required (d)
S_0 = Substrate (NH_4-N) concentration at time = 0 (mgN/L)
S_f = Substrate concentration at end of react period (mgN/L)

A comparison of actual times observed versus calculated reaction times provides a coarse indication of whether the tested reactors were performing at a level commensurate with typical SBRs operating at the target conditions.

3.6 ANALYSIS OF PHARMACEUTICAL COMPOUNDS

Results from the monitoring of PC's in wastewater have been reported extensively in the past, however some methods employed lead to a high degree of uncertainty in the data. To ensure that methods were used in which errors associated with sample preparation and the analytical process were minimized, an extensive review of sampling, sample preparation and analytical techniques utilized in prior studies was conducted. The methods and the references which informed the methods employed in the current study are discussed subsequently.

3.6.1 SAMPLE PREPARATION

Sample preparation methods were based on those published by Vanderford and Snyder (2006) and Trenholm et al., (2009) with some minor adjustments. Briefly, samples were first shaken within the bottle to ensure they were well mixed before a volume of approximately 150 mL was filtered using Whatman 0.45 μm glass fibre filter paper. This removed particulate contaminants that were likely to disrupt the analytical procedure. A volume of 100 mL of filtered sample was measured and placed within a labelled beaker. Sample preparation was conducted in sets of 12, each set containing 1 method blank (MB) and 2 matrix spikes (MSs) for quality control purposes. MBs consisted of 100 mL of Milli-Q water and were used to assess analyte carryovers associated with sample preparation and analysis. MSs were created using 100 mL of Milli-Q water spiked with 100 μL of a methanol solution containing ATEN, SMX, CBZ and TRIM at a concentration of 100 μg/L achieving a concentration of 100 ng/L within the sample. ACE was initially spiked at a concentration of 100 ng/L during Phase I, but due to signal issues was increased to 10 ug/L under Phase II/III to more closely match concentrations expected in the primary effluent. Each sample, with the exception of blank samples, was spiked with 100 uL of methanol solution containing isotopically labelled standards (ILSs) of ATEN, CBZ, SMX and TRIM at a concentration of 100 μg/L achieving a final sample concentration of 100 ng/L. An isotopically labelled ACE surrogate was also initially spiked at 100 ng/L during Phase I, but increased to 10 μg/L in Phase II/III. This was required to facilitate the use of the isotope dilution method as explained in subsequent sections.

ILSs, which included Atenolo-d_7, Trimethoprim-d_3, and N-(4-Hydroxyphenyl-2,3,5,6-d_4) Acetamide (deuterated ACE) were obtained from C/D/N Isotopes (Point-Claire, Quebec). Solutions were prepared by dissolving the entire mass of standards provided in methanol. Carbamazepine-d_6 and Sulfamethoxazole-d_{40}, which were provided by the Servos lab (University of Waterloo) and were already dissolved in methanol. These standards are understood to have been obtained from Sigma-Aldrich (Oakville, ON) and C/D/N Isotopes, respectively. All standards were kept at -20 °C prior to usage.

Due to the low concentrations present within the samples as well as the presence of co-eluting compounds, extraction was considered to be mandatory. This requirement was further investigated and confirmed based on preliminary testing discussed in subsequent sections. Many alternative extraction techniques exist, however solid

phase extraction (SPE) continues to be the most popular method (Richardson and Ternes, 2011). A variety of SPE cartridge material can be used to enhance the performance relative to specific groups of compounds, however multi-residue analytical methods used to analyze samples for a large number of PCs with a wide variety of physico-chemical properties are noted to consistently rely of hydrophilic-lipophilic-balanced (HLB) media. HLB has been shown to be preferable for sample concentration and clean-up in wastewater PC analysis and achieves optimum extraction efficiency for most compounds under a neutral sample pH (Petrovic et al., 2010). Hence, Waters Oasis HLB 500 mg 5cc cartridges were utilized exclusively for this investigation.

Samples were extracted using both manual and automated SPE methods. Extraction methods, including elutants, volumes and flowrates, utilized for both manual and automatic SPE extraction were as described in Vanderford and Snyder (2006). Briefly, manual extraction was conducted by first pre-conditioning the HLB cartridges using 5 mL of MTBE, 5 mL of methanol and 5 mL of HPLC grade water sequentially percolated through the cartridge under a gentle vacuum (20 mm Hg). Each sample, MS or MB was then introduced to the cartridges under vacuum at a target flow rate of 15 mL/min. Following sample introduction, approximately 25 mL of reagent grade water was added to the sample beaker to ensure that the entirety of the sample was evacuated from the beaker and the vacuum manifold. SPE cartridges were then dried under gentle vacuum for approximately 15 minutes. Samples were eluted from the cartridges by means of percolating 5 mL of methanol, followed by 5 mL of methanol/MTBE solution prepared at a ratio of 10:90 (v/v) and collecting the elutant in 85 mm test tubes. Automatic SPE was performed using a Dionex Autotrace 280, programmed to perform the same procedure as describe above, but was limited to 6 SPE cartridges per run.

The eluted samples that were collected in test tubes were then evaporated to dryness under either a gentle stream of nitrogen or using a Dionex Rocket vacuum evaporator. During preliminary and phase 1 investigations, samples were reconstituted using 2.5 mL of Acetonitrile based on the request of the chemist performing the analysis for the commercial lab. This resulted in sample concentration of 40 times. Subsequent analytical phases utilized 500 μL of methanol for reconstitution, resulting in a concentration factor of 200 times, which was found to produce better results. All samples were stored at -20 ℃ until analysis was performed.

3.6.2 SAMPLE ANALYSIS

The analysis of all samples for PCs was conducted using LC-MS/MS with electrospray ionization in positive mode (ESI+). Analysis was conducted in several phases: preliminary and phase 1 analysis was conducted by a commercial lab. The commercial lab was developing a proprietary procedure for PC analysis and the PC fate investigations forming the subject of this thesis were intended to assist in the method development. Analysis from the commercial lab was conducted using a Shimatzu HPLC coupled with an AB Sciex QTRAP 5500. The second and third phases of analysis were conducted at the University of Waterloo utilizing an Agilent 1200 series HPLC coupled to an AB Sciex QTRAP 3200. The methods utilized in both phases are described in subsequent sections.

Due to uncertainty with the analytical methods, as well as the requirement to maintain flexibility of the experimental design, sampling was scheduled to occur in 3 stages. Preliminary investigations were also conducted to assess the accuracy and precision of the analytical methods and inform the experimental design. The sampling schedule utilized for Phase 1, 2 and 3 investigations is provided in Table 3.5.

Table 3.5 - Experimental Conditions, Reactors and Sampling Dates Used for Phase 1, 2 and 3

Experimental Condition	Reactor	Sampling Date
ϴ = 20d, t = 18°C	K20	September 25 – 29, 2012
	B	September 25 – 29, 2012
ϴ = 20d, t = 12°C	A20	December 11 – 14, 2012
	C	January 14 -18, 2013
ϴ = 7d, t = 18°C	K7	January 14 -18, 2013
	E	January 14 -18, 2013
ϴ = 7d, t = 12°C	A7	January 14 -18, 2013
	D	January 14 -18, 2013

Notes:

1. Θ = target SRT, t = target temperature
2. Cells highlighted in salmon represent reactors characterized under phase 1 investigations
3. Cells highlighted in green represent reactors characterized under phase 2 investigations
4. Cells highlighted in purple represent reactors characterized under phase 3 investigations.

3.6.3 PRELIMINARY INVESTIGATIONS

The commercial lab selected to do the phase I investigations was noted to be in the development stages of PC analysis. In the interest of due diligence, several preliminary investigations were conducted to assess the robustness and capabilities of the development method. The analytical method was proprietary in nature, and as such limited information was made available regarding the procedures employed. Two initial investigations were completed to accomplish several goals:

- To determine if the HDPE carrier used for IFAS would result in PC losses due to sorption;
- To assess the capabilities of the laboratory method; and
- To determine the anticipated relative standard deviations associated with analysis to inform the experimental design.

The initial investigation, which aimed to investigate sorptive losses associated with the HDPE media, was conducted using tap water spiked with standards dissolved in methanol. The investigation was completed using a 12L glass pilot reactor, supplemented with 5 L of HDPE IFAS media which had not been exposed to wastewater. Carrier media was soaked in tap water for 48h. The presence of chlorine/chloramine residual was expected to ensure no biological growth was present and therefore no losses would occur from this removal pathway. Synthetic feed water, which was mixed in a HDPE pail, was pumped into the reactor via a peristaltic pump utilizing Teflon tubing. Influent samples

were collected directly from the HDPE bucket prior to pumping into the reactor. The remaining feed was then pumped into the bioreactor and a 205 minutes "react" period occurred. Following react, the bioreactor underwent a settle phase for 55 minutes. Effluent samples were then collected from the bioreactor directly. Several batches of feedwater were produced for the initial investigation using volumes of tap water collected from the WTC:

- 11 L of high concentration feed solution (simulating predicted influent concentrations); and
- 11 L of low concentration feed solution (simulating predicted effluent concentrations).

The experiment was run twice, once with high concentration feed and again with low concentration feed. Duplicate influent and effluent samples were collected for each investigation to assess the variability associated with the process. Samples collected under the high strength scenario were frozen for several days prior to analysis. Low strength samples were brought immediately to the commercial lab for analysis. Samples were transported in coolers using ice to maintain a temperature of 4 °C. The results of the analysis received from the lab pertaining to the high strength feedwater and low strength feedwater investigation can be found in **Appendix B**.

A high degree of precision between duplicate samples as well as influent and effluent samples, was noted in both tests, however the recovery of the spiked compounds under the high strength feedwater test were considered poor (ACE, SMX and TRIM <20%). The recoveries achieved under the low strength feedwater scenario were found to be significantly improved (>50%), however were still considered to be below the standards typically observed in the literature. As a baseline, EPA Method 1694 suggests that a recovery of between 55 and 108 percent for ACE, SMX and TRIM and between 23 and 123 for CBZ of the expected value can be considered as meeting EPA performance criteria. Relative standard deviations (R.S.D.s) of 30% or less are also required to meet minimum performance standards. However, results reported in the literature typically achieve a R.S.D of QA/QC samples of <20% (Santos et al., 2005; Gros et al., 2006; Lishman et al., 2006; Van Nuijs et al., 2010; Tarcomnicu et al., 2011).

The results obtained through the initial investigation were particularly concerning because the sample matrix was considered to contain only minor levels co-eluting compounds leading to signal suppression. It was not clear whether the poor recoveries could be attributed to experimental or analytical error and thus an investigation into the methods utilized was initiated. However, based on the results presented it was determined that sorption to HDPE media was unlikely to contribute to significant PC losses during the investigation.

Upon meeting with the chemist to discuss the results of this testing, it was determined that direct injection of samples (no sample preparation) into the LC-MS/MS was being practiced. This methodology is rarely reported in the literature as its accuracy and precision were noted to be poor relative to analysis using SPE. To further assess the capabilities of the direct injection method, a follow up investigation was completed in which a standard addition test was used to estimate the background concentrations in WTC primary effluent. Primary effluent samples were spiked with varying levels of PC standards. By subtracting the known amounts added to the sample, an assessment of the background concentrations present as well as the relative standard deviations (RSD) could be determined.

This investigation was completed using two different sample preparation scenarios: 5 samples underwent the SPE sample preparation procedure identified above, and 5 were analyzed by the direct injection method. Each method was assessed based on samples taken from the same 4L brown glass sampling vessel. The samples were spiked with both varying quantities of unlabelled compounds and a pre-made mix containing many deuterated standards which included the 4 compounds investigated. Each sample had a final concentration of 100 ng/L of deuterated standard for each compound of interest. By analyzing these 5 samples and subtracting the spiked amount, the background concentration of the 4 PCs selected for this investigation could be determined.

The average background sewage concentrations of the compounds of interest, as well as the relative standard deviations (RSD) were then estimated by subtracting the known amount of unlabelled compound spiked into the sample from the measurement reported by the lab (background + spike). Matrix spikes were submitted for comparison and consisted of 100 ng/L of each compound spiked into DI water. The results of both the SPE and direct injection analysis are provided in **Appendix B**.

The results obtained for the direct injection investigation were highly variable, with calculated RSD values which were approximately twice those typically encountered in the literature (Santos et al., 2005; Gros et al., 2006; Lishman et al., 2006; Van Nuijs et al., 2010; Tarcomnicu et al., 2011). Variability between samples appeared to be most significant for samples that were not spiked with any standards and under the highest spiked concentrations. This demonstrates poor linearity associated with the calibration curve using these methods. The results obtained using the direct injection method were considered to be unacceptable and this method was abandoned.

The results for the SPE analysis produced an acceptable RSD for SMX and CBZ, however, ATEN and TRIM were noted to demonstrate poor reproducibility at elevated concentrations, demonstrating poor linearity of the calibration curve. This was most notable for Atenolol that appeared to be subject to significant ion suppression when concentrations exceeded approximately 2 μg/L. Similarly, the MS recoveries for SMX and TRIM met the minimum acceptable criteria under EPA method 1694, but were found to be outside the typical recoveries reported in literature. It should be noted that despite the addition of isotopically labelled standards (ILSs), their concentrations were not quantified by the chemist. It was believed that the use of the isotope dilution method (Vanderford and Snyder, 2006) would result in better recoveries, and significantly reduced RSD values. It was therefore determined that use of the isotope dilution method would be an absolute requirement for all future analysis.

The RSD calculated from these analyses was used to determine the required number of samples to achieve what was considered an acceptable level of certainty in the test results. The software package G*power 3 (Faul et al., 2009) was used in an iterative manner to determine the required sample size (n) to achieve suitable statistical certainty (α = 0.1 and β = 0.2) between mean removal rates achieved between the control and IFAS reactor. The minimum differences between the mean removal rates, calculated for each of the 4 compounds analyzed under the SPE investigation, required in order to observe a statistically significant difference between the two processes is reported in Table 3.6.

Table 3.6 - Statistical Determination of Minimum Sample Size

Pharmaceutical Compound	Minimum difference between mean elimination efficiencies (%)		
	n = 8	n = 10	n =12
ATEN	22.9	20.2	18.2
CBZ	31.4	27.6	25.0
SMX	23.4	20.6	18.6
TRIM	49.3	43.4	39.3

Based on the results of the power analysis a minimum sample size of 12 was selected for the phase 1 investigation. It was anticipated that the use of the isotope dilution method as well as the reduced matrix effects associated with secondary effluent would improve the statistical certainty, making the above values representative of worst case analytical results.

3.6.4 PHASE I INVESTIGATIONS

Based on the preliminary investigation results, 12 samples were collected between September 25 to 29, 2012 from the IFAS reactor and control reactor operated at a temperature of 18° C and an SRT of 20 d (reactor K20 and B respectively). In addition, 4 matrix spikes and 2 blanks were analyzed for QA/QC purposes. Samples collected were preserved with sodium azide and ascorbic acid and refrigerated prior to being transported to the Servos Lab at the University of Waterloo in coolers for sample preparation. Sample preparation was conducted using the methods described in section 3.6.1, resulting in a final reconstituted sample volume of 2.5 mL in acetonitrile and a concentration factor of 40 times.

Due to the uncertainty identified with the commercial labs methods during preliminary testing, only 1 experimental condition was investigated during phase 1. The data collected from this investigation was intended to further inform the remaining sampling, allowing for changes to the experimental methodology if warranted. The analytical method used by the commercial lab was proprietary in use, and limited details regarding the analytical process were provided. The method was understood to be based on the EPA 1694 method (Eaton and Franson, 2005) using a shimatzu HPLC, operated in electrospray ionization positive (ESI+) mode, coupled with an AB Sciex QTRAP 5500 MS.

It was requested that the isotope dilution method (Vanderford and Snyder, 2006) be used for this analysis, or at minimum isotopically labelled standards (ILSs) be quantitated, as all samples with the exception of blanks, were spiked with ILSs. However the chemist performing the analysis used serial dilution of samples (ranging from no dilution to 2000 times) making quantitation of the added ILSs impossible. Unfortunately, this practice was not

communicated prior to the reporting of results. Additionally, the dilutions used by the commercial lab resulted in sample signals that were significantly below the lowest point of the calibration curve. Any data which was found to be outside the calibration curve range was removed from the data set. Dilution has been used in previous studies to control matrix effects, however, a dilution factor of 2 to 4 is typically used (Gros et al., 2006). It is noted that the extreme dilutions practiced by the commercial lab likely resulted in increased analytical error associated with measurement errors. Due to incompatibility between this analytical method and the isotope dilution method, which was considered an absolute requirement, it was decided that all subsequent analysis would be performed by the Servos Lab at the University of Waterloo.

An analysis of the Phase 1 samples was performed by the Servos Lab employing the isotope dilution method. Briefly, the isotope dilution method involves the addition of a known concentration of ILSs to both the calibration standard solutions used to prepare the calibration curve and the samples and matrix spikes analyzed. ILSs must be dosed at equivalent concentrations, after concentration factors are considered, to both the calibration curve standards and the samples for a suitable comparison. This allows for a direct assessment of the matrix effects, such as ion suppression or enhancement, as well as losses associated with sample preparation and measurement to be determined. The measurement of the ILSs allows for these errors to be compensated for by the method, resulting in greater accuracy, precision and confidence in the data. As ILSs were not measured during the analysis by the commercial lab, matrix effects and losses associated with sample preparation could not be quantitated. The calibration curve used for Phase I analysis by the Servos Lab was prepared using reference standards and ILSs versions of ATEN, CBZ, SMX and TRIM. An additional calibration curve, composed of both unlabelled standards and an ILS of ACE, was prepared by Servos Lab staff. This required that ACE be quantitated separately from the remaining PCs.

The U of W lab utilized analytical blanks as a means to reduce carryover (also termed memory effects) of PCs from prior samples analyzed. This was accomplished via injection of HPLC grade methanol into the analytical equipment to “purge” any residual PC’s present. Carryover contributes to background noise which can provide false positives during the analysis of subsequent samples. Several analytical blanks were injected prior to and following the introduction of effluent and influent samples as well as between each individual influent sample due to the high concentration of co-eluting compounds anticipated. The analyte signals associated with the blanks were measured using manual quantitation methods in an attempt to quantitate the level of background noise present in the analytical equipment. To allow for a clear delineation between background signals and true analyte signals associated with wastewater samples, all data obtained at the Servos Lab was screened based on the average background noise measured in analytical blanks injected concurrently with influent and effluent sample measurements. At the advice of the Servos Lab chemist, any wastewater sample which produced a signal that was less than 2 times the average background noise signal was not reported due to uncertainty.

The sample preparation methodology requested by the commercial lab resulted in some data obtained from the Servos Lab being inadmissible as a result of background noise concerns. Sample preparation procedures used by the Servos Lab typically involve sample preparation methods which result in a 200 times concentration factor. At the request of the commercial lab, a concentration factor of only 40 times was utilized for Phase I PC sample preparations. This reduced concentration factor resulted in poor peaks for ATEN, ACE and SMX during Servos Lab

analysis. Due to the elevated background noise signals, sample analyte signals did not meet the criteria required for reporting confidence. This data was discarded from the dataset at the request of the Servos Lab. Although the method used by the Servos Lab was determined to provide a better level of accuracy and precision based on the MSs, the unusable data required that results from the commercial lab be used for these three PCs during Phase I investigations.

A method quantitation limit (MQL) was not determined using typical methods (standard addition tests) due to limited analytical time and availability. An "ad hoc" procedure was utilized to estimate the MQLs associated with influent and effluent samples based on the screening criteria discussed above. While no ILS carryover was observed in the samples, measurable signals of non-labelled PCs were observed. To provide an estimation of the MQL, the average blank signals measured during the analysis of samples (prior to, following and during in the case of influent) were quantitated. To allow for quantitation using the isotope dilution method, the average ILS signal obtained for each of the wastewater samples was used to calculate a ratio of the unlabelled and labelled signals for comparison to the calibration curve. This is demonstrated by equation 3.7:

$$Background\ Noise\ Concentration\ = \frac{Average\ Signal\ of\ Non\text{−}labeled\ PC\ Compound\ in\ Blank}{Average\ Signal\ of\ ILS\ observed\ in\ wastewater} \quad (3.7)$$

where:
Background Noise Concentration is measured in ng/L; and
Non-labelled and ILS signals are reported as peak area (counts)

It should be noted that this value represents the average MQL value estimate. MQL estimations were calculated for CBZ and TRIM under Phase I analysis, and for CBZ, SMX, TRIM and ATEN under Phase II/III. In some instances, values below the MQL were reported if the non-labelled PC signals exceeded 2 times the background noise signal. This method was used to provide a coarse comparison of the analytical capabilities relative to individual compounds as well as the impacts of the matrix analyzed.

3.6.5 PHASE 2 INVESTIGATIONS

Phase 2 investigations involved the analysis of one additional experimental condition. IFAS reactor A and control reactor C, both operated at a temperature of 12℃ and an SRT of 20 d, were considered under Phase 2. It was initially intended that sampling of both reactors occur concurrently in December 2012, however due to a filamentous organism outbreak within reactor C, characterized by a significant loss of biomass and performance, sampling and analysis of this reactor was postponed until January 2013 as part of Phase 3 investigations.

Based on the high level of variability associated with the analytical data from Phase 1, the total number of samples was increased to 18 based on 6 discrete samples analyzed in triplicate. 18 samples were collected from IFAS reactor 'A' between December 11 and 14, 2012. Additionally, 2 MS and 1 blank were analyzed for QA/QC purposes. The samples were preserved with sodium azide and ascorbic acid and refridgerated prior to being transported to the

Servos Lab in coolers for sample preparation. Samples were prepared using the methods described in the above sections, resulting in a final eluted sample volume of 500 μL and a concentration factor of 200 times. The isotope dilution method was utilized to correct for matrix effects and losses occurring due to sample preparation.

3.6.6 PHASE 3 INVESTIGATIONS

Phase 3 investigations involved the collection and analysis of samples from 5 reactors, including reactor C, IFAS reactors A, D, K and E operated at temperatures of 12 and 18 ℃, respectively, and an SRT of 7d. 18 samples were collected from each reactor between January 14 and 18, 2013. Additionally, 18 matrix spikes and 10 method blanks were analyzed for QA/QC purposes. Due to the large number of samples, 10 days were needed to prepare all samples for analysis. Samples collected were preserved with sodium azide and ascorbic acid and refrigerated prior to being transported to the Servos Lab in coolers for sample preparation. Samples were prepared using the methods described in the above sections, resulting in a final eluted sample volume of 500 μL and a concentration factor of 200 times. The isotope dilution method was utilized to correct for matrix effects and losses occurring due to sample preparation.

4. RESULTS AND DISCUSSION

Chapter 4 provides a summary of observed data related to the current investigation as well as an analysis of the results obtained. PC investigations were conducted using a 2^2 factorial experimental design using a sequential analysis approach. This resulted in 3 phases of investigations. The following monitoring/analytical data is provided for each phase:

- results from monitoring of conventional pollutants to assess reactor performance;
- batch nitrification testing results to further determine the presence and activity of autotrophs;
- effluent and mixed liquor solids measurements as well as calculated operating SRTs;
- QA/QC data from PC analysis; and
- screened PC data and calculated transformation efficiencies.

A discussion of observed reactor performance, observed PC transformation efficiencies, as well as a comparison to past investigations has been included with the presented data. The results of statistical analyses performed on conventional data and PC transformation efficiencies are also provided.

4.1. *PHASE 1 INVESTIGATIONS*

Phase 1 investigations were completed using the high temperature (18°C) and high SRT (20d) experimental condition for both the IFAS equipped SBBR (identified as reactor K20) and a control SBR (identified as reactor B). Prior to testing, the IFAS SBBR was permitted to run for approximately 13 months to ensure that a biofilm had fully developed and was at pseudo steady state. Reactor B had been in operation for several years and had demonstrated good performance during this historical period. Conventional data was collected during a monitoring period equal to approximately 3 SRTs prior to PC sampling to characterize the performance of the reactors. Effluent TSS, MLSS and daily WAS volumes were monitored during this period to ensure that both reactors were operating within range of the target experimental conditions. The data was collected to confirm that both reactors were operationally stable and providing a level of biological treatment commensurate with that typically observed for well operating SBRs at the given SRT and temperature. This data is located in **Appendix C**.

Phase 1 PC sample collection was initiated on September 25, 2012 and was conducted over a 4 day period. 12 sample sets were collected which consisted of both an initial sample, collected at t = 0, that characterized the mixed liquor conditions at the beginning of the treatment cycle, as well as a sample of the treated effluent (final). The sample sets were each collected at different times and are representative of individual treatment cycles. The 12 sample sets collected for PC sampling can be further characterized as follows: 3 sets comprised of individual samples with no replicates (samples 1, 5 and 8), three sample sets in which both the initial and effluent samples were collected in duplicate (sample set 2/3, 4/6 and 7/9) and one sample set in which both initial and effluent samples were collected in triplicate (samples 10/11/12). The collected samples were analyzed for conventional parameters, including tCOD, TAN and NO_3-N to ensure that acceptable levels of organic and TAN removals were sustained

throughout the sampling period. The raw conventional data is located in **Appendix C.** The mean concentrations that were observed along with mean removal efficiencies of COD and TAN that were calculated are presented in Table 4.1.

Table 4.1 - Conventional Performance During PC Sampling – Phase 1

Reactor	Experimental Condition	Initial			Final			Removal Rate	
		COD_t (mg/L)	TAN (mg/L)	NO_3-N (mg/L)	COD_t (mg/L)	TAN (mg/L)	NO_3-N (mg/L)	COD_t (%)	TAN (%)
K	SRT = 20 days Temperature = 18℃	151.6 (20.7)	11.4 (1.0)	8.9 (1.2)	37.6 (15.9)	0.04 (0.02)	24.1 (1.7)	73.8 (12.6)	99.7 (0.1)
B		227.6 (16.6)	20.1 (4.1)	1.6 (0.6)	47.4 (22.5)	0.50 (0.43)	24.0 (4.6)	80.3 (8.5)	96.8 (2.3)

Notes:
Values shown represent mean concentrations. Values in parentheses are calculated standard deviations.

Due to the presence of IFAS media, all SBBRs were required to operate with a greater mixed liquor volume to avoid the exposure of the media to atmospheric conditions during decant. Both reactors K20 and B received approximately 13 L of primary effluent during the fill phase of every treatment cycle; however, reactor K20 had a larger operating volume (23.3 L) relative to reactor B (20 L) due to the presence of IFAS media. As a result, reactor K received a volumetric loading which was approximately 20% less than reactor B. This had the effect of increasing the HRT; Reactor K operated at an HRT of 10.6 hours whereas reactor B operated at an HRT of 9 hours. It was anticipated that the slight increase in HRT might result in some minor decreases in the measured effluent COD and TAN values.

Based on the measured concentrations at the beginning and end of each feed cycle and the volumetric loading rates known to have been received by each reactor, it was estimated that the average primary effluent received by reactors K20 and B contained tCOD concentrations that were within the range of 242 to 318 mg/L. The differences observed in the initial concentrations calculated for each reactor were expected to have been the result of minor variances in the volume of primary effluent received during each feed cycle and analytical error. The peristaltic pumps used for conveyance of feed into each reactor were found to demonstrate slight inconsistencies in pumping rates; however this minor variance was not anticipated to impact the PC transformation efficiency of either reactor.

Envirosim Associates Ltd. (2011) have reported that the following COD values and COD:BOD_5 ratio are considered typical for North American domestic raw influent and primary effluent:

- Raw influent tCOD = 600 mg/L
- Raw influent non-biodegradable soluble COD (nbsCOD) = 30 mg/L
- Primary effluent tCOD = 376 mg/L
- Primary effluent nbsCOD = 30 mg/L

- Primary effluent COD:BOD_5 = 1.87

The results presented in Table 4.1 indicate that on an average basis, the pilot reactors received primary effluent with COD concentrations which were approximately 60 and 135 mg/L lower than COD concentrations in typical domestic sewage. However, it is acknowledged that the primary effluent concentrations estimated by Envirosim Associates Ltd. (2011) were based on typical primary clarifier removal performance. The performance of the clarifier which provided primary treatment of flows conveyed to the pilot reactors was not measured and may have exceeded typical performance. Additionally, it had been anecdotally reported by Environment Canada staff that some COD removals occurred within the sewage forcemain which conveyed sewage from the Skyway WWTP to the WTC. It has been postulated that this was the result of extended HRTs provided within the pipeline as a result of operation under low flow conditions.

Based on the typical COD:BOD_5 ratio reported by Envirosim Associates Ltd. (2011), it was estimated that during Phase 1 PC sampling the mean BOD_5 concentrations within reactors K20 and B at the beginning of each cycle during phase 1 PC sampling were between 80 and 120 mg/L. This suggests that primary effluent received by reactors K and B had BOD_5 concentrations in the range of 129-170 mg/L. Based on the data presented by Envirosim Associates Ltd., typical primary effluent can be expected to contain a BOD_5 concentration of approximately 200 mg/L. However, the Design Guidelines for Sewage Works suggest that primary effluent within Ontario can be assumed to contain a BOD_5 concentration ranging from 105 to 140 mg/L (MOE, 2008). When viewed collectively it was deemed that the sewage received by the reactors during the PC sampling period was within the expected range of typical primary effluent observed at Ontario municipal WWTFs.

Reactors K20 and B produced average effluent tCOD concentrations of 37.7 mg/L and 44.0 mg/L, respectively. The tCOD concentrations measured in the final samples collected from reactors K20 and B were compared using student t-test statistical methods (**Appendix G**) to determine if a significant difference in final tCOD concentrations occurred. At a confidence level of 95% the tCOD concentrations within final samples collected from both reactors were not found to be statistically different. This indicates that both reactors were providing a consistent level of COD removal.

tCOD can be partitioned into both soluble and particulate fractions which are either biodegradable or non-biodegradable. This can be described by equation 4.1 (Metcalf and Eddy, 2003).

$$tCOD = nbpCOD + nbsCOD + bpCOD + bsCOD \quad (4.1)$$

where:
nbpCOD = non-biodegradable particulate COD
nbsCOD = non-biodegradable soluble COD
bpCOD = biodegradable particulate COD
bsCOD = biodegradable soluble COD

According to the typical primary effluent nbsCOD concentration reported by Envirosim Associated Ltd., it was estimated that reactors K20 and B received 16.8 and 20 mg/L of nbsCOD, respectively. This suggested that an additional source of COD, equal to approximately 20 to 25 mg/L, was present within the effluents of both reactors. No further testing was done on final samples collected during Phase 1 to characterize this remaining COD.

Metcalf and Eddy (2003) suggests that the soluble COD concentration within the effluent produced by an activated sludge process operated at SRTs greater than 4 days can be assumed to consist entirely of nbsCOD. It was therefore likely that the COD measured within final samples during phase 1 consisted of non-biodegradable COD. However, as filtered COD analysis was not conducted, the soluble and particulate COD fractions measured within final samples collected from reactors K20 and B could not be determined. It was therefore considered likely that the COD measured in the final samples, which was estimated to be approximately 20 to 25 mg/L greater than typical values, were reflective of particulate COD within reactor effluents or elevated nbsCOD concentrations within the primary effluent. On this basis, reactors K and B were considered to be achieving a suitable level of organic removal.

Based on the measured TAN values reported in Table 4.1, the mean TAN concentrations within primary effluent were estimated to be approximately 21 and 30 mg/L for reactors K and B, respectively. This suggests that on an average basis, reactor B was receiving approximately 150% of the TAN load received by reactor K20. As noted above, reactor B received a volumetric loading that was estimated to be approximately 20% higher than reactor K. A statistical analysis of the TAN concentrations measured in initial samples, which took into consideration the expected difference in TAN loadings, was conducted using a student t-test comparison and a confidence level of 95% (**Appendix G**). This analysis suggests that each reactor received significantly different TAN loadings ($p<0.05$).

Envirosim Associates Ltd. (2011) report that typical municipal primary effluent contains a TAN concentration of approximately 32.5 mg/L. However, the Design Guidelines for Sewage Works (MOE, 2008) suggest that typical municipal sewage in Ontario contains a TAN concentration ranging from 20 to 25 mg/L. Hence, the primary effluent received by both reactors during Phase 1 contained TAN concentrations that were within range of typical municipal sewage.

It was noted that both reactors contained equivalent concentrations of NO_3-N within their respective effluents, suggesting that both had nitrified a similar concentration of TAN. The effluent NO_3-N concentrations measured in the final samples from both reactors were found to not vary significantly based on a student t-test and a confidence limit of 95% (**Appendix G**). However, it was also noted that reactor K20 had an average initial NO_3-N concentration of 8.9 mg/L. In contrast, reactor B had an initial NO_3-N concentration of 1.6 mg/L. This may suggest that some level of de-nitrification was occurring within both reactors, most notably in reactor B. On the basis of the observed NO_3-N within final samples, it was considered likely that both reactors were receiving primary effluent with a consistent TAN concentration and the observed TAN concentration differences in initial samples were the result of analytical error associated with the measurement of this parameter.

The Design Guidelines for Sewage Works (MOE, 2008) suggest that activated sludge bioreactors operated under conditions which permit nitrification can be expected to produce effluent TAN concentrations below 3 mg/L. The mean effluent TAN concentrations measured during the monitoring period for reactors K and B were 0.03 and 0.61 mg/L, respectively. A statistical analysis, using the student t-test, was completed which compared the TAN concentrations measured within final samples collected from reactor K20 and B (**Appendix G**). At a confidence level of 95%, no significant difference between TAN concentrations within the final samples collected from both reactors was detected. Both reactors were therefore providing full nitrification during the Phase 1 sampling period, as evidenced by the TAN and NO_3-N concentrations presented in Table 4.1.

The average concentrations of MLSS, MLVSS and effluent TSS, as well as the WAS volume discharged per day, were measured to assess the operating SRT, calculated using equation 2.1.

$$\theta = \frac{\forall_{bioreactor} \times X_{MLSS}}{[Q_{WAS} \times X_{WAS}] + [Q_{EFF} \times X_{EFF}]} \quad (2.1)$$

where:

$\theta = SRT\ (d)$

$\forall$ = Volume (m^3)

X = Solids Concentration $\left(\frac{g}{m^3}\right)$

Q = Flow $\left(\frac{m^3}{d}\right)$

The ESS, MLSS and MLVSS measurements as well as WAS volumes discharged and calculated SRT are provided in Table 4.2. Samples from both reactors were collected on September 25, 2012, in the cycle prior to the initiation of Phase 1 PC sampling. The values presented were considered to be reflective of reactor operating conditions during the 4 days over which Phase 1 PC samples were collected.

Table 4.2 – Results of Solids Monitoring During PC Sampling – Phase 1

Reactor	Date Collected	MLSS (mg/L)	MLVSS (mg/L)	Effluent TSS (mg/L)	WAS Volume (mL/d)	SRT (days)
K20	25/09/12	2030	1640	16	1020	17.5
B	25/09/12	3070	2410	11	1120	19

From Table 4.2 it can be observed that both reactors were operating within 2.5 days of the target SRT of 20 days. This was consistent with data collected during the 3 SRT period prior to sampling. Precise SRT control was found to

be a challenge due to the limited accuracy associated with WAS flowrate adjustments. However, it is considered unlikely that a significantly different biomass composition, or associated contaminant removals, would result from reactor operation at an SRT of 17.5 versus 20 days. On this basis, the reactors were considered to have been operated within the target SRT for a sufficient duration to ensure that the biomass composition was at steady state in both reactors.

The MLSS values associated with the SBBR were approximately 25% lower than the control (Table 4.2). This was likely due to the presence of the biofilm and the competition for limited substrates between the suspended growth and fixed film (biofilm) phases. The Design Guidelines for Sewage Works suggests that a well operated secondary treatment process should be capable of producing an effluent containing TSS concentrations of 15 mg/L or less (MOE, 2008). Both reactors produced an effluent that was consistent with typical secondary effluent with regards to TSS.

Based on the initial COD concentrations and the subsequently estimated BOD_5 values presented above, reactors K20 and B were operated at Food:Microorganisms ratios (F/M) of 0.11 and 0.13 $gBOD_5/gMLVSS \cdot d$, respectively. Typically, SBRs are operated at an F/M ranging from 0.04 to 0.10 (Metcalf and Eddy, 20003). Additionally, it is noted that reactors K20 and B operated at HRTs of 10.6 and 9 hours, respectively. Typically, SBRs are designed with a target HRT between 15 and 40 hours (Metcalf and Eddy, 2003).

On the basis of all of the conventional monitoring data it was concluded that reactors K20 and B were performing well; both reactors achieved full nitrification, despite operating with reduced hydraulic retention times and slightly higher organic loading rates than suggested by design references. The final samples collected from both reactors were noted to contain COD concentrations which were higher than those reported as typical for municipal sewage; however it was suspected that this was due to the presence of pCOD or elevated nbsCOD within the effluent. On this basis, both reactors were believed to be achieving organic removal efficiencies typical of a well operated SBR.

The nitrification abilities of reactors K20 and B were assessed using batch nitrification testing to investigate performance and confirm steady-state conditions had been achieved. Nitrification testing was conducted on September 25, 2012, before Phase 1 PC sampling commenced. Expected nitrification rates were calculated for reactor B using equations 3.5 and 3.6 based on typical kinetic parameters for activated sludge reported in the literature; and, the reactor operating conditions. Due to the complexity associated with the presence of IFAS media, expected nitrification rates were not estimated for reactor K20. A summary of the nitrification rate testing results is presented in Table 4.3. Further raw data from the nitrification rate testing is located in **Appendix D**.

Table 4.3 - Nitrification Rate Testing Results – Phase 1

Reactor	Reactor MLSS	Reactor MLVSS	Calculated TAN Removal Rate	Measured TAN Removal Rate	Measured NOx Production Rate	Measured Specific TAN Removal Rate	Measured Specific NOx Production Rate
	(mg/L)	(mg/L)	(gN/d)	(gN/d)	(gN/d)	(mgN/gVSS/d)	(mgN/gVSS/d)
K20	2030	1603	-	5.36 (0.20)	6.46 (0.30)	140	169
B	3070	2410	3.65	3.34 (0.27)	3.38 (0.46)	69	70
Note: Values shown in parentheses represent the standard error associated with linear regression.							

A review of the data presented in Appendix D revealed that there was a poor nitrogen balance in the batch testing on Reactor K20. Additionally, it was noted that reactor K20 had a lower starting TAN concentration than anticipated. Nearly all TAN removed during nitrification is expected to be converted to NO_x-N under aerobic conditions. However, the data suggests that a measurement error occurred when testing samples collected from Reactor K20; more NO_x-N was generated during the testing than TAN was removed. In the testing anyhydrous NH_4Cl was added to each reactor to achieve a target starting concentration of 30 mg/L. The initial samples (t=0) collected from reactor K20 during testing were found to have a TAN concentration of 22 mg/L. In contrast, the initial samples collected from reactor B had a TAN concentration of 28 mg/L. However, the NO_x-N concentration in the final sample from K20, collected at the end of testing, had a concentration of 31 mg/L. As the creation of NO_x-N can be solely attributed to nitrification, this suggested that 30 mg/L or greater of TAN was removed during the nitrification rate test.

Reactor K20 demonstrated similar issues regarding TAN measurements within initial samples based on data collected during PC sampling (Table 4.1). On this basis, NO_x-N production was considered to be more reflective of nitrification performance and was utilized to assess the performance achieved by both reactors. The NOx-N removal rates measured for reactors K20 and B were compared using a student t-test on the regression slopes (**Appendix G**) to determine if a significant difference in the nitrification rates achieved by either reactor occurred. At a confidence level of 95%, the NO_x-N production rates were found to be statistically different ($p<0.05$). The results indicated that the IFAS reactor K20 achieved a nitrification rate which was approximately twice the nitrification rate observed for reactor B. This difference was attributed to the presence of the IFAS biomass, as both reactors were operating at the same temperature and the SRTs differed by only 1 d.

Maas et al., (2008) conducted nitrification rate testing using IFAS carriers obtained from a full scale WWTP. In-basin ammonia removal rates, which included the contributions of the mixed liquor, were reported to range from 0.3 to 1.2 $gN/m^2 \cdot d$. Based on the NOx-N production rate measured for reactor K20, and utilizing the same calculation methods as Maas et al., (2008), an in-reactor removal rate of 1.6 $gN/m^2 \cdot d$ was observed. This demonstrates that reactor K20

was providing nitrification performance which exceeded the performance of a typical full scale IFAS equipped bio-reactor.

The PC chemical analysis for the first phase investigation was conducted utilizing two laboratories: A commercial lab and the Servos Lab at the University of Waterloo. The labs utilized different analytical procedures and it was uncertain which lab's methodology would produce results that achieved a suitable level of accuracy and precision. Hence, a detailed assessment of the quality assurance/quality control (QA/QC) data that was collected is presented to establish the context in which the actual test sample values were determined. QA/QC was assessed through the analysis of Matrix spikes (MSs), method blanks as well as an evaluation of ILS recoveries through comparison to concentrations measured within MSs and wastewater samples to those measured within the calibration curve.

The inclusion of Quality Control (QC) data, in the form of spiked analyte recoveries, to demonstrate the capabilities of the analytical method has become common in PC analysis studies (Lee et al., 2003; Lishman et al., 2006; Hao et al., 2008; Rodil et al., 2009; Van Nuijs et al., 2010; Tarcomnicu et al., 2011). Many of these articles utilized de-ionized water, tap water or uncontaminated surface water as a QC matrix. To demonstrate the analytical abilities of LC-MS/MS methods when analyzing PCs, Van Nuijs et al., implemented QC criteria which required that target analyte recoveries were within ± 15% of the spiked amount for results to be considered satisfactory. Tarcomnicu et al., (2011) similarly used the same QC recovery criteria as a means of quality assurance, but also required that relative standard deviations (RSD) calculated for replicate QC samples were below 15%.

To ensure that the analytical method utilized achieved the highest level of accuracy and precision possible, and to recognize the limitations of data obtained during PC analysis, an estimate of the total analytical error was required. The total analytical error can be primarily attributed to analyte losses occurring during sample preparation and analysis as well as signal suppression/enhancement due to matrix effects. Additional losses associated with sample and analyte measurements, sorptive losses associated with sample contact to lab equipment and volatilization during sample evaporation were also expected to occur. However, these losses were expected to represent a lesser impact on the final data than the losses due to sample preparation and analysis (Vogeser and Seger, 2010, Hall et al., 2012). The measurement of isotopically labelled standards (ILSs), which were spiked into all samples following filtration, allowed for an assessment and correction of these systematic losses which occurred during sample preparation and analysis.

Four matrix spikes (MS) were prepared and analyzed using the same method as the authentic samples. The analysis of MSs was conducted to provide confirmation of the analytical measurement process via quantitation of a reference standard at a known concentration. As reported in Section 3.6, all MSs were spiked with reference standards for the 5 PCs to achieve a final concentration of 100 ng/L. If the analytical process was achieving satisfactory performance, it was expected that each of the 5 analytes would be reported at concentrations between 85 and 115 ng/L and would have RSD values less than 15%.

The data reported by both labs for CBZ in the MS1 and MS2 samples (**Appendix E**) suggested that a sample preparation error involving CBZ reference standards occurred. It is believed that this data was the result of ILS recovery issues, as the commercial lab data did not demonstrate the same variability or any consistent trends when compared to the data obtained from the Servos Lab. The CBZ and SMX results reported by the Servos Lab did not meet the QC recovery criteria proposed above, demonstrating poor analytical accuracy and precision. The data from the commercial lab were considered to be below the QC requirements for accuracy for all 5 analytes. However, it was noted that the commercial lab achieved acceptable levels of precision for SMX and TRIM. The reported recoveries of ACE indicated that neither lab could quantitate this analyte within the MSs at the spiked concentration of 100 ng/L. Based on these poor recoveries, MS and ILS concentrations for ACE were subsequently increased to 10 ug/L for Phase 2/3 analysis.

The analytical equipment was noted to be susceptible to contamination associated with sample injections, particularly when wastewater samples were analyzed. This contamination can result in background noise that can mask the analytical signals. Analytical blanks consisting of methanol were therefore injected periodically between samples to encourage a "flushing" of the column, reducing the level of contamination. Despite this flushing, background signals of unlabelled PCs were observed in both the method blanks as well as the analytical blanks. However, ILS signals were not detected in any of these samples.

To allow for a clear delineation between background signals and true results associated with wastewater samples, all data obtained at the U of W laboratory was screened based on the average level of background noise measured during the injection of analytical blanks during initial and final sample measurements. Any sample that produced a signal which was less than 2 times the average background noise signal was considered unreliable and was not reported. This was consistent with the quality assurance criteria suggested by the Servos Lab Chemist.

The sample preparation methodology employed for phase 1, which was modified from the standard procedure utilized by the Servos Lab, was implemented at the request of the commercial lab (Section 3.6.4). This method resulted in reduced concentration factors for all MSs and wastewater samples relative to the standard method used by the Servos Lab. According to Servos Lab staff, this resulted in low analyte signals recorded by the LC-MS/MS that did not meet the signal to background criteria outlined above for ATEN, ACE and SMX for all samples. Although it was determined that the Servos Lab method provided a better level of accuracy and precision, based on the analysis of the MSs, when compared to the commercial lab, no data was available from the Servos Lab for ATEN, ACE and SMX. In the absence of this data, results from the commercial lab were used to characterize the concentrations of ATEN, ACE and SMX within the initial and final samples collected in Phase I investigations. However, it is recognized that the method utilized by the commercial lab produced poor accuracy for these compounds, and poor precision for ATEN. The precision associated with the analysis of ACE could not be estimated as ACE could not be quantitated in the MSs by either lab at the spiked concentration of 100 ng/L.

Specific MQL estimates were determined for CBZ and TRIM using the method outlined in Section 3.7 and are presented for the initial and final samples separately in Table 4.4. The reported MQLs from the commercial lab are provided as well.

Table 4.4 – Calculated and Reported MQL's for Phase 1 Analysis

Sample ID	Calculated MQL		Reported MQL		
	CBZ	TRIM	SMX	ATEN	ACE
K20 Initial	34	76	<5	<5	<10
K20 Final	31	32	<5	<5	<10
B Initial	39	79	<5	<5	<10
B Final	34	40	<5	<5	<10

A study completed by Santos et al., (2005) estimated the MQLs for influent and effluent wastewater samples separately. The estimated MQLs for the various PCs studied ranged from 6.2 to 319.8 ng/L for influent samples and 3 to 160 ng/L for effluent samples. A similar trend can be observed for TRIM where calculated MQLs for initial samples are approximately twice those calculated for final samples (Table 4.4). However, CBZ appeared to be relatively unaffected by both matrices.

The MQL values reported by the commercial lab are understood to have been determined from the analysis of standards in methanol and did not account for the impacts of matrix effects associated with wastewater samples. Zhang et al., (2011) reported that calculated MQL's for wastewater influents and effluents were 2 to 10 times higher than those calculated for matrix free standards prepared in solvents. As the commercial lab failed to detect ACE in MSs at a concentration of 100 ng/L, is it believed that the MQL values reported by the lab were likely significantly overstated and may be unreliable in the context of wastewater analysis. The true MQL associated with SMX, ATEN and ACE analysis conducted by the commercial lab could not be estimated based on the data provided.

The MQLs achieved for the analysis were generally higher than those reported in other studies. Nurmi and Pellinen (2011) reported MQL's for SMX, TRIM, ATEN and ACE of 11, 13, 70 and 190 ng/L, respectively, based on a signal to noise ratio of 10 obtained through the analysis of spiked wastewater effluent samples. Radjenovic et al., (2009) calculated MQLs using the same methodology (signal to noise ratio) as Nurmi and Pellinen (2011) using primary effluent. CBZ, SMX, TRIM, ATEN and ACE MQL's were estimated at 15.8, 1.7, 5.5, 8.2 and 75.3, respectively. The estimated MQL's for CBZ and TRIM for Phase 1 were approximately 2 to 3 and 3 to 14 times higher, respectively, than the results reported in the referenced studies. This discrepancy may be the result of the approach used to calculate the MQL, which has only been used infrequently (Standley et al., 2008). However, this method is considered to be highly conservative and likely reflects a high confidence in data, when available.

A summary of screened PC data is provided in Table 4.5. The raw data obtained from Phase I PC analysis is provided in **Appendix E**. The methods employed by the commercial lab resulted in many of the SMX samples being diluted to levels outside the calibration curve range. These samples were removed from the data set due to their inherent uncertainty. Additionally, several samples that were stored at the U of W laboratory were accidently lost and thus no data was available for these samples. These losses affect the reported concentrations for CBZ and TRIM only.

Table 4.5 – Reported PC Concentrations for Phase I Investigation

Sample ID	Duplicate With:	Initial					Final				
		CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
K1		448	331	93	ND	47300	ND	542	ND	34	71
K2	K3	445	285	83	551	41500	523	ND	<MQL	32	11
K3	K2	ND	ND	ND	679	33000	260	ND	<MQL	26	<MQL
K4	K6	445	289	93	723	39200	448	210	<MQL	29	119
K5		465	297	82	660	35800	435	255	<MQL	39	<MQL
K6	K4	258	ND	92	1340	52600	283	ND	<MQL	20	<MQL
K7	K9	465	693	93	1170	78800	520	231	<MQL	ND	<MQL
K8		308	ND	92	679	36800	298	ND	<MQL	52	<MQL
K9	K7	278	ND	105	648	42200	313	ND	<MQL	ND	<MQL
K10	K11,K12	308	ND	111	986	58200	330	463	<MQL	62	<MQL
K11	K10,K12	313	ND	117	862	53400	320	ND	<MQL	55	<MQL
K12	K10,K11	315	ND	127	939	44900	305	287	<MQL	ND	<MQL
B1		480	607	156	1160	67800	460	748	143	216	<MQL
B2	B3	470	588	149	924	54600	450	ND	145	150	22
B3	B2	268	ND	199	1010	58700	308	592	152	151	<MQL
B4	B6	ND	ND	ND	1030	70800	ND	604	ND	162	23
B5		518	591	155	930	56200	498	ND	117	179	12
B6	B4	280	ND	194	1130	74400	358	ND	138	143	19
B7	B9	648	ND	182	636	76800	448	ND	141	168	19
B8		298	ND	181	632	45300	330	566	181	28	19
B9	B7	278	ND	210	517	39200	293	337	179	211	<MQL
B10	B11,B12	300	ND	190	ND	44500	255	ND	145	35	<MQL
B11	B10,B12	368	ND	185	1280	74800	ND	ND	ND	26	<MQL
B12	B10,B11	268	ND	194	1190	61900	278	481	217	38	<MQL

Notes:

1. <MQL – Signal was below the calculated or reported MQL value.
2. ND – Not data was available due to analytical error, sample destruction or failed outlier test.
3. Data obtained from the Servos Lab (UW) are highlighted in blue. Data obtained from the commercial lab are highlighted in green.

The removal of unsuitable SMX data resulted in no sample sets that included replicate measurements. However, it is noted that the initial and final concentrations ranged from 285 to 693 and 210 to 542, respectively, for reactor K. Similarly, initial and final concentrations for reactor B ranged from 588 to 607 and 337 to 748, respectively. As a result of the large variance in these measurements, as well as the inconsistencies between initial and final samples, there was limited confidence in this dataset.

It was noted that replicate sample measurements for CBZ in both initial and final samples, as well as ATEN and ACE in initial samples showed poor agreement. As these replicates were prepared using aliquots of collected wastewater from the same bottle, the variability demonstrated is considered to reflect challenges with sample preparation and analytical performance.

In the case of CBZ, it was noted that replicate samples followed a typical pattern in which the first sample analyzed had a concentration approximately 150 to 200 percent of the concentration reported for the second sample. This was observed for both the initial and effluent sample sets of K2/K3, K4/K6 and K7/K9 as well as samples B2/B3, B4/B6 and B7/B9, subject to data availability. In each of these sets, the initial sample has a reported concentration that generally ranged from 450 ng/L to 648 ng/L. The second samples in each set ranged between 260 and 313 ng/L. It is not clear why this consistent phenomenon occurred.

ATEN demonstrated a high variability within replicate initial samples collected from Reactor K only. Sample sets K2/K3, K4/K6, K7/K9 each contained one replicate that was reported to be 123, 185 and 181%, respectively, of the other sample. However, no trends between sample order and those characterized by elevated concentrations, as was observed for CBZ, were noted. By contrast, initial replicate samples collected from Reactor B were noted to be between 110 and 123 percent of each other, demonstrating improved precision. The reason for this trend is not known, however, based on the results of QA/QC data obtained for the commercial lab, poor analytical performance is suspected.

ACE demonstrated highly variable replicate measurements in a similar fashion to ATEN. Sample sets K2/K3, K4/K6, K7/K9 each contained one replicate that were 126, 134 and 187%, respectively, of the other sample. By contrast, initial sample sets collected from Reactor B contained replicate samples that were 108, 105 and 196% of the other, demonstrating an improvement for sample set B2/B3 and B4/B6, but reduced precision for set B7/B9. It was noted in the data obtained for both reactor K and B that the third sample set (7 and 9) had the largest variability. The reason for this trend is not known, however, poor analytical performance is suspected.

Based on the results presented in Table 4.5, transformation efficiencies (expressed as a percentage) as well as standard deviations for the transformation efficiencies were calculated. In these calculations effluent measurements that were below the calculated or reported MQL were considered to be equal to the MQL. This provided a level of conservatism within the transformation efficiency estimates. The calculated efficiencies are provided in Table 4.6.

Table 4.6 – Observed Transformation Efficiencies for Phase I Investigation

Reactor	Transformation Efficiency Observed (%)				
	CBZ	SMX	TRIM	ATEN	ACE
K1	ND	-64	65	ND	>99
K2/K3	12	ND	61	95	>99
K4/K6	-4	27	65	98	>99
K5	6	14	61	94	>99
K7/K9	-12	67	68	ND	>99
K8	3	ND	65	92	>99
K10/K11/K12	6	ND	73	94	>99
AVERAGE	2	11	66	95	-
STD DEV	9	55	4	2	-
B1	4	-23	9	81	>99
B2/B3	-3	-1	15	84	>99
B5	-28	ND	29	86	>99
B4/B6	4	ND	24	81	>99
B7/B9	20	ND	18	67	>99
B8	-11	ND	0	96	>99
B10/B11/B12	0	ND	0	97	>99
AVERAGE	-2	-12	14	85	-
STD DEV	15	ND	11	10	-

Notes:
ND – Insufficient data available to calculate transformation efficiency.

It can be observed from Table 4.6 that the calculated standard deviations for the data demonstrated an acceptable level of precision for CBZ, TRIM and ATEN as all demonstrated RSDs that were less than or equal to 15%. This is generally consistent with the criteria used to assess the precision of the analytical method to determine if adequate performance was being achieved. However, the SMX data demonstrated much higher variance. No sample sets with replicate samples were available and therefore it could not be determined if this variability was truly reflective of initial and final concentrations. ACE was transformed at an efficiency greater than 99% in both reactors and therefore no significant difference between the IFAS reactor and the control was observed.

The mean transformation efficiencies calculated for CBZ were in the range of 12 to -12 and 20 to -28 for reactor K and B, respectively, however the net transformation efficiencies suggests that no quantifiable transformation occurred in either reactor. A minor increase in the transformation efficiency for ATEN was demonstrated by the IFAS reactor relative to the control, as demonstrated by the mean transformation efficiency calculated. However, it is noted that the removal efficiencies calculated based on sample B8 and sample set B10/B11/B12 were consistent with those calculated for reactor K. TRIM demonstrated significantly increased transformation efficiency in the IFAS relative to the control, as evidenced by a 52% difference in the mean transformation efficiencies. A more detailed statistical comparison of the transformation efficiencies observed in the SBBR and SBR is provided in Section 4.4.

4.2. *PHASE 2 INVESTIGATIONS*

Phase 2 investigations were completed using the low temperature (12°C) and high SRT (20d) experimental condition for both the IFAS equipped SBBR (identified as reactor A20) and a control SBR (identified as reactor C). Prior to testing, the IFAS SBBR was permitted to run for approximately 14 months to ensure that a biofilm had fully developed and was at pseudo steady-state conditions. Reactor C had been in operation for several years and had demonstrated generally good performance during this historical period, with the exception of some seasonal (winter) process upsets. Conventional data was collected during a monitoring period of approximately 3 SRTs prior to PC sampling to characterize the performance of the reactors. Effluent TSS, MLSS and daily WAS volumes were monitored during this period to ensure that both reactors were operating within range of the target experimental conditions. This data was collected to confirm that both reactors were operationally stable and providing a level of biological treatment commensurate with that typically observed for well operating SBRs at the given SRT and temperature. This data is located in **Appendix C**.

Phase 2 PC sample collection from reactor A20 was initiated on December 11, 2012 and was conducted over a 4 day period. A total of 6 sampling events, consisting of: both an initial sample, collected at t = 0, which characterized the mixed liquor conditions at the beginning of the treatment cycle; as well as a sample of the treated effluent (final), were used to characterize PC transformation efficiencies. Each of the 6 sampling events included the collection of triplicate initial and final samples. The collected samples were analyzed for conventional parameters, including tCOD, TAN and NO_3-N to ensure that acceptable levels of organic and ammonia removals were sustained throughout the sampling period. Raw conventional data is located in **Appendix C**. The mean concentrations that were observed as well as mean removal efficiencies of COD and TAN that were calculated are presented in Table 4.7.

In Phase 2 reactor C underwent repeated and sustained process upsets causing significantly elevated effluent TSS at various times throughout the monitoring period. It was determined that these performance issues were related to the proliferation of filamentous organisms, as confirmed through microscope investigations of reactor C MLSS. The images obtained through microscopic investigations are located in **Appendix H**. These upsets resulted in poor nitrification performance at various times due to reactor operation significantly below the target SRT. As a result, PC sampling of reactor C, which was intended to be conducted in December, concurrent with reactor A20, was delayed due to these performance issues.

The performance of reactor C was restored after chemical treatment and biomass supplementation from reactor B in January 2013. Further details of process monitoring and the actions undertaken to correct performance issues are described in **Appendix C**. Phase 2 PC sample collection from reactor C was initiated on January 14, 2013 and was conducted over a 5 day period. Consistent with reactor A20, a total of 6 sampling events, each with an initial and final sample, were used to characterize PC transformation efficiencies. Each of these 6 sampling events included the collection of initial and final samples in triplicate. The collected samples were analyzed for conventional parameters, including tCOD, TAN and NO_3-N to ensure that acceptable levels of organic and TAN removals were sustained throughout the sampling period. Raw conventional data is located in **Appendix C.** The mean concentrations that were observed along with mean removal efficiencies of COD and TAN that were calculated are presented in Table 4.7.

Table 4.7 - Conventional Performance During Phase 2 PC Sampling

Reactor	Dates Collected	Initial			Final			Removal Efficiency	
		COD_t (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	COD_t (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	COD_t (%)	NH_3-N (%)
A20	11/12/12 to 14/12/12	194.2 (22.8)	15.0 (2.8)	4.7 (1.6)	105.4 (91.8)	0.58 (0.82)	14.5 (3.3)	45 (47)	96 (5)
C	14/01/13 to 18/01/13	204.7 (57.7)	10.4 (3.1)	1.5 (1.0)	66.2 (37.8)	0.11 (0.08)	15.9 (3.5)	67 (12)	99 (0.8)

Notes:
Values shown represent mean concentrations. Values in parentheses are calculated standard deviations.

As identified previously, IFAS SBBRs received a volumetric loading that was approximately 20% less than the conventional SBRs. Based on the measured concentrations at the beginning and end of each feed cycle and the volumetric loading rates known to have been received by each reactor, it was estimated that primary effluent received by reactors A20 and C contained tCOD concentrations which ranged from 265 to 280 mg/L. Based on typical COD concentrations reported for typical North American domestic raw influent and primary effluent (Envirosim Associates Ltd., 2011) and the results presented in Table 4.7, it was estimated that the pilot reactors received primary effluent with COD concentrations which were approximately 100 mg/L lower than COD concentrations in typical domestic sewage. However, these lower than anticipated results may have been partially explained by increased levels of COD removal achieved by the primary clarifier as well as minor levels of COD removal reported to occur within the sewage forcemain.

Based on COD:BOD_5 ratios reported for typical North American primary effluents (Envirosim Associates Ltd., 2011) it was estimated that the mean BOD_5 concentrations within reactors A20 and C at the beginning of each cycle during phase 2 PC sampling was between approximately 100 and 110 mg/L, respectively. On this basis, it was estimated that the primary effluent received by reactors A20 and C contained BOD_5 concentrations of 140 to 150 mg/L. Based on typical primary effluent COD concentrations and COD:BOD_5 ratios (Envirosim Associates Ltd., 2011), primary

effluent can be expected to contain a BOD_5 concentration of approximately 200 mg/L. However, the MOE Design Guidelines for Sewage Works suggest that primary effluent within Ontario can be assumed to contain a BOD_5 concentration ranging from 105 to 140 mg/L (MOE, 2008). When viewed collectively it was deemed that the sewage received by the reactors during the PC sampling period was within the expected range of typical primary effluent within Ontario municipal WWTFs.

Reactors A20 and C produced effluent tCOD concentrations of 105.4 mg/L and 66.2 mg/L, respectively. As discussed previously, and as demonstrated by equation 4.1, tCOD can be partitioned into both soluble and particulate fractions that are either biodegradable or non-biodegradable. According to the typical primary effluent nbsCOD concentration reported by Envirosim Associated Ltd. (2011), it was estimated that the final samples collected from reactors A20 and C contained average nbsCOD concentrations of approximately 16.8 and 20.0 mg/L, respectively. This suggested that an additional source of COD, equal to approximately 90 and 45 mg/L, respectively, was present within the effluents of reactor A20 and C.

As a result of the higher than expected effluent tCOD measurements observed during Phase 1, filtered COD testing was included as a conventional monitoring parameter for Phase 2. The results from fCOD analysis indicated that final samples collected from reactors A20 and C contained sCOD concentrations equal to 60.8 and 44.6 mg/L, respectively. Metcalf and Eddy (2003) suggests that the soluble COD concentration within the effluent produced by an activated sludge process operated at SRTs greater than 4 days can be entirely attributed to nbsCOD. This suggests that both reactors were receiving elevated nbsCOD concentrations within the primary effluent; however this was not confirmed with additional testing. On the basis of the measured tCOD and sCOD values for reactors A20 and C, the particulate COD (pCOD) concentrations within final samples were determined to be approximately 45 and 22 mg/L, respectively. This indicates that some level of biomass may have been present in the effluents discharged from both reactors.

The effluent tCOD, sCOD and pCOD values observed in the final samples collected from reactors A20 and C were investigated using a student t-test approach to determine if these results were significantly different. At a confidence level of 95%, none of the COD fractions within the effluents produced by both reactors were found to be statistically different. This indicates that no difference in the level of organic removals achieved by either reactor was observed. However, the pCOD values measured within the effluents of both reactors were indicative of minor settlement issues experienced by both reactors.

Based on the measured TAN values reported in Table 4.7, the mean primary effluent TAN concentrations received by reactors A20 and C were determined to have been 15.6 and 26.9 mg/L for reactors A20 and C, respectively. The TAN values within the primary effluent, as estimated based on TAN concentrations within initial and final samples collected from reactors A20 and C, were compared using a student t-test (Appendix G) to determine if a significant difference in concentrations occurred. At a confidence limit of 95%, the TAN concentrations within primary effluents were found to be statistically different. This suggests that reactor A20 received a higher TAN loading than reactor C.

However, samples were not collected concurrently, and therefore it was not known if this discrepancy was indicative of measurement and/or analytical error or was the result of temporal variability.

The Design Guidelines for Sewage Works (MOE, 2008) report that typical municipal sewage in Ontario contains a TAN concentration ranging from 20 to 25 mg/L. On this basis, the primary effluent received by reactor A20 during Phase 2 contained TAN concentrations that were between 25 and 40 percent lower than typical Ontario municipal sewage. It was not known if the additional TAN loading received by reactor C would have an influence on the level of PC transformation.

It was noted that both reactors contained similar concentrations of NO_3-N (difference of 1.4 mg/L) within their respective final samples despite the significantly different initial TAN concentrations received by each reactor during PC sampling. It was also noted that the nitrogen balance demonstrated poor agreement between initial and final samples when the concentrations of TAN and NO_3-N were considered. This may have been demonstrative of analytical error similar to what was observed in Phase 1. However, it was also noted that reactor A20 had an average initial NO_3-N concentration of 4.5 mg/L. In contrast, reactor C had an initial NO_3-N concentration of 1.5 mg/L. This may suggest that some level of de-nitrification was occurring within both reactors.

The effluent TAN concentrations measured during the monitoring period for reactors A20 and C were 0.58 and 0.11 mg/L, respectively. The effluent TAN and NO_3-N concentrations within final samples collected from reactors A20 and C were compared using a student t-test (Appendix G) to determine if they were statistically different. At a confidence limit of 95%, both the TAN and NO_3-N concentrations within primary effluents were found not to be statistically different. The Design Guidelines for Sewage Works (MOE, 2008) suggest that activated sludge bioreactors operated under conditions permitting nitrification can be expected to produce effluent TAN concentrations below 3 mg/L. On this basis, the effluent TAN concentrations as well as the NO_3-N data presented in Table 4.7 were considered to be demonstrative of full nitrification performance achieved by both reactors during the sampling period.

The average concentrations of MLSS, MLVSS and effluent TSS, as well as the WAS volume discharged per day, were measured to assess the operating SRT, calculated using equation 2.1. The ESS, MLSS and MLVSS measurements as well as WAS volumes discharged and calculated SRT are provided in Table 4.8. Samples from reactor A20 were collected on December 11, 2012, during the cycle prior to PC sampling was initiated. Samples from reactor C were collected on January 11, 2013, during the Friday prior to PC sampling commencement. The values reported in Table 4.8 were considered to be reflective of reactor operating conditions during the period in which phase 2 PC samples were collected.

Table 4.8 – Results of Solids Monitoring During PC Sampling

Reactor	Date Collected	MLSS (mg/L)	MLVSS (mg/L)	ESS (mg/L)	WAS Volume (mL/d)	SRT (days)
A20	11/12/12	1290	1060	6.8	1100	18.2
C	11/01/13	3300	2710	57.6	460	18.3

Both reactors were noted to have been operating within 2 days of the target SRT of 20 days (Table 4.8). This was consistent with data collected from reactor A20 during the 3 SRT period prior to sampling. Due to the filamentous issue, reactor C was operated at an average SRT of 11 days over the 3 SRT monitoring period. However, data collected over December and January indicate that reactor C partially recovered and was operated at an average SRT of 16 days for the 30 days prior to PC sampling. Despite this minor SRT shortfall, Reactor C was not considered to have been operated at conditions which were considerably different from Reactor A20. On this basis, the reactors were considered to have been operating within the target SRT for a sufficient duration to ensure that the biomass composition was at steady state in both reactors.

A well operated secondary treatment process should be capable of producing an effluent containing TSS concentrations of 15 mg/L or less (MOE, 2008). Based on the data presented in Table 4.8, reactor A20 was producing an effluent which was consistent with typical secondary effluent. Reactor C was noted to be producing an effluent with TSS concentrations which significantly exceeded typical secondary treatment. In order to maintain operation at the target SRT, WAS volumes removed from reactor C were reduced to compensate for lost biomass within the effluent.

The MLSS values associated with the SBBR were approximately 40% of those within the control (Table 4.8). This was likely due to the presence of the biofilm and the competition for limited substrates between the suspended growth and fixed film (biofilm) phases. Despite the biomass losses associated with Reactor C effluent, a significantly elevated MLSS concentration was maintained, demonstrating that the high effluent TSS had likely developed just prior to PC sampling and that adjusted WAS volumes were adequately accounting for biomass losses within effluent.

Based on the primary effluent COD concentrations and the subsequently estimated BOD_5 values presented above, reactors A20 and C were operated at Food:Microorganisms ratios (F/M) of 0.32 and 0.13 $gBOD_5/gMLVSS \cdot d$, respectively. Typically, SBRs are operated at an F/M ranging from 0.04 to 0.10 (Metcalf and Eddy, 2003). Additionally, it was noted that reactors A20 and C were operated at HRTs of 10.6 and 9 hours, respectively. Typically, SBRs are designed with a target HRT between 15 and 40 hours (Metcalf and Eddy, 2003).

On the basis of all of the conventional monitoring data it was concluded that reactors A20 and C performed well, providing full nitrification, despite operating with reduced hydraulic retention times and slightly higher organic loading

rates than are typically recommended for design in design references. The observed COD concentrations measured within the effluents of both reactors exceeded those reported to be typical for a well operating SBR. However this was believed to be the result of above average nbsCOD concentrations within the primary effluent.

The nitrification abilities of reactors A20 and C were assessed using batch nitrification testing to investigate performance and confirm steady-state conditions had been achieved. Nitrification testing for reactor A20 was conducted on December 12, 2012 prior to the initiation of phase 2 PC sampling for reactor A20. Nitrification testing for reactor C was conducted on January 11, 2013, on the Friday before phase 2 PC sampling of reactor C was commenced. Expected nitrification rates were calculated for reactor C based on typical kinetic parameters for activated sludge reported in the literature and the reactor operating conditions using equations 3.5 and 3.6. Due to the complexity associated with the presence of IFAS media, expected nitrification rates were not estimated for reactor A20. A summary of the nitrification rate testing results is presented in Table 4.9. Further raw data from the nitrification rate testing is located in **Appendix D**.

Table 4.9 - Nitrification Rate Testing Results – Phase 2

Reactor	Reactor MLSS	Reactor MLVSS	Calculated TAN Removal Rate	Measured TAN Removal Rate	Measured NOx Production Rate	Measured Specific TAN Removal Rate	Measured Specific NOx Production Rate
	(mg/L)	(mg/L)	(gN/d)	(gN/d)	(gN/d)	(mgN/gVSS/d)	(mgN/gVSS/d)
A20	1290	1060	-	3.10 (0.33)	2.48 (0.14)	125	100
C	3300	2710	1.54	2.84 (0.12)	3.00 (0.53)	53	56

The results of initial and final TAN and NOx-N measurements for testing on both reactors demonstrated a good nitrogen balance. Both reactors initially contained similar TAN concentrations (25.8 and 25.5 mg/L for reactors A20 and C, respectively) and achieved nearly identical removals of TAN (18.3 and 17.9 mg/L for reactors A20 and C, respectively). Additionally, reactors A20 and C started with a similar NO_x-N concentration (0.8 and 0.9 mg/L within reactors A20 and C, respectively), and generated similar levels of NO_x-N (14.5 and 15.9 mg/L for reactors A20 and C, respectively). However, as a result of the significantly reduced operating MLSS/MLVSS within reactor A20, specific nitrification and NO_x-N production rates within the IFAS SBBR were approximately twice those of the SBR control. This difference was attributed to the presence of the IFAS biomass, as both reactors were operating at the same temperature and SRT.

The TAN removal rates and NOx-N production rates measured for reactors A20 and C were compared using a student t-test on the regression slopes (**Appendix G**) to determine if the reactors achieved different nitrification performance. At a confidence level of 95%, both the TAN removal rates and NO_x-N production rates were found to not be statistically different ($p<0.05$). This indicates that both reactors achieved a similar nitrification rate under Phase

2 experimental conditions. However, it was noted that reactor C achieved a TAN removal rate which was approximately 185 percent of the calculated rate based on typical kinetic parameters at the selected operating conditions.

Maas et al., (2008) conducted nitrification rate testing using IFAS carriers obtained from a full scale WWTP. In-basin ammonia removal rates, which included the contributions of the mixed liquor, were reported to range from 0.3 to 1.2 $gN/m^2 \cdot d$. Based on the results presented in Table 4.9, and utilizing the same calculation methods as Maas et al., (2008), an in-reactor removal rate of 0.77 $gN/m^2 \cdot d$ was estimated for reactor A20. This demonstrates that reactor A20 was providing nitrification performance consistent with a full scale IFAS equipped bio-reactor.

The PC chemical analysis for the phase 2 investigation was conducted entirely at the Servos Lab at the University of Waterloo. Based on the recommendations of the Servos Lab chemist, the preparation methods typically utilized by the lab were re-instated, resulting in a concentration factor of 200 times. A detailed assessment of the quality assurance/quality control (QA/QC) data that was collected as part of the phase 2 analysis is presented to establish the context in which the actual test sample values were determined. As noted previously, samples collected from reactor C were analyzed at the same time as Phase 3 samples due to reactor performance issues which necessitated a delay in sample collection schedules. As reactor C samples were prepared and analyzed concurrently with phase 3 samples, QA/QC data corresponding to phase 3, provided in section 4.3, can be considered representative of the analytical accuracy and precision achieved during their analysis.

QA/QC was assessed through the analysis of Matrix spikes (MSs), method blanks as well as an evaluation of isotopically labelled standard (ILS) recoveries (**Appendix E**). To ensure that the analytical method utilized achieved the highest level of accuracy and precision possible, and to recognize the limitations of data obtained during PC analysis, an estimate of the total analytical error was determined. The total analytical error was primarily attributed to analyte losses occurring during sample preparation and analysis as well as signal suppression/enhancement due to matrix effects. Additional losses associated with sample and analyte measurements, sorptive losses associated with sample contact to lab equipment and volatilization during sample evaporation were also expected to have occurred. However, these losses were expected to have a lesser impact on the final data than the losses associated with sample preparation and analysis (Vogeser and Seger, 2010, Hall et al., 2012).

To provide an assessment of the accuracy and precision of the analytical method, two MSs were prepared and analyzed using the same method as the authentic samples collected from reactor A20. The analysis of MSs also provided confirmation of the analytical measurement process via quantitation of a reference standard at a known concentration. As reported in Section 3.6, all MSs were spiked with reference standards of CBZ, SMX, TRIM and ATEN to achieve a final concentration of 100 ng/L. In phase 2, samples were spiked with ACE reference standard to achieve a final concentration of 10 μg/L. If the analytical process was achieving satisfactory performance, it was expected that each of the 5 analytes would be reported at an average concentration between 85 and 115 percent of the spiked amount and would result in RSD values less than 15%.

The results for CBZ, SMX, TRIM and ATEN reported by the Servos Lab met both the accuracy and precision criteria outlined above. ACE was noted to have been 1 % below the accuracy criteria, but achieved an R.S.D. that was significantly better than the criteria established for precision. On this basis, the analytical methodology used for phase 2 analysis demonstrated significant improvements in both the accuracy and precision achieved when contrasted with phase 1 QA/QC results. These results were expected to provide a commensurate level of accuracy and precision during the analysis of wastewater samples as has been demonstrated in previous studies (Van Nuijs et al., 2010; Tarcomnicu et al., 2011).

The analytical equipment was noted during Phase 1 analysis to be susceptible to contamination as a result of sample injections, particularly when wastewater samples were analyzed. This contamination was observed at similar concentrations during phase 2 analysis and resulted in background noise that masked the analytical signals observed. Analytical blanks (methanol) were injected periodically between samples to encourage a “flushing” of the column, reducing the level of contamination. Despite this flushing, background signals which resulted in detectable concentrations of the 5 PCs were observed in both the method blanks as well as the analytical blanks. However, ILS signals were not detected in any of the blanks analyzed and therefore contamination was restricted to the unlabelled PCs. Utilizing the procedure described in Section 3.6.4, any sample that resulted in a non-labelled PC signal which was less than 2 times the average non-labelled PC signal in analytical blanks was removed from the dataset due to uncertainty.

Method quantitation limits (MQLs) were separately estimated for the initial and final samples collected from each reactor using the method described in Section 3.6.4. During the analysis of the A20 influent samples, an analytical error (mobile phase was permitted to run empty) occurred that resulted in unusable data for samples 6 through 15, inclusive. These samples were re-run at a later date after the LC column had been replaced with a virgin column. The estimated MQLs from both phase 2 analysis and the re-run analysis of reactor A20 samples are provided in Table 4.10. Due to the high signals associated with influent ACE concentrations, and the complete removals observed, the MQL was estimated based on the calibration curve response. The MQL was estimated based on the lowest calibration point that produced a concentration which was distinguishable from the peak measured for the 0 calibration point.

Table 4.10 - Calculated and reported MQL’s for Phase 2/Re-run Analysis

Sample ID	Calculated MQL				Predicted MQL
	CBZ	SMX	TRIM	ATEN	ACE
C Influent	27	1120	96	310	500
A20 Influent	43 (88)	1404 (237)	109 (62)	375 (302)	500
C Effluent	14	671	43	150	500
A20 Effluent	13	368	40	131	500
Note: Values shown in parentheses represent the MQL’s calculated for re-run analysis.					

As demonstrated in Table 4.10, the presence of significant background signals resulted in an estimated SMX MQL of 1404 ng/L, making initial sample quantitation impossible for A20. Similar high MQLs were noted for the initial samples collected from reactor C. It was observed that the concentrations of SMX in the analytical blank signals were significantly lower following column replacement, which permitted SMX quantitation in all but one of the re-submitted A20 initial samples. As demonstrated in Table 4.10, the estimated MQL for SMX during sample re-runs was reduced to 237 ng/L, or approximately one sixth of the MQL estimated for the earlier phase 2 analysis. It was therefore considered likely that the column utilized during phase 1, 2 and 3 analysis was contaminated, particularly with respect to SMX. It was also noted that TRIM and ATEN achieved slightly reduced MQLs, whereas CBZ MQLs demonstrated a slight increase following column replacement. It was not known why SMX was particularly susceptible to contamination in regards to the initial column used.

Hughes et al., (2007) reported that carryover effects can affect an individual sample or multiple samples in sequence, particularly if the analyte signals in samples exceed calibration ranges. The author postulated that carryover effects can be random, caused by late-eluting residues on chromatographic columns affecting sample analysis, even after several sample runs. Naegele et al., (2001) suggests that proteins from biological matrices can act as binding agents, resulting in incomplete elution from the stationary phase within the column. It is believed that the column used for phase 1, 2 and 3 analysis by the Servos Lab may have been contaminated as a result of prior usage (Zhang et al., 2011). Vogeser and Seger (2010) also note that the presence of conjugate metabolites can contribute to inaccurate measurements as these compounds can affect the signals observed for both target analytes and ILSs. The presence of conjugate compounds or TPs were not investigated as part of this study, however, it was possible that the presence of these compounds had an effect on the analysis.

It was observed that the calculated MQL's for reactor C were very similar to those calculated for reactor A20 during phase 2 investigations. It is therefore believed that the analytical equipment was performing consistently during both analytical runs. It was found that the background signals associated with SMX and ATEN for reactor C samples were similar to those observed for reactor A20, resulting in much higher MQLs than those calculated for CBZ and TRIM. The reason for these higher MQL's was believed to be related to the previously discussed contamination within the LC column or perhaps the result of conjugate compounds or TPs. No additional investigations were completed to determine why the column exhibited higher background levels of ATEN and SMX relative to CBZ and TRIM. ACE was also estimated to have a high MQL relative to CBZ and TRIM. However, as sample concentrations in the influent were in the low μg/L range, transformation efficiencies exceeding 99 percent were detected despite elevated ACE MQLs.

The MQLs achieved under phase 2 analysis were generally higher than those reported in other studies. Radjenovic et al., (2009) calculated MQLs based on a signal to noise ratio of 10 during the analysis of primary effluent using LC-MS/MS. CBZ, SMX, TRIM, ATEN and ACE MQL's were estimated at 15.8, 1.7, 5.5, 8.2 and 75.3, respectively. Nurmi and Pellinen (2011) reported MQL's for SMX, TRIM, ATEN and ACE of 11, 13, 70 and 190 ng/L, respectively, using the same methodology as Radjenovic et al., (2009) during the analysis of spiked wastewater effluent samples using LC coupled with Time-of-Flight MS. However, Petrovic et al., (2006) used ultra-performance liquid chromatography-

quadrupole-time-of-flight Mass Spectrometry and estimated that the MQL's for the analysis of CBZ, SMX, TRIM, ATEN and ACE in influent wastewater were 100, 150, 10, 50 and 50 ng/L, respectively. These results appear to demonstrate that little consistency between the MQLs achieved for individual compounds exists between studies. It is therefore likely that the ability to quantitate these PCs may depend on either the analytical instrument utilized or the composition of the matrix investigated.

The estimated MQL's for the 5 compounds analyzed in phase 2 ranged from being consistent with the reported MQLs to as much as 800 times (SMX) the results reported in the three referenced studies. It was considered that the discrepancy observed between the estimated MQLs based on phase 2 analysis and the other studies referenced may be due to the method use to calculate the MQL. Standley et al., 2008 utilized a similar approach for calculating specific MQL's as was utilized in this study. The study authors detected contamination of several PCs during the analysis of wastewater samples. Of particular note was the detected contamination of oxybenzone, which resulted in an estimated MQL of 940 ng/L. The MQL estimated for oxybenzone was noted to be approximately 25 to 2000 times higher than the MQLs calculated for the remaining 32 PCs analyzed. The method utilized during phase 2 analysis to calculate the MQLs was considered to be highly conservative and was anticipated to reflect a high confidence in the data obtained, when available.

A summary of the screened PC concentration data for reactors A20 and C is presented in Tables 4.11 and 4.12, respectively. Raw LC-MS/MS data obtained from phase 2 PC analysis is provided in **Appendix E**. The final sample collected for A20-4 failed to inject and thus no data was available for any of the 5 PCs.

Table 4.11 – Reported PC Concentrations for Reactor A20 Initial and Final Samples

Sample ID	Initial					Final				
	CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
A20-1	279	<MQL	158	665	ND	ND	357	101	324	<MDL
A20-2	275	<MQL	<MQL	ND	32850	231	321	97	351	<MDL
A20-3	ND	<MQL	125	695	33200	229	368	92	301	<MDL
A20-4	221	<MQL	134	955	42200	ND				
A20-5	219	<MQL	157	1415	44000	211	407	92	335	<MDL
A20-6	ND	386	121	1185	52500	260	ND	106	496	<MDL
A20-7	314	320	138	1100	40200	256	<MQL	90	375	<MDL
A20-8	312	265	95	800	40150	268	<MQL	ND	359	<MDL
A20-9	ND	416	121	735	ND	270	<MQL	93	354	<MDL
A20-10	255	479	250	1105	49000	228	510	112	464	<MDL
A20-11	350	1155	266	2380	51000	224	490	113	357	<MDL
A20-12	<MQL	<MQL	313	755	61500	240	488	109	397	<MDL
A20-13	421	740	212	1550	ND	237	384	96	292	<MDL
A20-14	303	<MQL	210	2215	44600	262	<MQL	114	399	<MDL
A20-15	334	550	ND	2750	45050	228	427	102	359	<MDL
A20-16	236	<MQL	170	1190	68000	247	437	100	438	<MDL
A20-17	282	<MQL	171	1180	70000	248	510	89	398	<MDL
A20-18	249	<MQL	ND	ND	62500	ND	472	106	465	<MDL

Notes:
1. ND – No data was available due to analytical error, sample destruction or failed outlier test.
2. MQL designates values which are below the method quantitation limit as defined above.
3. MDL designates no signal was detected for target analyte.
4. Data highlighted in blue reflects data obtained during re-run analysis.

Table 4.12 – Reported PC Concentrations for Reactor C Initial and Final Samples

Sample ID	Initial					Final				
	CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
C-1	116	<MQL	100	705	27550	110	<MQL	89	408	<MDL
C -2	114	<MQL	105	870	25350	143	<MQL	95	480	<MDL
C -3	112	<MQL	91	730	22700	117	<MQL	92	530	<MDL
C -4	149	<MQL	128	1390	47050	151	<MQL	110	650	<MDL
C -5	161	<MQL	136	835	49350	154	<MQL	119	580	<MDL
C -6	219	<MQL	131	945	46250	ND	<MQL	100	665	<MDL
C -7	208	<MQL	114	1070	47850	209	<MQL	ND	620	<MDL
C -8	209	<MQL	ND	930	46050	204	<MQL	114	705	<MDL
C -9	205	<MQL	115	870	47600	202	<MQL	118	655	<MDL
C -10	187	<MQL	171	1360	60000	214	<MQL	159	ND	<MDL
C -11	177	<MQL	151	1455	56500	175	<MQL	122	710	<MDL
C -12	182	<MQL	143	1070	59000	193	<MQL	136	715	<MDL
C -13	232	<MQL	151	1235	57000	222	<MQL	139	670	<MDL
C -14	237	<MQL	172	1200	52000	216	<MQL	119	600	<MDL
C -15	214	<MQL	122	1145	45450	217	<MQL	144	645	<MDL
C -16	159	<MQL	155	1275	48450	278	<MQL	133	745	<MDL
C -17	156	<MQL	140	ND	50500	315	<MQL	141	655	<MDL
C -18	ND	<MQL	166	1315	55000	ND	<MQL	144	670	<MDL

Notes:
1. ND – Not data was available due to analytical error, sample destruction or failed outlier test.
2. MQL designates values which are below the method quantitation limit as defined above.
3. MDL designates no signal was detected for target analyte.

Due to the nature of the analytical procedure, outliers were anticipated within the dataset. To screen the data against possible outliers, all data was subjected to the Grubbs' outlier test (Grubb, 1969), using α = 0.95. To apply Grubbs' Outlier Test, a minimum of three replicates were required. This testing procedure also required the assumption that the data for each set of triplicate samples was normally distributed. Measured concentrations that failed the Grubbs' outlier test have been removed from the data set and labelled "ND" in Tables 4.11 and 4.12.

Replicate sample measurements for CBZ for both initial and final samples collected from both reactors achieved a suitable degree of precision; the average RSD calculated for both reactors for the analysis of the initial samples was 13 and 6 percent for reactors A20 and C, respectively. Final samples achieved a similar level of precision in which R.S.D's ranged from 3 to 7 (μ = 5) and 1 to 14 (μ = 7) percent, with an average R.S.D. of 5 and 7 percent for reactors A20 and C, respectively. TRIM achieved similar levels of precision as that observed for CBZ. The analysis of initial samples from reactors A20 and C resulted in R.S.D.'s ranging from 12 to 18 (μ = 14) percent and 3 to 17 (μ = 9) percent respectively. Final samples achieved improved precision; initial sample R.S.D.'s ranged from 2 to 9 percent (μ = 6) and 3 to 13 (μ = 8) percent for reactors A20 and C, respectively.

The removal of unsuitable SMX data resulted in no data for initial samples from reactor C and only re-run results for reactor A20. It was noted that the replicate samples analyzed during re-runs demonstrated a similar level of variability as was observed during phase 1 analysis. A20-7, A20-8 and A20-9 (triplicate samples) demonstrated a range of concentrations from 265 to 416 ng/L that resulted in an RSD of 23 percent. Samples A20-10 and A20-11 (duplicate samples) were reported at concentrations of 479 and 1155 ng/L, respectively. As a result of the large variance in these measurements, there was limited confidence in this dataset. However, final samples of A20 were found to have significantly improved precision, with RSD ranging between 2.5 and 8 percent. Final samples collected from reactor C were all found to contain concentrations below the estimated MQL.

ATEN demonstrated the highest variability of the 4 PCs for which data was available. Both reactor A20 and C demonstrated highly variable replicate measurements with initial samples producing R.S.D.'s between 19 and 61 (μ = 32) and 4 and 28 (μ = 14) percent, respectively. It was noted that the analysis of initial samples collected from reactor A20 demonstrated a much greater variability than reactor C and was approximately 2 times the criteria used during MS analysis to assess acceptable precision. It was not evident why the analysis of reactor A20 samples achieved a reduced level of precision. However, data available from the original phase 2 analysis did not demonstrate the same degree of variance as was noted for the re-runs; A20-1 and A-20-3 only differed by approximately 5 percent and samples A20-16 and A20-17 differ by less than 1 percent. However, no triplicate samples were available from phase 2 analysis, and therefore a true comparison could not be achieved. The analysis of final samples demonstrated a significantly improved precision; R.S.D.'s ranged between 3 and 15 (μ = 9) percent and 6 and 13 (μ = 8) percent for reactors A20 and C, respectively. This demonstrated that a level of precision was achieved which was consistent with CBZ and TRIM.

ACE generally demonstrated a similar level of precision during the analysis of initial samples to what was observed for CBZ and TRIM. Initial samples achieved R.S.D.'s ranging from 6 to 12 percent and 2 to 11 percent for reactor A20

and C, respectively with average R.S.D's of 10 and 6 percent. An assessment of the precision achieved for the analysis of ACE within final samples could not be conducted as all samples were below the MDL.

Based on the results presented in Tables 4.11 and 4.12, transformation efficiencies (expressed as a percentage) as well as standard deviations for the transformation efficiencies were calculated. In these calculations effluent measurements that were below the calculated or reported MQL were considered to be equal to the MQL. This provided a level of conservatism within the transformation efficiency estimates. The calculated efficiencies are provided in Table 4.13.

Table 4.13 – Observed Transformation Efficiencies for Phase 2 Investigation

Reactor	Transformation Efficiency Observed (%)				
	CBZ	SMX	TRIM	ATEN	ACE
A20-1	17%	ND	32%	52%	>99
A20-2	-7%	-19%	28%	65%	>99
A20-3	15%	ND	22%	59%	>99
A20-4	24%	40%	60%	71%	>99
A20-5	31%	37%	51%	84%	>99
A20-6	3%	ND	42%	63%	>99
AVERAGE	14%	19%	39%	66%	-
STD DEV	14%	33%	14%	11%	-
C-1	-8%	ND	7%	39%	>99
C-2	13%	ND	17%	40%	>99
C-3	1%	ND	-1%	31%	>99
C-4	-7%	ND	10%	45%	>99
C-5	4%	ND	10%	47%	>99
C-6	-89%	ND	9%	47%	>99
AVERAGE	-14%	ND	9%	41%	-
STD DEV	37%	ND	6%	6%	-
Notes: ND indicates insufficient data is available to calculate a transformation efficiency					

It was deemed that the CBZ transformation efficiency calculated for the sixth Reactor C sample set was likely an outlier caused by analytical error as it failed Grubbs test at an α of 0.95. However, this analysis required an assumption that all sample sets could be treated as replicates, which is not accurate as each set was collected at different times. It should be noted that none of the reported CBZ concentrations within initial and final samples within set 6 failed the Grubbs' outlier test individually. It was therefore likely that the calculated transformation efficiency for sample set C-6 was reflective of poor accuracy and/or precision within both the initial and final measurements that resulted in additive errors. If this value were to be eliminated from the dataset, the average transformation efficiency

observed would have been reduced to 1% and the standard deviation would likewise be reduced to 9%. However, as the outlier status could not be confirmed, this data point was included in the data set. The mean transformation efficiencies calculated for CBZ were in the range of 31 to -7 and 13 to -89 for reactor A20 and C, respectively. The net transformation efficiencies suggest that no quantifiable transformation of CBZ occurred in either reactor.

It can be observed from Table 4.13 that calculated TRIM and ATEN removals demonstrated RSDs less than 15%. A minor increase in the transformation efficiency for ATEN was observed for the IFAS reactor relative to reactor C (25%), as demonstrated by the mean transformation efficiency calculated. It was noted in this instance that all transformation efficiencies calculated for the IFAS SBBR were consistently elevated in contrast to those calculated for the SBR. TRIM demonstrated a more significant increase in transformation efficiency (30%) in the IFAS relative to the control and demonstrated a similar trend. SMX data demonstrated high variability, suggesting poor analytical precision and was therefore not considered valid. ACE was transformed at an efficiency greater than 99% in both reactors and therefore RSDs could not be estimated. A more detailed statistical comparison of the transformation efficiencies observed for CBZ, TRIM and ATEN in the SBBR and SBR is provided in Section 4.4.

4.3. *PHASE 3 INVESTIGATIONS*

Phase 3 evaluated PC transformation efficiency under the two remaining experimental conditions: Reactors K7 and E were used to investigate PC transformation efficiencies at an SRT of 7 days and a mixed liquor temperature of 18℃ and reactors A7 and D were used to investigate PC transformation efficiencies at an SRT of 7 days and a mixed liquor temperature of 12℃. The data presented in Section 4.3 provides a comparative discussion of the primary effluent received by all four reactors during Phase 3. Comparisons are then drawn between the conventional performance achieved by each pair of reactors that were operated under the same experimental conditions (i.e., K7 and E, A7 and D) as well as collectively between all four reactors sampled as part of Phase 3. Data pertaining to PC concentrations and transformation efficiencies, as well as a discussion of this data, has been presented separately for each pair of reactors operated under the two experimental conditions investigated during Phase 3. An overview discussion of conventional performance and transformation efficiencies achieved under all 4 experimental conditions, as investigated in Phases 1, 2 and 3 of this study, will be presented in Section 4.4.

Following completion of Phase 2 sampling on December 14, 2012, the WAS flowrates for the IFAS reactors were increased to achieve the target SRT of 7 days. The IFAS reactors were provided 31 days (approximately 4 SRTs) to adjust to the new process conditions. Reactors D and E (conventional SBRs) had been in operation for several years and had demonstrated generally good performance during this historical period. Conventional data was collected during this transitional period for all reactors to assess when steady state performance had been achieved. Effluent TSS, MLSS and daily WAS volumes were monitored during this period to ensure that all reactors were operating within range of the target experimental conditions. Further, this data (**Appendix C**) was collected to confirm that all reactors were operationally stable and providing a level of biological treatment commensurate with that typically observed for well operating SBRs at the given SRT and temperatures.

Reactor D was observed to have undergone several process upsets in the months prior to phase 3 sampling. Poor settling was observed in Reactor D in late October, around the same time as similar settling issues were encountered in Reactor C. It was determined that these performance issues were related to the proliferation of filamentous organisms, as confirmed through microscope investigations of Reactor D MLSS (**Appendix H)**. Mitigative measures including biomass removal, chemical treatment and replenishing lost biomass through re-seeding with reactor E WAS were employed to correct this issue. Further details of the process monitoring and the actions undertaken to correct performance issues are described in **Appendix C**.

Based on effluent TSS measurements conducted in November and December, it was believed that the filamentous issue had been resolved. However, sampling conducted on January 3, 2013 indicated that the filamentous organisms were still present, as evidenced by higher than typical effluent TSS concentrations which resulted in reactor operation below the target SRT. However, as reactor D was not expected to provide nitrification at the target operating conditions, and with due consideration of lab timing constraints, sampling of reactor D was conducted on January 14, 2013 along with the other reactors comprising Phase 3 investigations. The minor deviations from the target SRT observed during the 3 SRT monitoring period were not anticipated to result in any significant changes in the bacterial consortia present within Reactor D.

Phase 3 PC sample collection from reactor K7, E, A7 and D was initiated on January 14, 2013 and was conducted over a 5 day period. A total of 6 sampling events, consisting of both an initial sample, collected at t = 0, that characterized the mixed liquor conditions at the beginning of the treatment cycle (initial), as well as a sample of the treated effluent (final) were used to characterize PC transformation efficiencies. Each of these 6 sampling events included the collection of initial and final samples in triplicate. The collected samples were analyzed for conventional parameters, including tCOD, TAN and NO_3-N to ensure that acceptable levels of organic and TAN removals were sustained throughout the sampling period. The raw conventional data is presented in **Appendix C.** The mean concentrations that were observed along with mean removal efficiencies of COD and TAN that were calculated are presented in Table 4.14.

Table 4.14 - Conventional Performance During PC Sampling – Phase 3

Reactor	Experimental Condition	Initial (mg/L)			Final (mg/L)			Removal Efficiency (%)	
		COD_t	NH_3-N	NO_3-N	COD_t	NH_3-N	NO_3-N	COD_t	NH_3-N
K7 (IFAS)	SRT = 7 days Temperature = 18°C	180 (57.9)	7.5 (2.2)	4.8 (1.6)	61.2 (44.5)	0.08 (0.09)	14.6 (2.7)	65 (21)	99 (1)
E (SBR)		212 (67.3)	10.6 (2.8)	1.1 (0.7)	40.0 (17.2)	0.17 (0.08)	15.8 (3.0)	90 (3)	99 (1)
A7 (IFAS)	SRT = 7 days Temperature = 12°C	188 (56.8)	7.4 (2.3)	3.1 (1.3)	49.8 (36.1)	0.05 (0.07)	12.5 (4.2)	88 (4)	99 (1)
D (SBR)		211 (68.4)	12.4 (4.5)	2.9 (4.2)	56.5 (30.1)	11.4 (4.75)	2.3 (1.0)	90 (3)	9 (10)

Notes:
Values shown represent mean concentrations. Values in parentheses are standard deviations.

As discussed previously, the IFAS SBBRs received a volumetric loading that was approximately 20% less than the conventional SBRs. Based on the measured concentrations at the beginning and end of each feed cycle and the volumetric loading rates received by each reactor, it was estimated that the primary effluent received by reactors K7, E, A7 and D contained tCOD concentrations that were within the range of 274 to 299 mg/L. Based on typical COD concentrations reported for North American domestic raw influent and primary effluent (Envirosim Associates Ltd., 2011) and the estimated primary effluent concentrations, it was estimated that the pilot reactors received primary effluent during Phase 3 sampling with COD concentrations which were between 75-100 mg/L lower than COD concentrations of typical domestic sewage.

Based on COD:BOD_5 ratios reported for typical North American primary effluents (Envirosim Associates Ltd., 2011) it was estimated that the mean BOD_5 concentrations within reactors K7, E, A7 and D at the beginning of each cycle during phase 3 PC sampling were between 96 and 113 mg/L. On this basis, it was estimated that the primary effluent received by reactors K7, E, A7 and D had BOD_5 concentrations between 147 and 160 mg/L. These concentrations were found to be within the range of typical values for primary effluent reported elsewhere (Envrosim Associated Ltd., 2011; MOE, 2008). When viewed collectively it was deemed that the sewage received by the reactors during the PC sampling period was within the expected range of typical primary effluent observed at Ontario municipal WWTFs.

From Table 4.14 it can be observed that reactors K7, E, A7 and D produced average effluent tCOD concentrations of 61.2, 40.0, 49.8 and 56.5 mg/L, respectively. The tCOD concentrations measured in the final samples collected from reactors K7, E, A7 and D were compared using ANOVA (Appendix G) to determine if a significant difference in final tCOD concentrations occurred. At a confidence level of 95%, the final concentrations of tCOD were found to not be statistically different. This indicated that all four reactors were providing a consistent level of organic removal.

As discussed previously, and as demonstrated by equation 4.1, tCOD can be partitioned into both soluble and particulate fractions that are either biodegradable or non-biodegradable. Using typical nbsCOD fractions for municipal primary effluents (Envirosim Associated Ltd., 2011) it was estimated that the IFAS SBBRs (reactor K7 and A7) received 16.8 mg/L and the conventional SBRs (reactor E and D) received 20 mg/L of nbsCOD. It was therefore considered likely that an additional source of COD equivalent to approximately 20 to 44 mg/L was present within the final samples collected from reactors K7, E, A7 and D.

The results from fCOD analysis indicated that the final samples contained average soluble COD concentrations that ranged between 36.6 and 59.3 mg/L. Hence, the average particulate COD concentrations (pCOD) within the effluent were determined to range between 1.9 and 9.5 mg/L and therefore almost all of the tCOD contained in the final samples was soluble. Metcalf and Eddy (2003) suggests that most of the COD contained within the effluents of activated sludge reactors operated at SRTs greater than 4 days can be assumed to be nbsCOD. On this basis, it would appear that all reactors were receiving elevated concentrations of nbsCOD. Based on the sCOD concentration of Phase 2 final samples, which were also within the range of 40 to 60 mg/L, it was likely that the primary effluent received by the reactors contained an elevated nbsCOD concentration.

Based on the measured TAN values reported in Table 4.14, the average primary effluent TAN concentrations were estimated to range between approximately 12.9 and 16.2 mg/L for reactors K7, E, A7 and D. The Design Guidelines for Sewage Works (MOE, 2008) suggest that typical municipal sewage in Ontario contains a TAN concentration ranging from 20 to 25 mg/L. Hence, the primary effluent received during Phase 3 contained TAN concentrations that were between 20 and 40 percent lower than typical Ontario municipal sewage. It was not known if the reduced TAN concentrations would have an effect on the level of PC removals achieved.

It was observed that all reactors which achieved substantial nitrification (K7, E and A7) contained similar concentrations of NO_3-N (maximum difference of 3 mg/L) within their respective final samples. It was also noted that the sum of the concentrations of TAN and NO_3-N within the initial and final samples from these reactors achieved good agreement, demonstrating a consistent nitrogen balance. However, it was noted that reactor E had an average initial NO_3-N concentration of just 1.1 mg/L. By contrast, reactors K7 and A7 had average initial NO_3-N concentrations of 4.8 and 3.1 mg/L, respectively. This may suggest that some level of de-nitrification was occurring within these reactors, particularly in reactor E.

The average TAN concentrations measured within the final samples collected from reactor K7, E and A7 during Phase 3 were 0.08, 0.17 and 0.05 mg/L, respectively. The TAN and NO_x-N concentrations measured in the final samples collected from reactor K7, E and A7 were compared through ANOVA. At a confidence limit of 95%, all TAN and NO_x-N concentrations within the final samples were found to not be statistically different and hence these reactors were achieving equivalent levels of nitrification performance. In contrast to reactors K7, E and A7, reactor D demonstrated very minor removal of TAN and this was attributed to limited nitrification, as demonstrated by the NO_x-N concentrations within final samples, and to uptake by heterotrophs for biomass synthesis.

The average concentrations of MLSS, MLVSS and effluent TSS, as well as the WAS volume discharged per day, were measured to assess the operating SRT, which was calculated using equation 2.1. The ESS, MLSS and MLVSS measurements as well as WAS volumes discharged and calculated SRTs are provided in Table 4.15. Reactor K7, E, A7 and D were all targeted for operation at an SRT of 7 days. Samples from each reactor were collected on January 11, 2013, during the Friday prior to PC sampling commencement. The values were considered to be reflective of reactor operating conditions during the period in which phase 3 PC samples were collected.

Table 4.15 – Results of Solids Monitoring During PC Sampling – Phase 3

Reactor	Date Collected	MLSS (mg/L)	MLVSS (mg/L)	Effluent TSS (mg/L)	WAS Volume (mL/d)	SRT (days)
K7	11/01/13	800	680	7.2	2760	7.7
E	11/01/13	1900	1560	5.2	3000	8.0
A7	11/01/13	1260	1060	8.4	2960	7.6
D	11/01/13	1220	1040	62	880	7.1

From Table 4.15 it can be observed that all reactors were operating within 1 day of the target SRT. This was consistent with data collected from reactor reactors K7, A7 and E during the 3 SRT period prior to sampling. Due to the filamentous issue, reactor D operated at an average SRT of 5.4 days over the 3 SRT monitoring period. However, as reactor D was not expected to nitrify under the target operating conditions, this minor SRT shortfall was not expected to have substantial impacts on achieving the experimental objectives. On this basis, the reactors were considered to have been operated within the target SRT for a sufficient duration to ensure that the biomass composition was at steady state in both reactors.

The MLSS values in reactor K7 were approximately 40% of those in reactor E (Table 4.15). This was attributed to the presence of the biofilm and the competition for limited substrates between the suspended growth and fixed film (biofilm) phases. In contrast, reactors A7 and D contained similar MLSS concentrations, however this may have been the result of biomass loss due to the filamentous issue. During operation prior to the on-set of filamentous issues, reactor D was noted to operate at an MLSS concentration of approximately 1550 mg/L.

The Design Guidelines for Sewage Works suggests that a well operated secondary treatment process should be capable of producing an effluent containing TSS concentrations of 15 mg/L or less (MOE, 2008). Table 4.15 demonstrates that reactors K7, E and A7 produced an effluent consistent with typical secondary effluent. Reactor D demonstrated elevated effluent TSS concentrations (62 mg/L) which occurred as a result of the filamentous issue

which was occurring during Phase 3 PC sampling. To compensate for the elevated biomass losses due to the poorly settling mixed liquor, WAS volumes were lowered to maintain the target SRT of 7 days.

Based on the COD concentrations within the initial samples and the subsequently estimated BOD_5 values presented above, reactors K7, E, A7 and D were operated at Food:Microorganisms ratios (F/M) of 0.32, 0.27, 0.33 and 0.40 $gBOD_5/gMLVSS \cdot d$, respectively. Typically, SBRs are operated at an F/M ranging from 0.04 to 0.10 (Metcalf and Eddy, 20003). Similar to Phase 1 and 2, the IFAS SBBRs and the SBR controls were operated at HRTs of 10.6 and 9 hours, respectively. These SRTs are much shorter than typical HRTs recommended for SBR design, which typically range between 15 and 40 hours (Metcalf and Eddy, 2003).

On the basis of all of the conventional monitoring data it was concluded that all of the reactors were performing well, with those expected to nitrify achieving full nitrification, despite operating with reduced hydraulic retention times and slightly higher organic loading rates than recommended for design in design references. The organic removal efficiency achieved for all reactors was noted to be slightly below the expected capabilities of well operating SBRs, however this was attributed to above average nbsCOD concentrations within the primary effluent. The cause of the elevated sCOD concentrations was not investigated further, but was not anticipated to affect the PC transformations achieved under Phase 3.

The nitrification abilities of reactors K7, E, A7 and D were assessed using batch nitrification testing to investigate performance and confirm steady-state conditions had been achieved. All nitrification testing was conducted on January 11, 2013, before Phase 3 PC sampling commenced. Expected nitrification rates were calculated for reactors D and E using equations 3.5 and 3.6 based on typical kinetic parameters for activated sludge reported in the literature and the reactor operating conditions. Due to the complexity associated with the presence of IFAS media, expected nitrification rates were not estimated for Reactors K7 or A7. A summary of the nitrification rate testing results is presented in Table 4.16. Further raw data from the nitrification rate testing is located in **Appendix D**.

Table 4.16 - Nitrification Rate Testing Results – Reactors K7, E, A7 and D

Reactor	Reactor MLSS	Reactor MLVSS	TAN Removal Rate	Calculated TAN Removal Rate	NOx Production Rate	Specific TAN Removal Rate	Specific NOx Production Rate
	(mg/L)	(mg/L)	(gN/d)	(gN/d)	(gN/d)	(mgN/gVSS/d)	(mgN/gVSS/d)
K7	803	683	3.54 (0.13)	-	4.60 (0.17)	223	289
E	1897	1556	3.84 (0.11)	1.32	3.23 (0.39)	123	104
A7	1260	1058	3.26 (0.51)	-	2.96 (0.18)	132	120
D	990	842	0.39 (0.11)	0.07	0.14 (0.009)	23	8

1. Values shown in parentheses are standard error of linear regression.
2. Cells highlighted in Orange represent reactors operated at an SRT of 7 days and a temperature of 18℃.
3. Cells highlighted in Blue represent reactors operated at an SRT of 7 days and a temperature of 12℃.

The TAN removal rates for reactors K7 and E were compared using a student t-test on the regression slopes (**Appendix G**) to determine if a significant difference in TAN rates occurred. At a confidence limit of 95%, the TAN rates were found to be statistically different ($p < 0.01$). This difference is less apparent in Table 4.16 as rates are provided based on volumetric removal rates. However, it was also noted that reactor K7 achieved a volumetric NOx-N production rate which was approximately 40 percent higher than reactor E. The difference between the NOx-N production rates for reactor K7 and E was also tested using a t-test at 95% confidence limit, based on the NO_x-N measurements during testing, and was found to not be significantly different.

It was noted that reactor K7 had a lower starting TAN concentration than anticipated. Anyhydrous NH_4Cl was added to each reactor to achieve a target starting concentration of 30 mg/L. Initial samples collected from reactor K7 during testing were found to have a TAN concentration of 20.6 mg/L. In contrast, initial samples collected from reactors E had TAN concentrations of 28.1 mg/L, respectively. However, NO_x-N was measured in final sample of K7, at the end of testing, at a concentration of 30.9 mg/L. As the creation of NO_x-N can be solely attributed to nitrification, this suggested that a greater concentration of TAN was removed during the nitrification rate test than TAN measurements suggested.

It was therefore concluded that a measurement error occurred during the analysis of TAN concentrations within reactor K7 samples. A similar discrepancy was noted during phase 1, in which lower than expected initial TAN concentrations were observed within the same reactor during previous nitrification rate testing. It was therefore considered that NO_x-N results likely provided a better estimate of the nitrification rates achieved and thus both reactor K7 and E were providing consistent nitrification performance. As a result of the significantly reduced operating

MLSS/MLVSS within reactor K7, specific nitrification and NOx-N production rates were approximately twice those of reactor E. This difference was attributed to the presence of the IFAS biomass in K7.

As expected, reactor D achieved very limited nitrification. The minor amounts of TAN removal that occurred within reactor D were attributed to heterotrophic uptake of TAN during biomass synthesis. The nitrification capacity demonstrated by reactor A7, which was not observed in reactor D, was attributed to the presence of IFAS. On a volumetric basis, reactor K7 and A7 achieved TAN removal rates of 4.60 g/d and 2.96 g/d, respectively; demonstrating an approximate 50% increase in the nitrification rate achieved by K7. Reactor K7 was operated at the same SRT but at mixed liquor temperature which were 6℃ higher than reactor A7. It was therefore likely that this increase was primarily the result of nitrification occurring within the mixed liquor of reactor K7. It was not anticipated that nitrification was occurring within the mixed liquor of reactor A7, as demonstrated by the negligible performance of reactor D.

Maas et al., (2008) conducted nitrification rate testing using IFAS carriers obtained from a full scale WWTP. In-basin ammonia removal rates, which included the contributions of the mixed liquor, were reported to range from 0.3 to 1.2 $gN/m^2 \cdot d$. Based on the NOx-N production rate results presented in Table 4.16, and utilizing the same calculation methods as Maas et al., (2008), in-reactor removal rates of 1.14 and 0.74 $gN/m^2 \cdot d$ were observed for reactors K7 and A7, respectively. This demonstrates that both reactors were providing nitrification performance consistent with a full scale IFAS equipped bio-reactor.

The PC chemical analysis for phase 3 investigations was conducted in the Servos Lab at the University of Waterloo. Based on the improved accuracy and precision observed during Phase 2, relative to Phase 1, a concentration factor of 200 times was again used in Phase 3. A detailed assessment of the quality assurance/quality control (QA/QC) data that was collected as part of the Phase 3 analysis is presented to establish the context in which the actual test sample values were determined. QA/QC was assessed through the analysis of Matrix spikes (MSs), method blanks as well as an evaluation of ILS recoveries. The approach utilized to assess QA/QC in Phase 3 was identical to that utilized in Phase 2. MSs, prepared in de-ionized and distilled water were used to assess the accuracy and precision achieved by the method. ILS recoveries were used to assess matrix effects associated with analysis of wastewater samples. QA/QC Data is located in **Appendix E**.

To ensure that the analytical method utilized achieved the highest level of accuracy and precision possible, and to recognize the limitations of data obtained during PC analysis, an estimate of the total analytical error was determined. Total analytical error was primarily attributed to analyte losses occurring during sample preparation and analysis as well as signal suppression/enhancement due to matrix effects. Additional losses associated with sample and analyte measurements, sorptive losses associated with sample contact to lab equipment and losses during sample evaporation were also expected to have occurred. However, these losses were expected to have a lesser impact on the final data than the losses associated with sample preparation and analysis (Vogeser and Seger, 2010, Hall et al., 2012). To provide an assessment of the accuracy and precision of the analytical method, 16 matrix spikes

(MSs) and eight method blanks were prepared and analyzed using the same method as the authentic samples collected from the reactors investigated under Phase 3.

The analysis of MSs provided confirmation of the analytical measurement process via quantitation of a reference standard at a known concentration. As reported in Section 3.6, all MSs were spiked with reference standards of CBZ, SMX, TRIM and ATEN to achieve a final concentration of 100 ng/L. MSs were again spiked with ACE reference standard to achieve a final concentration of 10 μg/L. If the analytical process was achieving satisfactory performance, it was expected that each of the 5 analytes would be reported at an average concentration between 85 and 115 percent of the spiked amount and would result in RSD values less than 15%.

The results for all 5 PCs reported by the Servos Lab met both the accuracy and precision criteria outlined above, after erroneous data was removed from the dataset. On this basis, the analytical methodology used for phase 3 analysis demonstrated accuracy and precision which was commensurate with that achieved during Phase 2 and significantly improved relative to Phase 1. As a result, the analytical process was expected to provide a consistent level of accuracy and precision during the analysis of wastewater samples with those demonstrated in previous studies (Van Nuijs et al., 2010; Tarcomnicu et al., 2011).

The analytical equipment was noted during Phase 1 and 2 analysis to be susceptible to contamination as a result of sample injections, particularly when wastewater samples were analyzed. This contamination was observed at similar concentrations during phase 3 analysis and resulted in background noise that masked the analytical signals observed (**Appendix E**). Analytical blanks consisting of methanol were injected periodically between samples to encourage a "flushing" of the column, reducing the level of contamination. Despite this flushing, background signals were observed in both the method blanks as well as the analytical blanks. However, ILS signals were not detected in any of the blanks analyzed, demonstrating that contamination was confined to non-labelled PCs only. Utilizing the procedure described in Section 3.6.4, any sample that resulted in a non-labelled PC signal which was less than 2 times the average non-labelled PC signal in analytical blanks was removed from the dataset due to uncertainty.

Method quantitation limits (MQLs) were separately estimated for the initial and final samples collected from each reactor using the method described in Section 3.6.4. The estimated MQLs are provided in Table 4.17. Due to the high signals associated with influent ACE concentrations, and the complete removals observed, the MQL was estimated based on the calibration curve response. The MQL for ACE was estimated based on the lowest calibration point that produced a signal which was distinguishable from the signal measured for the 0 calibration point.

Table 4.17 - Calculated and reported MQL's for Phase 3

Sample ID	Calculated MQL (ng/L)				
	CBZ	SMX	TRIM	ATEN	ACE
K7 Influent	23	771	100	356	500
E Influent	28	661	63	338	500
A7 Influent	24	642	105	424	500
D Influent	29	722	91	417	500
K7 Effluent	11	434	56	139	500
E Effluent	15	440	37	210	500
A7 Effluent	12	316	44	142	500
D Effluent	27	529	44	196	500

It was observed that the calculated MQL's for CBZ, TRIM and ATEN based on Phase 3 analysis were similar to those calculated under Phase 2 analysis. It was therefore believed that the analytical equipment was performing consistently during both phases. It was noted during Phase 2 that the IFAS SBBR achieved improved MQLs within final samples relative to the control SBR. This was not observed during Phase 3; reactor D demonstrated the highest MQLs for CBZ and SMX whereas reactor K7 demonstrated the highest MQL for TRIM and Reactor E demonstrated the highest MQL for ATEN. Hence, there was no evident trend between the MQLs achieved and the operating conditions. ACE was also estimated to have a high MQL relative to CBZ and TRIM. However, as sample concentrations in the influent were in the low μg/L range, transformation efficiencies exceeding 99 percent were detected despite elevated ACE MQLs.

As demonstrated in Table 4.17, the presence of significant background signals resulted in SMX MQLs that ranged from 642 to 771 ng/L, making initial sample quantitation impossible for all 4 reactors investigated. This issue also occurred during Phase 2 analysis and hence SMX data from initial samples was not available. However, samples analyzed under phase 2 had higher estimated MQLs for initial samples, that ranged from 1120 to 1404 ng/L. Phase 2 initial samples from reactor A20 that were re-run at a later date after the LC column was replaced achieved a much better MQL (237 mg/L) as a result of significant reductions in the background signals of SMX observed. It was not known why MQLs calculated for Phase 3 analysis of samples demonstrated significant reductions in the background signals measured relative to Phase 2 as both phases utilized the same LC column during analysis.

It is believed that the column used for phase 1, 2 and 3 analysis by the Servos Lab may have been contaminated as a result of prior usage (Zhang et al., 2011). Vogeser and Seger (2010) noted that the presence of conjugate metabolites can contribute to inaccurate measurements as these compounds can affect the signals observed for both

target analytes and ILSs. The presence of conjugate compounds or TPs were not investigated as part of this study, however, it was possible that phase 3 samples had a reduced presence of these compounds relative to phase 2 samples which resulted in reduced MQLs for SMX. However, it was also possible that the primary effluent received during each phase contained different concentrations of compounds which lead to varying matrix effects.

As previously identified in Phase 2, the MQLs achieved using this methodology were generally higher than those reported in other studies which used similar methods (Radjenovic et al., 2009; Nurmi and Pellinen 2011; Petrovic et al., 2006). However, the results reported in these studies appear to demonstrate that little consistency between the MQLs achieved for individual compounds exists between studies. When viewed collectively with the MQLs calculated for Phase 2 and 3 of this investigation it appears likely that the ability to quantitate the selected PCs may depend on either the analytical instrument utilized or the matrix investigated. The estimated MQL's for the 5 compounds analyzed in phase 3 ranged from being approximately in line with, or less than, the reported MQLs to as high as 450 times (SMX) the results reported in the three referenced studies.

It was considered likely that the discrepancy observed between the estimated MQLs based on phase 3 analysis and the other studies referenced may be due to the method used to calculate the MQL. As was discussed previously, Standley et al., 2008 utilized a similar approach in calculating specific MQL's due to contamination detected within the analytical instrument. The authors estimated MQL for oxybenzone, which was detected in blanks, was approximately 25 to 2000 times higher than the MQLs calculated for the remaining 32 PCs analyzed. The method utilized during phase 3 analysis to calculate the MQLs was considered to be highly conservative and was anticipated to reflect a high confidence in the data obtained, when available.

The data collected from each pair of reactors (K7 and E, A7 and D) which were operated at the same experimental conditions has been presented sequentially. This segregated approach has been utilized to permit an individual focus on the performance and transformation efficiencies observed for each IFAS SBBR relative to their respective SBR controls. Reactors K7 and E were operated at an SRT of 7 days and a temperature of 18℃ which reflects the third experimental condition investigated. A summary of the screened PC concentration data for reactors K7 and E is presented in Tables 4.18 and 4.19, respectively. Raw LC-MS/MS data obtained from phase 3 PC analysis is provided in **Appendix E**. Due to an error in the preparation method samples K7-1 and K7-2 could not be analyzed.

Table 4.18 – Reported PC Concentrations for Reactor K7 Initial and Final Samples

Sample ID	Initial					Final				
	CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
K7-1	ND					133	<MQL	<MQL	<MQL	<MDL
K7-2	ND					137	<MQL	<MQL	<MQL	<MDL
K7-3	126	<MQL	<MQL	500	22050	130	<MQL	<MQL	<MQL	<MDL
K7-4	151	<MQL	<MQL	725	37150	166	<MQL	<MQL	<MQL	<MDL
K7-5	154	<MQL	<MQL	685	36300	143	<MQL	<MQL	<MQL	<MDL
K7-6	ND	<MQL	<MQL	850	35950	185	<MQL	<MQL	<MQL	<MDL
K7-7	201	<MQL	<MQL	610	35700	189	<MQL	<MQL	<MQL	<MDL
K7-8	ND	<MQL	<MQL	610	37650	192	<MQL	<MQL	<MQL	<MDL
K7-9	202	<MQL	<MQL	<MQL	36200	195	<MQL	<MQL	<MQL	<MDL
K7-10	183	<MQL	113	990	48550	215	<MQL	<MQL	<MQL	<MDL
K7-11	185	<MQL	104	835	47000	203	<MQL	<MQL	<MQL	<MDL
K7-12	ND	<MQL	92	750	43750	195	426	<MQL	<MQL	<MDL
K7-13	220	<MQL	<MQL	850	40850	204	<MQL	65	247	<MDL
K7-14	223	<MQL	<MQL	580	39850	202	<MQL	68	227	<MDL
K7-15	ND	<MQL	91	805	ND	190	<MQL	67	181	<MDL
K7-16	173	<MQL	86	695	36400	196	395	<MQL	<MQL	<MDL
K7-17	168	<MQL	111	ND	34550	199	<MQL	<MQL	<MQL	<MDL
K7-18	174	<MQL	99	660	39100	288	<MQL	<MQL	<MQL	<MDL

Notes:
1. ND – data was not available due to analytical issues or failed Grubbs outlier test.
2. MQL designates values which are below the method quantitation limit as defined above.
3. MDL designates no signal was detected for target analyte.

Table 4.19 – Reported PC Concentrations for Reactor E Initial and Final Samples

Sample ID	Initial					Final				
	CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
E-1	103	<MQL	114	670	27150	123	<MQL	ND	261	<MDL
E-2	107	<MQL	102	560	25050	128	<MQL	102	246	<MDL
E-3	99	<MQL	94	830	23400	125	<MQL	101	223	<MDL
E-4	128	<MQL	111	920	45700	159	364	115	358	<MDL
E-5	126	<MQL	155	840	47400	145	<MQL	112	210	<MDL
E-6	ND	<MQL	129	1140	48050	188	<MQL	102	261	<MDL
E-7	225	<MQL	ND	1460	ND	215	<MQL	128	323	<MDL
E-8	183	<MQL	113	925	43800	219	<MQL	115	266	<MDL
E-9	199	<MQL	115	655	45000	205	<MQL	104	349	<MDL
E-10	176	<MQL	140	930	63000	168	570	140	265	<MDL
E-11	170	<MQL	ND	1085	64000	168	565	ND	279	<MDL
E-12	172	<MQL	137	1040	60000	ND	ND	139	308	<MDL
E-13	237	<MQL	167	1095	52500	219	<MQL	145	370	<MDL
E-14	223	<MQL	141	ND	50500	213	468	110	382	<MDL
E-15	217	<MQL	137	815	49250	216	<MQL	131	ND	<MDL
E-16	188	<MQL	153	990	53500	178	464	134	294	<MDL
E-17	174	<MQL	146	975	57000	176	422	137	268	<MDL
E-18	ND	<MQL	141	1010	52000	ND	453	129	331	<MDL

Notes:
1. ND – data was not available due to analytical issues or failed Grubbs outlier test.
2. MQL designates values which are below the method quantitation limit as defined above.
3. MDL designates no signal was detected for target analyte.

Due to the nature of the analytical procedure, and based on the analytical data obtained during phase 2, outliers were anticipated within the dataset. To screen the data against possible outliers, all data was subjected to the Grubbs' outlier test, using a confidence limit of 95%. To apply Grubbs' Outlier Test, a minimum of three replicates were required. This testing procedure also required the assumption that the data for each set of triplicate samples was normally distributed. Measured concentrations that failed the Grubbs' outlier test have been removed from the data set and labelled "ND" in Tables 4.26 and 4.27.

Replicate sample measurements for CBZ for both initial and final samples collected from both reactors achieved a suitable degree of precision; R.S.Ds calculated for reactor E during the analysis of the initial samples ranged from 2 to 10 percent with an average of 5 percent. For reactor K7, due to the removal of data that failed Grubbs's Outlier Test, only one RSD, equal to 2 percent, was calculated. However, the data removed from both reactors failed the outlier test due to the low standard deviation achieved during analysis; RSDs ranged from 2 to 9 percent prior to the removal of outliers. This demonstrates that an acceptable level of precision was achieved. Final samples achieved a similar level of precision in which R.S.D's ranged from 2 to 23 (μ = 8) and 1 to 13 (μ = 5) percent, respectively.

TRIM achieved similar levels of precision as that observed for CBZ. The analysis of initial samples from reactors K7 and E resulted in R.S.D.'s ranging from 10 to 13 (μ = 11) percent and 4 to 17 (μ = 10) percent, respectively. Final samples achieved similar precision; sample R.S.D.'s for reactor E ranged from 3 to 14 percent, with an average of 8 percent. Final TRIM concentrations within reactor K7 were all below the MQL with the exception of one sample set, which achieved an RSD of 2%. The removal of unsuitable SMX data resulted in no initial sample data for either reactor K7 or E. Similarly, no triplicate sets were available for final samples collected from reactor K7 and only one triplicate set of final samples collected from reactor E were available. An RSD of 5 percent was achieved for this set.

ATEN demonstrated the highest variability of the 4 PCs for which data was available. Both reactor K7 and E demonstrated highly variable replicate measurements with initial samples producing R.S.D.'s between 11 and 19 (μ = 15) and 2 and 40 (μ = 17) percent, respectively. It was noted that the analysis of initial samples collected from reactor E demonstrated a much greater variability than reactor K7 samples, however both reactors achieved a similar RSD which was within or slightly exceeded the criteria used during MS analysis to assess acceptable precision. The analysis of final samples demonstrated a similar level of precision; RSD's for reactor E ranged between 8 and 27 percent with an average R.S.D. of 13 percent. This demonstrated an acceptable level of precision based on the criteria used to assess precision. Due to the presence of concentrations that were below the MQL, final samples collected from reactor K7 only contained one sample set comprised of triplicate samples. An RSD of 16 percent was achieved. Despite this minor exceedance, the level of precision achieved was considered acceptable.

ACE demonstrated a similar level of precision during the analysis of initial samples to what was observed for CBZ and TRIM. Initial samples achieved R.S.D.'s ranging from 2 to 6 percent and 3 to 8 percent for reactor K7 and E, respectively, with average R.S.D's of 4 percent achieved for both reactors. An assessment of the precision achieved for the analysis of ACE within final samples could not be conducted as all samples were below the MDL.

Based on the results presented in Tables 4.26 and 4.27, transformation efficiencies (expressed as a percentage) as well as standard deviations for the transformation efficiencies were calculated. In these calculations effluent measurements that were below the calculated or reported MQL were considered to be equal to the MQL. This provided a level of conservatism within the transformation efficiency estimates. The calculated efficiencies are provided in Table 4.20.

Table 4.20 – Observed Transformation Efficiencies for Phase 3 Investigation – K7 and E

Reactor	Transformation Efficiencies Observed (%)				
	CBZ	SMX	TRIM	ATEN	ACE
K7-1	-6	ND	ND	72	>99
K7-2	-8	ND	ND	82	>99
K7-3	5	ND	ND	77	>99
K7-4	-11	ND	45	84	>99
K7-5	10	ND	27	71	>99
K7-6	-33	ND	43	79	>99
AVERAGE	-7	-	39	77	-
STD DEV	15	-	10	5	-
E-1	-22	ND	2	65	>99
E-2	-29	ND	17	71	>99
E-3	-5	ND	-2	69	>99
E-4	3	ND	-1	72	>99
E-5	4	ND	13	61	>99
E-6	2	ND	9	70	>99
AVERAGE	-8	-	6	68	-
STD DEV	14	-	8	4	-
Notes: ND indicates insufficient data is available to calculate a biotransformation rate					

It can be observed from Table 4.20 that the calculated CBZ, TRIM and ATEN removals demonstrated RSDs less than 15%. As no initial SMX data was available, transformation efficiencies could not be determined. ACE was transformed at an efficiency greater than 99% in both reactors and therefore RSDs could not be estimated.

The mean transformation efficiencies calculated for CBZ were in the range of 10 to -33 and 4 to -29 for reactors K7 and E, respectively. The net transformation efficiencies suggest that no quantifiable transformation of CBZ occurred in either reactor operated at an SRT of 7 days and a temperature of 18℃. SMX data was not available and therefore transformation efficiencies could not be calculated. TRIM demonstrated an increase in transformation efficiency (33%) in the IFAS relative to the control, as demonstrated by the mean transformation efficiencies calculated. A

minor increase in the transformation efficiency for ATEN was observed for the IFAS reactor relative to reactor C (9%). ACE was transformed at an efficiency greater than 99% in both reactors and thus no differentiation of the performance achieved by either reactor could be made. A more detailed statistical comparison of the transformation efficiencies observed for CBZ, TRIM and ATEN in the SBBR and SBR is provided in Section 4.4.

Reactors A7 and D were operated at an SRT of 7 days and a temperature of 12℃ which reflects the final experimental condition investigated. A summary of the screened PC concentration data for reactors A7 and D is presented in Tables 4.21 and 4.22, respectively. Raw LC-MS/MS data obtained from phase 3 PC analysis is provided in **Appendix E**. Due to an error in the preparation method samples A7-1, A7-2 and A7-3 could not be analyzed.

Table 4.21 – Reported PC Concentrations for Reactor A7 Initial and Final Samples

Sample ID	Initial					Final				
	CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
A7-1	ND					127	<MQL	52	186	<MDL
A7-2	ND					132	<MQL	52	186	<MDL
A7-3	ND					137	<MQL	52	ND	<MDL
A7-4	146	<MQL	<MQL	735	35150	173	303	63	ND	<MDL
A7-5	148	<MQL	<MQL	735	34650	135	276	48	214	<MDL
A7-6	ND	<MQL	<MQL	ND	35950	186	<MQL	60	212	<MDL
A7-7	185	<MQL	94	625	33900	182	<MQL	62	226	<MDL
A7-8	185	<MQL	92	655	ND	186	307	58	240	<MDL
A7-9	219	<MQL	<MQL	670	34200	203	<MQL	57	196	<MDL
A7-10	173	<MQL	125	1200	44650	186	500	68	ND	<MDL
A7-11	174	<MQL	116	910	44200	184	505	75	256	<MDL
A7-12	ND	<MQL	108	955	ND	ND	ND	63	261	<MDL
A7-13	229	<MQL	98	1060	35350	258	370	<MQL	<MQL	<MDL
A7-14	ND	<MQL	119	ND	50000	204	377	<MQL	<MQL	<MDL
A7-15	227	<MQL	94	950	31600	224	<MQL	<MQL	<MQL	<MDL
A7-16	189	<MQL	119	ND	38800	174	342	71	209	<MDL
A7-17	169	<MQL	140	765	34600	172	380	71	254	<MDL
A7-18	228	<MQL	96	820	37350	ND	<MQL	71	234	<MDL

Notes:
1. ND – data was not available due to analytical issues or failed Grubbs outlier test.
2. MQL designates values which are below the method quantitation limit as defined above.
3. MDL designates no signal was detected for target analyte.

Table 4.22 – Reported PC Concentrations for Reactor D Initial and Final Samples

Sample ID	Initial					Final				
	CBZ	SMX	TRIM	ATEN	ACE	CBZ	SMX	TRIM	ATEN	ACE
D-1	120	<MQL	113	975	26500	117	<MQL	94	935	<MDL
D-2	134	<MQL	130	1375	27850	125	<MQL	108	955	<MDL
D-3	114	<MQL	108	1145	23750	115	<MQL	86	ND	<MDL
D-4	149	<MQL	127	865	48200	153	<MQL	125	890	<MDL
D-5	153	<MQL	147	1210	50500	171	<MQL	154	945	<MDL
D-6	172	<MQL	113	1135	47100	180	<MQL	132	950	<MDL
D-7	209	<MQL	135	ND	45150	222	<MQL	123	1115	<MDL
D-8	203	<MQL	128	1160	45050	219	<MQL	152	1045	<MDL
D-9	201	<MQL	106	1155	ND	212	<MQL	103	1020	<MDL
D-10	167	<MQL	166	1595	56500	175	630	ND	1270	<MDL
D-11	184	<MQL	184	1580	59000	164	<MQL	140	1175	<MDL
D-12	175	<MQL	151	ND	55000	179	444	139	1270	<MDL
D-13	ND	<MQL	180	1085	ND	ND	<MQL	150	1280	<MDL
D-14	202	<MQL	162	1390	40150	227	<MQL	170	980	<MDL
D-15	203	<MQL	157	1555	39950	227	<MQL	137	1300	<MDL
D-16	301	<MQL	158	1030	48250	179	<MQL	138	1190	<MDL
D-17	261	<MQL	162	1370	43250	163	<MQL	145	1115	<MDL
D-18	ND	<MQL	ND	1500	45650	ND	<MQL	161	1015	<MDL

Notes:
1. ND – data was not available due to analytical issues or failed Grubbs outlier test.
2. MQL designates values which are below the method quantitation limit as defined above.
3. MDL designates no signal was detected for target analyte.

Data from reactors A7 and D were screened for outliers using the Grubbs' outlier test, at a confidence limits of 95%. To apply Grubbs' Outlier Test, a minimum of three replicates were required. This testing procedure also required the assumption that the data for each set of triplicate samples was normally distributed. Measured concentrations that failed the Grubbs' outlier test have been removed from the data set and labelled "ND" in Tables 4.21 and 4.22.

Replicate sample measurements for CBZ for both initial and final samples collected from both reactors achieved a suitable degree of precision; R.S.Ds calculated for reactor A7 and D ranged from 10 to 15 percent and 2 to 8 percent with an average of 13 and 6 percent, respectively. Final samples achieved a similar level of precision in which R.S.D's ranged from 4 to 16 and 2 to 8 percent, with an average R.S.D. of 9 and 5 percent for reactors A7 and D, respectively. The removal of unsuitable SMX data resulted in no initial sample data for either reactor A7 or D. Similarly, no triplicate sets were available for final samples collected from either reactor. TRIM demonstrated increased variability when compared to CBZ. The analysis of initial samples from reactors A7 and D resulted in R.S.D.'s ranging from 7 to 19 percent and 7 to 13 percent respectively. Initial TRIM data demonstrated average R.S.D.'s of 13 and 11 percent for reactors A7 and D, respectively. Final samples achieved similar precision; sample R.S.D.'s ranged from 0 to 14 and 8 to 20 percent, with an average of 5 and 12 percent, respectively.

ATEN demonstrated a slightly increased level of variability when compared to CBZ and TRIM. Both reactor A7 and D initial samples resulted in R.S.D.'s between 4 and 15 and 17 and 18 percent, respectively. The average R.S.D. for the analysis of initial samples from reactor A7 and D was 15 and 17 percent, respectively. It was noted that the analysis of initial samples collected from reactor D demonstrated greater variability than reactor A7 samples, however both reactors achieved a similar RSD which was within or slightly exceeding the criteria used during MS analysis to assess acceptable precision. The analysis of final samples demonstrated a similar level of precision; R.S.D.'s for reactor D ranged between 4 and 15 percent with an average R.S.D.'s of 7 percent. Only two final sample sets with triplicate measurements were available for reactor K7 which both achieved an RSD of 10 percent. This demonstrates an acceptable level of precision was achieved based on the criteria used to assess precision during the analysis of MSs. ACE demonstrated a similar level of variability to TRIM. Initial samples achieved R.S.D.'s ranging from 2 to 25 percent and 4 to 8 percent for reactor K7 and E, respectively, with average R.S.D's of 11 and 5 percent achieved for both reactors. An assessment of the precision achieved for the analysis of ACE within final samples could not be conducted as all samples were below the MDL.

Based on the results presented in Tables 4.29 and 4.30, transformation efficiencies (expressed as a percentage) as well as standard deviations for the transformation efficiencies were calculated. In these calculations measurements of final samples that were below the calculated MQL were considered to be equal to the MQL. This provided a level of conservatism within the transformation efficiency estimates. The calculated efficiencies are provided in Table 4.23.

Table 4.23 – Observed Transformation Efficiencies for Phase 3 Investigation – A7 and D

Reactor	Transformation Efficiency Observed (%)				
	CBZ	SMX	TRIM	ATEN	ACE
A7-1	ND	ND	ND	ND	>99
A7-2	-12	ND	ND	71	>99
A7-3	3	ND	37	66	>99
A7-4	-6	ND	41	75	>99
A7-5	0	ND	57	86	>99
A7-6	12	ND	40	71	>99
AVERAGE	-1	-	44	74	-
STD DEV	9	-	9	7	-
D-1	3	ND	18	19	>99
D-2	-6	ND	-6	13	>99
D-3	-7	ND	-2	8	>99
D-4	2	ND	17	22	>99
D-5	-12	ND	8	12	>99
D-6	39	ND	7	15	>99
AVERAGE	3	-	7	15	-
STD DEV	19	-	10	5	-
Notes: ND indicates insufficient data is available to calculate a biotransformation rate					

It was considered likely that the CBZ transformation efficiency calculated for the sixth Reactor D sample set was an outlier caused by analytical error as it failed Grubbs test at an α of 0.95. However, this analysis required an assumption that all sample sets could be treated as replicates, which is not accurate as each set was collected at different times. It should be noted that the dataset used to calculate the transformation efficiencies were screened for outliers and both the initial and final samples within sample set D-6 included outliers which were removed. It is therefore likely that the calculated transformation efficiency for sample set D-6 was reflective of poor accuracy and/or precision within both the initial and final measurements that resulted in additive errors. If this value were to be eliminated from the dataset, the average transformation efficiency observed would have been reduced to -4% and the standard deviation would likewise be reduced to 6%. However, as the outlier status could not be confirmed, this data point was included in the data set.

The mean transformation efficiencies calculated for CBZ were in the range of 12 to -12 and 39 to -12 for reactor A7 and D, respectively. The net transformation efficiencies suggest that no quantifiable transformation of CBZ occurred in either reactor. Calculated TRIM and ATEN removals demonstrated RSDs less than 15%. A significant increase in the transformation efficiency for ATEN was observed for the IFAS reactor A7 relative to reactor D (59%), as

demonstrated by the mean transformation efficiencies calculated. TRIM similarly demonstrated an increase in transformation efficiency (37%) in the IFAS relative to the control. SMX data was not available and therefore transformation efficiencies could not be calculated. ACE was transformed at an efficiency greater than 99% in both reactors and therefore RSDs could not be estimated. A more detailed statistical comparison of the transformation efficiencies observed for CBZ, TRIM and ATEN in the SBBR and SBR is provided in Section 4.4.

4.4. SUMMARY AND STATISTICAL ANALYSIS OF RESULTS

To provide further context of the conventional removal performance and PC transformation efficiencies reported, an overview of the data and general trends observed throughout phases 1, 2 and 3 is presented in the following section. This comparison focuses on the following:

- The conventional performance achieved by each of the reactors;
- Operational parameters (MLSS, MLVSS, ESS)
- The concentrations of the 5 PCs measured within initial samples
- The transformation efficiencies achieved for each PC

Observed trends were investigated through statistical means to confirm their significance, where warranted. Additionally, comparisons between the results obtained through this investigation and those reported in previous studies is provided as a means of assessing the consistency of the data obtained with prior work.

4.4.1. CONVENTIONAL PARAMETERS

As described in the previous sections PC sampling was conducted in three phases that spanned a period of 5 months. Temporal variability within the raw sewage conveyed to the Wastewater Technology Centre, and the subsequently treated primary effluent conveyed to the pilot reactors, was anticipated. However, despite varying feed composition, the removal of organic matter was found to generally be consistent between all reactors throughout all phases. Similarly, each IFAS reactor was found to provide full nitrification under each of the four experimental conditions. The control reactors were found to be fully nitrifying under all conditions except when operated at an SRT of 7 days and a temperature of 12 °C.

Organic removals were assessed through the measurement of tCOD and sCOD within final samples, which characterized the effluent from each treatment cycle. Final samples collected from the IFAS reactors K20, A20, K7 and A7, were found to produce mean tCOD concentrations of 38, 105, 61 and 50 mg/L, respectively. Despite a noted difference in tCOD concentrations, analysis using ANOVA (**Appendix G**) indicated that the final samples collected from the IFAS reactors were not statistically different at a confidence level of 95%. Final samples from the corresponding control reactors B, C, E and D, contained average tCOD concentrations of 47, 66, 40 and 56 mg/L. Analysis using ANOVA indicated that all control reactors produced effluents which contained tCOD concentrations which were not statistically different. ANOVA testing was also conducted on the combined dataset (both IFAS and

control reactors) and indicated that none of the final sample sets demonstrated a statistically significant difference from the others, suggesting that all reactors provided a similar level of organic removal.

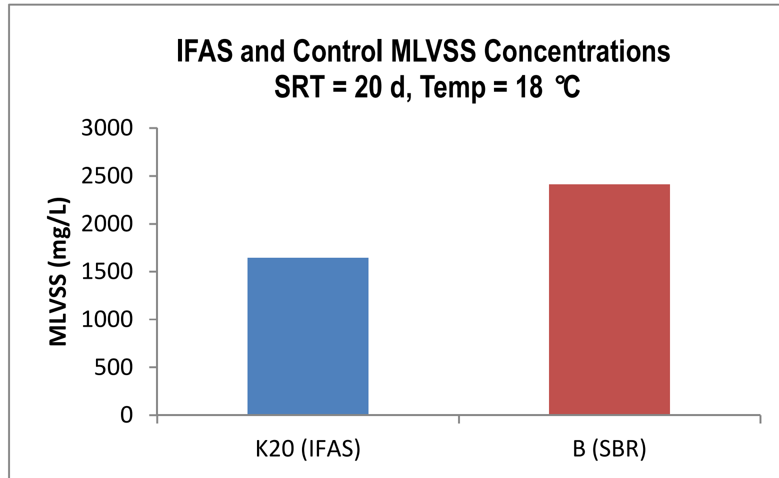
The apparent differences in effluent tCOD values were likely related to measurement errors and varying contributions of pCOD fractions within the effluent. sCOD measurements conducted in phase 2 and 3 demonstrated that the final samples collected from IFAS reactors A20, K7 and A7 contained average sCOD concentrations equal to 61, 59 and 48 mg/L, respectively while the effluents collected from the control reactors C, E and D contained sCOD concentrations of 38, 36 and 47 mg/L, respectively. ANOVA testing completed on the final sCOD IFAS dataset, the control dataset as well as a combined dataset found that all values were not statistically different. This further demonstrated that all reactors provided a consistent level of organic removal through Phases 1, 2 and 3.

A previous investigation was conducted at the Wastewater Technology Centre (same facility as this study) which used pilot scale SBRs operated under a variety of SRTs and temperatures (Pileggi, 2007). All SBRs received the same primary effluent (primary treatment of raw sewage received from the Skyway WWTP) as feed and tCOD was measured using the same methods as this investigation. The author reported that the mean effluent tCOD values ranged from 15 to 55 mg/L. Based on the consistency of the results of this study with those reported by Pileggi (2007), it was considered likely that the raw sewage feed from the Skyway WWTP contains an elevated concentration of nbsCOD relative to those reported as typical by Envirosim Associates (2011). On this basis, it was believed that all reactors were achieving complete removal of biodegradable COD consistent with a typical AS reactor operated under sufficient SRT.

It was noted that the IFAS reactors achieved full nitrification under each of the four experimental conditions investigated. Similarly, the control reactors provided full nitrification under all but the low temperature, low SRT condition (t = 12°C and SRT = 7 days). The effluent TAN concentrations within the final samples collected from the seven reactors that provided nitrification (K20, B, A20, C, K7, E and A7) were analyzed using ANOVA (**Appendix G**). At a confidence limit of 95%, the effluent TAN concentrations were found to not be statistically different. This suggests that these seven reactors provided similar nitrification performance. It was anticipated at the experimental design stage that Reactor D would not provide a significant level of nitrification based on the specified experimental conditions. This condition provided a unique opportunity to not only assess the nitrification capabilities provided by the inclusion of IFAS biomass, but also to investigate the impacts of autotrophic bacteria on the PC transformation efficiencies.

MLVSS was analyzed in the IFAS and control reactors as a means of assessing the suspended growth biomass present under each of the experimental conditions. Figures 4.1 to 4.4 provide a graphical summary of the MLVSS concentrations measured in each IFAS and control reactor at the four experimental conditions investigated during phase 1, 2 and 3. From these figures it can be seen that the MLVSS concentrations were consistently lower in the IFAS reactors, with the exception of reactor A7, which was operated under low SRT and low temperature conditions. In all other cases, the MLVSS concentrations within the IFAS reactors were approximately 30 to 60 percent lower than their paired control. This was attributed to the presence of the biofilm within the IFAS media and the limited

substrates available for bacterial growth within the suspended growth phase. It was therefore demonstrated that the IFAS reactors provided consistent performance with their paired controls while operating with significantly less suspended growth biomass.



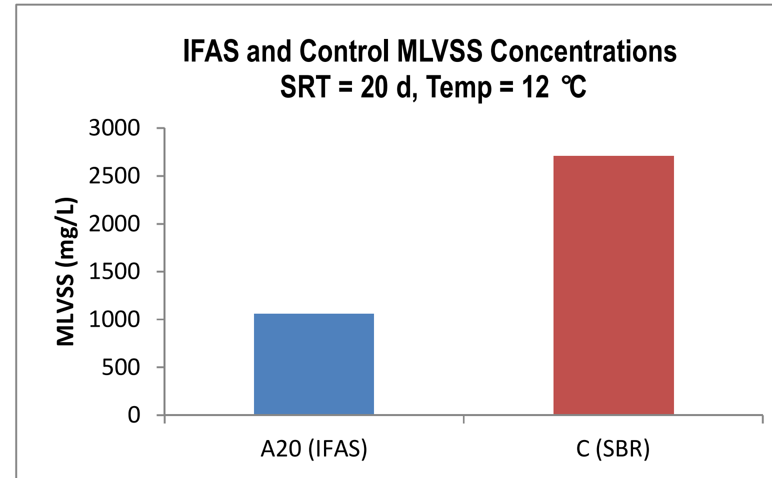
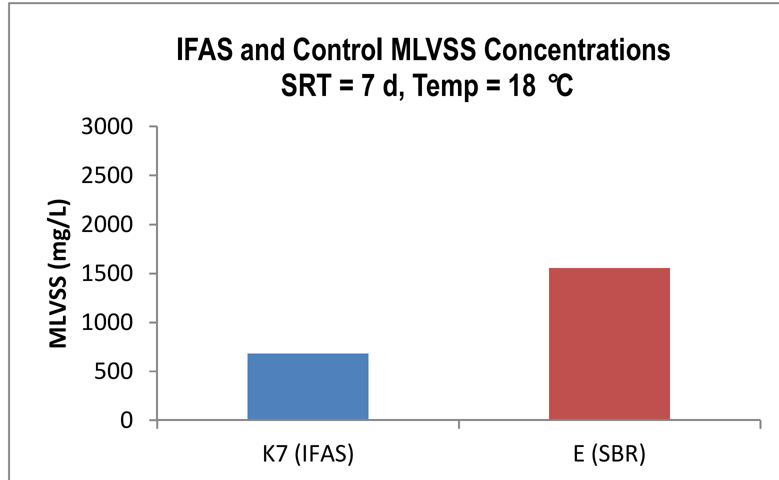


Figure 4.1 – MLVSS concentrations within reactor K20 and B

Figure 4.2 - MLVSS concentrations within reactor A20 and C



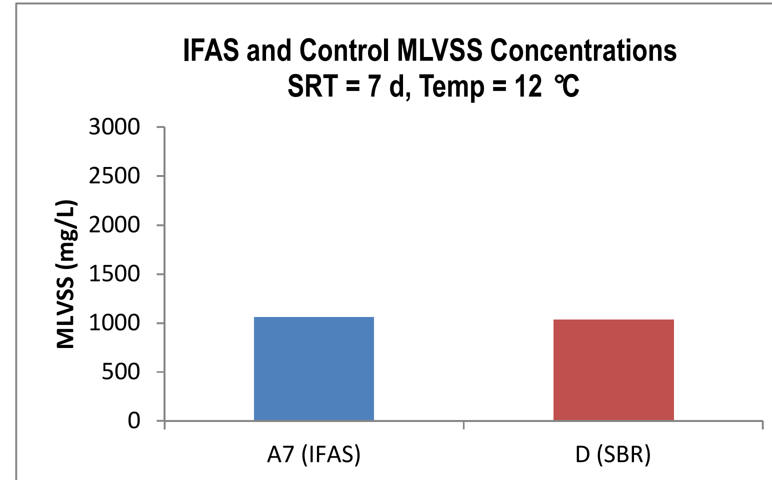


Figure 4.3 – MLVSS concentrations within reactor K7 and E

Figure 4.4 - MLVSS concentrations within reactor A7 and D

The similar MLVSS concentrations observed in the IFAS and control reactors under the low SRT, low temperature condition was likely the result of filamentous issues that were encountered just prior to the initialization of phase 3 PC sampling. Under this experimental condition, reactor A7 produced a mean effluent TSS of 8.4 mg/L. In contrast, reactor D produced a mean effluent TSS concentration of 62 mg/L. This was approximately four times the effluent TSS concentration reported as typical for well operating activated sludge reactors operating under the target experimental conditions (Metcalf and Eddy, 2003). It was therefore likely that the MLVSS concentration measured within reactor D may have been reduced compared to what would have occurred if the filamentous occurrence was not occurring. However, as this reactor was not expected to provide nitrification, the SRT shortfall was not considered to have significant impacts on the level of PC transformation efficiencies achieved. Reactor D achieved organic removals consistent with those observed at the remaining experimental conditions and was therefore considered to be performing as expected.

Nitrification rate testing was also utilized to determine if the pilot reactors were at steady state conditions and if the IFAS SBBRs provided nitrification rate improvements when compared to their respective control SBRs. Table 4.24 summarizes the nitrification performance observed during phases 1, 2 and 3. Under all conditions except the high SRT, low temperature conditions (reactors A20 and C), the IFAS reactors were found to provide statistically different (increased) nitrification rates at a confidence limit of 95%. This difference is further demonstrated by the specific nitrification rates, which represent the nitrification rate normalized on the basis of reactor MLVSS concentrations. As can be observed, the specific nitrification rates were considerably higher for the IFAS reactors as a result of operation with reduced MLVSS concentrations.

Table 4.24 – Nitrification Rate Testing Summary

Reactor	Measured TAN Removal Rate	Measured NOx Production Rate	Measured Specific TAN Removal Rate	Measured Specific NOx Production Rate
	(gN/d)	(gN/d)	(mgN/gVSS/d)	(mgN/gVSS/d)
K20	5.36 (0.20)	6.46 (0.30)	140	169
B	3.34 (0.27)	3.38 (0.46)	69	70
A20	3.10 (0.33)	2.48 (0.14)	125	100
C	2.84 (0.12)	2.14 (0.27)	53	56
K7	3.54 (0.13)	4.60 (0.17)	223	289
E	3.84 (0.11)	3.23 (0.39)	123	104
A7	3.26 (0.51)	2.96 (0.18)	132	120
D	0.39 (0.11)	0.14 (0.009)	23	8

1. Values shown in parentheses are the standard error of linear regression.

It has been postulated that co-metabolism may be the primary biological mechanism responsible for the transformation of PCs (Ternes et al., 2004; Clara et al., 2005; Göbel et al., 2007; Stasinakis et al., 2010). Investigations have attributed PC transformation to both heterotrophic (Majewksy et al., 2011) and autotrophic activity (Kreuzinger et al., 2004; Perez et al., 2005; Eichhorn et al., 2005). Organic removals achieved by the IFAS SBBRs and control SBRs were found to be statistically consistent across all experimental conditions investigated during phase 1, 2 and 3 of this study. Similarly, seven of the eight reactors investigated were found to produce an effluent with TAN concentrations which were not statistically different. Only the low SRT, low temperature condition allowed for a direct assessment of the role of autotrophs; under these conditions it was expected that no significant autotroph population would be present.

Only minor differences between the IFAS and control reactors were observed based on the removals of conventional parameters. However, it was noted during the literature review that the presence of a biofilm environment, with extended SRTs provided for the bacteria within the biofilm, may allow for a greater diversity within the bacterial consortia present. Based on the nitrification rate testing completed, it was found that the IFAS reactors provided significantly improved nitrification kinetics when compared to their respective controls. This may indicate that a more diverse autotrophic population was present within the IFAS reactors and therefore may provide an explanation for any improvements in PC transformation efficiencies observed.

4.4.2. PC ANALYSIS

PC concentrations within the primary effluent were calculated based on the results of phase 1, 2 and 3 analysis of initial and final samples and were compared to the results of previous studies. Based on the PC concentrations observed, transformation efficiencies were calculated. To provide a graphical demonstration of the PC transformation efficiencies observed under each experimental condition, box and whisker plots were generated for CBZ, TRIM and ATEN using the transformation efficiencies calculated under Phase 1, 2 and 3 investigations. To confirm the statistical significance of any apparent trends observed through the generated box and whisker plots, ANOVA testing was conducted.

Statistical analysis utilizing a three factor ANOVA approach (α = 0.95) was conducted using Minitab 17 software (Minitab Inc. PA, USA). Factors were considered significant if the calculated p-value was less than 0.05 for that factor. The ANOVA results and all outputs from the Minitab software are presented in **Appendix G**. The calculated transformation efficiencies for all 3 PCs were expected to demonstrate some skewing, due to the limitations (truncation of data) imposed by the effluent MQLs. While this skewing of transformation efficiency data likely resulted in a slightly non-parametric distribution for some reactors, normality was assumed as the MQLs only affected a limited number of transformation efficiency data.

ACETAMINOPHEN

ACE concentrations within the primary effluent were estimated based on the measured values within initial samples, the known volume of primary effluent conveyed to each reactor during each cycle and an assumed concentration of zero within the mixed liquor at the end of the react cycle. Concentrations of ACE were found to vary between approximately 59 and 141 μg/L for Phase 1, 34 and 125 μg/L for Phase 2, and 35 and 96 μg/L for Phase 3. Lavén et al., (2009) reported similar findings in which the mean ACE concentration within influent wastewater was found to be 84 μg/L. Gros et al., (2006) found that ACE concentrations varied significantly; influent concentrations ranged between 130 ng/L to approximately 26 μg/L. However, Gómez et al., (2007) reported ACE concentrations which ranged from 29 to 246 μg/L. When viewed collectively, the ACE concentrations in this study were found to be within the range of those reported in the referenced studies.

ACE was observed to have been transformed in all reactors, under all experimental conditions at an efficiency greater than 99%. Therefore no comparison between reactor performances could be conducted. Similar

observations have been reported by Majewsky et al., (2011) in which ACE experienced transformation efficiencies of ~100% regardless of the operating SRT and the activities of autotrophs and heterotrophs. Kreuzinger et al., 2004 reported similar results, in which the readily biodegradable PC ibuprofen was found to be transformed at 100% efficiency across a range of SRTs investigated. Hence, no box and whisker plots were generated for this PC.

SULFAMETHOXAZOLE

Only limited and unreliable data was available to characterize SMX transformation efficiencies and therefore no performance comparisons between the IFAS and control reactors could be made for this compound. Hence, box and whisker plots for SMX were not generated. Additionally, due to the limited analytical results available to characterize SMX concentrations within initial and final samples, no assessment of SMX concentrations within the primary effluent were made.

CARBAMAZEPINE

The influent concentrations of CBZ were noted to significantly vary between each phase of analysis. Phase 1 initial concentrations were noted to range between 260 and 650 ng/L with an average concentration of approximately 375 ng/L in phase 1. Phase 3 initial concentrations ranged between 100 and 300 ng/L with an average concentration of 185 ng/L. The initial concentrations in reactor A20, that was sampled as part of Phase 2, ranged between 220 and 420 ng/L, with an average concentration of 290 ng/L. Initial samples from reactor C, that was sampled concurrently with Phase 3, had concentrations that ranged from 110 to 240 with an average of 180 ng/L for reactor C. Based on ANOVA, the influent concentrations were found to be statistically different at a confidence limit of 95% (**Appendix G**). The cause of this temporal variance was not determined.

Miège et al., (2008) reported that CBZ concentrations within influents range from 100 ng/L to as high as 1900 ng/L. Concentrations within effluents were reported to range between 180 and 2300 ng/L. This demonstrates that de-conjugation, which leads to negative transformation efficiencies and higher effluent values, is a common occurrence. Miao and Metcalfe (2003) reported influent CBZ concentrations at an Ontario WWTP of approximately 370 ng/L. The mean concentrations observed during phase 1 and reactor A20 PC sampling were consistent with these values. It was noted by Miao and Metcalfe (2003) that a significant portion of the influent CBZ load was present in conjugated forms. It is possible that the variability within the initial samples reflects varying level of de-conjugation occurring prior to conveyance to the pilot reactors. On this basis, the concentrations found within the initial and final samples were considered to be consistent with results reported in prior investigations.

Box and whisker plots were generated to provide a visual indication of the PC transformation occurring within the IFAS and control reactors under the four experimental conditions. The box and whisker plots generated for CBZ transformation efficiencies at each of the four experimental conditions are presented as Figures 4.5 to 4.8. The ANOVA results (**Appendix G**) suggested that for CBZ, neither SRT, temperature or IFAS had a statistically significant effect on the reported transformation efficiency at a confidence limit of 95%. The normality of the residual errors (**Appendix G)** from the linear regression were found to be generally well behaved, and appeared to be consistent with stochastic error, with the exception of a single suspected outlier (-0.75). Hence, the regression model

was considered to have been appropriate to describe the data. It was also noted that the general linear model used to describe CBZ transformation achieved under the various experimental conditions had an R^2 value of only 18%. This suggests that an additional significant source of variability existed within the data that was not attributed to the parameters investigated. Due to the normality of the errors, it was considered likely that the variability within the data that was not explained by the model could be attributed to analytical variance.

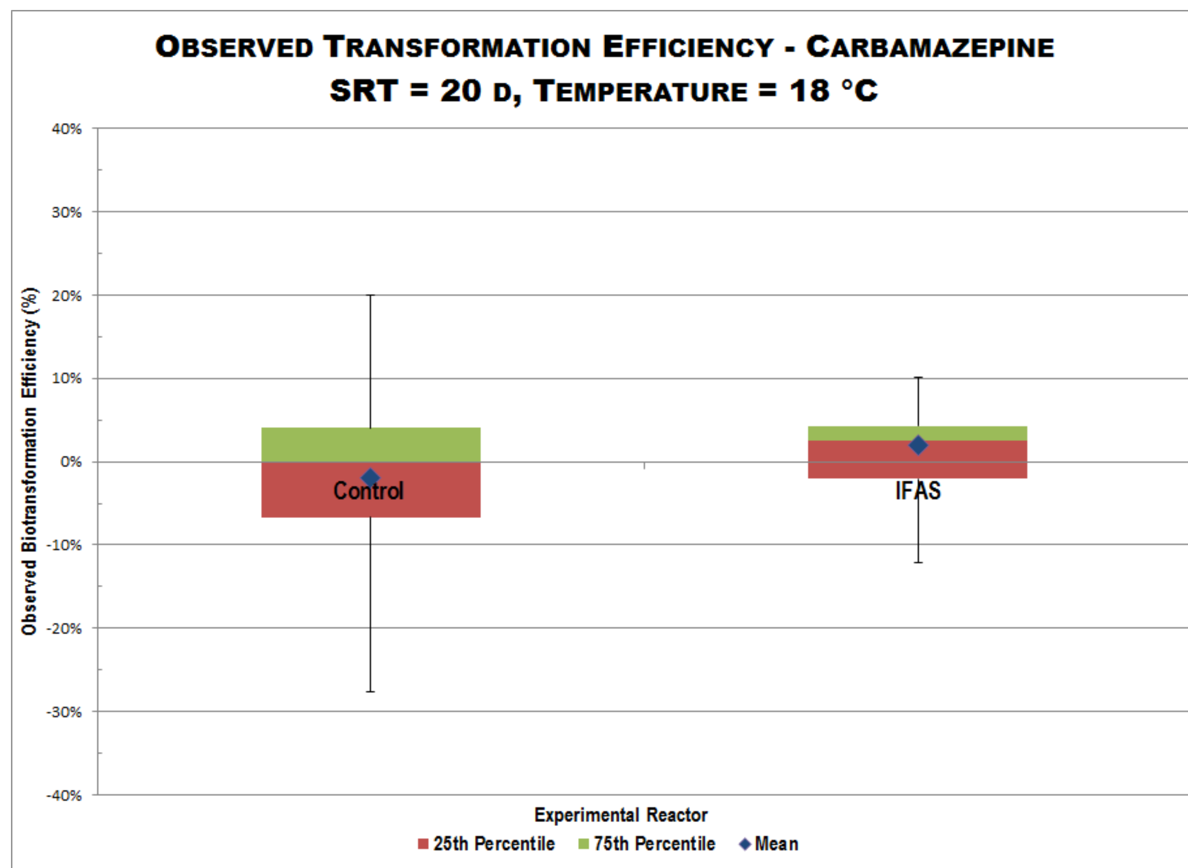


Figure 4.5 – CBZ transformation efficiency for IFAS and Control at 20 d SRT and 18°C

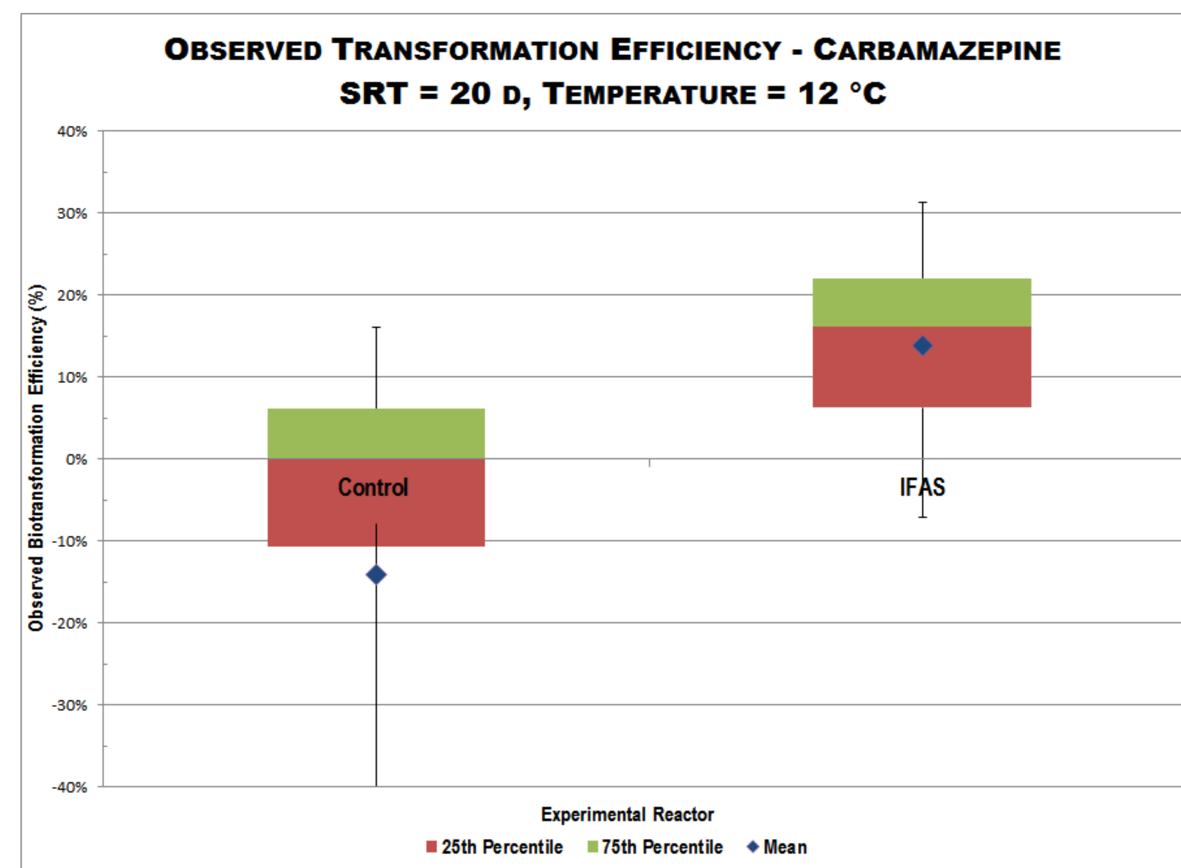


Figure 4.6 – CBZ transformation efficiency for IFAS and Control at 20 d SRT and 12°C

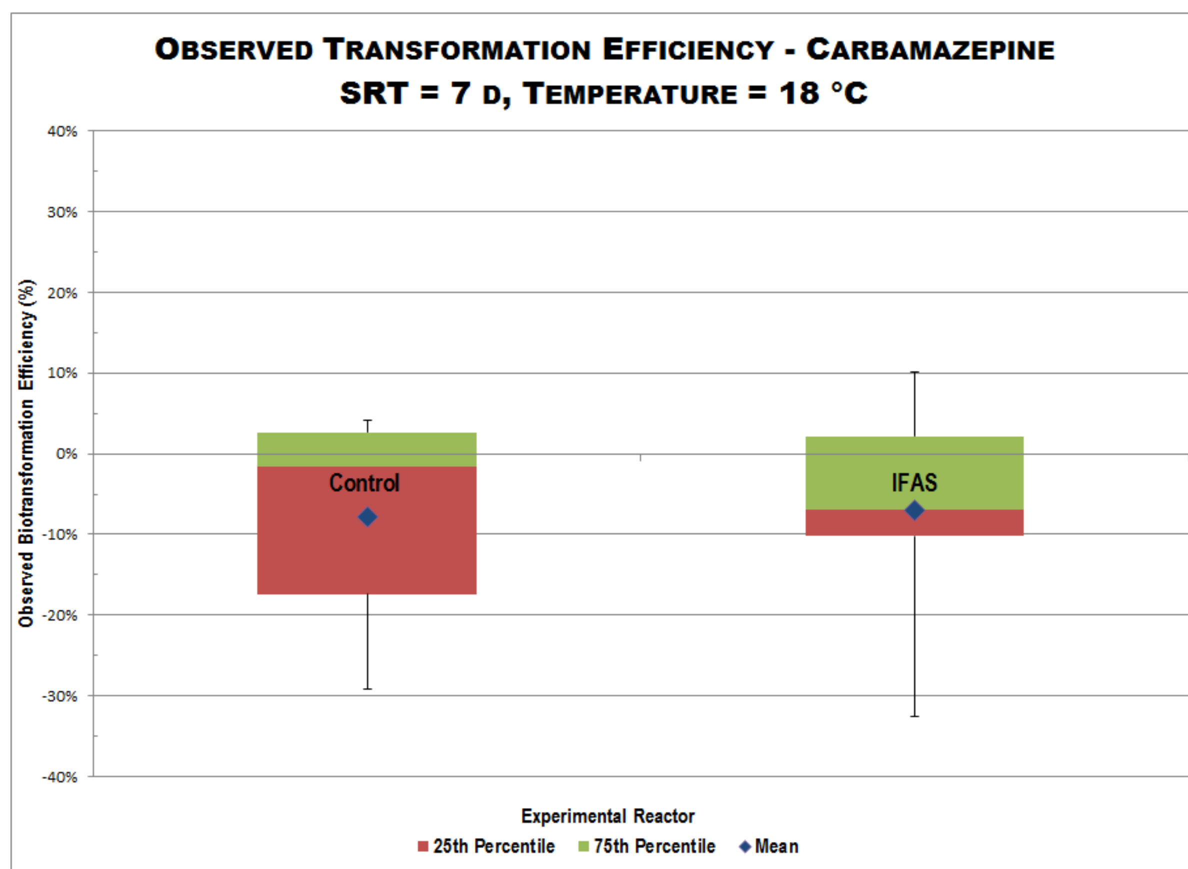


Figure 4.7 – CBZ transformation efficiency for IFAS and Control at 7 d SRT and 18°C

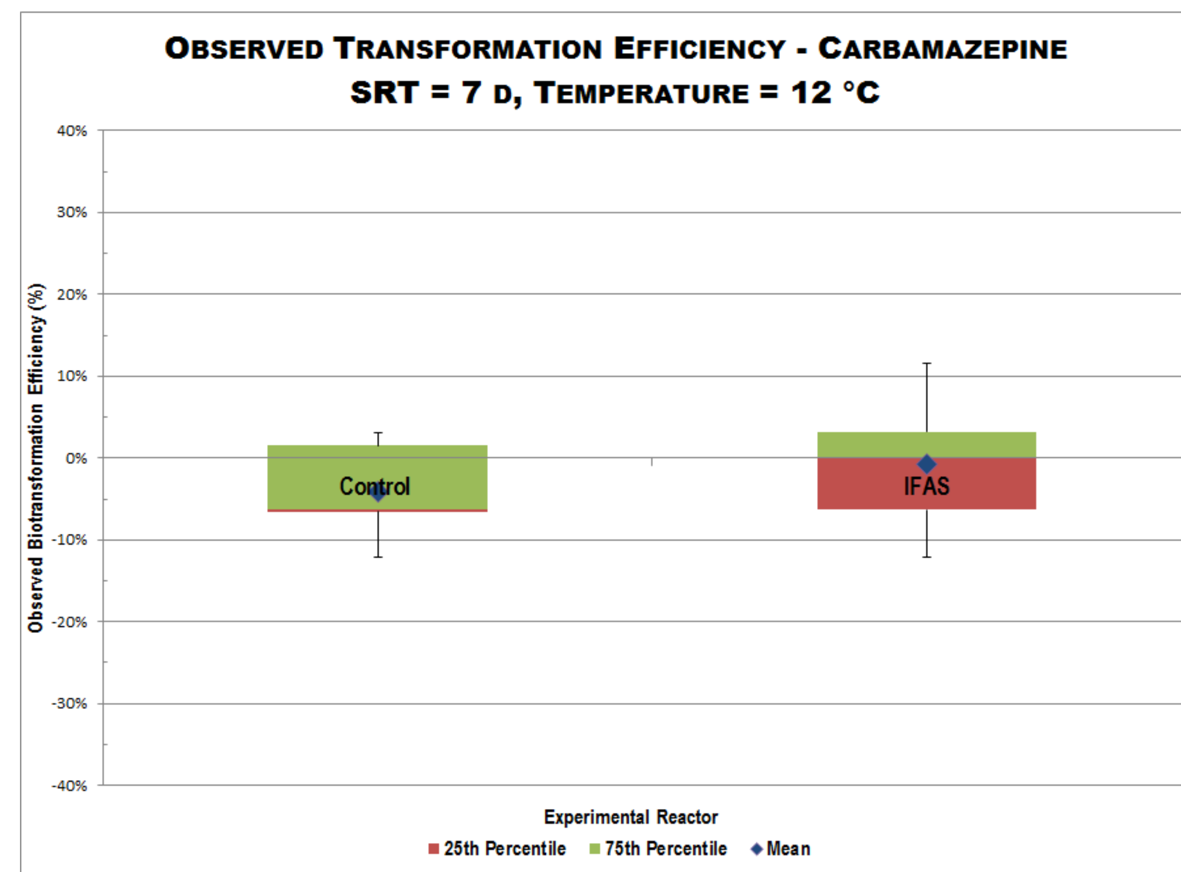


Figure 4.8 – CBZ transformation efficiency for IFAS and Control at 7 d SRT and 12°C

All transformation efficiency data was combined and plotted as a histogram to determine the most consistent transformation efficiencies observed. This analysis indicated that essentially all transformation efficiencies from both IFAS and control reactors and across all conditions were clustered between 0 and -10 percent (**Appendix G**). This data suggests that no quantifiable transformation of CBZ occurred in either the IFAS or control reactors at the four conditions investigated. Similar results have been reported in previous studies (Radjenovic et al., 2009; Clara et al., 2005b; Kreuzinger et al., 2004). In these studies, only very minor (<15%), or in some cases negative, removals were reported regardless of the biological treatment process or operating conditions employed.

TRIMETHOPRIM

TRIM concentrations within the primary effluent were estimated based on the measured concentrations within the initial and final samples as well as the known volumes of primary effluent conveyed to each reactor as feed, and the mixed liquor remaining at the end of each treatment cycle. The final samples collected from the IFAS reactors were found to have concentrations that were below the MQL and therefore the primary effluent concentrations were estimated based on the initial and final samples collected from the control. The mean TRIM concentrations in the primary effluent during Phases 1, 2 and 3 were estimated to be 194, 250 and 142, respectively. An evaluation using ANOVA and a confidence limit of 95% (**Appendix G**) found that the estimated primary effluent concentrations were statistically different during the three periods in which samples were collected.

Metcalfe et al., (2003) reported that TRIM was detected in Canadian WWTP effluents at concentrations ranging from 9 to 271 ng/L. Gobel et al., (2004) reported similar concentrations of 200 ng/L within primary effluent at a Swiss WWTP. The concentrations of TRIM estimated to have been present within the primary effluent were generally in line with the reported results of these prior investigations. The cause for the temporal variance was not determined, but was consistent with the temporal variance observed for CBZ. It is acknowledged that approximately 60% of TRIM excreted from the body is in the parent form (Vree et al., 1978). It was considered possible that reduced de-conjugation was occurring during Phase 3. However, as these metabolites were not quantitated, this suspicion could not be confirmed.

Box and whisker plots were created to provide a visual indication of the TRIM transformations occurring within the IFAS and control reactors under the four experimental conditions. These plots are presented as Figures 4.9 to 4.12. The box and whisker plots were noted to demonstrate consistent improvements in TRIM transformation efficiency for the IFAS reactors across all four experimental conditions when compared with their respective controls. TRIM demonstrated the most significant transformation efficiency improvement for IFAS under an SRT of 20 days and a temperature of 18 °C. It was noted that all other conditions resulted in similar transformation efficiency differences between the IFAS and control SBRs.

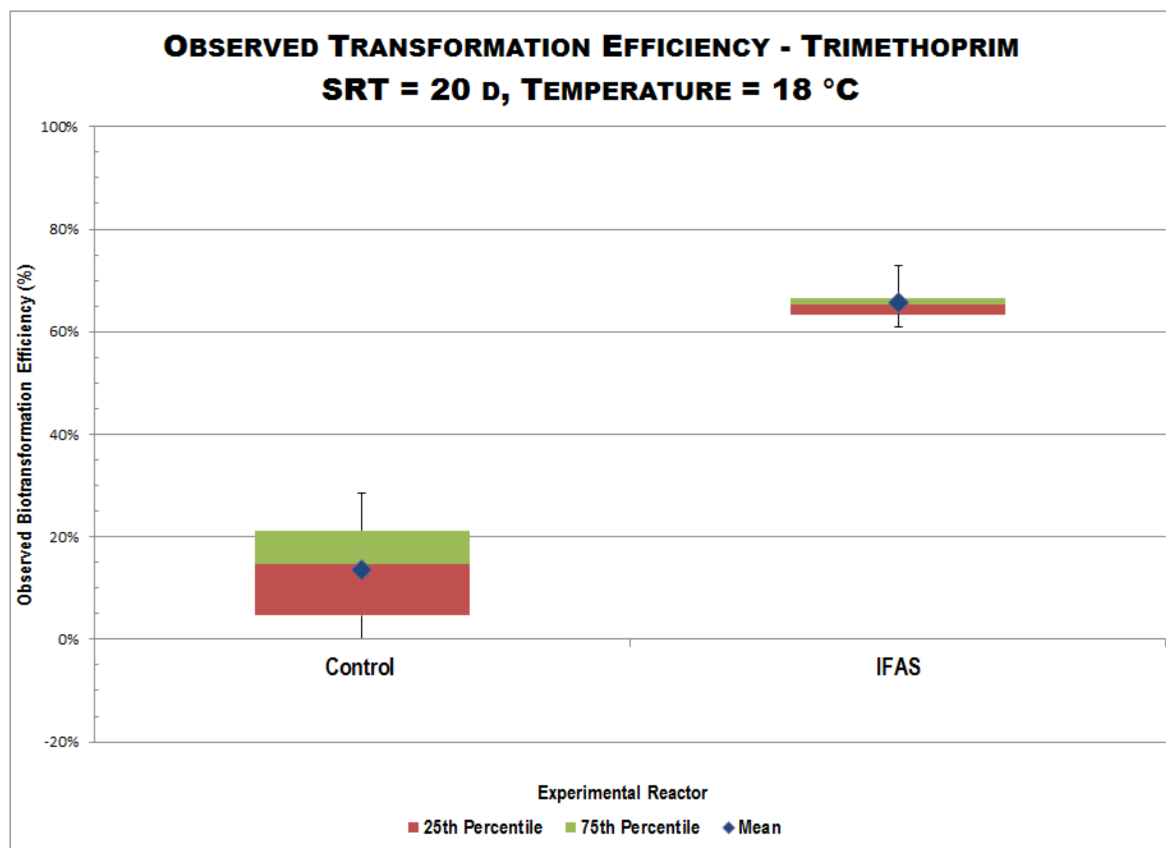


Figure 4.9 – TRIM transformation efficiency for IFAS and Control at 20 d SRT and 18°C

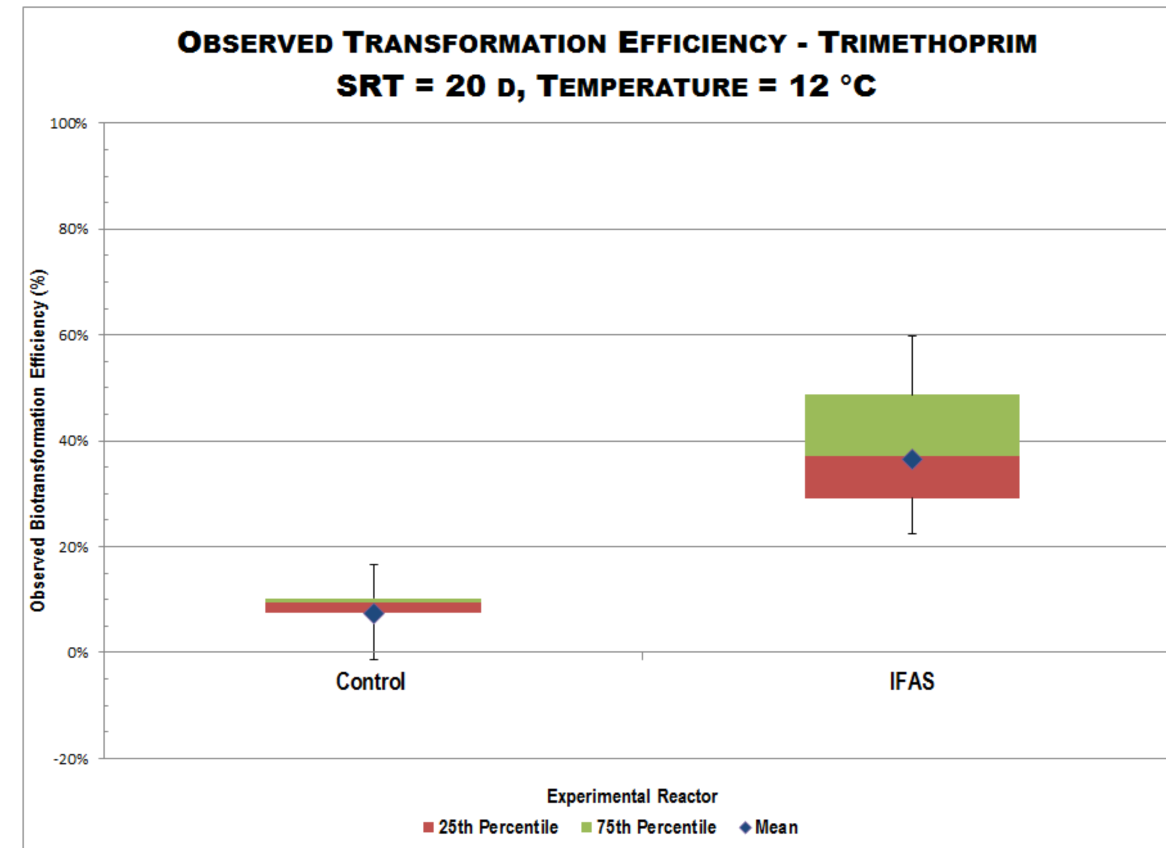


Figure 4.10 – TRIM transformation efficiency for IFAS and Control at 20 d SRT and 12°C

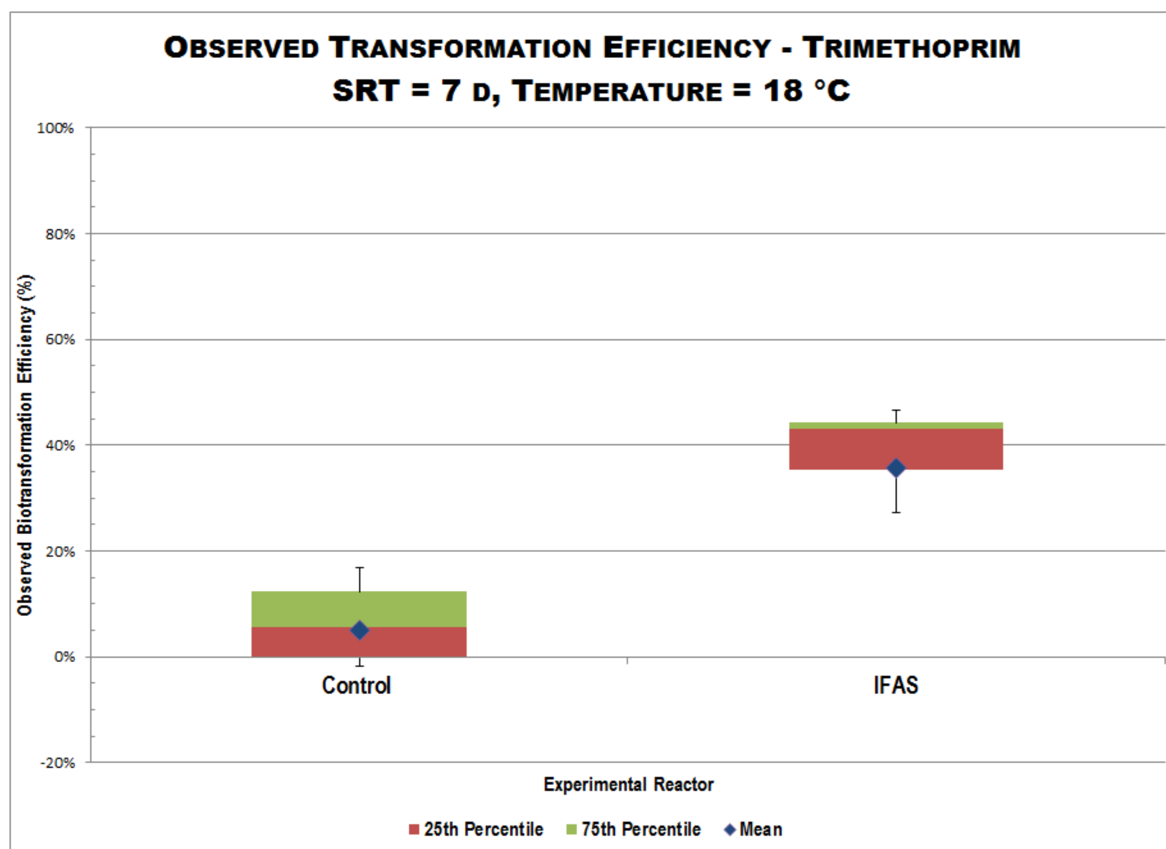


Figure 4.11 – TRIM transformation efficiency for IFAS and Control at 7 d SRT and 18°C

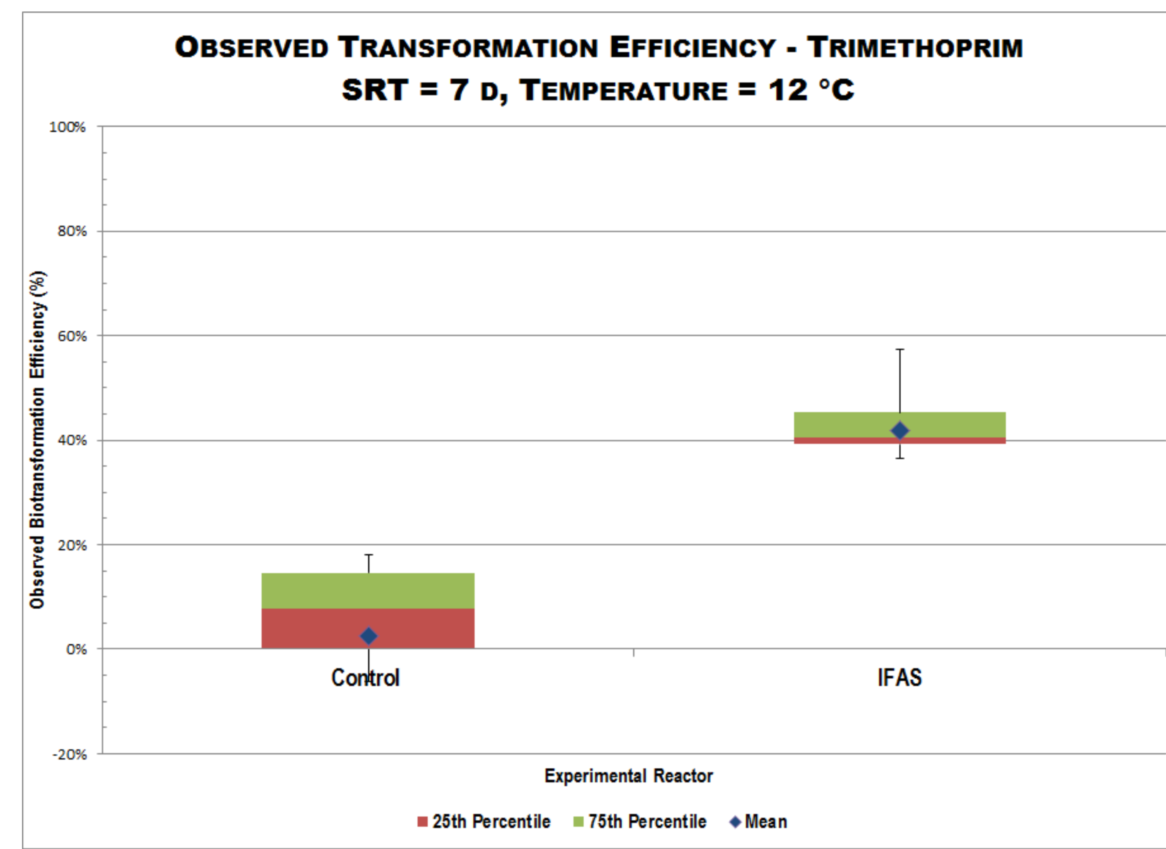


Figure 4.12 – TRIM transformation efficiency for IFAS and Control at 7 d SRT and 12°C

All TRIM transformation efficiency data was combined and plotted as a histogram to determine the most consistent transformation efficiencies observed. This data demonstrated no particular trend, indicating that the transformation efficiencies achieved by the reactors under the experimental conditions were highly variable. The transformation efficiencies observed during this study ranged from – 10 percent to 70 percent. It should be noted that transformation efficiencies may have exceeded 70%, however this could not be reported with certainty due to MQL limitations. This range of transformation efficiencies was noted to be consistent with results reported in the literature (Gobel et al., 2007; Batt et al., 2007; Radjenovic et al., 2009; Rosal et al., 2010). In the referenced studies, removals were reported to range from 5% to over 90% under a variety of processes.

It was demonstrated through ANOVA that SRT, temperature and IFAS had statistically significant effects on the reported transformation efficiencies for TRIM at a confidence limit of 95% (**Appendix G**). A significant two-way and three-way interaction was noted for SRT*Temperature and SRT*Temperature*IFAS which suggests that these factors likely combined to result in improved transformation efficiencies.

The general linear model used to describe TRIM transformation was noted to result in an R^2 value of 87%. This demonstrated that the three factors considered in the analysis contributed a significant amount of the observed variability to the data. The residuals within the model were generally well behaved, and appear to have been consistent with stochastic error. The errors also appear to have been approximately normally distributed and thus the regression model was considered to have been appropriate to describe the data. Based on the residuals analysis, which demonstrated the normality of the errors not characterized by the model, it was considered likely that the remaining error within the model could be attributed to analytical error.

Eichhorn et al., (2005) postulated that increased transformation rates can be attributed to the presence of ammonia monooxygenase enzyme, produced by AOBs, which has been shown to transform a variety of compounds with aromatic rings. The author also noted that the involvement of AOBs has been demonstrated through experiments using AOB inhibitors in which significantly reduced transformations occurred as a result of their addition. SRT was noted to have been a statistically significant factor in the transformation efficiencies achieved throughout this experiment. However, as Figure 4.11 and 4.12 demonstrate TRIM removals from reactors E and D, which achieved full nitrification and no nitrification, respectively, it appears that nitrification performance did not noticeably contribute to TRIM transformation efficiencies. Both reactors E and D achieved mean TRIM transformation efficiencies which were below 10%.

In the current study the F value calculated for IFAS was noted to be significantly larger than for SRT and temperature, suggesting that the presence of IFAS was the most significant contributor to variability within the transformation efficiency data. This was noted to be similar to the findings reported by Göbel et al., (2007) in which the relationship of SRT was found to be process specific. Göbel et al., (2007) found enhanced eliminations were achieved in an MBR pilot when operated under elevated SRTs (approximately 90% at θ = 60 to 80d) but did not find that SRT influenced the transformation rates achieved through CAS.

ATENOLOL

ATEN concentrations within the primary effluent were estimated based on the measured concentrations within the initial and final samples as well as the known volumes of primary effluent conveyed to each reactor as feed, and the mixed liquor remaining at the end of each treatment cycle. Mean primary effluent ATEN concentrations during Phase 1, 2 and 3 were estimated to be 1360, 2060 and 1310, respectively. An evaluation using ANOVA and a confidence limit of 95% (**Appendix G**) found that the estimated primary effluent concentrations of ATEN were statistically different during the three periods in which samples were collected.

Nikolai et al., (2006) measured ATEN in Canadian WWTP influents and effluents and reported a range of concentrations from 650 to 1100 ng/L and 160 to 775 ng/L, respectively. The ATEN concentrations measured within the initial and final samples collected during this investigation were noted to be consistent with the values reported by Nikolai et al., (2006). Estimated mean primary effluent concentrations were therefore elevated above the ATEN concentrations reported for influents. However, Radjenovic et al., (2009) reported ATEN concentrations within primary effluent which ranged between 840 and 2800 ng/L. When viewed collectively, the ATEN concentrations estimated to have been present in the primary effluent were considered to be within the range of those reported by other studies.

According to results published by Escher et al., (2006) ATEN is excreted from the body largely unmetabolized (70-96%) with a small percentage of atenolol-glucuronide (<5%) and hydroxyatenolol (<5%). This may have accounted for the reduced temporal variability observed for ATEN during Phase 1 and 3 when compared to CBZ and TRIM. However, the cause of the significant increase in ATEN concentrations within primary effluent during phase 2 remains uncertain.

Box and whisker plots were created to provide a visual indication of the ATEN transformations occurring within the IFAS and control reactors under the four experimental conditions. These plots are presented as Figures 4.13 to 4.16. ATEN demonstrated improved transformation efficiencies under all four experimental conditions for the IFAS reactors when compared to their respective controls. However, the most significant transformation efficiency difference between the IFAS and control SBRs was observed under the low SRT, low temperature condition. This condition was noted to be the only condition in which nitrification was not achieved for the control SBR. A similar relationship between the activity of nitrifying bacteria and ATEN removal has been observed previously (Sathyamoorthy et al., 2013). However, a linkage between ATEN removal and the activity of heterotrophs was reported in the prior study.

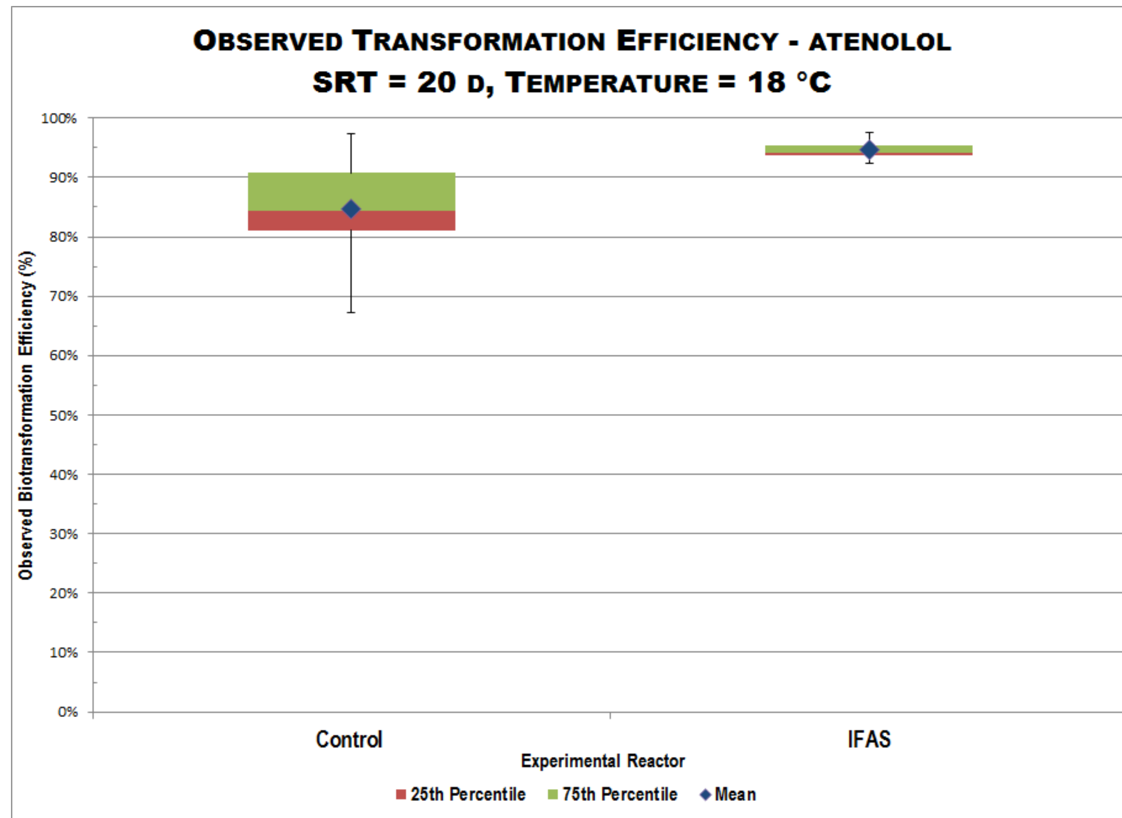


Figure 4.13 – ATEN transformation efficiency for IFAS and Control at 20 d SRT and 18°C

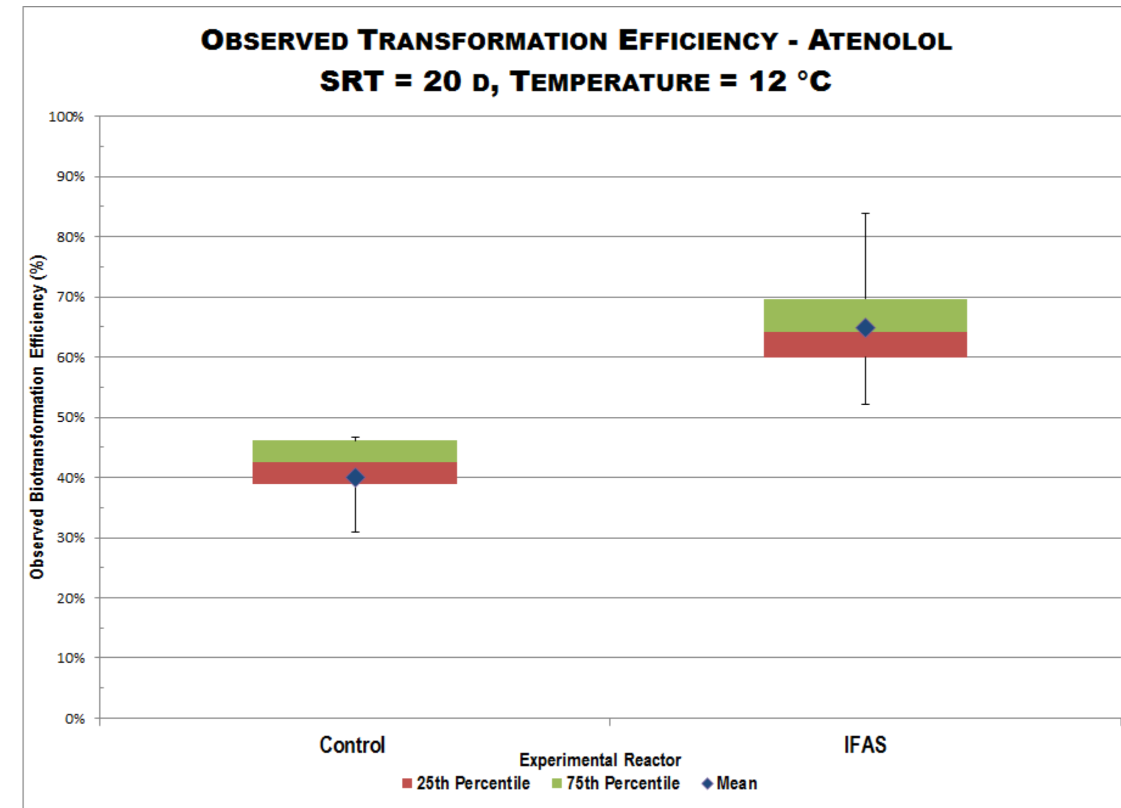


Figure 4.14 – ATEN transformation efficiency for IFAS and Control at 20 d SRT and 12°C

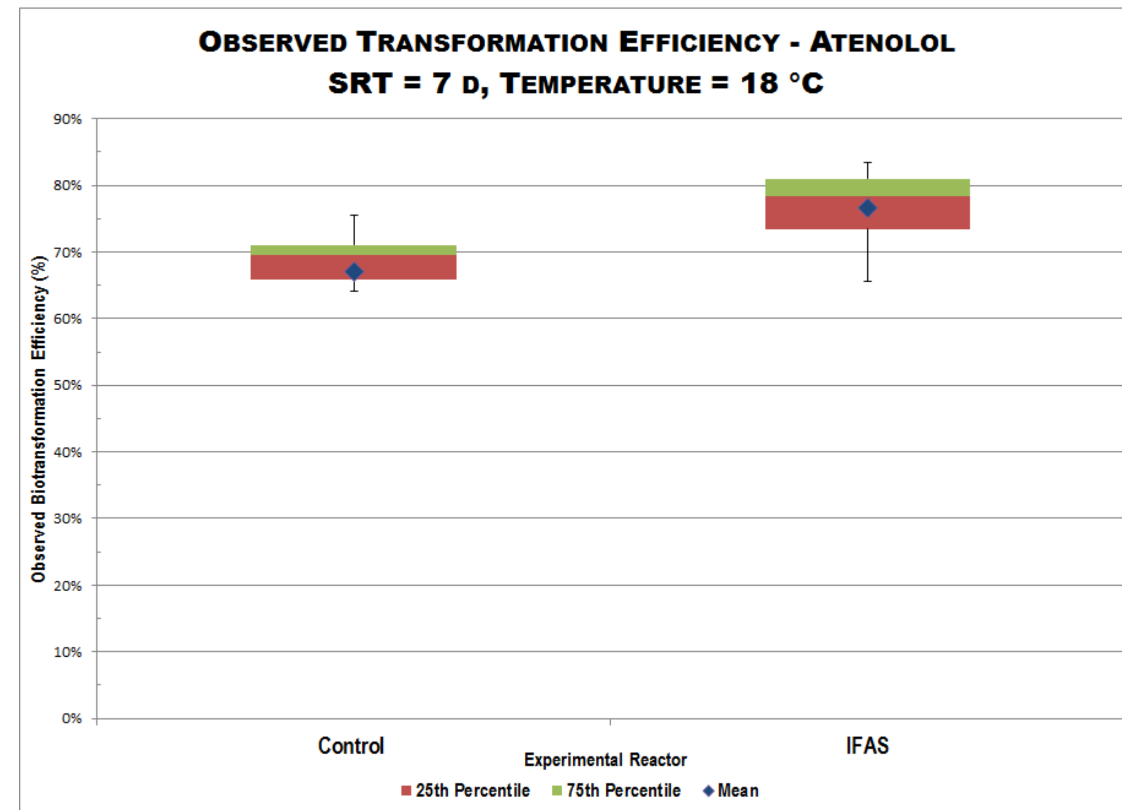


Figure 4.15 – ATEN transformation efficiency for IFAS and Control at 7 d SRT and 18°C

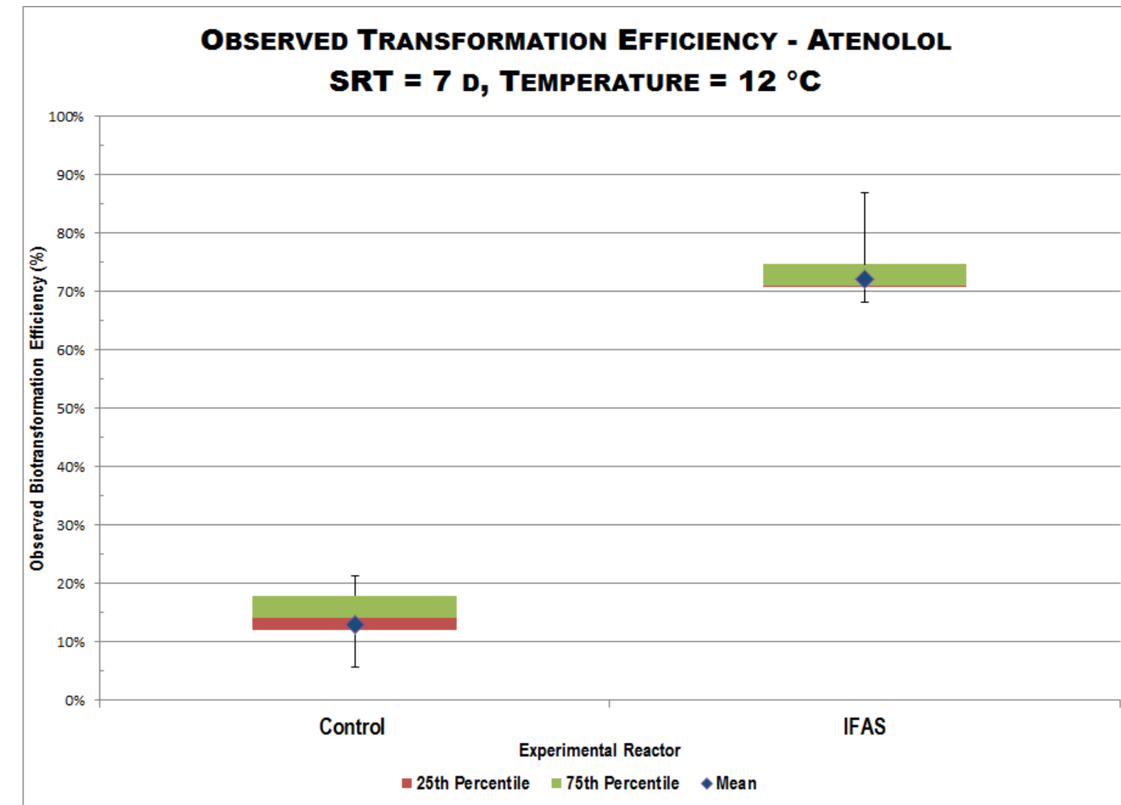


Figure 4.16 – ATEN transformation efficiency for IFAS and Control at 7 d SRT and 12°C

All ATEN transformation efficiency data was combined and plotted as a histogram to determine the most consistent transformation efficiencies observed. The transformation efficiency data was noted to demonstrate a general clustering around 50%, however it was noted that transformation efficiencies ranged between 0 and >90%. Due to the high concentration of influent ATEN, the effluent MQLs resulted in a reduced truncation of the transformation data. This range of transformation efficiencies was consistent with previous results reported in the literature (Radjenovic et al., 2007; Maurer et al., 2007; Radjenovic et al., 2009; Rosal et al., 2010). In these studies, removals were reported to range from <10% to approximately 80% under a variety of processes.

Based on ANOVA analysis, SRT, temperature and the presence of IFAS were found to be statistically significant influences of the transformation efficiencies achieved for ATEN. Based on the reported F values, the most significant contribution to the variability within the transformation data was temperature, with IFAS contributing slightly less than temperature, yet demonstrated a much greater effect than SRT. Two significant two-way and one significant three-way interaction were noted for SRT*IFAS and temperature*IFAS as well as SRT*Temperature*IFAS which suggests that these factors likely combined to result in improved transformation efficiencies. Based on the effects plot (**Appendix G**) it was observed that a constant increase in the ATEN transformation efficiency occurred as a result of increases of SRT and temperature as well as the inclusion of IFAS media. This further demonstrates that all factors contributed to the transformation efficiencies achieved.

The general linear model used to describe ATEN transformation was noted to result in an R^2 value of 93%. This demonstrated that the three factors considered in the analysis contributed a significant amount of the observed variability to the data. The residuals within the model were generally well behaved, and appear to be consistent with stochastic error, with the exception of one minor outlier (0.18). The errors also appeared to be approximately normally distributed and thus the regression model was considered to have been appropriate to describe the data. Based on the residual analysis, it was considered likely that the remaining error within the model was the result of analytical error.

Castiglioni et al., (2006) reported that transformation efficiencies at Italian WWTPs demonstrated significant seasonal variance. Under winter conditions, ATEN transformation efficiencies were observed to range from 0 to 21%, whereas under summer conditions transformations efficiencies were increased to between 36 and 76%. This was consistent with the results of this investigation in which temperature was found to generate the largest F value, which suggested that temperature was the most significant contributor to variability within the transformation efficiency data. HRT has also been cited as a potential operational factor influencing transformation rates of ATEN (Maurer et al., 2007). The IFAS SBBRs and the SBR controls were operated at HRTs of 10.8 and 9 hours, respectively. As the presence of IFAS was found to also be statistically significant, it is possible that the effects of increased HRT were captured by this factor.

Radjenovic et al., (2008) conducted a study which elucidated one biotic pathway (hydrolysis of amide bond) which results in the conversion of ATEN to Atenololic Acid. This paper also indicated that both the parent compound and the formed atenololic acid were not detected after 2 days and 20 days from the batch test using MBR sludge whereas

atenololic acid remained in reactors containing CAS sludge. Additionally, a higher conversion of atenololic acid appeared to occur from the MBR sludge (up to 90%) whereas CAS sludge only achieved a conversion of 40-60%. As atenololic acid was not investigated under this study, it cannot be determined whether a similar result occurred and whether this may explains the higher removals observed in the IFAS SBBRs.

5. CONCLUSIONS AND RECOMMENDATIONS

Four IFAS SBBRs and four control SBRs were operated with over a range experimental conditions in a 2^2 factorial design to investigate whether IFAS processes can enhance PC removals when compared to conventional activated sludge. The experimental conditions involved operation at different combinations of SRT and mixed liquor temperature. The reactors were investigated under three phases under which the performance of the reactors was investigated through the measurement of the following:

- Conventional parameters (tCOD, sCOD, TAN, NO_3-N) within the initial and final samples;
- Operational parameters (MLSS, MLVSS, ESS); and
- The transformation efficiencies achieved for 5 PC (CBZ, SMX, TRIM, ATEN and ACE).

During all three phases of PC sampling, the pilot reactors were found to have been performing as anticipated with respect to conventional contaminant removals. Organic removals were found to be statistically similar between the IFAS and control reactors across all four experimental conditions. Full nitrification was observed for all reactors with the exception of the control SBR operated under the low SRT, low temperature condition. The IFAS SBBRs were found to demonstrate improved nitrification kinetics when compared to their respective controls operated under the same experimental conditions. This was believed to be related to the more diverse bacterial consortia present as a result of the IFAS biofilms. All reactors were generally believed to be operating at steady state and were within an acceptable range of the target operating conditions.

Due to complications associated with the analysis of samples, only CBZ, TRIM, ATEN and ACE could be successfully quantitated. CBZ was found to not have been transformed to any appreciable level across all conditions investigated through either the IFAS SBBRs or control SBRs. ACE was transformed at efficiencies greater than 99% under all conditions and in both IFAS and control reactors and therefore no comparison could be made. TRIM and ATEN demonstrated improved transformation efficiencies under all conditions within the IFAS reactors. The presence of IFAS media, SRT and temperature were all found to be statistically significant effects through ANOVA using a confidence limit of 95%. It is recommended that the transformation efficiencies of SMX be further investigated using properly functioning analytical equipment to confirm whether the IFAS process provides improved transformation efficiencies as predicted and confirmed for TRIM and ATEN.

Based on the observed performance regarding conventional contaminants, the IFAS process demonstrated a significant improvement over a similarly sized activated sludge reactor. Additionally, the activated sludge reactors operated under low temperature conditions both developed filamentous organisms which resulted in poor settling and biomass losses. In the case of the control operated at an SRT of 20 days, nitrification was lost as a result of biomass washout. The IFAS reactors, which were operated under the same experimental conditions and received the same primary effluent, did not appear to be similarly affected. Based on the conventional data observed, the IFAS process appears to provide a robust treatment process which provided improved nitrification capabilities under most

conditions. However, the increased energy requirements associated with bioreactor operation at a D.O. of 4 mg/L compared to 2 mg/L may warrant fiscal analysis for suitability.

The conditions under which the reactors were operated were considered atypical relative to most full-scale IFAS systems. IFAS systems are generally operated at an SRT ranging from 2 to 8 days. It is therefore recommended that additional research be completed to further characterize the PC transformation efficiencies achievable under more realistic operational conditions. Similarly, an investigation into the specific contributions of the biofilms to both organic removal and nitrification is warranted. The contributions of heterotrophs and autotrophs were not specifically investigated under the current study. Additional investigations involving conditions conducive to the selective culturing of these species would provide useful information regarding the role of each in achieving the PC transformations observed.

Transformation efficiencies were found to be affected by SRT, temperature and IFAS however transformation products were not investigated as part of this study. It is not known what form ACE, TRIM and ATEN was transformed to, nor the toxicity of these transformation products on the aquatic environment. As a result, it cannot be concluded that either reactor investigated achieved reduced toxicity to aquatic organisms which is the fundamental goal of wastewater treatment. As methods develop and reference standards become available, an assessment of the toxicity of the effluents produced by both the control and IFAS process on sensitive aquatic organisms warrants additional investigation.

6. REFERENCES

1. Artan, N., & Orhon, D. (2005). *Mechanism and design of sequencing batch reactors for nutrient removal* (No. 19). Iwa Publishing.

2. Ayarza, J. M., Guerrero, L. D., & Erijman, L. (2010). Nonrandom assembly of bacterial populations in activated sludge flocs. *Microbial ecology*, *59*(3), 436-444.

3. Baker, D. R., & Kasprzyk-Hordern, B. (2011). Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography–mass spectrometry. *Journal of Chromatography A*, *1218*(44), 8036-8059.

4. Batt, A. L., Kim, S., & Aga, D. S. (2007). Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere*, *68*(3), 428-435.

5. Bedner, M., & MacCrehan, W. A. (2006). Transformation of acetaminophen by chlorination produces the toxicants 1, 4-benzoquinone and N-acetyl-p-benzoquinone imine. *Environmental science & technology*, *40*(2), 516-522.

6. Benotti, M. J., Trenholm, R. A., Vanderford, B. J., Holady, J. C., Stanford, B. D., & Snyder, S. A. (2008). Pharmaceuticals and endocrine disrupting compounds in US drinking water. *Environmental Science & Technology*, *43*(3), 597-603.

7. Berk, V., Fong, J. C., Dempsey, G. T., Develioglu, O. N., Zhuang, X., Liphardt, J., ... & Chu, S. (2012). Molecular architecture and assembly principles of Vibrio cholerae biofilms. *Science*, *337*(6091), 236-239.

8. Bisceglia, K. J., Yu, J. T., Coelhan, M., Bouwer, E. J., & Roberts, A. L. (2010). Trace determination of pharmaceuticals and other wastewater-derived micropollutants by solid phase extraction and gas chromatography/mass spectrometry. *Journal of chromatography A*, *1217*(4), 558-564.

9. Bjornberg, C., Lin, W., & Zimmerman, R. (2010). Kinetic evaluation and model simulation of temperature impact on biofilm growth and nitrification in a full-scale MBBR system. *Proceedings of the Water Environment Federation*,*2010*(12), 4146-4171.

10. Boltz, J.P., Leaf, W.R., MCquarrie, J.P., Menniti, A., Daigger, G.T. (2012). *Overcoming Hydraulic Limitations of the Integrated Fixed-Film Activated Sludge and Moving Bed Biofilm Reactor Process.* Florida Water Resources Journal. January 2012, 22-32.

11. Bryers, J. D. (2000). Biofilm formation and persistence. *Biofilms II: Process Analysis and Applications. Wiley-Liss Inc., New York*.

12. Busetti, F., Linge, K. L., Blythe, J. W., & Heitz, A. (2008). Rapid analysis of iodinated X-ray contrast media in secondary and tertiary treated wastewater by direct injection liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, *1213*(2), 200-208.

13. Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., & Zuccato, E. (2006). Removal of pharmaceuticals in sewage treatment plants in Italy.*Environmental Science & Technology*, *40*(1), 357-363.

14. Celiz, M. D., Tso, J., & Aga, D. S. (2009). Pharmaceutical metabolites in the environment: analytical challenges and ecological risks. *Environmental toxicology and chemistry*, *28*(12), 2473-2484.

15. Clara, M., Kreuzinger, N., Strenn, B., Gans, O., & Kroiss, H. (2005). The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, *39*(1), 97-106.

16. Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., & Kroiss, H. (2005b). Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water research*, *39*(19), 4797-4807.

17. Da Silva, M. F., Tiago, I., Veríssimo, A., Boaventura, R. A., Nunes, O. C., & Manaia, C. M. (2006). Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. *FEMS microbiology ecology*, *55*(2), 322-329.

18. Daims, H., Nielsen, J. L., Nielsen, P. H., Schleifer, K. H., & Wagner, M. (2001). In Situ Characterization ofNitrospira-Like Nitrite-Oxidizing Bacteria Active in Wastewater Treatment Plants. *Applied and Environmental Microbiology*, *67*(11), 5273-5284.

19. Daims, H., Purkhold, U., Bjerrum, L., Arnold, E., Wilderer, P., & Wagner, M. (2001b). Nitrification in sequencing biofilm batch reactors: lessons from molecular approaches. *Water Science & Technology*, *43*(3), 9-18.

20. Daughton, C. G., & Ternes, T. A. (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change?. *Environmental health perspectives*, *107*(Suppl 6), 907.

21. Doman, J. (1929). Results of operation of experimental contact filter with partially submerged rotating plates. *Sewage works journal*, 555-560.

22. Eaton, A. D., & Franson, M. A. H. (Eds.). (2005). *Standard methods for the examination of water & wastewater*.

23. Edwards, M., Topp, E., Metcalfe, C. D., Li, H., Gottschall, N., Bolton, P., ... & Lapen, D. R. (2009). Pharmaceutical and personal care products in tile drainage following surface spreading and injection of dewatered municipal biosolids to an agricultural field. *Science of the total environment*, *407*(14), 4220-4230.

24. Eichhorn, P., Ferguson, P. L., Pérez, S., & Aga, D. S. (2005). Application of ion trap-MS with H/D exchange and QqTOF-MS in the identification of microbial degradates of trimethoprim in nitrifying activated sludge. *Analytical chemistry*,*77*(13), 4176-4184.

25. Englert, B. (2007). Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. *US Environmental Protection Agency (EPA)*, 1-72.

26. Envirosim Associates Ltd. (2011). *Impact of Primary Settling on Wastewater Characteristics. The Biowin Advantage 2(4), 1-13.*

27. Escher, B. I., Bramaz, N., Richter, M., & Lienert, J. (2006). Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites using a mode-of-action-based test battery and a QSAR approach.*Environmental science & technology*, *40*(23), 7402-7408.

28. Escher, B. I., & Fenner, K. (2011). Recent advances in environmental risk assessment of transformation products. *Environmental science & technology*,*45*(9), 3835-3847.

29. Falås, P., Baillon-Dhumez, A., Andersen, H. R., Ledin, A., & la Cour Jansen, J. (2012). Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals. *Water research*, *46*(4), 1167-1175.

30. Fatta-Kassinos, D., Meric, S., & Nikolaou, A. (2011). Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and bioanalytical chemistry*, *399*(1), 251-275.

31. Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses.*Behavior research methods*, *41*(4), 1149-1160.

32. Freeman, C., & Lock, M. A. (1995). The biofilm polysaccharide matrix: A buffer against changing organic substrate supply?. *Limnology and Oceanography*,*40*(2), 273-278.

33. Gabb, D. M. D., Ekama, G. A., & Jenkins, D. (1989). Incidence of< i> Sphaerotilus Natans</i> in Laboratory Scale Activated Sludge Systems. *Water Science & Technology*, *21*(4-5), 29-41.

34. Gieseke, A., Purkhold, U., Wagner, M., Amann, R., & Schramm, A. (2001). Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. *Applied and Environmental Microbiology*, *67*(3), 1351-1362.

35. Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., & Giger, W. (2007). Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment*, *372*(2), 361-371.

36. Golet, E. M., Xifra, I., Siegrist, H., Alder, A. C., & Giger, W. (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental Science & Technology*, *37*(15), 3243-3249.

37. Gómez, M. J., Petrović, M., Fernández-Alba, A. R., & Barceló, D. (2006). Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography–tandem mass spectrometry analysis in hospital effluent wastewaters. *Journal of Chromatography A*, *1114*(2), 224-233.

38. Gómez, M. J., Martinez Bueno, M. J., Lacorte, S., Fernández-Alba, A. R., & Agüera, A. (2007). Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast.*Chemosphere*, *66*(6), 993-1002.

39. Gómez, M. J., Gómez-Ramos, M. M., Malato, O., Mezcua, M., & Férnandez-Alba, A. R. (2010). Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river waters using liquid chromatography–quadrupole-time-of-flight mass spectrometry with an accurate-mass database. *Journal of Chromatography A*, *1217*(45), 7038-7054.

40. Gros, M., Petrović, M., & Barceló, D. (2006). Development of a multi-residue analytical methodology based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta*, *70*(4), 678-690.

41. Grote, M., Schwake-Anduschus, C., Michel, R., Stevens, H., Heyser, W., Langenkamper, G., ... & Freitag, M. (2007). Incorporation of veterinary antibiotics into crops from manured soil. *Landbauforschung Volkenrode*, *57*(1), 25.

42. Grubbs, F. E. (1969). Procedures for detecting outlying observations in samples. *Technometrics*, *11*(1), 1-21.

43. Hall, T. G., Smukste, I., Bresciano, K. R., Wang, Y., McKearn, D., & Savage, R. E. (2012). Identifying and Overcoming Matrix Effects in Drug Discovery and Development. *Intech Europe*, *18*, 389-420.

44. Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P. F., Ingerslev, F., Holten Lützhøft, H. C., & Jørgensen, S. E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment-a review. *Chemosphere*, *36*(2), 357-393.

45. Hapeshi, E., Lambrianides, A., Koutsoftas, P., Kastanos, E., Michael, C., & Fatta-Kassinos, D. (2013). Investigating the fate of iodinated X-ray contrast media iohexol and diatrizoate during microbial degradation in an MBBR system treating urban wastewater. *Environmental Science and Pollution Research*,*20*(6), 3592-3606.

46. Harries, J. E., Sheahan, D. A., Jobling, S., Matthiessen, P., Neall, P., Sumpter, J. P., ... & Zaman, N. (1997). Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout.*Environmental Toxicology and Chemistry*, *16*(3), 534-542.

47. Helbling, D. E., Hollender, J., Kohler, H. P. E., & Fenner, K. (2010). Structure-based interpretation of biotransformation pathways of amide-containing compounds in sludge-seeded bioreactors. *Environmental science & technology*,*44*(17), 6628-6635.

48. Hem, L. J., Rusten, B., & Ødegaard, H. (1994). Nitrification in a moving bed biofilm reactor. *Water Research*, *28*(6), 1425-1433.

49. Hernando, M. D., Petrovic, M., Fernández-Alba, A. R., & Barceló, D. (2004). Analysis by liquid chromatography–electrospray ionization tandem mass spectrometry and acute toxicity evaluation for β-blockers and lipid-regulating agents in wastewater samples. *Journal of Chromatography A*, *1046*(1), 133-140.

50. Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., Jansen, J. L. C., & Andersen, H. R. (2011). Determination of sorption of seventy-five pharmaceuticals in sewage sludge. *Water research*, *45*(15), 4470-4482.

51. Hughes, N. C., Wong, E. Y., Fan, J., & Bajaj, N. (2007). Determination of carryover and contamination for mass spectrometry-based chromatographic assays. *The AAPS journal*, *9*(3), E353-E360.

52. Hwang, J. H., & Oleszkiewicz, J. A. (2007). Effect of cold-temperature shock on nitrification. *Water Environment Research*, *79*(9), 964-968.

53. Ingerslev, F., & Halling-Sørensen, B. (2000). Biodegradability properties of sulfonamides in activated sludge. *Environmental Toxicology and Chemistry*,*19*(10), 2467-2473.

54. Janke, D., & Fritsche, W. (1985). Nature and significance of microbial cometabolism of xenobiotics. *Journal of basic microbiology*, *25*(9), 603-619.

55. Jenkins, D., Richard, M. G., & Daigger, G. T. (Eds.). (2004). *Manual on the causes and control of activated sludge bulking, foaming, and other solids separation problems*. IWA publishing.

56. Jones, R., Lambert, R., Manoharan, R., Crane, R., & Campbell, H. (1999, April). Demonstration of an integrated fixed film activated sludge (IFAS) system for enhanced nitrification using a new free moving media. In *Proceedings of the 28th Annual WEAO Technical Symposium, Toronto, Ont., Session* (Vol. 8).

57. Joss, A., Keller, E., Alder, A. C., Göbel, A., McArdell, C. S., Ternes, T., & Siegrist, H. (2005). Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water research*, *39*(14), 3139-3152.

58. Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C. S., ... & Siegrist, H. (2006). Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water Research*,*40*(8), 1686-1696.

59. Joss, A., Siegrist, H., & Ternes, T. A. (2008). Are we about to upgrade wastewater treatment for removing organic micropollutants?. *Water Science & Technology*, *57*(2).

60. Kaewpipat, K., & Grady Jr, C. P. L. (2002). Microbial population dynamics in laboratory-scale activated sludge reactors. *Water Science & Technology*, *46*(1-2), 19-27.

61. Kern, S., Fenner, K., Singer, H. P., Schwarzenbach, R. P., & Hollender, J. (2009). Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. *Environmental science & technology*, *43*(18), 7039-7046.

62. Kim, H. S., Pei, R., Gunsch, C., Gellner, J. W., Boltz, J. P., Freudenberg, B., ... & Schuler, A. J. (2009). Trace Organic Chemical Profiles in Nutrient Removal Systems With and Without Integrated Fixed Film Activated Sludge.*Proceedings of the Water Environment Federation*, *2009*(17), 704-711.

63. Kimura, K., Hara, H., & Watanabe, Y. (2007). Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. *Environmental science & technology*, *41*(10), 3708-3714.

64. Kosma, C. I., Lambropoulou, D. A., & Albanis, T. A. (2010). Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece. *Journal of Hazardous Materials*, *179*(1), 804-817.

65. Kreuzinger, N., Clara, M., Strenn, B., & Kroiss, H. (2004). Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater.*Water Science & Technology*, *50*(5), 149-156.

66. Lam, M. W., Young, C. J., Brain, R. A., Johnson, D. J., Hanson, M. A., Wilson, C. J., ... & Mabury, S. A. (2004). Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environmental Toxicology and Chemistry*, *23*(6), 1431-1440.

67. Larcher, S., & Yargeau, V. (2011). Biodegradation of sulfamethoxazole by individual and mixed bacteria. *Applied microbiology and biotechnology*, *91*(1), 211-218.

68. Lavén, M., Alsberg, T., Yu, Y., Adolfsson-Erici, M., & Sun, H. (2009). Serial mixed-mode cation-and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry. *Journal of Chromatography A*, *1216*(1), 49-62.

69. Lee, H. B., Sarafin, K., Peart, T. E., & Svoboda, M. L. (2003). Acidic pharmaceuticals in sewage-Methodology, stability test, occurrence, and removal from Ontario samples. *Water Quality Research Journal of Canada*, *38*(4), 667-682.

70. Leu, S. Y., Chan, L., & Stenstrom, M. K. (2012). Toward long solids retention time of activated sludge processes: Benefits in energy saving, effluent quality, and stability. *Water Environment Research*, *84*(1), 42-53.

71. Lewandowski, Z., Webb, D., Hamilton, M., & Harkin, G. (1999). Quantifying biofilm structure. *Water Science and Technology*, *39*(7), 71-76.

72. Lewandowski, Z., & Beyenal, H. (2003). Biofilm monitoring: a perfect solution in search of a problem. *Water Science & Technology*, *47*(5), 9-18.

73. Lishman, L., Smyth, S. A., Sarafin, L., Kleywegt, S., Toito, JO., Peart, T., Lee, H.B., Servos, M., Beland, M., Seto, P. (2006). *Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada, Science of the Total Environment*, 367: 544-558

74. Lundström, E., Adolfsson-Erici, M., Alsberg, T., Björlenius, B., Eklund, B., Lavén, M., & Breitholtz, M. (2010). Characterization of additional sewage treatment technologies: Ecotoxicological effects and levels of selected pharmaceuticals, hormones and endocrine disruptors. *Ecotoxicology and environmental safety*, *73*(7), 1612-1619.

75. Maas, C. L., Parker, W. J., & Legge, R. L. (2008). Oxygen uptake rate tests to evaluate integrated fixed film activated sludge processes. *Water Environment Research*, *80*(12), 2276-2283.

76. Majewsky, M., Gallé, T., Yargeau, V., & Fischer, K. (2011). Active heterotrophic biomass and sludge retention time (SRT) as determining factors for biodegradation kinetics of pharmaceuticals in activated sludge. *Bioresource technology*, *102*(16), 7415-7421.

77. Margot, J., Kienle, C., Magnet, A., Weil, M., Rossi, L., de Alencastro, L. F., ... & Barry, D. A. (2013). Treatment of micropollutants in municipal wastewater: Ozone or powdered activated carbon?. *Science of the Total Environment*, *461*, 480-498.

78. Matuszewski, B. K., Constanzer, M. L., & Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry*, *75*(13), 3019-3030.

79. Maurer, M., Escher, B. I., Richle, P., Schaffner, C., & Alder, A. C. (2007). Elimination of β-blockers in sewage treatment plants. *Water research*,*41*(7), 1614-1622.

80. Martins, A. M., Pagilla, K., Heijnen, J. J., & van Loosdrecht, M. (2004). Filamentous bulking sludge—a critical review. *Water Research*, *38*(4), 793-817.

81. McAvoy, D. C., Schatowitz, B., Jacob, M., Hauk, A., & Eckhoff, W. S. (2002). Measurement of triclosan in wastewater treatment systems. *Environmental toxicology and chemistry*, *21*(7), 1323-1329.

82. McMahon, K. D., Martin, H. G., & Hugenholtz, P. (2007). Integrating ecology into biotechnology. *Current opinion in biotechnology*, *18*(3), 287-292.

83. McQuarrie, J. P., & Boltz, J. P. (2011). Moving bed biofilm reactor technology: process applications, design, and performance. *Water Environment Research*,*83*(6), 560-575.

84. Metcalf and Eddy Inc., (2003). Wastewater Engineering: Treatment and Reuse, Fourth Edition McGraw-Hill Inc., New York, NY

85. Miao, X. S., & Metcalfe, C. D. (2003). Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. *Analytical chemistry*, *75*(15), 3731-3738.

86. Miège, C., Choubert, J. M., Ribeiro, L., Eusèbe, M., & Coquery, M. (2008). Removal efficiency of pharmaceuticals and personal care products with varying wastewater treatment processes and operating conditions--conception of a database and first results. *Water Science & Technology*, *57*(1).

87. MOE. (2008). Design Guidelines for Sewage Works, Ministry of the Environment, Ontario.

88. Monroe, D. (2007). Looking for chinks in the armor of bacterial biofilms. *PLoS biology*, *5*(11), e307.

89. Morgan-Sagastume, F., & Allen, D. G. (2003). Effects of temperature transient conditions on aerobic biological treatment of wastewater. *Water research*,*37*(15), 3590-3601.

90. Müller, N. (1998). Implementing biofilm carriers into activated sludge process—15 years of experience. *Water science and technology*, *37*(9), 167-174.

91. Naegele, E., Buckenmaier, S., Frank, M. (2010). Achieving lowest carry-over with Agilent 1290 Infinity LC and LC/MS systems. Agilent Technologies.

92. Namkung, E., Stratton, R. G., & Rittmann, B. E. (1983). Predicting removal of trace organic compounds by biofilms. *Journal (Water Pollution Control Federation)*, 1366-1372.

93. Nikolai, L. N., McClure, E. L., MacLeod, S. L., & Wong, C. S. (2006). Stereoisomer quantification of the-blocker drugs atenolol, metoprolol, and propranolol in wastewaters by chiral high-performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1131*(1), 103-109.

94. Nödler, K., Licha, T., Barbieri, M., & Pérez, S. (2012). Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. *Water research*, *46*(7), 2131-2139.

95. Nurmi, J., & Pellinen, J. (2011). Multiresidue method for the analysis of emerging contaminants in wastewater by ultra performance liquid chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A*, *1218*(38), 6712-6719.

96. Nutt, S., Devlin, P., Thomas, K., Nazareth, V., (2011). Using the IFAS process to Re-Rate the Peterborough WWTP. Water Environmental Association of Ontario.

97. Oaks, J. L., Gilbert, M., Virani, M. Z., Watson, R. T., Meteyer, C. U., Rideout, B. A., ... & Khan, A. A. (2004). Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature*, *427*(6975), 630-633.

98. Odegaard, H., Rusten, B., & Westrum, T. (1994). A NEW MOVING-BED BIOFILM REACTOR-APPLICATIONS AND RESULTS (VOL 29, PG 157, 1994). *Water science and Technology*, *30*(11), R1-R1.

99. Onesios, K. M., Jim, T. Y., & Bouwer, E. J. (2009). Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review.*Biodegradation*, *20*(4), 441-466.

100. Ort, C., & Gujer, W. (2006). Sampling for representative micropollutant loads in sewer systems. *Water Science & Technology*, *54*(6-7), 169-176.

101. Ort, C., Lawrence, M. G., Rieckermann, J., & Joss, A. (2010). Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review.*Environmental science & technology*, *44*(16), 6024-6035.

102. Osenbrück, K., Gläser, H. R., Knöller, K., Weise, S. M., Möder, M., Wennrich, R., ... & Strauch, G. (2007). Sources and transport of selected organic micropollutants in urban groundwater underlying the city of Halle (Saale), Germany. *Water Research*, *41*(15), 3259-3270.

103. Paredes, D., Kuschk, P., Mbwette, T. S. A., Stange, F., Müller, R. A., & Köser, H. (2007). New aspects of microbial nitrogen transformations in the context of wastewater treatment–a review. *Engineering in Life Sciences*, *7*(1), 13-25.

104. Patel, A., Nakhla, G., & Zhu, J. (2005). Detachment of multi species biofilm in circulating fluidized bed bioreactor. *Biotechnology and bioengineering*, *92*(4), 427-437.

105. Pérez, S., Eichhorn, P., & Aga, D. S. (2005). Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. *Environmental Toxicology and Chemistry*, *24*(6), 1361-1367.

106. Petrovic, M., Gros, M., & Barcelo, D. (2006). Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography–quadrupole–time-of-flight mass spectrometry. *Journal of chromatography A*,*1124*(1), 68-81.

107. Petrovic, M., Farré, M., De Alda, M. L., Perez, S., Postigo, C., Köck, M., ... & Barcelo, D. (2010). Recent trends in the liquid chromatography–mass spectrometry analysis of organic contaminants in environmental samples.*Journal of Chromatography A*, *1217*(25), 4004-4017.

108. Pileggi, V. (2007). *Adsorption and partitioning behaviour of selected trace polycyclic synthetic musks in a suspended growth aerobic activated sludge system. Department of Chemical Engineering, Ryerson University, Toronto, ON.*

109. Plósz, B. G., Nopens, I., Rieger, L., Griborio, A., De Clercq, J., Vanrolleghem, P. A., ... & Takács, I. W. (2012). J., Ekama, GA 2012. In *A critical review of clarifier modelling: state-of-the-art and engineering practices. In: Proceedings of 3rd IWA/WEF Wastewater Treatment Modelling Seminar WWTmod* (pp. 26-28).

110. Radjenović, J., Petrović, M., & Barceló, D. (2009). Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water research*, *43*(3), 831-841.

111. Rahman, M. F., Yanful, E. K., Jasim, S. Y., Bragg, L. M., Servos, M. R., Ndiongue, S., & Borikar, D. (2010). Advanced oxidation treatment of drinking water: Part I. Occurrence and removal of pharmaceuticals and endocrine-disrupting compounds from Lake Huron water. *Ozone: Science & Engineering*,*32*(4), 217-229.

112. Ramanathan, R. (Ed.). (2011). *Mass spectrometry in drug metabolism and pharmacokinetics*. John Wiley & Sons.

113. Regional Municipality of Halton Wastewater Treatment Systems 2010 Performance Report (2011). Halton Region.

114. Renew, J. E., & Huang, C. H. (2004). Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography A*, *1042*(1), 113-121.

115. Richardson, M. L., & Bowron, J. M. (1985). The fate of pharmaceutical chemicals in the aquatic environment. *Journal of Pharmacy and Pharmacology*,*37*(1), 1-12.

116. Richardson, S. D., & Ternes, T. A. (2005). Water analysis: emerging contaminants and current issues. *Analytical Chemistry*, *77*(12), 3807-3838.

117. Richardson, S. D., & Ternes, T. A. (2011). Water analysis: emerging contaminants and current issues. *Analytical Chemistry*, *83*(12), 4614-4648.

118. Rodarte-Morales, A. I., Feijoo, G., Moreira, M. T., & Lema, J. M. (2011). Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. *World Journal of Microbiology and Biotechnology*, *27*(8), 1839-1846.

119. Rodil, R., Quintana, J. B., López-Mahía, P., Muniategui-Lorenzo, S., & Prada-Rodríguez, D. (2009). Multi-residue analytical method for the determination of emerging pollutants in water by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*,*1216*(14), 2958-2969.

120. Rosal, R., Rodríguez, A., Perdigón-Melón, J. A., Petre, A., García-Calvo, E., Gómez, M. J., ... & Fernández-Alba, A. R. (2010). Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Research*, *44*(2), 578-588.

121. Santos, J. L., Aparicio, I., Alonso, E., & Callejón, M. (2005). Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors. *Analytica Chimica Acta*, *550*(1), 116-122.

122. Santos, J. L., Aparicio, I., Callejón, M., & Alonso, E. (2009). Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). *Journal of hazardous materials*, *164*(2), 1509-1516.

123. Sathyamoorthy, S., Chandran, K., & Ramsburg, C. A. (2013). Biodegradation and Cometabolic Modeling of Selected Beta Blockers during Ammonia Oxidation. *Environmental science & technology*, *47*(22), 12835-12843.

124. Scheurer, M., Ramil, M., Metcalfe, C. D., Groh, S., & Ternes, T. A. (2010). The challenge of analyzing beta-blocker drugs in sludge and wastewater. *Analytical and bioanalytical chemistry*, *396*(2), 845-856.

125. Schwarzenbach, R. P., Gschwend, P. M., & Imboden, D. M. (2005).*Environmental organic chemistry*. John Wiley & Sons.

126. Servos, M. R., Smith, M., McInnis, R., Burnison, B. K., Lee, B. H., Seto, P., & Backus, S. (2007). The Presence of Selected Pharmaceuticals and the Antimicrobial Triclosan in Drinking Water in Ontario, Canada. *Water Quality Research Journal of Canada*, *42*(2).

127. Shao, B., Chen, D., Zhang, J., Wu, Y., & Sun, C. (2009). Determination of 76 pharmaceutical drugs by liquid chromatography–tandem mass spectrometry in slaughterhouse wastewater. *Journal of Chromatography A*, *1216*(47), 8312-8318.

128. Simonich, S. L., Federle, T. W., Eckhoff, W. S., Rottiers, A., Webb, S., Sabaliunas, D., & De Wolf, W. (2002). Removal of fragrance materials during US and European wastewater treatment. *Environmental Science & Technology*,*36*(13), 2839-2847.

129. Smith, D. L., Dushoff, J., & Morris Jr, J. G. (2005). Agricultural antibiotics and human health. *PLoS Medicine*, *2*(8), e232.

130. Sobeck, D. C., & Higgins, M. J. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. *Water Research*, *36*(3), 527-538.

131. Sriwiriyarat, T., Pittayakool, K., Fongsatitkul, P., & Chinwetkitvanich, S. (2008). Stability and capacity enhancements of activated sludge process by IFAS technology. *Journal of Environmental Science and Health Part A*, *43*(11), 1318-1324.

132. Standley, L. J., Rudel, R. A., Swartz, C. H., Attfield, K. R., Christian, J., Erickson, M., & Brody, J. G. (2008). Wastewater-contaminated groundwater as a source of endogenous hormones and pharmaceuticals to surface water ecosystems. *Environmental toxicology and chemistry*, *27*(12), 2457-2468.

133. Stasinakis, A. S., Kordoutis, C. I., Tsiouma, V. C., Gatidou, G., & Thomaidis, N. S. (2010). Removal of selected endocrine disrupters in activated sludge systems: Effect of sludge retention time on their sorption and biodegradation.*Bioresource technology*, *101*(7), 2090-2095.

134. Steen, R. J., Hogenboom, A. C., Leonards, P. E., Peerboom, R. A., Cofino, W. P., & Brinkman, U. A. T. (1999). Ultra-trace-level determination of polar pesticides and their transformation products in surface and estuarine water samples using column liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A*, *857*(1), 157-166.

135. Struijs, J., Stoltenkamp, J., & Van de Meent, D. (1991). A spreadsheet-based box model to predict the fate of xenobiotics in a municipal wastewater treatment plant. *Water Research*, *25*(7), 891-900.

136. Stumpf, M., Ternes, T. A., Wilken, R. D., Rodrigues, S. V., & Baumann, W. (1999). Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *Science of the Total Environment*, *225*(1), 135-141.

137. Tarcomnicu, I., van Nuijs, A. L., Simons, W., Bervoets, L., Blust, R., Jorens, P. G., ... & Covaci, A. (2011). Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. *Talanta*, *83*(3), 795-803.

138. Ternes, T. A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. *Water research*, *32*(11), 3245-3260.

139. Ternes, T. A. (2001). Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. *TrAC Trends in Analytical Chemistry*, *20*(8), 419-434.

140. Ternes, T. A., Joss, A., & Siegrist, H. (2004). Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment.*Environmental Science & Technology*, *38*(20), 392A-399A.

141. Ternes, T. A., & Joss, A. (Eds.). (2006). *Human pharmaceuticals, hormones and fragrances: the challenge of micropollutants in urban water management*. IWA publishing.

142. Thompson, A., Griffin, P., Stuetz, R., & Cartmell, E. (2005). The fate and removal of triclosan during wastewater treatment. *Water environment research*, 63-67.

143. Togola, A., & Budzinski, H. (2008). Multi-residue analysis of pharmaceutical compounds in aqueous samples. *Journal of Chromatography A*, *1177*(1), 150-158.

144. Trenholm, R. A., Vanderford, B. J., & Snyder, S. A. (2009). On-line solid phase extraction LC–MS/MS analysis of pharmaceutical indicators in water: a green alternative to conventional methods. *Talanta*, *79*(5), 1425-1432.

145. Trovó, A. G., Nogueira, R. F., Agüera, A., Sirtori, C., & Fernández-Alba, A. R. (2009). Photodegradation of sulfamethoxazole in various aqueous media: persistence, toxicity and photoproducts assessment. *Chemosphere*, *77*(10), 1292-1298.

146. Vanderford, B. J., & Snyder, S. A. (2006). Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry.*Environmental science & technology*, *40*(23), 7312-7320.

147. Vanderford, B. J., Mawhinney, D. B., Trenholm, R. A., Zeigler-Holady, J. C., & Snyder, S. A. (2011). Assessment of sample preservation techniques for pharmaceuticals, personal care products, and steroids in surface and drinking water. *Analytical and bioanalytical chemistry*, *399*(6), 2227-2234.

148. Van Der Gast, C. J., Ager, D., & Lilley, A. K. (2008). Temporal scaling of bacterial taxa is influenced by both stochastic and deterministic ecological factors. *Environmental microbiology*, *10*(6), 1411-1418.

149. Van Nuijs, A. L., Tarcomnicu, I., Simons, W., Bervoets, L., Blust, R., Jorens, P. G., ... & Covaci, A. (2010). Optimization and validation of a hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the determination of 13 top-prescribed pharmaceuticals in influent wastewater.*Analytical and bioanalytical chemistry*, *398*(5), 2211-2222.

150. Vogeser, M., & Seger, C. (2010). Pitfalls associated with the use of liquid chromatography–tandem mass spectrometry in the clinical laboratory. *Clinical chemistry*, *56*(8), 1234-1244.

151. Von Sperling, M. (2007). *Activated sludge and aerobic biofilm reactors* (Vol. 5). IWA publishing.

152. Vree, T. B., Hekster, Y. A., Baars, A. M., Damsma, J. E., & Van Der Kleijn, E. (1978). Determination of trimethoprim and sulfamethoxazole (co-trimoxazole) in body fluids of man by means of high-performance liquid chromatography.*Journal of Chromatography B: Biomedical Sciences and Applications*, *146*(1), 103-112.

153. WEF. (2010). Manual of Practice No. 35: Biofilm Reactors, The Water Environment Federation, Alexandria, VA.

154. WHO. (2012). Pharmaceuticals in Drinking Water, World Health Organization Press, Geneva, Switzerland

155. Wilderer, P. A. (1992). Sequencing batch biofilm reactor technology.*Harnessing biotechnology for the 21st century*, 475-479.

156. Wilderer, P. A. (1995). Technology of membrane biofilm reactors operated under periodically changing process conditions. *Water Science and Technology*, *31*(1), 173-183.

157. Wilderer, P., & McSwain, B. (2004). The SBR and its biofilm application potentials. *Water Science & Technology*, *50*(10), 1-10.

158. Wobus, A., & Röske, I. (2000). Reactors with membrane-grown biofilms: Their capacity to cope with fluctuating inflow conditions and with shock loads of xenobiotics. *Water Research*, *34*(1), 279-287.

159. Wolfaardt, G. M., Lawrence, J. R., Robarts, R. D., & Caldwell, D. E. (1998). In situ characterization of biofilm exopolymers involved in the accumulation of chlorinated organics. *Microbial ecology*, *35*(3-4), 213-223.

160. Wunder, D. B., Bosscher, V. A., Cok, R. C., & Hozalski, R. M. (2011). Sorption of antibiotics to biofilm. *Water research*, *45*(6), 2270-2280.

161. Zhang, X., Oakes, K. D., Luong, D., Metcalfe, C. D., & Servos, M. R. (2011). Solid-phase microextraction coupled to LC-ESI-MS/MS: evaluation and correction for matrix-induced ionization suppression/enhancement for pharmaceutical analysis in biological and environmental samples. *Analytical chemistry*, *83*(17), 6532-6538.

162. Zupanc, M., Kosjek, T., Petkovšek, M., Dular, M., Kompare, B., Širok, B., ... & Heath, E. (2013). Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation and UV treatment. *Ultrasonics sonochemistry*, *20*(4), 1104-1112.

Appendix A

Profiles of 5 PCs Selected

A.1. Acetaminophen

Acetaminophen (ACE) is an over the counter non-steroidal anti-inflamatory drug which is commonly detected in influent wastewaters in the μg/L range. Yargeua et al. (2007) detected ACE in Quebec surface water samples, illustrating that it is an environmental contaminant of concern within Canada. Table A1 presents some of the relevant physicochemical parameters of ACE.

Table A1 – Physico-chemical Parameters for ACE

Compound	K_H ($pa \cdot m^3 \cdot mol^{-1}$)	K_d (L/gSS)	Log K_{ow}[1]	pKa
Acetaminophen (ACE)	1.27×10^{-11} [(a)]	0.0004[(b)] <0.03[(e)]	0.46 [(a)]	1.72, 9.82[(d)]

Notes:

a) Values obtained from Jones et al. 2006.
b) Calculated based on Kow by Jones et al. 2002.
c) Measured Sorption Coefficient for inactivated Activated sludge. Biodegradation occurred rapidly limiting sorption measurements to fresh activated sludge. (Stevens-Garmon et al. 2011)
d) Ternes and Joss, 2006.

Based on the henry's law coefficient and reported sorption coefficients, losses due to volatilization and sorption to solids is expected to be insignificant.

Past studies have reported that Acetaminophen is very infrequently detected in wastewater effluents (Ternes 1998, Gros et al. 2006, Rosal et al. 2010) leading to the common belief that it is rapidly biotransformed or "readily biodegradable". However, Gros et al. (2010) completed a 3 year study of PPCP fate through seven WWTPs and found that ACE was detected in WWTP effluents 93% of the time and in river waters receiving WWTP effluents 89% of the time out of 74 sampling events. Edwards et al. (2009) observed detectable concentrations of ACE in tile drainage approximately 100 d after land application of biosolids occurred, suggesting a potential for groundwater contamination.

Table A2 provides a brief summary of typical ACE transformation rates for full-scale and pilot wastewater treatment processes encountered in the literature.

TABLE A2 – ACE TRANSFORMATION IN LITERATURE		
Pilot/Full Scale/Batch	**Process**	**Removal rate**
Full Scale	CAS, θ = 10d	>99 %[(a)]
Pilot	Flat sheet MBR	>99 %[(a)]
Pilot	Hollow Fibre MBR	>99 %[(a)]
Full Scale	CAS	99 %[(b)]
Full Scale	BNR	>99%[(c)]
Full Scale	CAS, SBR, A_2O, MLE	>95% [(d)]

a) Radjenovic et al. 2009
b) Gomez et al. (2007)
c) Yu et al. (2006)
d) Sim et al. (2010)

Based on the published results, it would appear that the transformation of ACE is a rather rapid process which is not dependent on the operational conditions, such as redox, SRT or HRT. Majewsky et al. (2011) completed a recent transformation batch study in which ACE was found to display transformation kinetics approximately 4 times faster in a CAS process (θ = 6d , τ= 17h) in comparison to an EA process (θ = 54d, τ = 58h). This observation led the author to the conclusion that ACE transformations can be attributed primarily to heterotrophs. However, these experiments were conducted using synthetic feed and non-adapted biomass and therefore may not be reflective of real world conditions.

Prescott (1980) reported that ACE is largely excreted from the body in metabolized forms, approximately 55% as glucuronide conjugates and 30% as sulphate conjugates. Sunkara and Wells (2010) investigated the occurrence of metabolites of ACE in wastewater influents and effluents and detected consistent influent concentrations of sulphate and an additional TP in influents. Effluent results were too variable to be considered conclusive. Several authors have observed transformation products arising from incomplete biodegradation of ACE. Chiron et al. (2010) used in vitro assays and concluded that the nitration and the presence of nitric oxide within nitrifying activated sludge lead to the creation of 3-nitro-ACE and 3-Chloro-5-nitro-ACE. In bioreactor influent, 3-OH-ACE and 3-Chloro-ACE were noted to be present at 27% and 7 to 15%, respectively, relative to the measured ACE concentration. These compounds were not detected in nitrified effluent, indicating that they were transformed.

ACE was selected as a PPCP that is highly hydrophobic and represents a readily transformable compound which is expected to be almost entirely transformed (undetected in effluent). TPs of ACE were not considered in this investigation as there was no access to reference standards or adequate analytical equipment to perform analysis.

A.2. Atenolol

Atenolol (ATEN) is one of the most widely used cardioselective andrenergic blockers (β blocker) used for the treatment of cardiovascular diseases for its antihypertensive, antianginal and antiarrhythmic properties. Nikolai et al. (2006) measured ATEN in Canadian WWTP influents and effluents and found they ranged from 650 to1100 ng/L and 160 to 775 ng/L, respectively. Table A3 presents some of the relevant physicochemical parameters of ATEN.

Table A3 – Physico-chemical Parameters for ATEN

Compound	K_H ($pa \cdot m^3 \cdot mol^{-1}$)	K_d (L/gSS)	Log K_{ow}[1]	pKa
Atenolol (ATEN)	1.38×10^{-18} [a]	0.16 [b] <0.1 [a,c,d]	0.16 [a]	9.67 [a]

Notes:
a) Küster et al. 2010
b) Horsing et al. 2011
c) Stevens-Garmon et al. 2011
d) Radjenovic et al. 2009

Despite the low measured K_d values for ATEN for both primary and secondary sludge, Horsing et al. (2011) estimated that only 85% of ATEN will be in the liquid phase within a bioreactor utilizing a long SRT. A study investigating the effects of land application of biosolids found that ATEN was detected in agricultural tile drains 9 months after biosolids were land applied (Edwards et al. 2009) demonstrating a persistent behaviour, increasing risk of groundwater contamination. Due to the extremely low henry's law constant, contaminant losses due to volatilization can be ignored.

Atenolol is frequently detected in WWTP effluents and in the waters receiving WWTP effluents. Gros et al. (2010) conducted a 3 year study on contaminant fate from seven WWTPs and found that ATEN was detected in wastewater effluents 93% of the time and in the receiving waters 89% of the time. Benotti et al. (2008) found that ATEN was one of the more frequently detected PC's in US drinking water, demonstrating that unintentional uptake is occurring as a result of contamination within the environment.

Table A4 provides a brief summary of typical ATEN transformation rates for full-scale and pilot wastewater treatment processes encountered in the literature.

TABLE A4 – ATEN TRANSFORMATION IN LITERATURE		
Pilot/Full Scale/Batch	**Process**	**Removal rate**
Full Scale	CAS, θ = 8-10d	79%[b]
Full Scale	CAS, θ = 15d	73%[b]
Full Scale	A_2O	14.4%[c]
Full Scale	CAS, θ = 10d	61%[a]
Pilot Scale	FS MBR	77%[a]
Pilot Scale	HF MBR	70%[a]
Full Scale	CAS	<10%[d,e]
Full Scale	CAS	63%[f]
Full Scale	Denit/Nit	37%[f]
Full Scale	Oxidation Ditch	77%[f]
Full Scale (6 WWTPS)	CAS	Winter: 0 to 21%[g] Summer: 36 to 76%[g]

a) Radjenovic et al. 2009
b) Maurer et al. 2008
c) Rosal et al. (2010)
d) Paxeus et al. (2003)
e) Radjenovic et al. (2007)
f) Vieno et al. (2007)
g) Castiglioni et al. (2006)

Based on the data presented in the above table, it appears that ATEN transformation rates are variable and may depend on the treatment process employed. Results published by Vineo et al. (2007) and Rosal et al. (2010) suggest that redox conditions may affect transformation rates as those utilizing de-nitrification process appear to generally observe lower transformations. Additionally, results published by Castiglioni et al. (2006) (included in table above) appear to suggest that temperature is an important factor in the removal of ATEN, suggesting a biological mechanism is responsible. HRT has also been cited as a potential operational factor influencing transformation rates of ATEN (Maurer et al. 2007). Radjenovic et al. (2009) performed sorption experiments using primary and secondary sludges. Primary and CAS sludge appeared to contain a significantly higher sorbed fraction of ATEN relative to MBR or digested sludges.

According to results published by Escher et al. (2006) Atenolol is excreted from the body largely unmetabolized (70-96%) with a small percentage of atenolol-glucuronide (<5%) and hydroxyatenolol (<5%).

Radjenovic et al. (2008) conducted a study which elucidated one biotic pathway (hydrolysis of amide bond) which results in the conversion of ATEN to Atenololic Acid. This paper also indicated that both the parent compound and the formed atenololic acid were not detected after 2 days and 20 days from the batch test using MBR sludge where as atenololic acid remained in the reactors with CAS sludge. Additionally, a higher conversion of atenololic acid appeared to occur from the MBR sludge (up to 90%) whereas CAS sludge only achieved a conversion of 40-60%, suggesting additional TPs may exist. Figure A1 demonstrates the observed results of the batch tests.

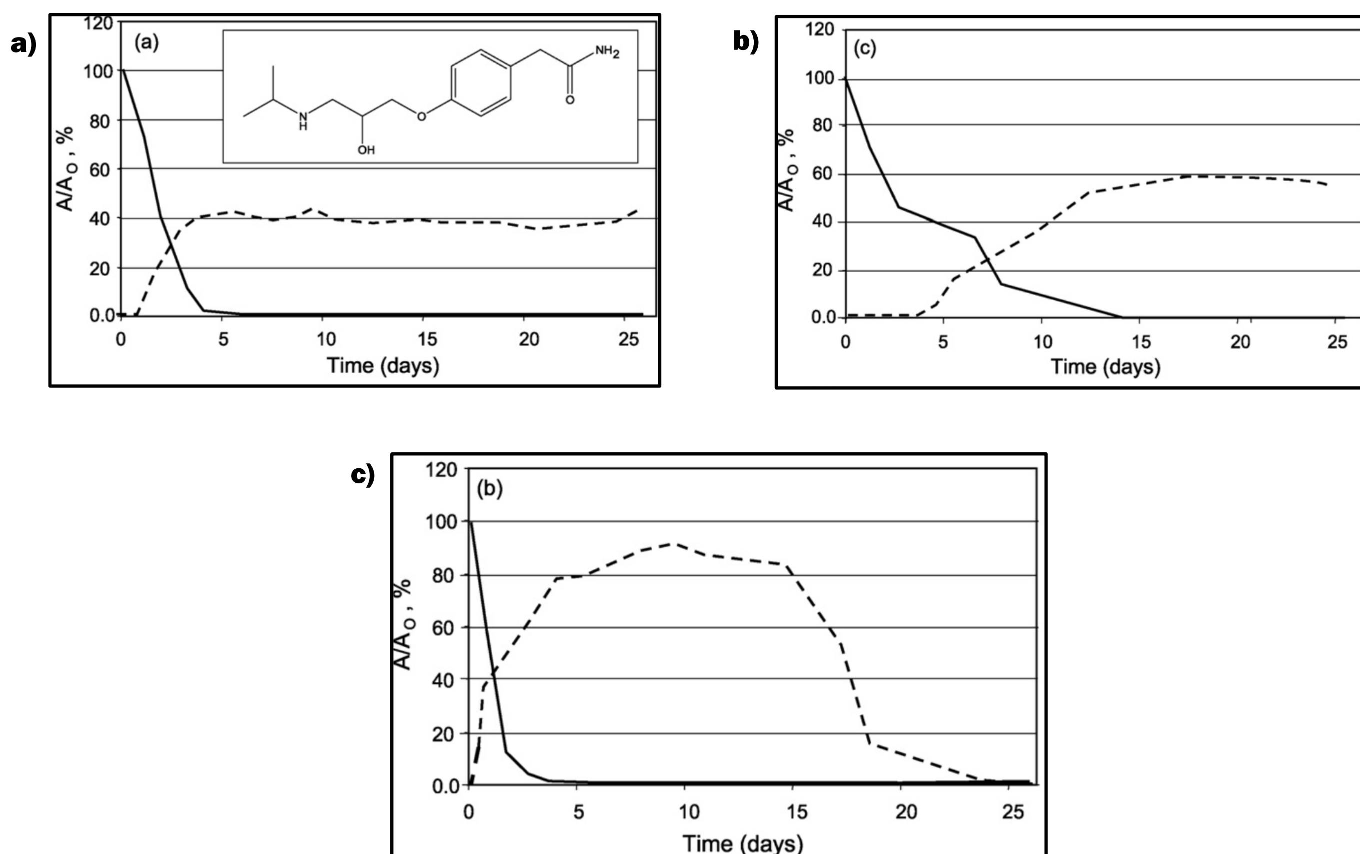


Figure A1 – Measured Concentrations of ATEN (solid line) and Atenololic Acid (dashed line) vs. time from batch testing of a) CAS sludge w/ an initial ATEN concentration of 10 mg/L b) CAS sludge w/ an initial ATEN concentration of 50 µg/L and c) MBR sludge w/ an initial ATEN concentration of 10 mg/L (Radjenovic et al. 2008)

Barbieri et al. (2012) conducted ATEN testing using an aquifer under nitrate reducing conditions and found that a combination of abiotic and biotic mechanisms led to approximately 65% removal of ATEN. However, an overall mass balance conducted on ATEN and atenololic acid suggests that no measurable mineralization occurred throughout the entire 90 d experiment.

ATEN was selected as a PPCP that is largely hydrophobic and represents a high degree of variability in transformation rates achieved depending on process conditions. Atenololic acid was not analyzed as reference standard was not available nor was access to required analytical equipment.

A.3. Carbamazepine

Carbamazepine (CBZ) is an antiepileptic drug which is prescribed to treat psychomotor epilepsy but is also effective in the treatment of trigeminal neuralgia. It is also used to treat bipolar depression (Clara et al. 2004). CBZ has been reported in Canadian wastewater influents and effluents by Metcalfe et al. (2003) at concentrations of approximately 700 ng/L. Yargeau et al. (2007) reported that CBZ was detected in Quebec surface waters at concentrations as high as 106 ng/L.

Table A5 presents some of the relevant physicochemical parameters of CBZ.

Table A5 – Physico-chemical Parameters for CBZ

Compound	K_H (pa·m^3·mol^{-1})	K_d (L/gSS)	Log K_{ow}[1]	pKa
Carbamazepine (CBZ)	1.55 x 10^{-10} [(a)]	<0.135 [(b,c)] ND[(,d)]	2.45[(a)]	13.9 [(a)]

Notes:
a) Ternes and Joss, 2006
b) Stevens-Garmon et al. 2011
c) Radjenovic et al. (2009)
d) Horsing et al. (2011)

Horsing et al. (2011) estimated that all CBZ will be present entirely in the liquid phase of the bioreactor operating under a long SRT. Clara et al. (2004) conducted extensive testing related to the fate of CBZ both in batch and full scale systems. Sorption of CBZ to activated sludge accounted for less than 1% of the total amount dosed to the batch reactors. Sorption to solids in this study can therefore be ignored as a potential removal mecahanism. Due to the extremely low henry's law constant, contaminant losses due to volatilization can also be ignored.

CBZ is frequently detected in WWTP effluents and in the waters receiving WWTP effluents. Gros et al. (2010) conducted a 3 year study on contaminant fate from seven WWTPs and found that CBZ was detected in wastewater effluents 100% of the time and in the receiving waters 100% of the time. Edwards et al. (2009) observed detectable concentrations of CBZ in tile drainage approximately 100 d after land application of biosolids occurred demonstrating its persistence. Benotti et al. (2008) found that CBZ was one of the more frequently detected PPCP's in US drinking water, demonstrating that unintentional uptake is occurring as a result of contamination within the environment. A study completed by Loos et al. (2010) reported CBZ was detected in 43% of groundwater samples extracted from 23 European countries at concentrations as high as 390 ng/L. Clara et al. (2004) also investigated a WWTP that utilizes subsurface discharge of treated effluents and found that Carbamazepine was detected in the groundwaters proximal to the discharge point. Reduced concentrations were believe to be related to dilution of wastewater only.

Table A6 provides a brief summary of typical CBZ transformation rates for full-scale and pilot wastewater treatment processes encountered in the literature.

TABLE A6 – CBZ TRANSFORMATION IN LITERATURE

Pilot/Full Scale/Batch	Process	Removal rate
Full Scale	CAS	~7%[a]
Full Scale	CAS, θ = 2 to 237d	-43 to 14%[b]
Pilot Scale	MBR	ND[b]
Full Scale	A_2O	9.5%[c]
Full Scale	CAS	-122 to 24% [d]
Full Scale	CAS	<10 – 53 % [e]
Pilot Scale	MBR	12[b]
Pilot Scale	MBR, θ= 11d	11[f]
Pilot Scale	MBR, θ= 20d	-8[f]
Pilot Scale	MBR, θ= 41d	9[f]

a) Radjenovic et al. 2009
b) Clara et al. 2005b
c) Rosal et al. (2010)
d) Nakada et al. (2006)
e) Paxeus et al. (2003)
f) Kreuzinger et al. (2004)

The overwhelming majority of studies suggest that CBZ is not transformed to any significant degree and it undergoes very limited biotic and abiotic transformations in the wastewater treatment process. Clara et al. (2004) conducted an investigation using batch reactors operating at a variety of SRTs and found that no transformation occurred at any process condition. Monitoring of 8 full scale WWTPs confirmed that no significant transformations were occurring regardless of process or operational conditions.

Many studies demonstrate that CBZ undergoes negative "removals" which can likely be attributed to de-conjugation reactions occurring within the secondary treatment process, in which the conjugated form undergoes cleavage and the parent compound is released. Small amounts of "removals" can likely be attributed to errors within the data arising from sampling and analytical procedures. CBZ is therefore anticipated to undergo very limited transformation in the wastewater treatment process.

33 metabolites of CBZ have been identified based on investigations analyzing human urine (Lertratanangkoon and Horning, 1982). The main metabolic pathway of carbamazepine is oxidation in the liver followed by conjugation with glucuronide (Miao and Metcalfe, 2003). Only 1-2% of CBZ is excreted from the human body in the parent form, with 10,11-epoxide CBZ being the major metabolite in addition to glucuronide conjugates (Ternes and Daughton, 1999). Miao and Metcalfe (2003) completed a study on metabolites of CBZ and noted that very little transformation occurs for all forms of CBZ throughout the wastewater treatment process. A summary of their findings is provided in Table A7.

TABLE A7 – CBZ TPS DETECTED THROUGH WWTP BY MIAO AND METCALFE, 2003

Compound	Influent Concentration (ng/L)	Effluent Concentration (ng/L)	Surface Water Concentration (ng/L)
CBZ	368.9 ± 5.3	426.2 ± 6.1	0.7 ± 0.0
CBZ-Epoxide	47.2 ± 1.8	52.3 ± 1.2	nd
DBZ-diOH	1571.7 ± 31.0	1325.0 ± 12.2	2.2 ± 0.3
CBZ-2OH	121.0 ± 1.6	132.3 ± 2.1	nd
CBZ-3OH	94.8 ± 2.2	101.5 ± 0.3	nd
CBZ-10OH	8.5 ± 0.6	9.3 ± 0.4	nd

nd – not detected

CBZ was selected as a PPCP that is highly hydrophobic and is consistently found to undergo no transformation, other than de-conjugation, regardless of process conditions. TPs of CBZ were not analyzed as reference standards were not available nor was access to applicable analytical equipment.

A.4. Sulfamethoxazole

Sulfamethoxazole (SMX) is a frequently prescribed sulfonamide antibiotic which is typically taken by the patient with a lower dosage of Trimethoprim (TRIM). SMX is also used in veterinary medicine. Miao et al. (2004) reported that SMX was present in Canadian wastewater effluents at a concentration of 243 ng/L. Yargeua et al. (2007) measured SMX at concentrations as high as 578 ng/L in Quebec surface waters.

Table A8 presents some of the relevant physicochemical parameters of SMX.

Table A8 – Physico-chemical Parameters for SMX

Compound	K_H (pa·m^3·mol^{-1})	K_d (L/gSS)	Log K_{ow}[1]	pKa
Sulfamethoxazole (SMX)	6.42×10^{-13} [(a)]	0.256 [(b)] <0.150[(c)]	0.89[(a)]	5.81 1.4 [(a)]

Notes:
a) Ternes and Joss, 2006
b) Göbel et al., 2005
c) Stevens-Garmon et al. 2011

Horsing et al. (2011) estimated that 97% of SMX will be contained in the liquid phase within a bioreactor operating under a long SRT. Göbel et al. (2004) projected that at a full scale WWTP approximately 1 to 2% of total influent SMX mass present will be sorbed to solids. Due to the extremely low henry's law constant, contaminant losses due to volatilization can be ignored.

SMX is reported to undergo limited attenuation through wastewater treatment and is frequently detected in environmental matrices. Gros et al. (2010) conducted a 3 year study on contaminant fate from seven WWTPs and found that SMX was detected in wastewater effluents 100% of the time and in the receiving waters 100% of the time. This has raised concerns over the proliferation of antibiotic resistant bacteria resulting from chronic exposure to sub-lethal dosages of antibiotic compounds (Richardson and Ternes, 2011). Antibiotic resistant bacteria have been reportedly found in the air and soil around farms as well as in surface and groundwater which lead to a ban on antibiotic usage to promote growth in retail meat and poultry within the European Union (Smith et al. (2005). Ferreira

da Silva et al. (20005) investigated anti-biotic resistant bacteria in a WWTP and found that strains of antibiotic resistant enterococci were not being eliminated by wastewater treatment process and certain resistant bacteria were measured at increased levels in wastewater effluent relative to influent. Benotti et al. (2008) found that SMX was the most frequently detected PPCP's in a US drinking water study (at concentrations exceeding 10 ng/L), demonstrating that unintentional uptake is occurring as a result of contamination within the environment.

Table A9 provides a brief summary of typical SMX transformation rates for full-scale and pilot wastewater treatment processes encountered in the literature.

TABLE A9 – SMX TRANSFORMATION IN LITERATURE

Pilot/Full Scale/Batch	Process	Removal rate
Full Scale	A_2O	17%[a]
Full Scale	CAS	[b]
Full Scale	CAS	60%[c]
Full Scale	CAS	-138 to 60 [d]
Full Scale	Biostyr (biofilter)	-61 to 29%[d]
Pilot Scale	MBR HF	80%
Full Scale	CAS-N	75%[e]
Full Scale	Extended Air	75%[e]
Full Scale	Rotating biological contactor	35%[e]
Full Scale	Pure Oxygen AS	48%[e]
Pilot Scale	MBR	99%[f]

a) Rosal et al. (2010)
b) Ternes 2000
c) Carballa et al. 2004
d) Gobel et al. 2007
e) Batt et al. 2007
f) Carballa et al. 2007

Based on the data presented in the above table, it appears that SMX transformation rates are variable and may depend on the treatment process employed. Early studies investigating the biodegradation of SMX were flawed, utilizing activated sludge samples without proper understanding of autotrophic and heterotrophic functions and ignoring the requirements to promote co-metabolism (Ingerslev and halling-Sorensen, 2000). This led to the assumption that activated sludge exposed to mixed liquor required an "adaptation period" of 7-10 d before any significant transformation occurred, but these observations may have been the result of endogenous decay releasing substrates for co-metabolism or the recovery/growth of autotrophs.

Larcher and Yargeua (2011) performed more informed biodegradation studies using pure cultures of typical rodococcus and pseudomonas species typically found in wastewater treatment bioreactors. Two species, R. equi and P. aeruginosa were observed to transform SMX. When glucose was provided as a secondary substrate as well as key nutrients, SMX biotransformation rates of these bacteria increased between 2 and 4 times from what was observed without glucose addition, suggesting co-metabolism is occurring. A transformation product of unknown composition was also detected. However, when present in a mixed culture with 6 other bacteria, the mixed consortia resulted in reduced biotransformation, demonstrating no synergistic effects. Both species are noted to be a facultative anaerobes (Meeuse et al. 2007, Comolli and Donohue, 2002). The author notes that both species were capable of producing a common enzyme (arylamine N-acetyltransferase) which has a specificity for aromatic amines and this may explain the observed transformation of SMX.

Majewsky et al. (2011) recently performed batch experiments with 2 activtated sludges samples, the first from a CAS plant with 6-7d SRT and the second from an EA plant with an SRT of 54d. SMX transformation were observed to occur at relatively similar rates when normalized for active heterotroph populations, leading the researcher to conclude that SRT is not an important operational parameter influencing SMX transformations. Castiglioni et al. (2007) completed a study which investigated the seasonal removal efficiencies of SMX in Spain and found that a WWTP operating in winter “removed” 17% of SMX while in the summer was able to “remove” 71%, suggesting a biotic transformation mechanism. This result could be masked however as the study also observed that SMX concentrations in influents spiked significantly in the winter period. McArdell et al. (2003) also observed a similar trend for macrolide antibiotics.

SMX is excreted from the body predominantly in the metabolized form N^4-Acetylsulfamethoxazole (50%) and only approximately 15% in the parent form along with minor fractions of glucuronide and sulfate conjugates (Vree et al., 1994). Göbel et al. (2004) completed an investigation into both SMX and N^4-Acetyl-SMX and found that the metabolized form was present at approximately 3.25 times higher than the parent form in influent, which is in good agreement with the observations reported by Vree et al. This would suggest that N^4-Acetyl-SMX does not undergo significant transformation prior to secondary treatment. The results of a sampling campaign in which Raw, Primary Effluent, Secondary Effluent and Tertiary Effluent were characterized is provided in Table A10 below.

Table A10 – SMX and N⁴-Acetyl-SMX Fate in 2 WWTPs (Göbel et al. 2004)				
Compound	**Raw Influent Concentration (ng/L)(a)**	**Primary Effluent Concentration (ng/L)(b)**	**Secondary Effluent Concentration (ng/L)(b)**	**Tertiary Effluent Concentration (ng/L)(b)**
SMX	430	430	280	290
N^4-Acetyl-SMX	1400	890	40(c)	10(c)

a) Concentrations are median values based on nine samples
b) Concentrations are median values based on 15 samples
c) Eight of 15 tertiary effluent samples and six of 15 secondary effluent samples were below LOQ (~20 ng/L). These were included in the data set as 0.5 x LOQ.

It would appear based on the results presented above that N^4-Acetyl-SMX is almost entirely removed by typical wastewater treatment processes. SMX however is anticipated to undergo only partial transformation attributed to secondary treatment.

Nodler et al. (2012) elucidated an abiotic transformation pathway occurring under denitrifying conditions within a pilot aquifer experiment. 4-nitro-smx and desamino-smx were detected in the experiment, increasing in concentration concurrent with a decreasing concentration of SMX. This TP appears to be reversible, as a sample obtained after 87 days demonstrated that the parent compound was detected at approximately 50% of the initial concentration. A similar result was achieved by Barbieri et al. (2012) utilizing a similar set up and confirming the mechanism under concentrations of 1 ug/L and 1 mg/L. Figure A2, below, demonstrates the observed concentrations of SMX and 4-nitro-SMX during the experiment.

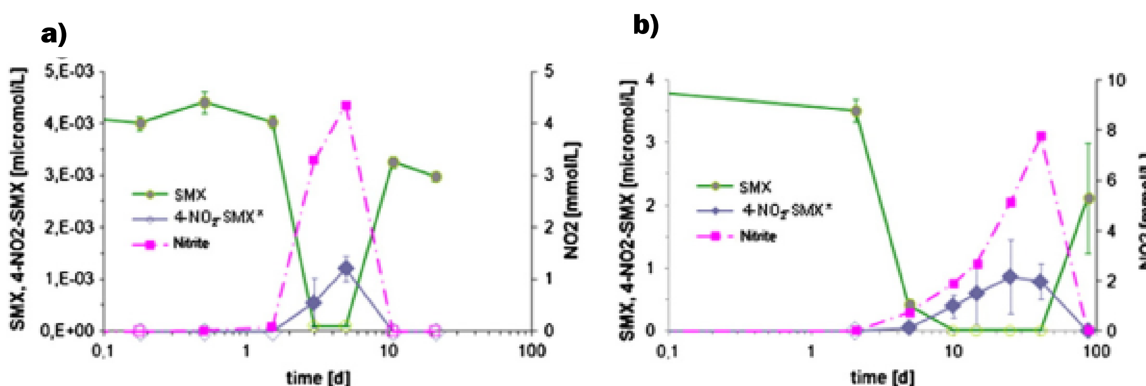


Figure A2 – Results of investigations completed by Barbieri et al. (2012) into N-nitro-SMX in the presence of nitrite at concentrations of a) 1 µg/L and b) 1 mg/L.

SMX was selected as a PPCP that is highly hydrophobic and represents a high degree of variability in transformation rates achieved and uncertainty regarding process conditions affecting these rates. TP's of SMX were not analyzed as reference standards were not available nor was access to applicable analytical equipment.

A.5. Trimethoprim

Trimethoprim (TRIM) is an antimicrobial compound that is almost exclusively taken in conjunction with sulfonamides. A ratio of 1:5 is typically used with SMX and this relationship appears to be more or less observed in raw wastewater received at WWTPs (Göbel et al. 2005). TRIM inhibits enzyme required for folate synthesis and results in a bactericidal effect when taken with SMX resulting in increased efficiency (Poe, 1976). Metcalfe et al. (2003) reported TRIM was detected in Canadian WWTP effluents at concentrations ranging from 9 to 271 ng/L.

Table A11 presents some of the relevant physicochemical parameters of TRIM.

Table A11 – Physico-chemical Parameters for TRIM

Compound	K_H ($pa \cdot m^3 \cdot mol^{-1}$)	K_d (L/gSS)	Log K_{ow}[1]	pKa
Trimethoprim (TRIM)		0.191 to 0.251 [(a)] 0.350[(b)]	0.91	7.12

Notes:
a) Stevens-Garmon et al. 2011
b) Horsing et al. 2011

Horsing et al. (2011) estimated that 96% of TRIM will be in the liquid phase within a bioreactor utilizing a long SRT. Göbel et al. (2004) projected that within a full scale WWTP approximately 1 to 2% of total influent TRIM will be sorbed to solids. Due to the extremely low henry's law constant, contaminant losses due to volatilization can be ignored.

TRIM is reported to undergo highly variable rates of attenuation through wastewater treatment and is frequently detected in environmental matrices. Gros et al. (2010) conducted a 3 year study on contaminant fate from seven WWTPs and found that TRIM was detected in wastewater effluents 96% of the time and in the receiving waters 86% of the time. In a similar fashion to SMX, this has raised concerns over the proliferation of antimicrobial resistant bacteria resulting from chronic exposure to sub-lethal dosages of antimicrobial compounds. Benotti et al. (2008) found that TRIM was one the more frequently detected PPCP's in source waters in a drinking water study, however it

was not detected in treated water. Therefore it can be concluded that unintentional uptake may be occurring as a result of contamination within the environment.

Table A12 provides a brief summary of typical TRIM transformation rates for full-scale and pilot wastewater treatment processes encountered in the literature.

TABLE A12 – TRIM TRANSFORMATION IN LITERATURE		
Pilot/Full Scale/Batch	**Process**	**Removal rate**
Full Scale	A_2O	5%[a]
Full Scale	CAS	20%[b]
Full Scale	Biostyr (biofilter)	25% [b]
Pilot Scale	MBR	80%[b]
Full Scale	CAS-N	70% [c]
Full Scale	Extended Aeration	96% [c]
Full Scale	Rotating Biological Contactor	77%[c]
Full Scale	Pure Oxygen AS	82%[c]
Full Scale	CAS	40%[d]
Pilot Scale	MBR FS	67%[d]
Pilot Scale	MBR HF	47.5%[d]
a) Rosal et al. (2010) b) Gobel et al. (2007) c) Batt et al. (2007) d) Radjenovic et al. (2009)		

Based on the data presented in the above table, it appears that TRIM transformation rates are highly variable and may depend on the treatment process employed. Junker et al. (2006) performed batch testing with ^{14}C labelled TRIM and found that over a period of 21 d no mineralization had occurred with 95% of the parent form being recovered in the soluble phase at approximately 95% of the input. Perez et al. (2005) performed similar testing using sludge/effluent collected from various process units (Primary, non-nitrifying CAS, nitrifying CAS, and chlorinated effluent). TRIM was observed to undergo very little transformation in the simulated Primary and CAS conditions, but experienced rather rapid transformation through the nitrification stage. Eichhorn et al. (2005) postulated that increased transformation rates were due to the presence of ammonia monooxygenase enzyme, produced by AOBs, which has been shown to transform a variety of compounds with aromatic rings. The author also notes that the involvement of AOB's has been demonstrated through experiments using AOB inhibitors in which significantly reduced transformations occured.

Gobel et al. (2004) characterized the fate of TRIM within two WWTPs through analysis of Raw influent, Primary Effluent, Secondary Effluent and Tertiary Effluent. The results of this analysis are provided below, in Table A13.

TABLE A13 – TYPICAL TRIM CONCENTRATIONS MEASURED IN SEQUENTIAL PROCESS UNITS AT 2 WWTPS (GÖBEL ET AL. 2004)

Compound	Raw Influent Concentration (ng/L)[(a)]	Primary Effluent Concentration (ng/L)[(b)]	Secondary Effluent Concentration (ng/L)[(b)]	Tertiary Effluent Concentration (ng/L)[(b)]
TRIM	290	230	200	70

a) Concentrations are median values based on nine samples
b) Concentrations are median values based on 15 samples
c) Eight of 15 tertiary effluent samples and six of 15 secondary effluent samples were below LOQ (~20 ng/L). These were included in the data set as 0.5 x LOQ.

A second investigation completed by Göbel et al. (2007) found enhanced elimination in an MBR pilot when operated under increasing SRT (approximately 90% at θ = 60 to 80d) but did not find that SRT influenced the transformation rates associated with CAS. Numerous negative "eliminations" were observed in CAS reactors, suggesting de-conjugation processes were occurring.

TRIM is excreted from the body approximately 60% in the parent form (Vree et al. 1978). Eichhorn et al. (2005) found 2 TPs of TRIM were found under batch tests utilizing AOB cultures: α-hydoxy-TRIM and a second TP which was attributed to oxidation of the aromatic ring. The concentrations observed by Eichhorn et al. (2005) for TRIM and it's two TP's during the batch testing is presented below as Figure A3.

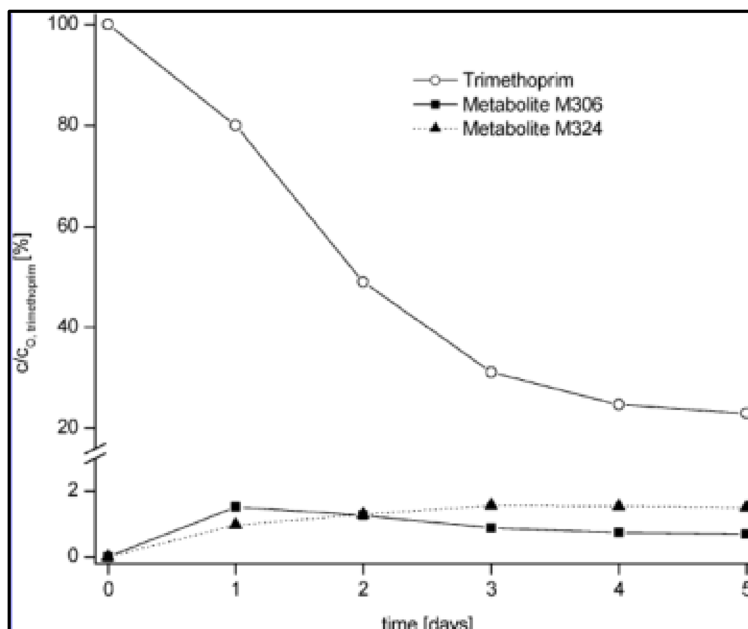


Figure A3 – TRIM and 2 TP's observed during batch testing of AOB culture (Eichhorn et al. 2005)

TRIM was selected as a PPCP that is highly hydrophobic and represents a high degree of variability in transformation rates achieved. It is expected that TRIM's transformation may be variable depending on process conditions. TP's of TRIM were not analyzed as reference standards were not available nor was access to applicable analytical equipment.

Appendix B

Preliminary Investigations

B.1. STATISTICAL CONSIDERATIONS

Investigating PC Transformation Efficiencies

For this experiment, the following simplified model can be used to express the net removal (transformation) of the influent pharmaceutical compounds:

$Y_{PC\ Trans} = \beta_{temp} + \beta_{SRT} + \beta_{IFAS} + \varepsilon$

If we were to use t-testing, we could look at the results from the 4 treatment levels and compare the mean "removal" of each compound at each of the four levels. This would involve t-tests for five compounds at all four levels. The t-test would be based on the following:

$H_{0:}$ $\mu_1 \neq \mu_2$
H_1: $\mu_1 = \mu_2$

Where μ_1 = mean removal of contaminant via control treatment process
μ_2 = mean removal of contaminant via IFAS treatment process

Table B1 provides an explanation of the errors associated with t-testing.

Table B1 - T-testing errors

Case	Error 1	Error 2
Null is true	**Fail to reject Null** (The two means are statistically different when they are truly different) (1- α)	**Reject Null** (The two means are statistically different when they are truly not) α
Null is false	**Reject Null Correctly** The two means are not statistically different when they are truly the same (1- β)	**Fail to reject Incorrectly** The two means are not statistically different when they are truly different β

However, if we utilize a factorial design, we can analyze the data using ANOVA to determine if any of the 3 factors contribute significantly to the variability observed. A factorial design would optimize the experimental capabilities while minimizing sampling requirements.

Power Analysis

We want to first try an a priori analysis to determine, under ideal conditions, how many samples would be required. This testing is only used to compare the means of an experiment at a single level (i.e. two or one tailed t-test).

We would need to first determine the effect size. This can be accomplished by comparing the results obtained for conventional systems and MBBR systems or MBR systems. If we assume that IFAS may have a similar effect size difference, we can narrow down on our required samples.

Effect size for comparison of means is calculated as follows:

$d = (\mu_1 + \mu_2)/\sigma$

Another uncertainty is whether the testing can follow a one tailed t-test.

It is likely that we will have to perform a compromise power analysis due to budget limitations. If we know the sample size and the effect we want to detect we can iterate on alpha and beta error tolerances. We need to define the relative seriousness of the alpha and beta probabilities. In the case of our experiment, we can likely put less emphasis on the beta error as it is unlikely that our control will perform better than the IFAS. In a compromise power analysis we need to define the statistic q which is simply a ratio of beta/alpha.

B.2. Preliminary Investigations of Commercial Lab

The commercial lab selected to do the phase I investigations was noted to be in the development stages of PC analysis. In the interest of due diligence, several preliminary investigations were conducted to assess the robustness and capabilities of the development method. The analytical method was proprietary in nature, and as such limited information was made available regarding the procedures employed. Two initial investigations were completed to accomplish several goals:

- To determine if the HDPE carrier used for IFAS would result in PC losses due to sorption;
- To assess the capabilities of the laboratory method; and
- To determine the anticipated relative standard deviations associated with analysis to inform the experimental design.

B.2.1. Results from Sorption Experiment

The initial investigation, which aimed to investigate sorptive losses associated with the HDPE media, was conducted using tap water spiked with standards dissolved in methanol. The investigation was completed

using a 12L glass pilot reactor, supplemented with 5 L of HDPE IFAS media which had not been exposed to wastewater. Carrier media was soaked in tap water for 48h. The presence of chlorine/chloramine residual was expected to ensure no biological growth was present and therefore no losses would occur from this removal pathway. Synthetic feed water, which was mixed in a HDPE pail, was pumped into the reactor via a peristaltic pump utilizing Teflon tubing. Influent samples were collected directly from the HDPE bucket prior to pumping into the reactor. The remaining feed was then pumped into the bioreactor and a 205 minutes "react" period occurred. Following react, the bioreactor underwent a settle phase for 55 minutes. Effluent samples were then collected from the bioreactor directly. Several batches of feedwater were produced for the initial investigation using volumes of tap water collected from the WTC:

- 11 L of high concentration feed solution (simulating predicted influent concentrations); and
- 11 L of low concentration feed solution (simulating predicted effluent concentrations).

The experiment was run twice, once with high concentration feed and again with low concentration feed. Duplicate influent and effluent samples were collected for each investigation to assess the variability associated with the process. Samples collected under the high strength scenario were frozen for several days prior to analysis. Low strength samples were brought immediately to the commercial lab for analysis. Samples were transported in coolers using ice to maintain a temperature of 4 °C. The results of the analysis received from the lab pertaining to the high strength feedwater and low strength feedwater investigation are provided below in Table B2 and B3.

Table B2 - Analytical results for simulated influent samples

Compound	Dosed Conc. (ng/L)	INF 1 (ng/L)	INF2 (ng/L)	EFF 1 (ng/L)	EFF 2 (ng/L)	Std. Dev. INF	Std. Dev. EFF	% Recovery (average)
Acetaminophen	2,400	123	118	116	124	3.5	5.7	5 %
Atenolol	1,000	851	864	869	885	9.2	11.3	86 %
Carbamazepine	600	418	403	411	414	10.6	2.1	68 %
Sulfamethoxazole	450	<1	<1	<1	<1	-	-	~0 %
Trimethroprim	500	79	77	77	77	1.4	-	16 %
EE2	20	<2	<2	<2	<2	-	-	<10 %

Table B3 Analytical results for simulated effluent samples

Compound	Dosed Conc. (ng/L)	INF 1 (ng/L)	INF2 (ng/L)	EFF 1 (ng/L)	EFF 2 (ng/L)	Std. Dev. INF	Std. Dev. EFF	% Recovery (average)
Acetaminophen	35	19	20	26	24	0.7	1.4	56 %
Atenolol	400	401	414	415	405	9.2	7.1	102 %
Carbamazepine	220	164	166	169	166	1.4	2.1	75 %
Sulfamethoxazole	85	66	68	68	63	1.4	3.5	79 %
Trimethroprim	250	220	219	217	220	0.7	2.1	88 %
EE2	5	2.1	2.3	2.7	2.6	0.1	0.1	44 %

A high degree of precision between duplicate samples as well as influent and effluent samples, was noted in both tests, however the recovery of the spiked compounds under the high strength feedwater test were considered poor (ACE, SMX and TRIM <20%). The recoveries achieved under the low strength feedwater scenario were found to be significantly improved (>50%), however were still considered to be below the standards typically observed in the literature. As a baseline, EPA Method 1694 suggests that a recovery of between 55 and 108 percent for ACE, SMX and TRIM and between 23 and 123 for CBZ of the expected value can be considered as meeting EPA performance criteria. Relative standard deviations (R.S.D.s) of 30% or less are also required to meet minimum performance standards. However, results reported in the literature typically achieve a R.S.D of QA/QC samples of <20% (Santos et al. 2005; Gros et al. 2006; Lishman et al. 2006; Van Nuijs et al. 2010; Tarcomnicu et al. 2011).

The results obtained through the initial investigation were particularly concerning because the sample matrix was considered to contain only minor levels co-eluting compounds leading to signal suppression. It was not clear whether the poor recoveries could be attributed to experimental or analytical error and thus an investigation into the methods utilized was initiated. However, based on the results presented it was determined that sorption to HDPE media was unlikely to contribute to significant PC losses during the investigation.

B.2.2. DIRECT INJECTION VS. SPE TESTING

Upon meeting with the chemist to discuss the results of the initial sorption testing, it was determined that direct injection of samples (no sample preparation) into the LC-MS/MS was being practiced. This methodology is rarely reported in the literature as its accuracy and precision were noted to be poor relative to analysis using SPE. To further assess the capabilities of the direct injection method, a follow up investigation was completed in which a standard addition test was used to estimate the background concentrations in WTC primary effluent. Primary effluent samples were spiked with varying levels of PC standards. By subtracting the known amounts added to the sample, an assessment of the background concentrations present as well as the relative standard deviations (RSD) could be determined.

This investigation was completed using two different sample preparation scenarios: 5 samples underwent the SPE sample preparation procedure identified above, and 5 were analyzed by the direct injection method. Each method was assessed based on samples taken from the same 4L brown glass sampling vessel. The samples were spiked with both varying quantities of unlabelled compounds and a pre-made mix containing many deuterated standards which included the 4

compounds investigated. Each sample had a final concentration of 100 ng/L of deuterated standard for each compound of interest. By analyzing these 5 samples and subtracting the spiked amount, the background concentration of the 4 PCs selected for this investigation could be determined.

The average background sewage concentrations of the compounds of interest, as well as the relative standard deviations (RSD) were then estimated by subtracting the known amount of unlabelled compound spiked into the sample from the measurement reported by the lab (background + spike). Matrix spikes were submitted for comparison and consisted of 100 ng/L of each compound spiked into DI water. The results of both the SPE and direct injection analysis are provided in Tables B4 and B5.

TABLE B4 – RESULTS OF DIRECT INJECTION INVESTIGATION

Sample ID	Volume	Sulfamethoxazole	|Δ|	Trimethroprim	|Δ|	Atenolol	|Δ|	Carbamazepine	|Δ|
Measured (ng/L)									
DI1	100 mL	596		161		952		249	
DI2	100 mL	1420		387		1920		628	
DI3	100 mL	1430		496		2340		677	
DI4	100 mL	1720		884		4400		1210	
DI5	100 mL	2050		1570		5870		2080	
Dosed (ng/L)									
DI1		0		0		0		0	
DI2		10		20		200		20	
DI3		50		100		1000		100	
DI4		250		500		5000		500	
DI5		500		1000		10000		1000	
Background Estimation (ng/L)									
DI1		596		161		952		249	
DI2		1410	814	367	206	1720	768	608	359
DI3		1380	30	396	29	1340	380	577	31
DI4		1470	90	384	12	-600	1940	710	133
DI5		1550	80	570	186	-4130	3530	1080	370
AVERAGE		1281		376		1337		645	
Std. Dev		389		145		384		298	
Std. Dev Rel to mean		30.3%		38.7%		28.7%		46.3%	

*** NOTE: Atenolol - First 3 Only considered

TABLE B5 – RESULTS OF SPE INVESTIGATION

Sample ID	Volume	Sulfamethoxazole	|Δ|	Trimethroprim	|Δ|	Atenolol	|Δ|	Carbamazepine*	|Δ|
Measured (ng/L)									
SP0	100 mL	724		220		1540		Deleted Value (3000)	
SP1	100 mL	904		293		1960		663	
SP2	100 mL	967		337		2320		619	
SP3	100 mL	1170		613		4300		1080	
SP4	100 mL	1600		1170		7680		1810	
MS1	100 mL	63		64		77		109	
Dosed (ng/L)									
SP0		0		0		0		0	
SP1		10		20		200		20	
SP2		50		100		1000		100	
SP3		250		500		5000		500	
SP4		500		1000		10000		1000	
MS1		100		100		100		100	
Background Estimation (ng/L)									
SP0		724		220		1540			
SP1		894	170	273	53	1760	220	643	
SP2		917	23	237	36	1320	440	519	124
SP3		920	3	113	124	-700	2020	580	61
SP4		1100	180	170	57	-2320	1620	810	230
MS1									
Average		911		203		1540		638	
Std. Dev		133		62		220		125	
RSD		14.6%		30.8%		14.3%		19.6%	

*** NOTE: Atenolol - First 3 Only considered

The results obtained for the direct injection investigation were highly variable, with calculated RSD values which were approximately twice those typically encountered in the literature (Santos et al. 2005; Gros et al. 2006; Lishman et al. 2006; Van Nuijs et al. 2010; Tarcomnicu et al. 2011). Variability between samples appeared to be most significant for samples that were not spiked with any standards and under the highest spiked concentrations. This demonstrates poor linearity associated with the calibration curve using these methods. The results obtained using the direct injection method were considered to be unacceptable and this method was abandoned.

The results for the SPE analysis produced an acceptable RSD for SMX and CBZ, however, ATEN and TRIM were noted to demonstrate poor reproducibility at elevated concentrations, demonstrating poor linearity of the calibration curve. This was most notable for Atenolol that appeared to be subject to significant ion suppression when concentrations exceeded approximately 2 µg/L. Similarly, the MS recoveries for SMX and TRIM met the minimum acceptable criteria under EPA method 1694, but were found to be outside the typical recoveries reported in literature. It should be noted that despite the addition of isotopically labelled standards (ILSs), their concentrations were not quantified by the chemist. It was believed that the use of the isotope dilution method (Vanderford and Snyder, 2006) would result in better recoveries, and significantly reduced RSD values. It was therefore determined that use of the isotope dilution method would be an absolute requirement for all future analysis.

The RSD calculated from these analyses was used to determine the required number of samples to achieve what was considered an acceptable level of certainty in the test results. The software package G*power 3 (Faul et al. 2009) was used in an iterative manner to determine the required sample size (n) based on a variety of levels of certainty. Figure B1 to B4 demonstrate the required sample sizes based on the variability observed through SPE testing and a variety of levels of statistical certain for α and β for ATEN, TRIM, SMX and CBZ.

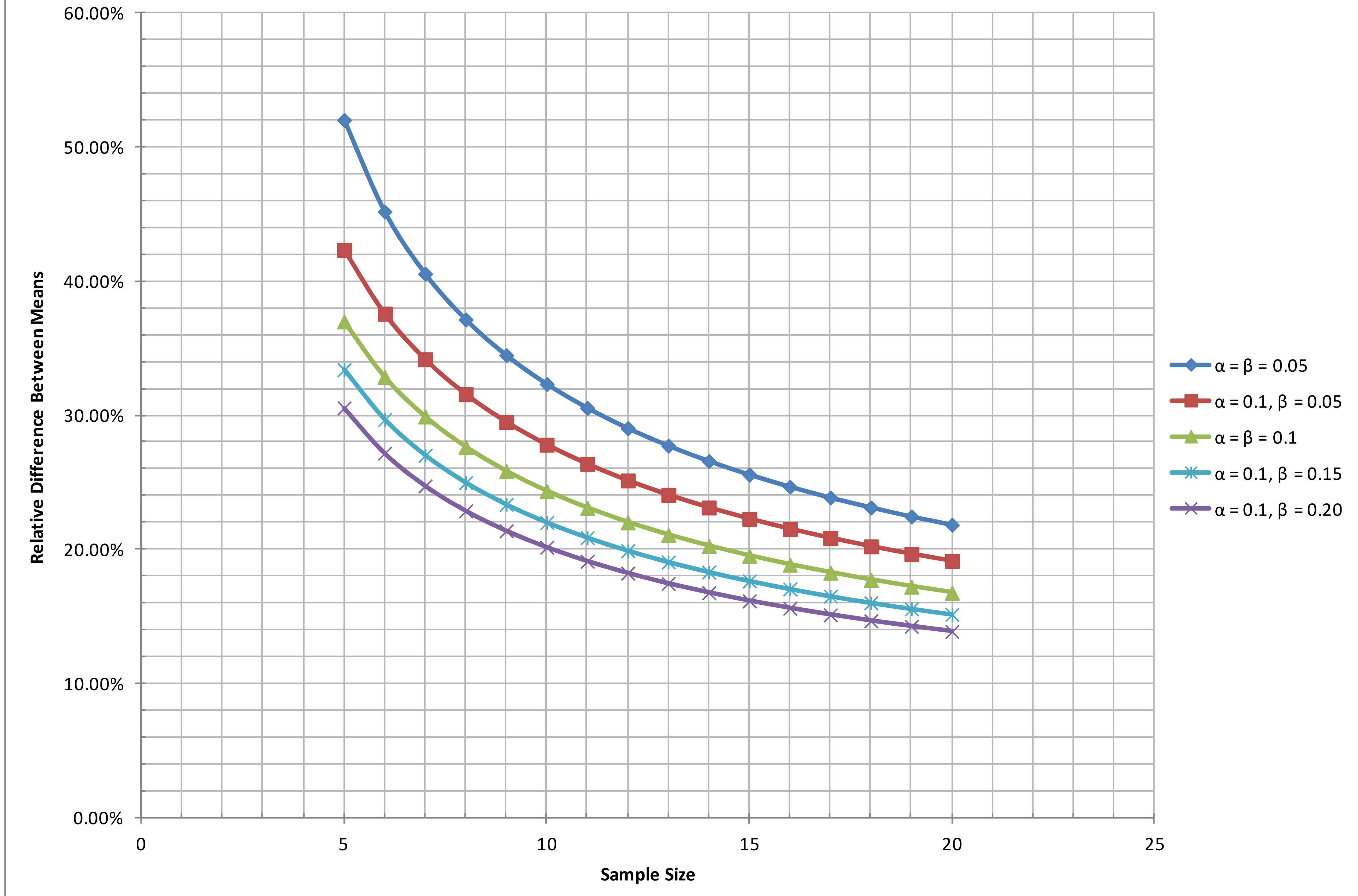


Figure B1 - Minimum Sample Size vs. Difference in Transformation Efficiencies - ATEN

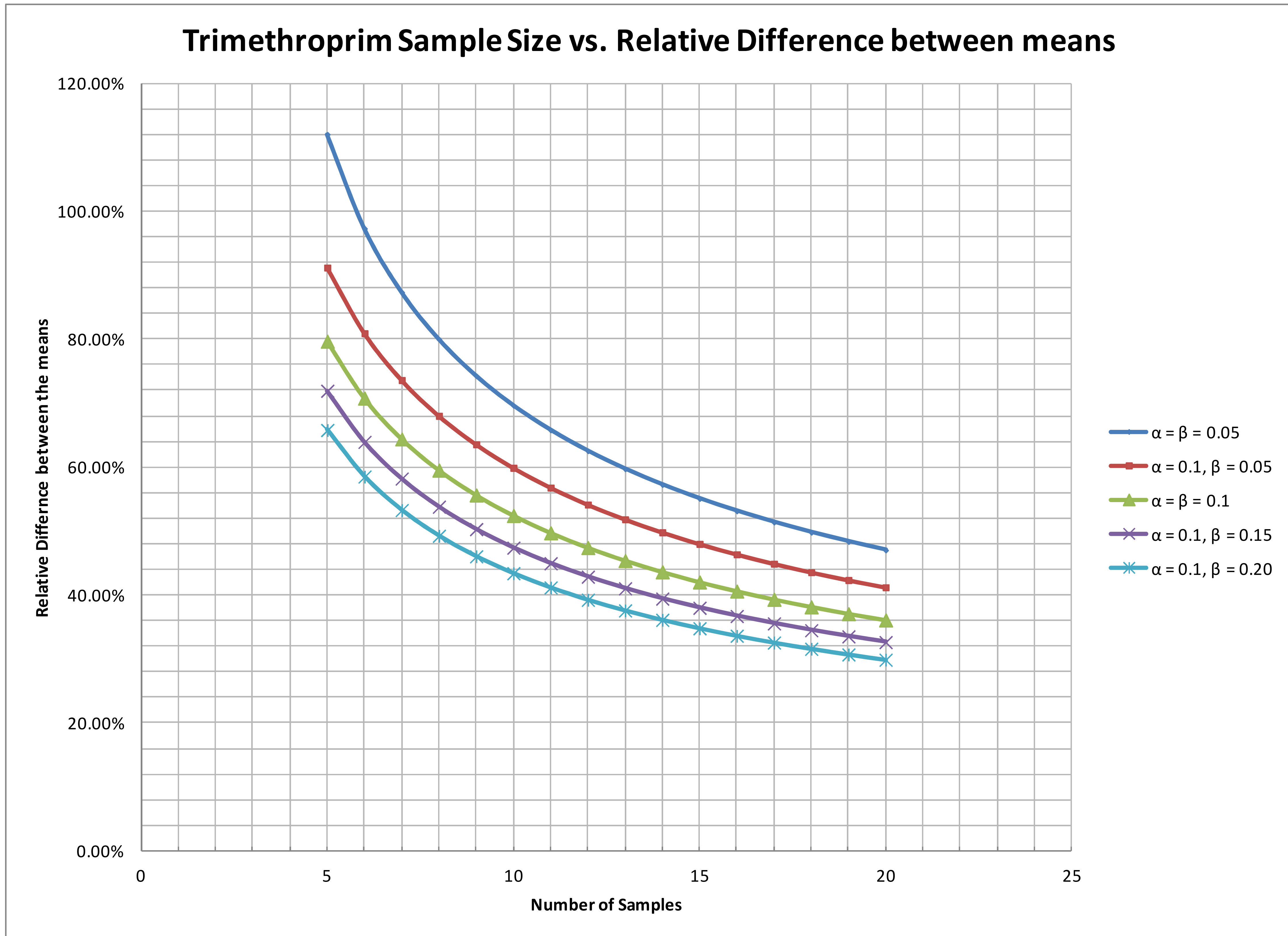


Figure B2 - Minimum Sample Size vs. Difference in Transformation Efficiencies - TRIM

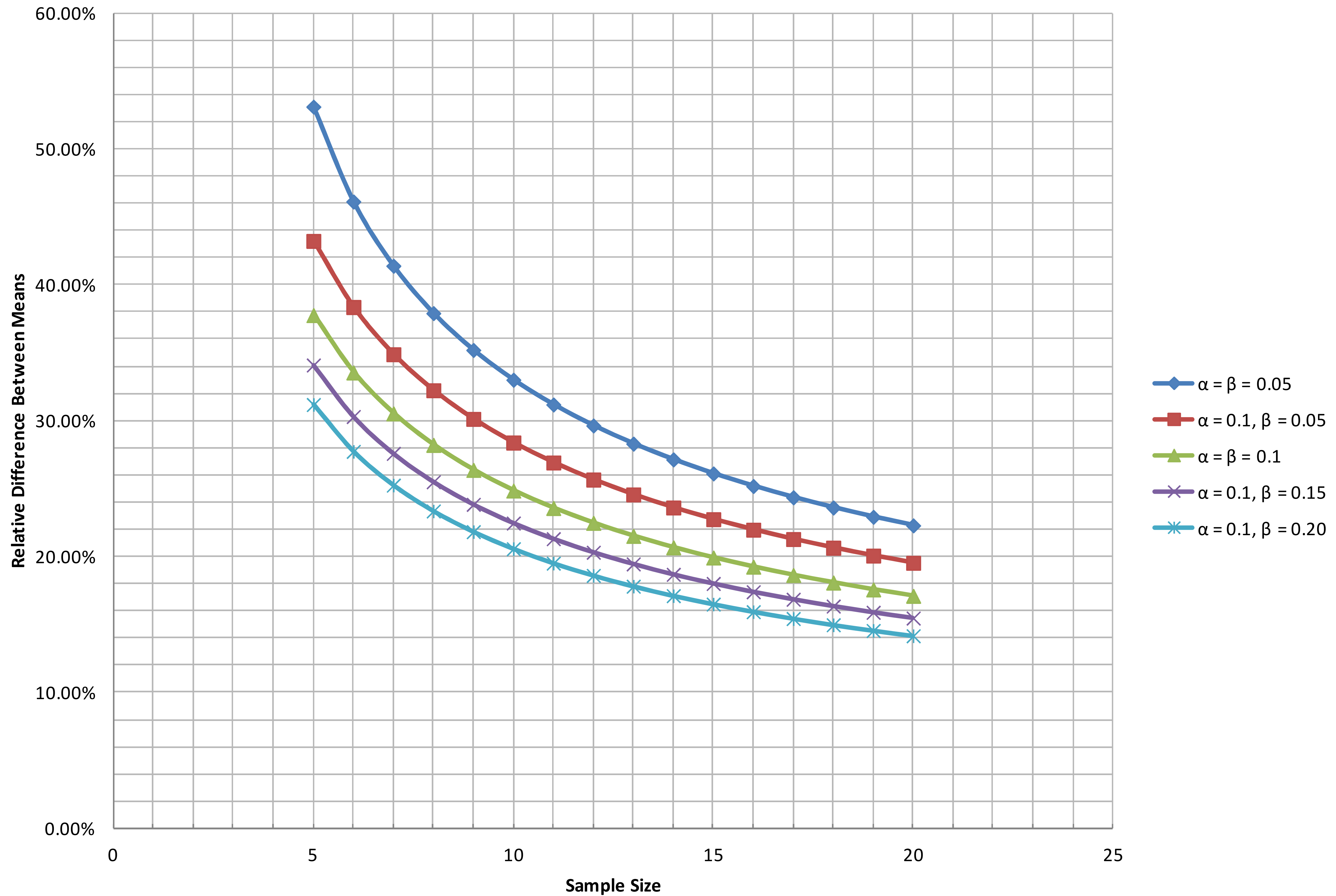


Figure B3 - Minimum Sample Size vs. Difference in Transformation Efficiencies - SMX

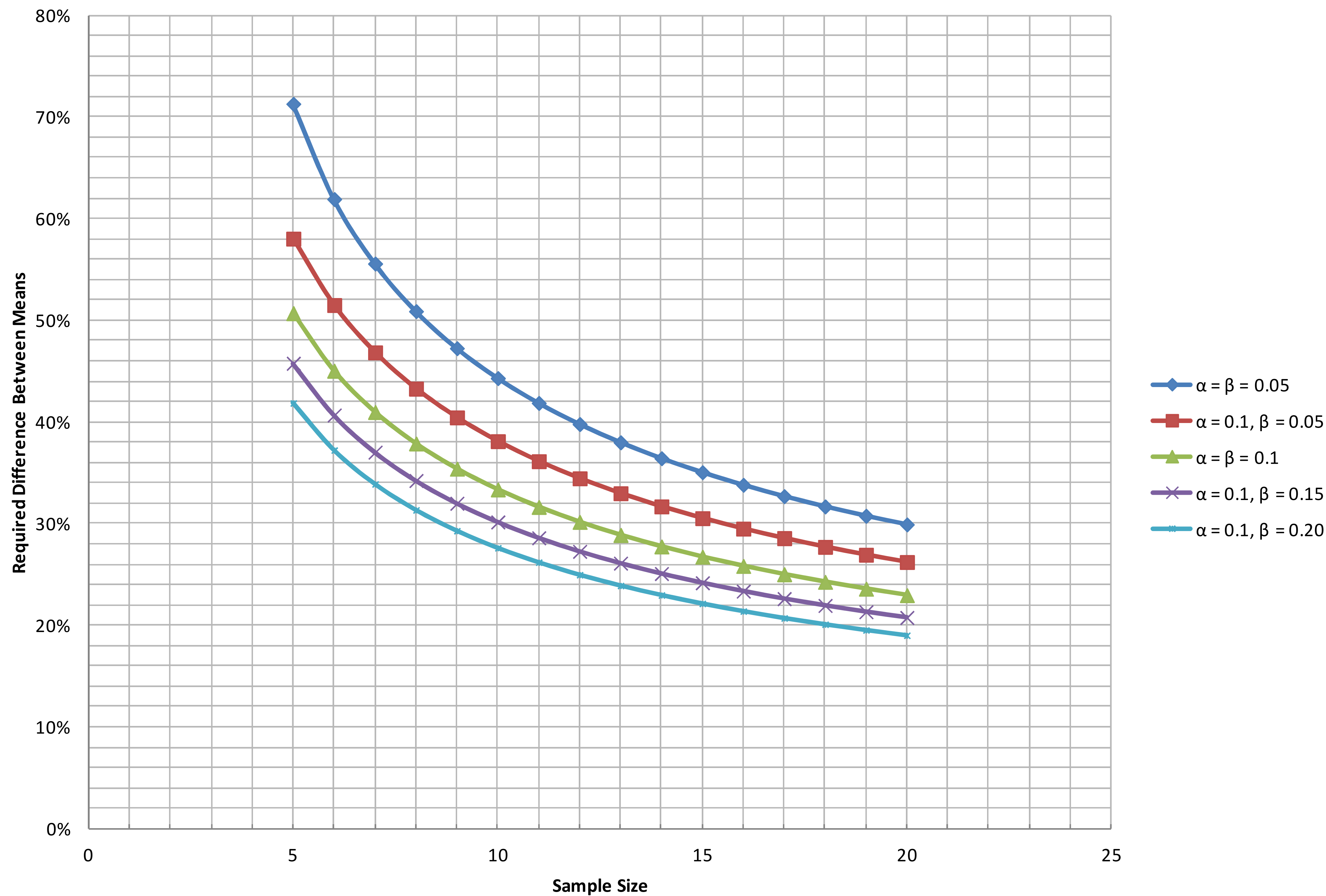


Figure B4 - Minimum Sample Size vs. Difference in Transformation Efficiencies - CBZ

to achieve suitable statistical certainty ($\alpha = 0.1$ and $\beta = 0.2$) between mean removal rates achieved between the control and IFAS reactor. The minimum differences between the mean removal rates, calculated for each of the 4 compounds analyzed under the SPE investigation, required in order to observe a statistically significant difference between the two processes are reported in Table B6.

Table B6 - Statistical Determination of Minimum Sample Size

Pharmaceutical Compound	Minimum difference between mean elimination efficiencies (%)		
	n = 8	n = 10	n =12
ATEN	22.9	20.2	18.2
CBZ	31.4	27.6	25.0
SMX	23.4	20.6	18.6
TRIM	49.3	43.4	39.3

Based on the results of the power analysis a minimum sample size of 12 was selected for the phase 1 investigation. It was anticipated that the use of the isotope dilution method as well as the reduced matrix effects associated with secondary effluent would improve the statistical certainty, making the above values representative of worst case analytical results.

Appendix C

Conventional Data

C.1. PHASE 1

Phase I investigations were completed using the high temperature (18°C) and high SRT (20d) conditions for both an IFAS reactor (indentified as reactor K20) and a control SBR (identified as reactor B). Prior to testing, the IFAS reactor was permitted to run for approximately 13 months to ensure that a biofilm had fully developed and was at pseduo steady state. Reactor B had been in operation for several years as a result of prior research conducted by others and had demonstrated good performance during this historical period. Data was collected prior to PC sampling to characterize the performance of the reactors and to ensure that they were providing biological treatment commensurate with that typically observed from a well operated SBR operated at the given SRT and temperature. Data presented in Table C1 demonstrates some of the observed effluent and operational parameters observed during a monitoring period of 3 SRTs prior to sampling for PC analysis. Raw Conventional Data is presented at the end of this appendix.

Table C1 - Process Monitoring Results Collected during 3 SRTs Prior to PC Sampling: Phase I

Reactor		Effluent tCOD (mg/L)	Effluent TAN (mg/L)	Effluent TSS (mg/L)	MLSS (mg/L)	MLVSS:MLSS (%)	Calculated SRT (d)
K20	Average	21.3	0.13	17.3	2214	81%	17.0
	Std Dev.	5.8	0.11	3.5	195		1.5
B	Average	29.1	0.10	14.6	3268	82%	20.7
	Std Dev.	26.7	0.13	3.4	196		2.0

The effluent tCOD, TAN and TSS concentrations measured during the monitoring period for both reactors are considered to be consistent with typical SBR performance under the target operating parameters. The SBBR and SBR provided efficient settlement of solids and full nitrification. The effluent tCOD values likely reflect the presence of non biodegradable organic material or small amounts of unsettled biomass. It should be noted that the effluent tCOD values were analyzed using unfiltered effluent samples, and may be somewhat elevated due to the presence of small concentrations of pin floc.

During the 3 SRT period prior to sampling, reactor K20 was noted to have been operated at an average SRT of 17.0 d. Reactor B was noted to have been operated at an average SRT of 20.7 d. SRT control was found to be a challenge due to the limited accuracy associated with WAS flowrate adjustments. Figure C1, presented below, demonstrates the mixed liquor suspended solids and operating SRT for both reactors prior to the collection of samples for Phase I investigations. Each of the 3 SRT periods prior to sampling are colorized.

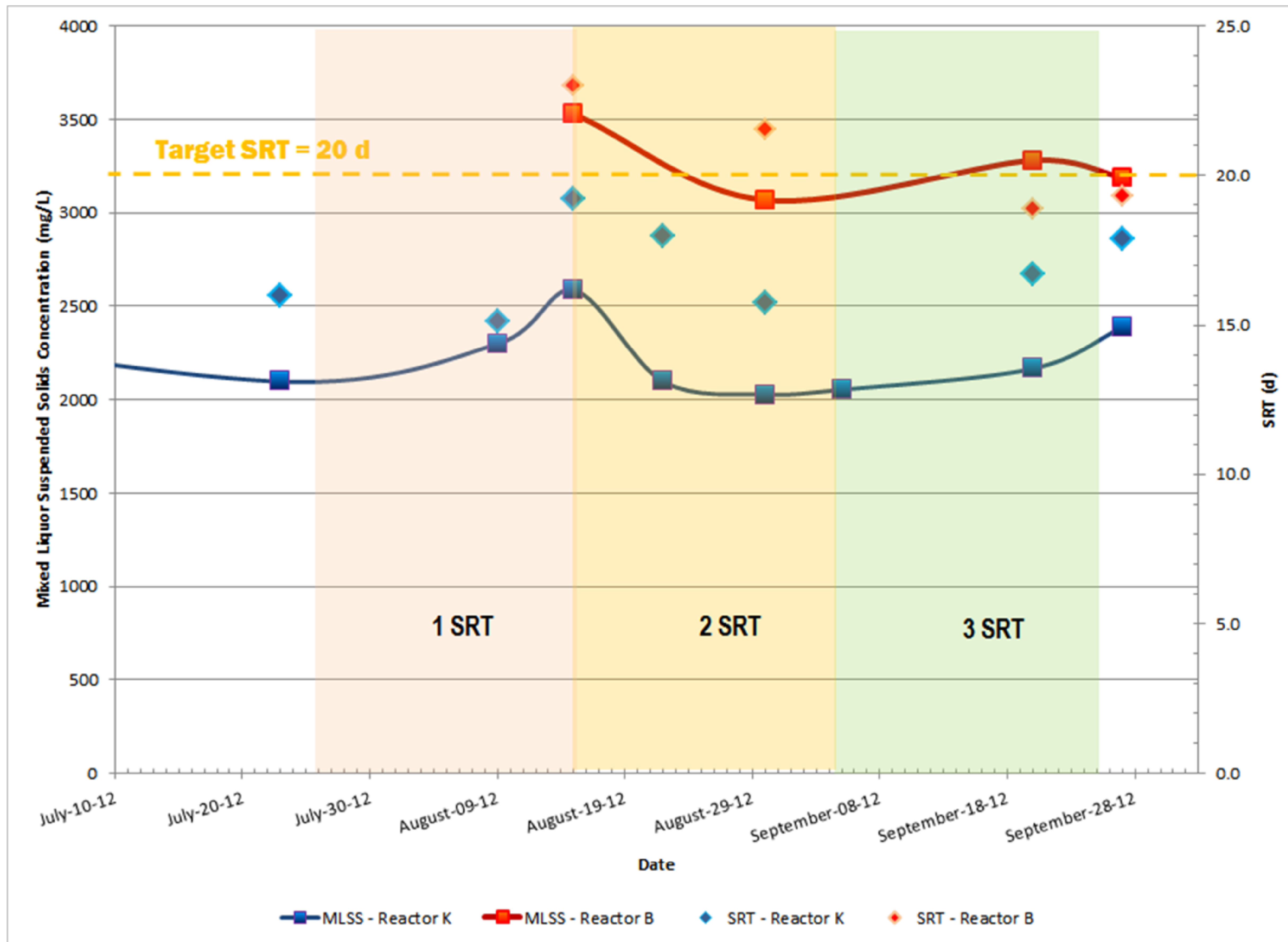


Figure C1– Mixed Liquor Suspended Solids and Operating SRT – Phase I – Reactor K20 and B

C.2. Phase II

Phase II investigations were completed using the low temperature (12°C) and high SRT (20d) conditions for both an IFAS reactor (identified as reactor A20) and a control SBR (identified as reactor C). Prior to testing, the IFAS reactor was permitted to run for approximately 14 months to ensure that a biofilm had fully developed and was at pseduo steady state. Reactor C had been in operation for several years as a result of prior research conducted by others and had demonstrated generally good performance during this historical period, with the exception of some seasonal (winter) process upsets. Data was collected to characterize the performance of the reactors to ensure that they were providing biological treatment commensurate with what is typically observed from a well functioning SBR operated at the given SRT and temperature. Data presented in Table C2 demonstrates some of the observed effluent and operational parameters observed during a monitoring period of 3 SRTs prior to sampling for PC analysis was conducted.

Table C2 - Process Monitoring Results Collected during 3 SRTs Prior to PC Sampling: Phase II

Reactor		Effluent tCOD (mg/L)	Effluent TAN (mg/L)	Effluent TSS (mg/L)	MLSS (mg/L)	MLVSS:MLSS (%)	Calculated SRT (d)
A20	Average	23.2	2.1	14.4	2084	82	17.1
	Std Dev.	13.6	6.5	5.8	350		1.7
C	Average	72.2	11.5	67.5	2455	79	11.8
	Std Dev.	65.2	12.3	48.3	676		5.4

The effluent COD, TAN and TSS concentrations measured during the monitoring period for the IFAS SBBR is considered to be generally consistent with typical SBR performance under the target operating parameters. The SBBR provided efficient settlement of solids and full nitrification during the monitoring period, with the exception of one process upset. This upset involved permitting the alkalinity tank to run empty prior to a sampling event conducted on November 14, 2012. This resulted in an effluent TAN concentration of 20.5 mg/L. After sufficient alkalinity dosing was resumed, full nitrification returned within a 2 week period. It should be noted that all other TAN measurements in this dataset were less than 0.3 mg/L.

Reactor C was observed to be undergoing sustained process upset causing significantly elevated effluent TSS throughout the monitoring period. Initially, the cause of the process upset was not clear as other reactors appeared to be unaffected. In late September, it was observed that unusually poor nitrification performance was occurring within Reactor C, demonstrating that target SRTs were not being maintained. This result prompted an investigation into effluent solids concentrations, which were noted to have increased to over 100 mg/L, explaining the loss of nitrification.

At this time, poorly settling mixed liquor was removed from the reactor on multiple occasions in early October in an attempt to eliminate all bulking sludge. This was accomplished by permitting reactor C to settle for 20 minutes, and all

mixed liquor which was not within the sludge blanket was removed manually. An effluent TSS sample collected on October 25, 2012 demonstrated that adequate settling had been restored. However, nitrification testing conducted on reactor C on October 11th and 25th confirmed that nitrifiers had been washed out. As a result of unstable conditions, PC sampling from reactor C which was originally intended for December was postponed until SRT issues could be resolved. Due to timing constraints, reactor A20 was sampled as part of Phase II investigations recognizing that a direct comparison of performance between the SBBR and SBR under identical feed conditions would not be achieved.

High effluent TSS in Reactor C effluent was again observed throughout November. Additional biomass removal using the procedure identified above was performed in November, with marginal improvements to the effluent TSS. Due to the sustained process upset, it was suspected that the cause of poor settling sludge within reactor C could be due to the proliferation of filamentous organisms. In response to the suspected filamentous issue, all in-reactor equipment (probes, tubing, air diffuser stones) from all reactors were thoroughly cleaned. All external tubing associated with reactor C, as well as the feed tank apparatus, were chemically cleaned by circulation of hydrogen peroxide for a period of several hours. In response to the lost biomass, reactor C was then re-seeded by supplementing with WAS from reactor B for a period of one week in an attempt to restore lost biomass. However, effluent TSS values remained high, prompting additional investigation.

Microscopic analysis was performed on December 4 and 5, 2012 to determine if a filamentous issue was the cause of poor performance within reactor C. WAS samples were collected from Reactor C, B, K and E on December 4, 2012 and investigated. WAS samples from reactors B and E were investigated for comparison as these processes were performing well. Microscope images captured during the investigation of Reactor C WAS are provided in **Appendix G**. This investigation confirmed that filamentous organisms were present which were causing biomass settlement issues and the associated loss of nitrification performance.

Based on operational suggestions found within Jenkins et al (2004) reactor C mixed liquor was supplemented with hydrogen peroxide to obtain a concentration of 30 mg/L in an attempt to eradicate all filamentous organisms. Reactor C was then reseeded with WAS from reactor B for several weeks prior to the Christmas Holiday period. Despite the loss of biomass, Reactor C appeared to provide some nitrification as well as COD removal during the monitoring period. Effluent samples collected on January 4, 2013 demonstrate that the reactor had regained the ability to nitrify; producing an effluent with a TAN concentration of 0.42 mg/L. Elevated effluent TSS was still occurring and WAS rates were adjusted to compensate for these solids losses.

During the 3 SRT period prior to sampling, reactor A20 was noted to have been operated at an average SRT of 17.1 d. SRT control was found to be a challenge due to the limited accuracy associated with WAS flowrate adjustments. Reactor C had a highly variable SRT during the monitoring period due to high effluent TSS values. However, prior to sampling Reactor C was operating at an SRT of 18 d.

Mixed liquor suspended solids concentrations also demonstrate that the Reactor C was gaining biomass as concentrations increased during the last 40 days of the monitoring period. Figure C2 and C3 demonstrate the mixed liquor suspended solids and operating SRT for Reactors A20 and C, respectively, prior to the collection of samples for Phase II investigations. Each of the 3 SRT periods prior to sampling is colorized.

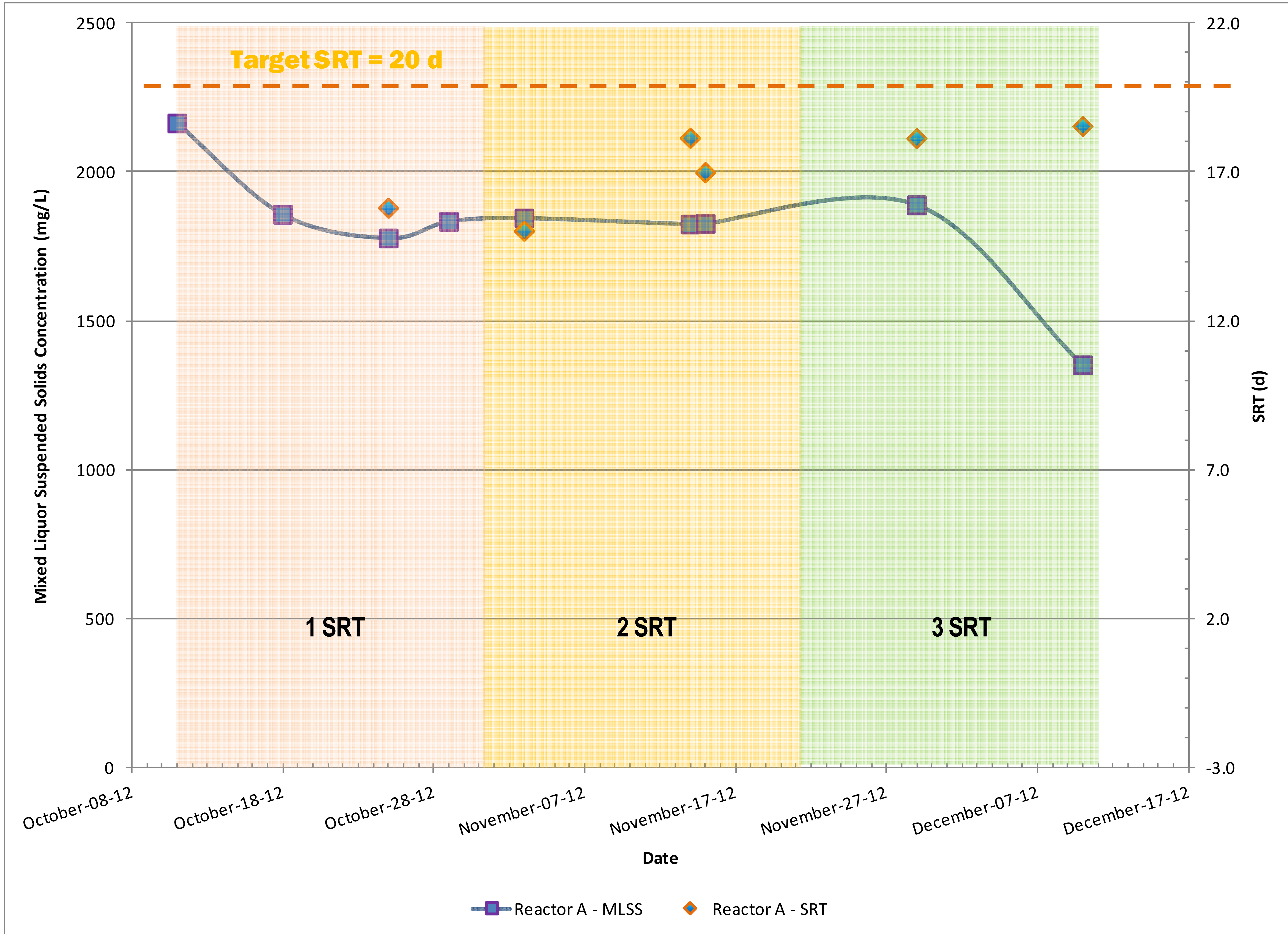


Figure C2 - Mixed Liquor Suspended Solids and Operating SRT – Phase II – Reactor A20

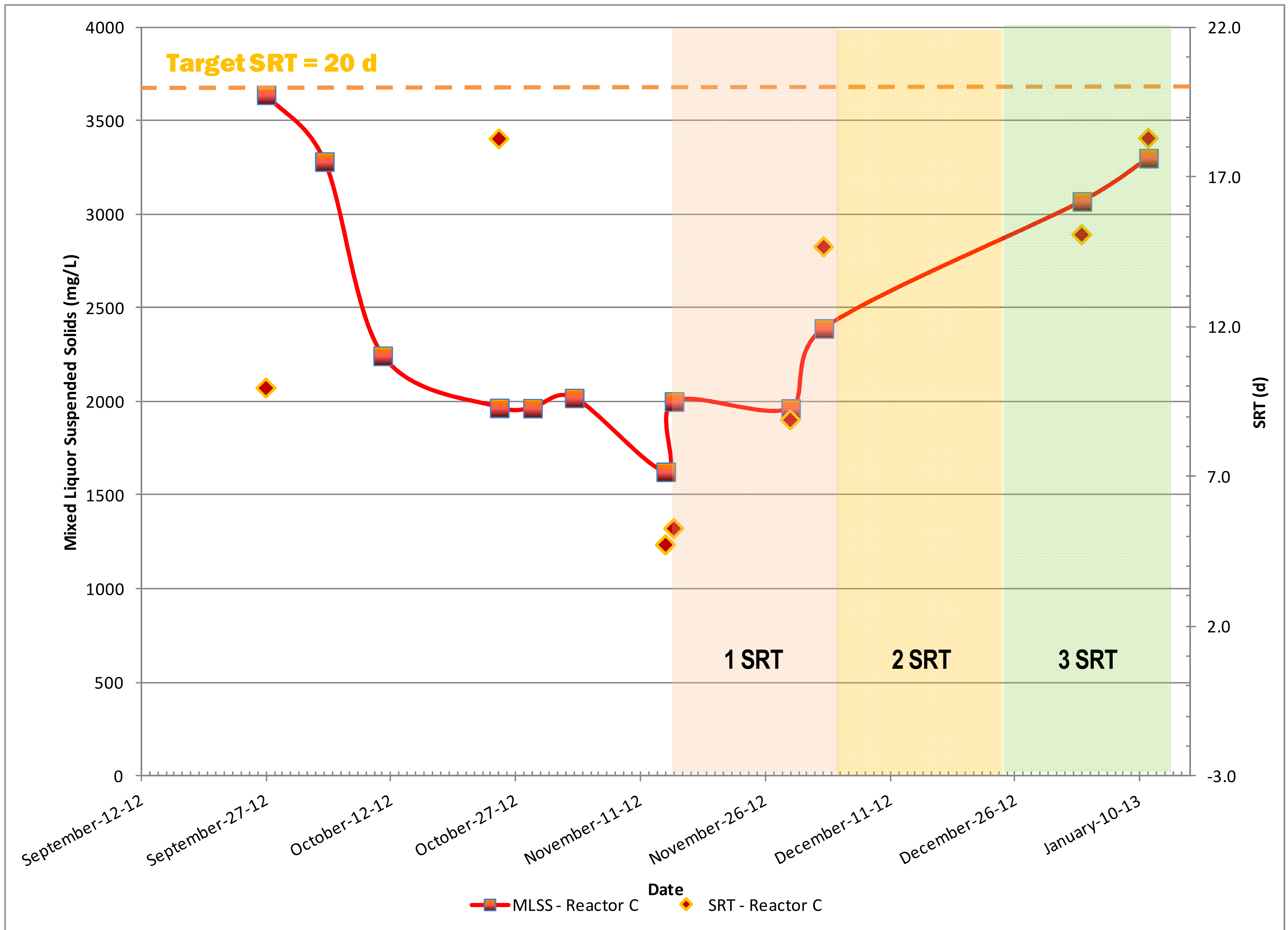


Figure C3 - Mixed Liquor Suspended Solids and Operating SRT – Phase II – Reactor C

C.3. Phase III

Phase III investigations were completed using reactors operating under the low SRT (7d) condition at both high (18°C) and low (12°C) conditions for two IFAS reactor (identified as reactor K7 and A20, respectively) and two control SBRs (identified as reactor E and D, respectively). Following completion of Phase II sampling on December 14, 2012, the WAS rates for the IFAS reactors were increased to achieve a target operating conditions of 7 days SRT based on the suspended growth phase. The reactors were provided approximately 31 days (approximately 4 SRTs) to adjust to these new process conditions. Reactor D and E had been in operation for several years as a result of prior research conducted by others and had demonstrated generally good performance during this historical period.

Data was collected to characterize the performance of the reactors to ensure that they were providing biological treatment commensurate with what is typically observed from a well performing SBR operated at the given SRT and temperature. Data presented in Table C3 demonstrates some of the observed effluent and operational parameters observed during the monitoring period of 4 SRTs prior to sampling for PC analysis was conducted.

Table C3 - Process Monitoring Results Collected during 4 SRTs Prior to PC Sampling: Phase III

Reactor		Effluent tCOD (mg/L)	Effluent TAN (mg/L)	Effluent TSS (mg/L)	MLSS (mg/L)	MLVSS:MLSS (%)	Calculated SRT (d)
K7	Average	28	0.16	11.1	982	83	7.6
	Std Dev.	16.6	0.13	4.0	167	-	0.1
E	Average	26.3	0.34	16.4	1660	82	7.6
	Std Dev.	14.2	0.17	9.8	206		0.3
A7	Average	27	0.16	8.8	1181	84	7.5
	Std Dev.	12.1	0.13	0.4	166	-	0.1
D	Average	51	17.6	49.8	1255	85	6.2
	Std Dev.	16.5	3.9	35.7	285	-	2.3

The effluent COD, TAN and TSS concentrations measured during the monitoring period for the K7 SBBR and the control reactor E are considered to be generally consistent with typical SBR performance under the target operating parameters. The A7 SBBR, as a result of the inclusion of IFAS media, provided nitrification which would not have been achieved under these conditions in a conventional SBR. The results observed for Reactor D, which demonstrate relatively minimal nitrification, demonstrate these limitations. Both SBBRs and Reactor E provided efficient settlement of solids and full nitrification during the monitoring period.

Reactor D was observed to have undergone a process upset following the Christmas Holiday period. Poor settling was observed in Reactor D in late October, concurrent with similar filamentous issues within Reactor C. It was suspected that the cause of poor settling sludge within reactor D could be due to the proliferation of filamentous organisms. These suspicions were confirmed through microscope analysis. The images obtained during the microscopic investigation are provided in **Appendix G**.

Mitigative measures similar to what was performed on Reactor C were implemented including: thorough cleaning of all equipment; chemical cleaning of all feed tubing by circulation of hydrogen peroxide solution; and, the removal of poorly settling biomass. To replenish the lost biomass, reactor D was re-seeded by supplementing with WAS from reactor E for a period of a week in an attempt to restore lost biomass. Based on effluent TSS measurements conducted in November and December, it was believed that the filamentous issue had been resolved. Sampling conducted on January 3, 2013 indicated that the filamentous issue had returned, resulting in high effluent TSS concentrations and operating SRTs below targets. WAS from reactor E was again added and WAS rates were significantly reduced to account for lost biomass in the effluent.

During the 4 SRT period prior to sampling, All reactors were noted to have been operated within +/- 1 day of the target SRT. SRT control was found to be a challenge due to the limited accuracy associated with WAS flowrate adjustments. With the exception of the filamentous occurrence which took place over the Christmas holidays, all reactors maintained consistent operating conditions over the monitoring period. However, at the time of sampling, it was noted that reactor D was operating at the target SRT of 7 days. Figures C3 and C4 demonstrates the mixed liquor suspended solids and operating SRT for Reactors K7 and E and A7 and D, respectively, prior to the collection of samples for Phase II investigations. Each of the 4 SRT periods prior to sampling is colorized.

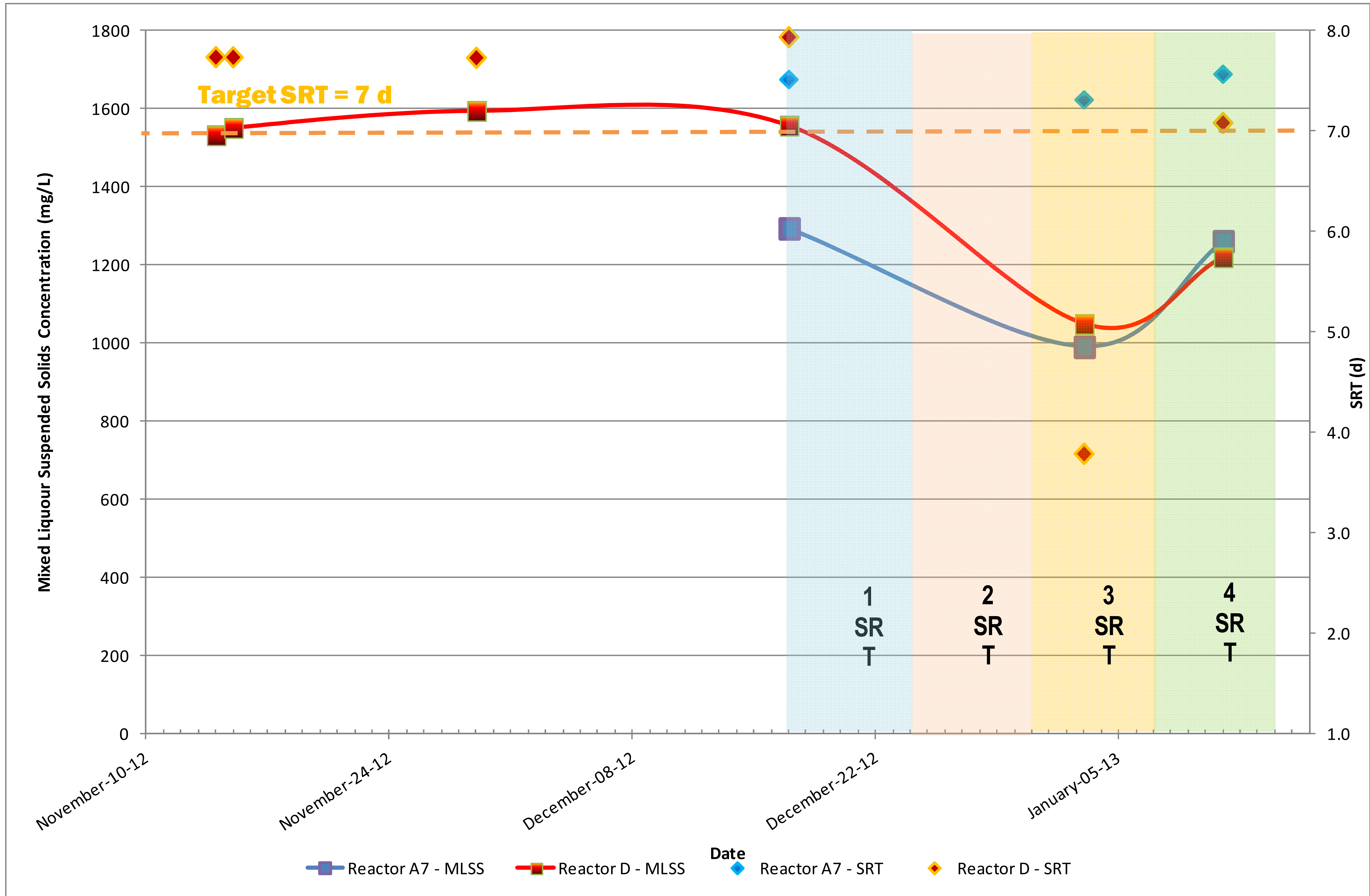


Figure C3 – Mixed Liquor Suspended Solids and Operating SRT – Phase III – Reactor A7 and D

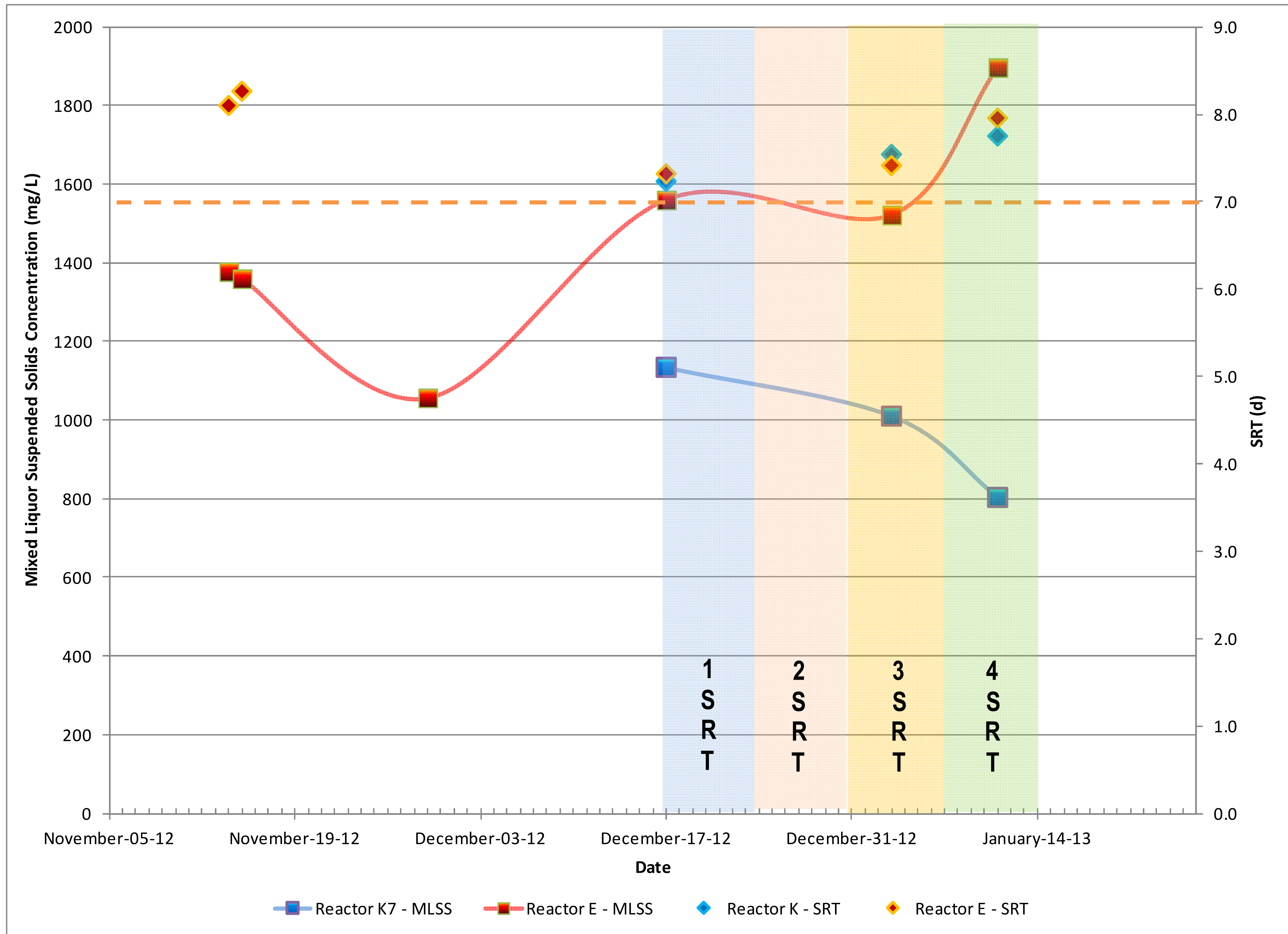


Figure C4 - Mixed Liquor Suspended Solids and Operating SRT – Phase III – Reactor K7 and E

SRT = 20d, Temp = 18 degrees

	Rep?	Date Collected	Volume	Influent Time Collected	CODt	fCOD	NH3-N	NO3-N	Effluent Time Collected	CODt	fCOD	NH3-N	NO3-N	NO2-N	Notes
IFAS-K1		25/09/2012	500 mL	1:30:00 PM	161	64	10	9.9	7:30:00 PM	24		0.04	21.6		
IFAS-K2		25/09/2012	500 mL	7:30:00 PM	139	51	11.8	8.5	1:30:00 AM	38		0.02	24.8		
IFAS-K3	Rep of K2	25/09/2012	500 mL	7:30:00 PM					1:30:00 AM						
IFAS-K4		26/09/2012	500 mL	1:30:00 PM	154	56	11.7	7.6	7:30:00 PM	23		0.05	23.5		
IFAS-K5		26/09/2012	500 mL	7:30:00 PM	119	59	12.6	8.2	1:30:00 AM	62		0.06	26.2		
IFAS-K6	Rep of K4	26/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
IFAS-K7		27/09/2012	500 mL	1:30:00 PM	185	82	10.9	10.5	7:30:00 PM	41		0.03	24.4		
IFAS-K8		27/09/2012	500 mL	7:30:00 PM					1:30:00 AM						
IFAS-K9	Rep of K7	27/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
IFAS-K10		28/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
IFAS-K11	Rep of K10	28/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
IFAS-K12	Rep of K10	28/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
Average					151.6	62.4	11.4	8.9		37.6		0.04	24.10		
Control-B1		25/09/2012	500 mL	1:30:00 PM	227	106	17.5	1.6	7:30:00 PM	43		0.25	29.6		
Control-B2		25/09/2012	500 mL	7:30:00 PM	203	92	19.4	0.9	1:30:00 AM	39		1.24	19.6		*limited nit likely due to base malfunction
Control-B3	Rep of K2	25/09/2012	500 mL	7:30:00 PM					1:30:00 AM						
Control-B4		26/09/2012	500 mL	1:30:00 PM	230	106	18.6	1.1	7:30:00 PM	32		0.54	19.3		
Control-B5		26/09/2012	500 mL	7:30:00 PM	250	96	27.3	2	1:30:00 AM	36		0.22	27.5		
Control-B6	Rep of K4	26/09/2012	500 mL	1:30:00 PM					7:30:00 PM						*oxygen diffuser stones replaced
Control-B7		27/09/2012	500 mL	1:30:00 PM	228	85	17.5	2.3	7:30:00 PM	87		0.25	24.1		
Control-B8		27/09/2012	500 mL	7:30:00 PM					1:30:00 AM						*base feed corrected
Control-B9	Rep of K7	27/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
Control-B10		28/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
Control-B11	Rep of K10	28/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
Control-B12	Rep of K10	28/09/2012	500 mL	1:30:00 PM					7:30:00 PM						
Average					227.6	97	20.06	1.58		47.4		0.5	24.02		

SRT = 20d, Temp = 12 degrees

	Date Collected	Influent Time Collected	CODt	fCOD	NH3-N	NO3-N	Effluent Time Collected	CODt	fCOD	NH3-N	NO3-N	NO2-N	Notes
IFAS-A1	11-Dec-12	8:30 PM	163	78	17.5	6.3	2:00 AM	42	27	1.84	18.3	1.41	
IFAS-A2													
IFAS-A3													
IFAS-A4													
IFAS-A5		2:30 PM	206	116	10.3	4.7	7:30 PM	136	61	0.04	11.8	<MDL	
IFAS-A6													
IFAS-A7		8:30 PM	186	100	15.4	5.7	2:30 AM	78	70	1	16.2	0.155	
IFAS-A8													
IFAS-A9													
IFAS-A10	12-Dec-12	3:00 PM	192	85	16.4	2.2	9:00 PM	250	85	0.03	11.8	0.031	
IFAS-A11													
IFAS-A12								21		0.01			
IFAS-A13		9:30 PM	224	104	15.3	4.4				0.09			
IFAS-A14													
IFAS-A15													
IFAS-A16					10.4	3.9				0.04	3.8		
IFAS-A17													
IFAS-A18													
AVerage			194.2	96.6	14.2	4.5		105.4	60.8	0.44	12.38	0.532	
Control-C1							Control-C4						
Control-C2							Control-C5						
Control-C3			264	45	5.6	1.3	Control-C6	122	68	0.1	11.3	0.035	
Control-C7							Control-C10						
Control-C8							Control-C11						
Control-C9			207	69	8.1	2.6	Control-C12	87	68	<MDL	13.4	0.005	
Control-C13							Control-C16						
Control-C14							Control-C17						
Control-C15			193	64	12.1	1.4	Control-C18	39	34	0.02	18.9	0.032	
Control-C19							Control-C22						
Control-C20							Control-C23						
Control-C21			142	85	11.8	0.4	Control-C24	31	25	0.06	17	0.033	
Control-C25							Control-C28						
Control-C26							Control-C29						
Control-C27			144	69	14.3	0.7	Control-C30	52	28	0.2	20.5	0.0833	
Control-C31							Control-C34						
Control-C32							Control-C35						
Control-C33			278	50	10.3	2.7	Control-C36	<MDL	5	0.18	14.5	0.028333	
AVERAGE			**204.7**	**63.7**	**10.4**	**1.5**		**66.2**	**38.0**	**0.1**	**15.9**	**0.04**	

SRT = 7d, Temp = 20 degrees											
	Influent					Effluent					Notes
IFAS-K7-1					IFAS-K7-4						
IFAS-K7-2					IFAS-K7-5						
IFAS-K7-3	234	90	4.2	3.6	IFAS-K7-6	140	126	0.03	10.2	0.035	
IFAS-K7-7					IFAS-K7-10						
IFAS-K7-8					IFAS-K7-11						
IFAS-K7-9	202	127	6.5	4.8	IFAS-K7-12	80	91	0.02	12.5	0.015	
IFAS-K7-13					IFAS-K7-16						
IFAS-K7-14					IFAS-K7-17						
IFAS-K7-15	170	50	8.8	3.8	IFAS-K7-18	<MDL	52		17	0.04	
IFAS-K7-19					IFAS-K7-22						
IFAS-K7-20					IFAS-K7-23						
IFAS-K7-21	108	69	7.9	3.6	IFAS-K7-24	54	35	0.03	15.5	0.05	
IFAS-K7-25					IFAS-K7-28						
IFAS-K7-26					IFAS-K7-29						
IFAS-K7-27	119	58	10.6	4.8	IFAS-K7-30	51	37	0.23	16.4	0.106667	
IFAS-K7-31					IFAS-K7-34						
IFAS-K7-32					IFAS-K7-35						
IFAS-K7-33	246	55	7.1	7.9	IFAS-K7-36	22	15	0.16	16.2	0.128333	
AVERAGE	**179.8**	**74.8**	**7.5**	**4.8**		**69.4**	**59.3**	**0.1**	**14.6**	**0.1**	
Control-E1					Control-E4						
Control-E2					Control-E5						
Control-E3	311	37	6.1	1.5	Control-E6	58	52	0.11	11.7	0.04	
Control-E7					Control-E10						
Control-E8					Control-E11						
Control-E9	181	60	9.7	1.1	Control-E12	46	50	<MDL	13.2	0.03	
Control-E13					Control-E16						
Control-E14					Control-E17						
Control-E15	211	49	12.4	1.2	Control-E18	43	39	0.1	17.9	0.078	
Control-E19					Control-E22						
Control-E20					Control-E23						
Control-E21	159	84	11.1	0.2	Control-E24	41	39	0.15	16.7	0.037	
Control-E25					Control-E28						
Control-E26					Control-E29						
Control-E27	137	69	14.3	0.6	Control-E30	45	34	0.28	19.9	0.718333	
Control-E31					Control-E34						
Control-E32					Control-E35						
Control-E33	271	50	10.2	2.2	Control-E36	7	4	0.23	15.4	0.04	
AVERAGE	**211.7**	**58.2**	**10.6**	**1.1**		**40.0**	**36.3**	**0.2**	**15.8**	**0.2**	

SRT = 7d, Temp = 12 degrees											
	Influent					Effluent					Notes
IFAS-A7-1					IFAS-A7-4						
IFAS-A7-2					IFAS-A7-5						
IFAS-A7-3	239	70	4.3	2.1	IFAS-A7-6	112	102	0.02	7.3	0.015	
IFAS-A7-7					IFAS-A7-10						
IFAS-A7-8					IFAS-A7-11						
IFAS-A7-9	184	86	6.1	3	IFAS-A7-12	21	47	0.01	8.7	0	
IFAS-A7-13					IFAS-A7-16						
IFAS-A7-14					IFAS-A7-17						
IFAS-A7-15	221	55	8.7	2.2	IFAS-A7-18	56	54	0.03	14.3	0.003	
IFAS-A7-19					IFAS-A7-22						
IFAS-A7-20					IFAS-A7-23						
IFAS-A7-21	121	59	8.5	2.2	IFAS-A7-24	58	42	0.03	13.3	0.008333	
IFAS-A7-25					IFAS-A7-28						
IFAS-A7-26					IFAS-A7-29						
IFAS-A7-27	118	61	10.6	3.4	IFAS-A7-30	43	36	0.17	19.1	0.07	
IFAS-A7-31					IFAS-A7-34						
IFAS-A7-32					IFAS-A7-35						
IFAS-A7-33	243	40	6	5.5	IFAS-A7-36	9	6	<MDL	12.5	0.045	
AVERAGE	**187.7**	**61.8**	**7.4**	**3.1**		**49.8**	**47.8**	**0.1**	**12.5**	**0.0**	
Control-D1					Control-D4						
Control-D2					Control-D5						
Control-D3	302	33	6.1	1.7	Control-D6	102	94	5.5	3.8	0.898	
Control-D7					Control-D10						
Control-D8					Control-D11						
Control-D9	192	72	8.3	2.3	Control-D12	68	66	6.8	3.3	0.802	
Control-D13					Control-D16						
Control-D14					Control-D17						
Control-D15	193	63	15.8	<MDL	Control-D18	44	37	13.8	2	0.752	
Control-D19					Control-D22						
Control-D20					Control-D23						
Control-D21	139	71	14.3	10.3	Control-D24	40	37	11.5	2	0.788	
Control-D25					Control-D28						
Control-D26					Control-D29						
Control-D27	151	75	17.9	0.1	Control-D30	70	34	18.5	1.2	0.616667	
Control-D31					Control-D34						
Control-D32					Control-D35						
Control-D33	286	52	12.1	0.2	Control-D36	15	14	12.3	1.6	0.635	
AVERAGE	**210.5**	**61.0**	**12.4**	**2.9**		**56.5**	**47.0**	**11.4**	**2.3**	**0.7**	

Phase 1 - Effluent Measurement Data

Date	Reactor	Parameter	
August-09-12	K	TAN	0.28
August-09-12	K	CODt	28
August-15-12	K	CODt	18
August-15-12	K	TAN	0.07
September-07-12	K	TAN	0.12
20-Sep-12	K	CODt	17.9
20-Sep-12	K	NH3-N	0.04
20-Sep-12	K	pH	7.4
20-Sep-12	K	NO2-N	0.05

AVERAGE CODt	21.3
STDEV	5.8
AVERAGE TAN	0.13
STDEV	0.11

Date	Reactor	Parameter	
09-Aug-12	B	NH3-N	0.04
09-Aug-12	B	CODt	13
15-Aug-12	B	NH3-N	0.04
15-Aug-12	B	CODt	69
07-Sep-12	B	NH3-N	0.02
20-Sep-12	B	CODt	17.2
20-Sep-12	B	NH3-N	0.3
20-Sep-12	B	pH	7.44
20-Sep-12	B	NO2-N	0.263
20-Sep-12	B	CODt	17.2

AVERAGE CODt	29.1
STDEV	26.7
AVERAGE TAN	0.10
STDEV	0.13

Phase 2 - Effluent Measurement Data

Date	Reactor	Parameter	
August-15-12	A	TAN	0.06
August-15-12	A	CODt	15
September-07-12	A	TAN	<MDL
August-09-12	A	TAN	0.3
August-09-12	A	CODt	49
20-Sep-12	A	CODt	31.1
20-Sep-12	A	NH3-N	0.05
20-Sep-12	A	pH	7.54
20-Sep-12	A	NO2-N	0.063
25-Oct-12	A	NH3-N	<MDL
25-Oct-12	A	CODf	20
25-Oct-12	A	CODt	30
25-Oct-12	A	NO3-N	20.6
14-Nov-12	A	NH3-N	20.5
14-Nov-12	A	CODt	28.0
14-Nov-12	A	NO3-N	6.2
14-Nov-12	A	CODf	20
29-Nov-12	A	NH3-N	0.02
29-Nov-12	A	CODt	11
29-Nov-12	A	NO3-N	13.1
03-Dec-12	A	NH3-N	0.02
03-Dec-12	A	CODt	2
03-Dec-12	A	NO3-N	17.2
11-Dec-12	A	NH3-N	0.28
11-Dec-12	A	CODt	20
11-Dec-12	A	NO3-N	9.5
11-Dec-12	A	NO2-N	0.016

AVERAGE COD	23.3
STDEV	14.5
AVERAGE TAN	2.4
STDEV	6.8

Date	Reactor	Parameter	
25-Oct-12	C	NH3-N	6.10
25-Oct-12	C	CODf	29
25-Oct-12	C	CODt	84
25-Oct-12	C	NO3-N	9.6
14-Nov-12	C	NH3-N	28.3
14-Nov-12	C	CODt	184.0
14-Nov-12	C	NO3-N	1.4
14-Nov-12	C	CODf	27
22-Nov-12	C	NH3-N	16.5
29-Nov-12	C	NH3-N	1.3
29-Nov-12	C	CODt	24
29-Nov-12	C	NO3-N	0.3
03-Dec-12	C	NH3-N	0.7
03-Dec-12	C	CODt	17
03-Dec-12	C	NO3-N	0.7
December-11-12	C	CODt	101
December-11-12	C	NH3-N	26.8
December-11-12	C	NO3-N	11.0
December-11-12	C	NO2-N	0.8
04-Jan-13	C	NH3-N	0.42
04-Jan-13	C	CODt	23
04-Jan-13	C	NO3-N	8.4
04-Jan-13	C	NO2-N	0.084
11-Jan-13	C	CODt	101

AVERAGE COD	72.2
STDEV	65.2
AVERAGE TAN	11.4
STDEV	12.3

Phase 3 - Effluent Measurement Data

Date	Reactor	Parameter		Date	Reactor	Parameter	
17-Dec-12	K	CODt	16	17-Dec-12	E	CODt	11.0
17-Dec-12	K	NH3-N	<MDL	17-Dec-12	E	NH3-N	0.42
04-Jan-13	K	CODt	21	04-Jan-13	E	CODt	29
04-Jan-13	K	NH3-N	0.3	04-Jan-13	E	NH3-N	0.45
04-Jan-13	K	NO3-N	15.8	04-Jan-13	E	NO3-N	12.3
04-Jan-13	K	NO2-N	0.06	04-Jan-13	E	NO2-N	0.24
11-Jan-13	K	CODt	47	11-Jan-13	E	CODt	39
11-Jan-13	K	NH3-N	0.22	11-Jan-13	E	NH3-N	0.15

AVERAGE COD	28	AVERAGE COD	26.3
STDEV	16.6	STDEV	14.2
AVERAGE TAN	0.16	AVERAGE TAN	0.34
STDEV	0.13	STDEV	0.17

Date	Reactor	Parameter		Date	Reactor	Parameter	
17-Dec-12	A	CODt	20.0	17-Dec-12	D	CODt	34.0
17-Dec-12	A	NH3-N	<MDL	17-Dec-12	D	NH3-N	19.9
04-Jan-13	A	CODt	20	04-Jan-13	D	CODt	67
04-Jan-13	A	NH3-N	0.28	04-Jan-13	D	NH3-N	19.9
04-Jan-13	A	NO3-N	9.5	04-Jan-13	D	NO3-N	2.0
04-Jan-13	A	NO2-N	0.02	04-Jan-13	D	NO2-N	1.42
11-Jan-13	A	CODt	41	11-Jan-13	D	CODt	51
11-Jan-13	A	NH3-N	0.18	11-Jan-13	D	NH3-N	13.1

AVERAGE COD	27.0	AVERAGE COD	50.7
STDEV	12.1	STDEV	16.5
AVERAGE TAN	0.16	AVERAGE TAN	17.6
STDEV	0.13	STDEV	3.93

Date	Source	Effluent TSS (mg/L)	MLSS	MLVSS		WAS Volume	SRT
Phase 1							
April-21-12	K	12	2756			270	19.1
May-11-12	K	17	2786			300	16.5
12-Jun-12	K	6	2460				
July-23-12	K	22	2097			255	16.0
August-09-12	K	20	2300			300	15.1
August-15-12	K	11	2585	2120	82%	270	19.2
August-22-12	K	15	2097			255	18.0
September-05-12	K		2054				
September-20-12	K	17	*2166*	*1762*	81%	270	16.7
September-25-12	K	16.0	*2028*	1643		255	17.5
Average	K	15.2	2333		82%		17.3
STDEV	K	4.8	293.4				1.6
3 SRTS averages		16.9	2190				17.1
STDEV	K	3.9	196.1				1.5
August-15-12	B	18	*3531*	*3023*	86%	205	18.4
August-30-12	B	*17*	*3190*			218	17.4
September-20-12	B	13	*3283*			280	15.1
September-25-12	B	11	3068	2408		218	19.0
Average	B	14.6	3267.9		86%		17.5
STDEV	B	3.4	196.1				1.7
3 SRTS averages		14.6	3267.9				17.5
STDEV	B	3.4	196.1				1.7

Phase 2							
April-21-12	A	5	2416			400	14.6
May-11-12	A	18	2229			270	16.7
July-23-12	A	13	2346			255	19.1
August-09-12	A	20	2360			210	19.5
August-15-12	A	20.8	1843	1462	79%	270	15.0
August-22-12	A	15	1927	*1604*	83%	255	17.5
August-30-12	A		*2346*				
September-05-12	A		2595				
September-20-12	A	13.4	*2671*	*2153*	81%	272	18.5
September-27-12	A	24.0	2516	*2188*	87%	280	15.5
October-11-12	A		2162				
October-18-12	A		*1856*	*1456*	78%		
October-25-12	A	15.2	*1776*			285	15.8
October-29-12	A		1831				
November-03-12	A	20.8	1843			270	15.0
November-14-12	A	11.2	*1822*			265	18.1
November-15-12	A	11	1825			290	17.0
November-29-12	A	8	*1887*			290	18.1
December-10-12	A	6.8	1350			272	18.5
A							
Average		14.4	2084.2		82%		17.1
STDEV		5.8	350.2				1.7
September-27-12	C	118.0	3638	*2838*	78%	207	9.9
October-04-12	C		*3281*	*2559*	78%		
October-11-12	C		2244			220	
October-25-12	C	20.0	*1966*			210	18.3
October-29-12	C		1964				
November-03-12	C		*2019*				
November-14-12	C	139	*1624*			215	4.7
November-15-12	C	140	2000			280	5.3
November-29-12	C	70.8	*1963*			235	8.9
December-03-12	C	35.2	2390			235	14.7
December-12-12	C	38					
December-13-12	C	9.6					
January-03-13	C	47.2	*3069*	*2515*	82%	215	15.1
January-11-13	C	57.6	*3300*			115	18.3
C							
Average		67.5	2454.9		79%		11.1
STDEV		48.3	676.3				5.4

Phase 3									
December-17-12	A7	9.2		1293				740	7.5
January-03-13	A7	8.8		*990*		*834*	0.842466	740	7.3
January-11-13	A7	8.4		*1260*				740	7.6
A7									
Average			8.8		1180.9		84%		7.4
STDEV			0.4		166.3				0.1
December-17-12	K7	15.2		1134				690	7.2
January-03-13	K7	10.8		*1010*		*833*	0.825083	690	7.5
January-11-13	K7	7.2		*803*				690	7.7
K7									
Average			11.1		982.4				7.6
STDEV			4.0		167.0				0.1
September-27-12	D	8.2		1411				708	8.0
October-04-12	D			*1167*					
October-25-12	D	99.2		*826*				710	2.8
October-29-12	D			995					
November-03-12	D			*1164*					
November-14-12	D	11.6		*1531*				710	7.7
November-15-12	D	12		1550				708	7.7
November-29-12	D	12.4		*1594*				708	7.7
December-03-12	D	5.6							
December-17-12	D	9.6		*1556*				708	7.9
January-03-13	D	77.6		*1048*				690	3.8
January-11-13	D	62.2		*1219*		*1038*	0.851563	220	7.1
D									
Average			49.8		1274.3				5.4
STDEV			35.7		258.5				2.2
September-20-12	E	4							
October-04-12	E			*1528*					
October-25-12	E	8.4		*1272*				665	8.3
October-29-12	E			1164					
November-03-12	E			*1344*					
November-14-12	E	7.6		*1378*				700	8.1
November-15-12	E	8		1360				680	8.3
November-29-12	E	17.6		*1057*					
December-03-12	E	8							
December-17-12	E	20.9		1560				680	7.3
January-03-13	E	23.2		*1522*		*1244*	0.817248	645	7.4
January-11-13	E	5.2		*1897*				750	8.0
E									
Average			16.4		1659.5				7.7
STDEV			9.8		206.3				0.3

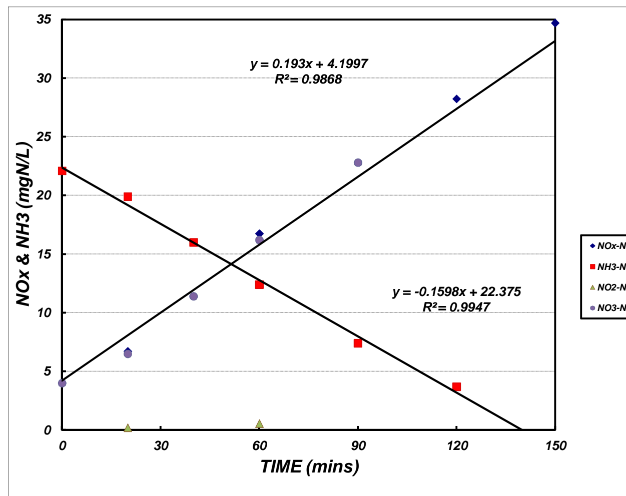
Appendix D

Nitrification Rate Testing

N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0		4.0	4.0	22.1	18	7.2	1.5
2	2:20:00 PM	20	0.203	6.5	6.7	19.9	18	7.2	1.5
3	2:40:00 PM	40		11.4	11.4	16.0	18	7.2	1.5
4	3:00:00 PM	60	0.552	16.2	16.8	12.4	18	7.2	1.5
5	3:30:00 PM	90		22.8	22.8	7.4	18	7.2	1.5
6	4:00:00 PM	120	0.736	27.5	28.2	3.7	18	7.2	1.5
7	4:30:00 PM	150		34.7	34.7		18	7.2	1.5
8	5:00:00 PM	180		36.4	36.4		18	7.2	1.5
						Averages	18.0	7.20	1.5

REACTOR K - 25 Sept - 2012



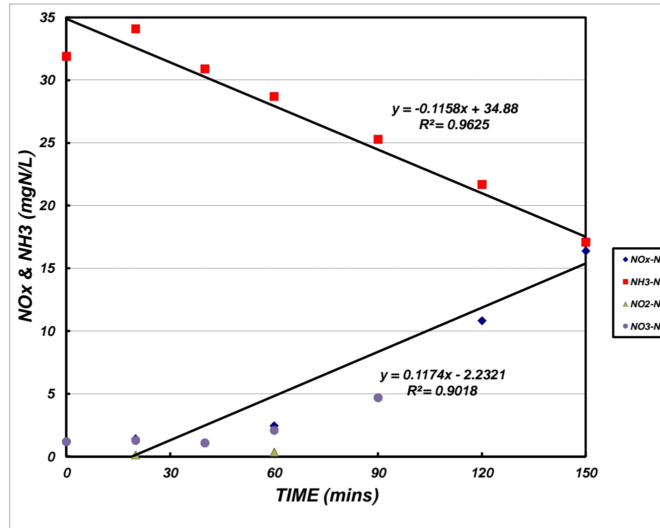
Overall Results

Batch Test VSS (mg/L)	1643
ARR (mg/L/min)	0.16
SARR (mg/gVSS/hr)	5.84
NPR (mg/L/min)	0.193
SNPR (mg/gVSS/hr)	7.05
20 deg C SNPR (mg/gVSS/h	8.10

N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0		1.2	1.2	31.9	18	7.2	1.5
2	2:20:00 PM	20	0.142	1.3	1.4	34.1	18	7.2	1.5
3	2:40:00 PM	40		1.1	1.1	30.9	18	7.2	1.5
4	3:00:00 PM	60	0.368	2.1	2.5	28.7	18	7.2	1.5
5	3:30:00 PM	90		4.7	4.7	25.3	18	7.2	1.5
6	4:00:00 PM	120	0.842	10.0	10.8	21.7	18	7.2	1.5
7	4:30:00 PM	150		16.4	16.4	17.1	18	7.2	1.5
8	5:00:00 PM	180	0.885	20.6	21.5	12.9	18	7.2	1.5
12									
						Averages	18.0	7.20	1.5

REACTOR B - 25 Sept - 2012



Overall Results

Batch Test VSS (mg/L)	2408
ARR (mg/L/min)	0.1158
SARR (mg/gVSS/hr)	2.89
NPR (mg/L/min)	0.1174
SNPR (mg/gVSS/hr)	2.93
20 deg C SNPR (mg/gVSS/hı	3.36

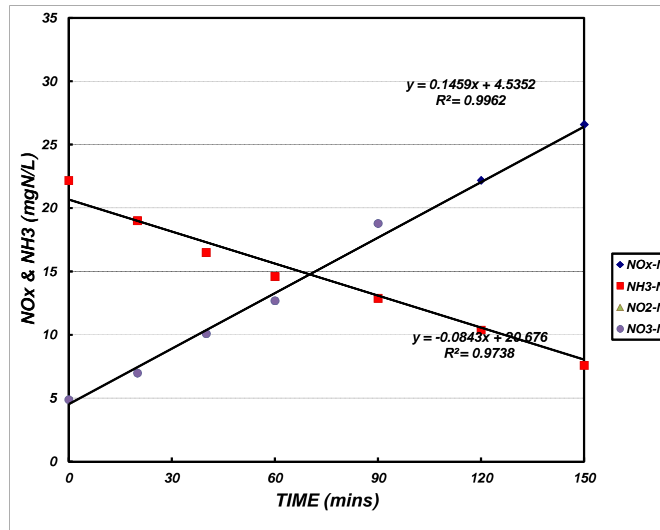
N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0		4.9	4.9	22.2	12	7.2	3
2	2:20:00 PM	20		7.0	7.0	19.0	12	7.2	3
3	2:40:00 PM	40		10.1	10.1	16.5	12	7.2	3
4	3:00:00 PM	60		12.7	12.7	14.6	12	7.2	3
5	3:30:00 PM	90		18.8	18.8	12.9	12	7.2	3
6	4:00:00 PM	120		22.2	22.2	10.4	12	7.2	3
7	4:30:00 PM	150		26.6	26.6	7.6	12	7.2	3
8	5:00:00 PM	180		30.3	30.3	6.6	12	7.2	3
9									
10									
11									
12									
						Averages	12.0	7.20	3.0

Overall Results

Batch Test VSS (mg/L)	1751
ARR (mg/L/min)	0.0843
SARR (mg/gVSS/hr)	2.89
NPR (mg/L/min)	0.1459
SNPR (mg/gVSS/hr)	5.00
20 deg C SNPR (mg/gVSS/hı	8.72

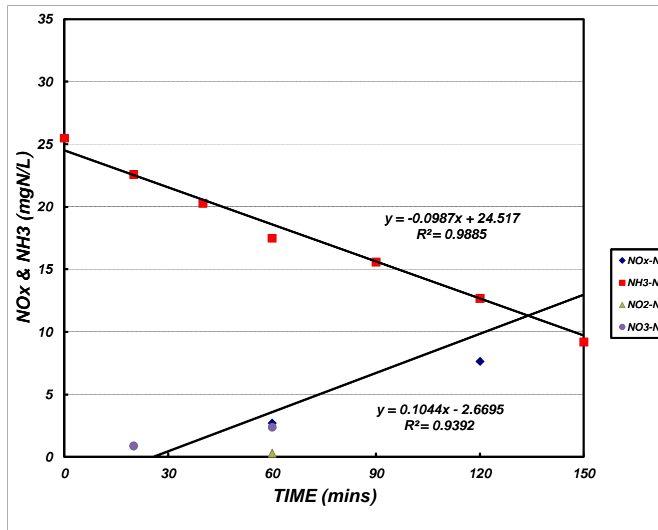
Reactor A20 - October 11, 2012



N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0				25.5	12	7.2	3
2	2:20:00 PM	20		0.9	0.9	22.6	12	7.2	3
3	2:40:00 PM	40				20.3	12	7.2	3
4	3:00:00 PM	60	0.320	2.4	2.7	17.5	12	7.2	3
5	3:30:00 PM	90				15.6	12	7.2	3
6	4:00:00 PM	120	0.450	7.2	7.7	12.7	12	7.2	3
7	4:30:00 PM	150				9.2	12	7.2	3
8	5:00:00 PM	180	0.915	16.8	17.7	7.6	12	7.2	3
12									
						Averages	12.0	7.20	3.0

Reactor C - January 11, 2013



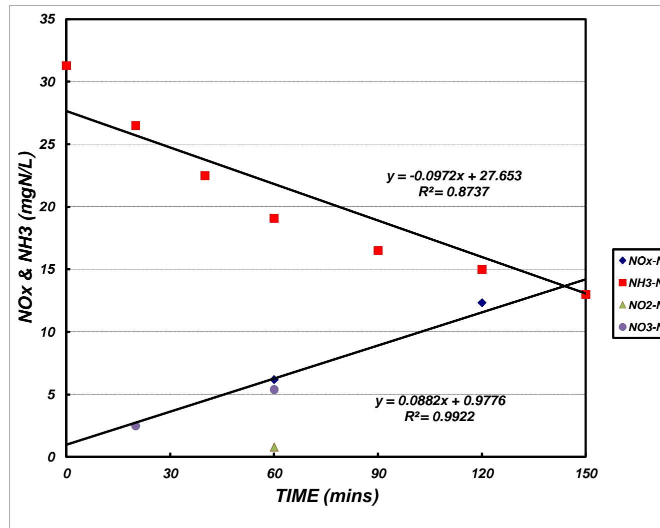
Overall Results

Batch Test VSS (mg/L)	2706
ARR (mg/L/min)	0.0987
SARR (mg/gVSS/hr)	2.19
NPR (mg/L/min)	0.1044
SNPR (mg/gVSS/hr)	2.31
20 deg C SNPR (mg/gVSS/hı	4.04

N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0				31.3	12	7.2	3
2	2:20:00 PM	20		2.5	2.5	26.5	12	7.2	3
3	2:40:00 PM	40				22.5	12	7.2	3
4	3:00:00 PM	60	0.800	5.4	6.2	19.1	12	7.2	3
5	3:30:00 PM	90				16.5	12	7.2	3
6	4:00:00 PM	120	0.940	11.4	12.3	15.0	12	7.2	3
7	4:30:00 PM	150				13.0	12	7.2	3
8	5:00:00 PM	180	0.980	15.4	16.4	13.2	12	7.2	3
12									
						Averages	12.0	7.20	3.0

Reactor A7 - January 11, 2013



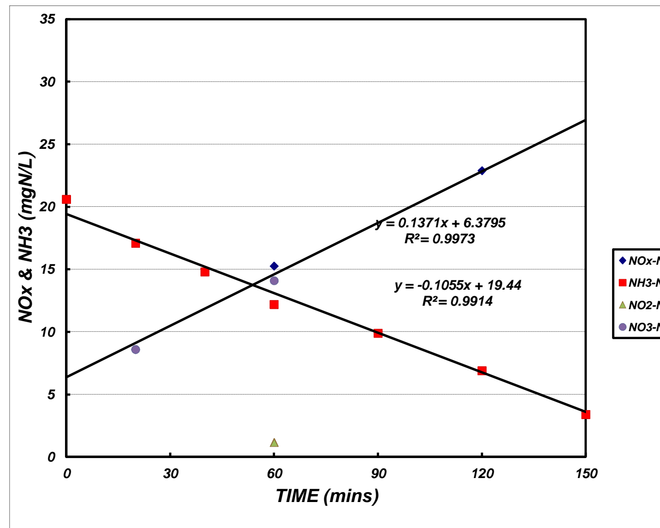
Overall Results

Batch Test VSS (mg/L)	1058
ARR (mg/L/min)	0.0972
SARR (mg/gVSS/hr)	5.51
NPR (mg/L/min)	0.0882
SNPR (mg/gVSS/hr)	5.00
20 deg C SNPR (mg/gVSS/hı	8.72

N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0				20.6	18	7.2	3
2	2:20:00 PM	20		8.6	8.6	17.1	18	7.2	3
3	2:40:00 PM	40				14.8	18	7.2	3
4	3:00:00 PM	60	1.170	14.1	15.3	12.2	18	7.2	3
5	3:30:00 PM	90				9.9	18	7.2	3
6	4:00:00 PM	120	1.200	21.7	22.9	6.9	18	7.2	3
7	4:30:00 PM	150				3.4	18	7.2	3
8	5:00:00 PM	180	1.155	29.7	30.9	1.0	18	7.2	3
12									
						Averages	18.0	7.20	3.0

Reactor K7 - January 11, 2013



Overall Results

Batch Test VSS (mg/L)	683
ARR (mg/L/min)	0.1055
SARR (mg/gVSS/hr)	9.27
NPR (mg/L/min)	0.1371
SNPR (mg/gVSS/hr)	12.05
20 deg C SNPR (mg/gVSS/hı	13.85

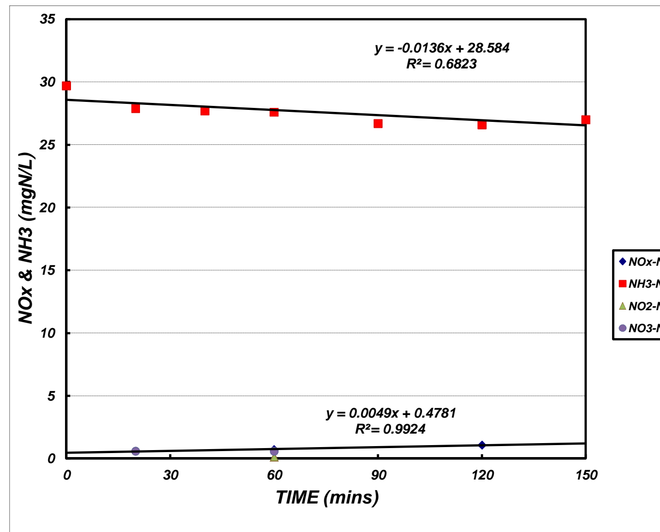
N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0				29.7	12	7.2	3
2	2:20:00 PM	20		0.6	0.6	27.9	12	7.2	3
3	2:40:00 PM	40				27.7	12	7.2	3
4	3:00:00 PM	60	0.130	0.6	0.7	27.6	12	7.2	3
5	3:30:00 PM	90				26.7	12	7.2	3
6	4:00:00 PM	120	0.185	0.9	1.1	26.6	12	7.2	3
7	4:30:00 PM	150				27.0	12	7.2	3
8	5:00:00 PM	180	0.358	1.0	1.4	26.5	12	7.2	3
12									
						Averages	12.0	7.20	3.0

Overall Results

Batch Test VSS (mg/L)	842
ARR (mg/L/min)	0.0136
SARR (mg/gVSS/hr)	0.97
NPR (mg/L/min)	0.0049
SNPR (mg/gVSS/hr)	0.35
20 deg C SNPR (mg/gVSS/hı	0.61

Reactor D - January 11, 2013



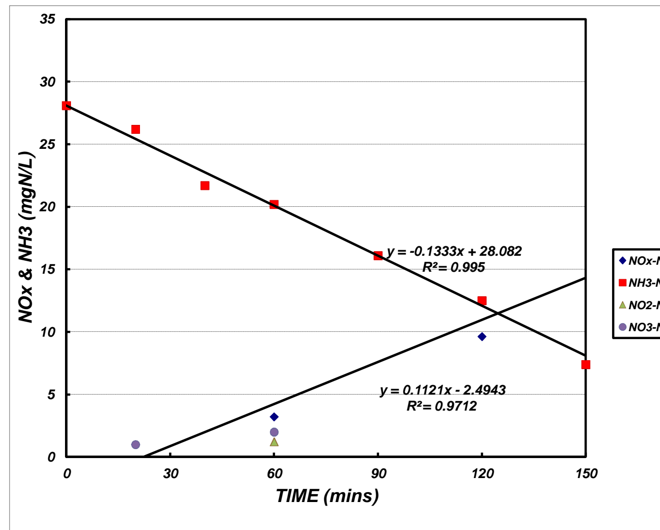
N-Species Analysis on Samples Periodically Taken from Batch Reactor Over Duration of Test

Sample #	Sample Time	Elapsed Time (min)	NO2 (mgN/L)	NO3 (mgN/L)	NOx (mgN/L)	NH3 (mgN/L)	Test Temp (deg C)	Test pH	Test DO
1	2:00:00 PM	0				28.1	18	7.2	3
2	2:20:00 PM	20		1.0	1.0	26.2	18	7.2	3
3	2:40:00 PM	40				21.7	18	7.2	3
4	3:00:00 PM	60	1.223	2.0	3.2	20.2	18	7.2	3
5	3:30:00 PM	90				16.1	18	7.2	3
6	4:00:00 PM	120	2.140	7.5	9.6	12.5	18	7.2	3
7	4:30:00 PM	150				7.4	18	7.2	3
8	5:00:00 PM	180	2.263	16.5	18.8	4.5	18	7.2	3
12									
						Averages	18.0	7.20	3.0

Overall Results

Batch Test VSS (mg/L)	1556
ARR (mg/L/min)	0.1333
SARR (mg/gVSS/hr)	5.14
NPR (mg/L/min)	0.1121
SNPR (mg/gVSS/hr)	4.32
20 deg C SNPR (mg/gVSS/hı	4.97

Reactor E - January 11, 2013



Appendix E

QA/QC Data

E.1. Phase 1

The PC chemical analysis for the first phase investigation was conducted utilizing two laboratories: A commercial lab and the SERVOS lab at the University of Waterloo. The labs utilized different analytical procedures and it was uncertain which lab's methodology would produce results that achieved a suitable level of accuracy and precision. Hence, a detailed assessment of the quality assurance/quality control (QA/QC) data that was collected is presented to establish the context in which the actual test sample values were determined. QA/QC was assessed through the analysis of Matrix spikes (MSs), method blanks as well as an evaluation of ILS recoveries through comparison to concentrations measured within MSs and wastewater samples to those measured within the calibration curve.

The inclusion of Quality Control (QC) data, in the form of spiked analyte recoveries, to demonstrate the capabilities of the analytical method has become common in PC analysis studies (Lee et al. 2003; Lishman et al. 2006; Hao et al. 2008; Rodil et al. 2009; Van Nuijs et al. 2010; Tarcomnicu et al. 2011). Many of these articles utilized de-ionized water, tap water or uncontaminated surface water as a QC matrix. To demonstrate the analytical abilities of LC-MS/MS methods when analyzing PCs, Van Nuijs et al. implemented QC criteria which required that target analyte recoveries were within ± 15% of the spiked amount for results to be considered satisfactory. Tarcomnicu et al. (2011) similarly used the same QC recovery criteria as a means of quality assurance, but also required that relative standard deviations (RSD) calculated for replicate QC samples were below 15%.

Alternatively, some researchers provide an assessment of method performance by reporting the recovery of spiked standards within wastewater samples. However due to the impacts of matrix effects, poor recoveries have been reported. Radjenovic et al. (2009) utilized spiked wastewater recoveries as a means of demonstrating the analytical capabilities of the method used in their study. Recoveries ranged from 35.4 to 127% of the spiked amounts, with an average recovery of 70% reported. Similarly, Gomez et al. (2010) utilized synthetic wastewater as a QC matrix in the analysis of almost 400 trace contaminants and reported recoveries that were generally greater than 70%, although some were less than 50%.

Poor linearity was observed during the initial investigations in which PC standards were spiked into primary effluent to assess the analytical capabilities of the commercial lab (Section 3.6.3). As a result, it was decided that QC would be carried out using deionized water to minimize noise during analysis and provide a simplified assessment of sample preparation performance. During Phase 1, 2 and 3 analysis, the analytical process was considered to be achieving an acceptable level of accuracy if the average MS recovery for each PC was within ±15% of the spiked concentration. Similarly, an acceptable level of precision was considered to be achieved if the RSD for the MS recoveries was below 15% for each PC. These benchmarks are commensurate with the procedure utilized by Tarcomnicu et al. (2011) and are considered reflective of good analytical performance.

To ensure that the analytical method utilized achieved the highest level of accuracy and precision possible, and to recognize the limitations of data obtained during PC analysis, an estimate of the total analytical error was required. The total analytical error can be primarily attributed to analyte losses occurring during sample preparation and analysis as well as signal suppression/enhancement due to matrix effects. Additional losses associated with sample

and analyte measurements, sorptive losses associated with sample contact to lab equipment and volatilization during sample evaporation were also expected to occur. However, these losses were expected to represent a lesser impact on the final data than the losses due to sample preparation and analysis (Vogeser and Seger, 2010, Hall et al. 2012). The measurement of isotopically labelled standards (ILSs), which were spiked into all samples following filtration, allowed for an assessment and correction of these systematic losses which occurred during sample preparation and analysis.

The SERVOS lab (UW) utilized the isotope dilution method, as outlined in section 3.6, in which ILSs were quantitated for each sample. As a result, the MS and sample results reported reflect analyte concentrations that had undergone correction for the total analytical error via the measured ILS recovery. The commercial lab utilized a proprietary method of which only limited information was made available. In an attempt to control the effects of co-eluting compounds, which cause matrix effects in wastewater samples, the commercial lab used a method which relied on serial dilution of samples to minimize their impacts. As a result of this method, the commercial lab was not able to quantitate the ILSs that were added to the MSs and samples and therefore the reported values were considered to be absolute measurements of the analytes without any corrections applied for the losses occurring during sample preparation and analysis. No estimate of the total analytical error could be obtained using this method, nor could any corrections be made.

Four matrix spikes (MS) were prepared and analyzed using the same method as the authentic samples. The analysis of MSs was conducted to provide confirmation of the analytical measurement process via quantitation of a reference standard at a known concentration. As reported in Section 3.6, all MSs were spiked with reference standards for the 5 PCs to achieve a final concentration of 100 ng/L. If the analytical process was achieving satisfactory performance, it was expected that each of the 5 analytes would be reported at concentrations between 85 and 115 ng/L and would have RSD values less than 15%. The measured MS values reported by both labs are provided in Table E1.

Table E1 – Measured Concentrations of 5 Analytes Within MSs - Phase 1

Sample ID	Reported Concentration of Target Analytes ng/L)				
	CBZ	SMX	TRIM	ATEN	ACE
SERVOS Lab Results					
MS1	212	221	93	99	ND
MS2	330	236	98	119	ND
MS3	120	180	92	117	ND
MS4	117	320	94	93	ND
Average	195	239	94	107	
R.S.D	52	25	3	12	
Commercial Lab Results					
MS1	255	69	63	60	<10
MS2	395	76	78	52	<10
MS3	103	73	62	39	<10
MS4	103	71	77	64	<10
Average	214	72	70	54	
R.S.D	66	4	12	21	

Note:
ND – No Data Available

The data reported by both labs for CBZ in the MS1 and MS2 samples suggests that a sample preparation error involving CBZ reference standards occurred. The SERVOS lab reported CBZ at concentrations equal to 212 and 330% of the spiked concentrations. This was consistent with the CBZ concentrations reported from the commercial lab that reported similar concentrations which were 255% and 395% of the spiked concentrations. As both labs reported similar results while utilizing different methods, it is unlikely that this error could be attributed to analytical equipment. Similarly, the reported concentrations for SMX from the SERVOS lab ranged from 180 to 320 percent of the expected value for all four MSs. It is believed that this data was the result of ILS recovery issues, as the commercial lab data did not demonstrate the same variability or any consistent trends when compared to the data obtained from the SERVOS lab. It is not clear why CBZ and SMX were the only analytes that demonstrated concentrations which were 2 to 3 times what was expected, as a common spiking solution with all 5 PCs present at the same concentrations was used to prepare each MS.

The CBZ and SMX results reported by the SERVOS lab did not meet the QC recovery criteria proposed above, demonstrating poor analytical accuracy and precision. The data from the commercial lab were considered to be below the QC requirements for accuracy for all 5 analytes. However, it was noted that the commercial lab achieved acceptable levels of precision for SMX and TRIM. The reported recoveries of ACE indicated that neither lab could

quantitate this analyte within the MSs at the spiked concentration of 100 ng/L. Based on these poor recoveries, MS and ILS concentrations for ACE were subsequently increased to 10 ug/L for Phase 2/3 analysis.

Based on the methods employed by Matuszewski et al. (2003), the recovery of ILSs within MSs was used to provide an estimate of the losses associated with sample preparation. This was achieved through comparison of the measured signals of ILSs for the 5 PCs within the MSs to the ILS signals measured in the calibration curves. The ILS signals associated with the calibration curves were used as a baseline, representing 100% recovery of the added ILS concentration. However, the calibration curve concentrations were noted to be impacted by measurement errors and some minor analytical error and therefore represent a best estimate of the expected concentration only. As such, this comparative method was noted to only provide a qualitative indication of losses and not absolute measurements. Data reported by the SERVOS laboratory for ILS recoveries from each of the 4 MSs are provided in Table E2. ACE ILS values were not reported as they were considered too low for reliable quantitation.

Table E2 – ILS recoveries for MS Phase I

Sample ID	Recovery of ILS (%)			
	CBZ	SMX	TRIM	ATEN
U of W Laboratory Results				
MS1	166	56	99	33
MS2	106	57	101	30
MS3	99	68	80	19
MS4	100	41	87	31

The results presented in Table E2 demonstrate a reduced recovery of ATEN and SMX relative to CBZ and TRIM. As reported previously by Gros et al. (2006), basic compounds such as ATEN demonstrate reduced recoveries through SPE with Oasis HLB cartridges when sample pH is acidic. This result has been similarly duplicated by others (Shao et al. 2009). Additionally, Gros et al. (2006) reported recoveries equal to 93%, 50%, 83%, 96% and 50% for CBZ, SMX, TRIM, ATEN and ACE, respectively, when spiked at a concentration of 100 ng/L into effluent wastewater samples and extracted using HLB SPE methods at neutral pH. This demonstrates a similar reduced recovery of SMX (50%), however ATEN in this study was recovered at 96%. Scheurer et al. (2010) investigated the recovery of ATEN through the SPE procedure. Spiked samples were extracted using c18 SPE cartridges and recoveries of 20%, 40% and 58% were reported for wastewater influent, effluent and surface water samples extracted under neutral pH. This suggests that ATEN is subject to reduced extraction efficiency dependent on the sample matrix. However, as the ILS recoveries for the MSs were obtained through analysis of de-ionized and distilled water, it is unlikely that matrix effects impacted the recovery. It is possible that the SPE cartridges (HLB) demonstrated differing sorption affinities related to pH for each of the 4 PCs analyzed as sample pH was not measured.

The importance of using the isotope dilution method can be demonstrated by the reported concentration of ATEN from MS3. The commercial lab did not quantitate the ILS of ATEN spiked into MS3 and therefore no correction for losses which occurred during the SPE procedure or the matrix effects associated with the sample analysis were made. The commercial lab reported an ATEN concentration equal to 39% of the spiked concentration. In contrast, the SERVOS lab, which utilized the isotope dilution method, reported a concentration equal to 117% of the spiked concentration, which reflects an accuracy improvement of 44%. As the analysis was conducted by both labs using aliquots prepared from the same sample, this improved accuracy was attributed to the method used by the SERVOS lab, which provided correction through the quantitation of the ILS standard spiked during sample preparation.

The concentrations reported for SMX by the SERVOS lab for the 4 MSs were noted to be closest to the expected value when ILS recoveries were highest. MS3, which had a reported SMX concentration of 180 ng/L, is also noted to have achieved the highest recovery of SMX ILS (68%). Similarly, MS4, which achieved the lowest SMX ILS recovery (41%), reported an SMX concentration of 320 ng/L. MS4 had the poorest accuracy achieved of the 4 MSs analyzed. These results suggest that the recovery of ILSs within each sample had an effect on the accuracy achieved. However, due to the limited sample size, these results are considered speculative.

Based on the methods employed by Matuszewski et al. (2003), the recovery of ILSs within samples were used to provide an estimate of the losses associated with wastewater sample preparation and analysis. The analytical method utilized by the SERVOS lab allowed for the determination of the ILS signals within the test samples collected from the reactors. As all samples were prepared using the same methods, the recovery of the spiked ILSs within wastewater samples relative to MSs provided an indicative measure of the matrix effects associated with the initial and final (treated) wastewater. As discussed in section 3.6.1, matrix effects can result in reduced recoveries of analytes through the SPE procedure as well as ion suppression and enhancement during analysis. Table E3 provides average ILS recoveries observed, normalized based on the average ILS signal measured for the calibration curves, for each of the 4 PCs.

Table E3 – Average Recovery of ILS from Wastewater Samples

Sample ID	Recovery of ILS (%)			
	CBZ	SMX	TRIM	ATEN
K20 Initial	108	23	67	6
B Initial	68	15	42	4
K20 Final	119	40	109	33
B Final	126	36	104	35

Based on the data presented in Table E3, the recovery of ILSs from the initial samples was generally between two and seven times lower than the final samples, with the poorest recovery achieved for ATEN. Effluent samples were

noted to achieve similar ILS recoveries to MSs. It was also noted that the recovery from reactor B initial samples was reduced when compared to reactor K20 initial samples. The cause of this was not clear; however it is suspected that reactor B had a greater concentration of co-eluting compounds which increased signal suppression due to elevated initial COD loadings, as presented previously. This effect was not observed in the effluents; both reactors demonstrated highly consistent ILS recoveries (within 10%).

The reduced level of recovery of ILSs within the initial samples was likely related to elevated matrix effects associated with the primary effluent wastewater. Renew and Huang (2004) used LC-MS/MS methods to investigate several fluoroquinolone and sulfonamide antibiotics as well as TRIM in wastewater samples. The authors observed that the signal suppression for every antibiotic investigated increased linearly in relation to the organic carbon content of the sample matrix. The author also noted that SMX recoveries were between 20 and 40 percent lower than those observed for fluoroquinolones and TRIM. Gros et al. (2006) reported that for effluent wastewater samples, ion suppression ranging from 40 to 60% was encountered. Similarly, influent samples demonstrated ion suppression which generally exceeded 60%, with ATEN demonstrating ion suppression as high as 90%. The ILS data is noted to be generally consistent with the results from these previously reported studies.

It was noted that SMX and ATEN ILSs were only recovered in initial samples at approximately 20 and 5 percent, respectively, of their spiked amounts. It is not known if these low recoveries of ILSs achieved for SMX and ATEN would have had an effect on the accuracy and precision achieved using the isotope dilution method. Anecdotally, SERVOS lab staff have indicated that data obtained using the isotope dilution method in which recoveries of ILSs are below 30% is considered questionable. Limited information involving ILS recoveries has been reported in the literature for comparison. However, Scheurer et al. (2010) observed recoveries of ATEN as low as 20% and this was attributed primarily to ion suppression. The authors noted that the usage of ILSs improved the recoveries to greater than 75%, drastically improving the analytical accuracy achieved. It is therefore considered that the accuracy achieved for Phase 1 investigations was likely consistent with these previous investigations.

As ILSs behave in an identical fashion to their non-labelled counterpart in the context of contaminant fate processes, the ILS recovery data suggests that measurements of the unlabelled PCs would have been similarly reported at approximately 20 and 5 percent of the true concentration within initial samples if the isotope dilution method were not used. However, the commercial lab was noted to have used a methodology that employed dilution of sample extracts to reduce the impacts of matrix effects. Due to the nature of this method, no measure of these errors could be made. Similarly, this method was shown during preliminary investigations to produce calibration curves with very limited range. Due to the increased uncertainty associated with this method it was considered critical that the isotope dilution method be used in all future PC analysis. This was consistent with recommendations made in previous studies investigating wastewater (Hao et al. 2008; Shao et al. 2009; Tarcomnicu et al. 2011).

E.2. Phase 2

The PC chemical analysis for the phase 2 investigation was conducted entirely at the SERVOS lab at the University of Waterloo. Based on the recommendations of the SERVOS lab chemist, the preparation methods typically utilized by the lab were re-instated, resulting in a concentration factor of 200 times. A detailed assessment of the quality assurance/quality control (QA/QC) data that was collected as part of the phase 2 analysis is presented to establish the context in which the actual test sample values were determined. As noted previously, samples collected from reactor C were analyzed at the same time as Phase 3 samples due to reactor performance issues which necessitated a delay in sample collection schedules. As reactor C samples were prepared and analyzed concurrently with phase 3 samples, QA/QC data corresponding to phase 3, provided in section 4.3, can be considered representative of the analytical accuracy and precision achieved during their analysis.

QA/QC was assessed through the analysis of Matrix spikes (MSs), method blanks as well as an evaluation of isotopically labelled standard (ILS) recoveries. ILS recoveries were used to provide a coarse (qualitative) assessment of matrix effects. This was conducted by comparing the ILS signals measured for each PC, as reported by the analytical instrument during the analysis of MSs and wastewater samples, to the ILS signals measured for each PC within the calibration curve. As the MSs, the wastewater samples and the calibration curve each received an equivalent mass of ILS the ILSs injected into each of the points on the calibration curve provide the best estimate of the "true" ILS concentration as calibration curves were prepared in methanol, without any sample preparation (losses), and thus provide the best estimate of the ILS concentration common to all three matrices. As discussed previously in section 4.1, it was decided that the assessment of accuracy and precision would be carried out using MSs prepared with distilled and de-ionized water to minimize noise during analysis and provide a simplified assessment of sample preparation method performance. The sample preparation and the analytical processes were considered to be achieving an acceptable level of accuracy if the average MS recovery for each PC was within ±15% of the spiked concentration. Similarly, an acceptable level of precision was considered to be achieved if the RSD for the MS recoveries was less than 15% for each PC. These benchmarks are commensurate with the procedures utilized in recent PC analyses (Van Nuijs et al. 2010; Tarcomnicu et al., 2011).

To ensure that the analytical method utilized achieved the highest level of accuracy and precision possible, and to recognize the limitations of data obtained during PC analysis, an estimate of the total analytical error was determined. The total analytical error was primarily attributed to analyte losses occurring during sample preparation and analysis as well as signal suppression/enhancement due to matrix effects. Additional losses associated with sample and analyte measurements, sorptive losses associated with sample contact to lab equipment and volatilization during sample evaporation were also expected to have occurred. However, these losses were expected to have a lesser impact on the final data than the losses associated with sample preparation and analysis (Vogeser and Seger, 2010, Hall et al. 2012). The measurement of ILSs, which were spiked into all wastewater samples following filtration, allowed for an assessment and correction of these systematic losses. Phase 2 analysis was carried out using the isotope dilution method, as outlined in section 3.6, in which ILSs were spiked and quantitated in

each sample. As a result, the MS and initial and final sample results reported reflect analyte concentrations that have undergone correction for the total analytical error via the measured ILS recovery.

To provide an assessment of the accuracy and precision of the analytical method, two MSs were prepared and analyzed using the same method as the authentic samples collected from reactor A20. The analysis of MSs also provided confirmation of the analytical measurement process via quantitation of a reference standard at a known concentration. As reported in Section 3.6, all MSs were spiked with reference standards of CBZ, SMX, TRIM and ATEN to achieve a final concentration of 100 ng/L. In phase 2, samples were spiked with ACE reference standard to achieve a final concentration of 10 µg/L. If the analytical process was achieving satisfactory performance, it was expected that each of the 5 analytes would be reported at an average concentration between 85 and 115 percent of the spiked amount and would result in RSD values less than 15%. The measured MS values, expressed as a percentage of the spiked amounts (recovery), are provided in Table E4. All raw LC-MS/MS data is located in **Appendix E**.

Table E4 - QA/QC Results - Phase 2

Sample ID	Measured Recovery (%)				
	CBZ	SMX	TRIM	ATEN	ACE
MS1	99	87	90	108	78
MS2	101	99	98	117	89
AVERAGE	100	93	94	113	84
R.S.D.	1	9	6	6	9

The results for CBZ, SMX, TRIM and ATEN reported by the SERVOS lab met both the accuracy and precision criteria outlined above. ACE was noted to have been 1 % below the accuracy criteria, but achieved an R.S.D. that was significantly better than the criteria established for precision. On this basis, the analytical methodology used for phase 2 analysis demonstrated significant improvements in both the accuracy and precision achieved when contrasted with phase 1 QA/QC results. These results were expected to provide a commensurate level of accuracy and precision during the analysis of wastewater samples as has been demonstrated in previous studies (Van Nuijs et al. 2010; Tarcomnicu et al., 2011).

Based on the methods employed by Matuszewski et al. (2003), the recovery of ILSs within MSs was used to provide an estimate of the losses associated with sample preparation. This was achieved through a comparison of the measured signals of ILSs for the 5 PCs within the MSs to the ILS signals measured in the calibration curves. The ILS signals associated with the calibration curves were used as a baseline, representing 100% recovery of the spiked ILS concentration. However, the reported concentrations for the calibration curve were impacted by measurement errors during preparation and some minor analytical error and therefore represent a best estimate of the expected concentration only. As such, this method was noted to only provide a qualitative indication of losses and not absolute

measurements. Data reported by the SERVOS lab for ILS recoveries from both MSs are provided in Table E5. ACE ILS values were not reported as the methodology used did not allow for a direct assessment of the recovery of this compound.

Table E5 – Average Recovery of ILS from MSs

Sample ID	Recovery of ILS (%)			
	CBZ	SMX	TRIM	ATEN
MS1	121	52	92	47
MS2	121	47	66	25

The results presented in Table E5 demonstrate a reduced recovery of ATEN and SMX was achieved relative to CBZ and TRIM. This behaviour was similar to that observed during phase 1 analysis, as was presented in Table E1. As discussed previously, basic compounds such as ATEN demonstrate reduced recoveries through SPE with Oasis HLB cartridges when the sample pH is acidic (Gros et al. 2006). Additionally, ATEN has been shown to demonstrate a variable recovery through SPE in which the recovery achievable is highly dependent on the sample matrix extracted (Scheurer et al. 2010). However, as the ILS recoveries for the MSs were obtained through analysis of de-ionized and distilled water, it is unlikely that matrix effects impacted the recoveries presented in Table E5. It is possible that the SPE cartridges (HLB) demonstrated differing sorption affinities related to pH for each of the 4 PCs analyzed as sample pH was not measured.

The ILS recoveries achieved in phase 2 MSs were consistent with those achieved during phase 1 analysis. However, it was noted that the MS values reported for phase 2 (Table E5) demonstrated considerable improvement in both accuracy and precision. The Phase 1 MSs were noted to have achieved particularly poor accuracy and precision for SMX and 2 out of 4 MSs demonstrated poor accuracy and precision for CBZ, with concentrations reported at approximately 2 to 3 times the spiked amounts (Table E1). When viewed comparatively, the ILS data from both phases suggests that the errors could be attributed to difficulty in the quantitation of the non labelled reference standards as ILS recoveries show only minor variability between both phase 1 and 2 analyses. This may have been the result of reduced concentration factors during phase 1, which may have resulted in greater impacts from background signals caused by contamination. Based on the data provided in Table E5, the magnitude of the errors observed during phase 1 do not appear to have been present during analysis of the phase 2 MSs. The cause of the errors observed during phase 1 remains unclear.

Based on the methods employed by Matuszewski et al. (2003), the recovery of ILSs within samples were used to provide an estimate of the losses associated with wastewater sample preparation and analysis. As all samples were prepared using the same methods, the recovery of the spiked ILSs within wastewater samples relative to MSs provided an indicative measure of the matrix effects associated with the initial and final (treated) wastewater. As

discussed in section 3.6.1, matrix effects can result in reduced recoveries of analytes through the SPE procedure as well as ion suppression or enhancement during analysis. Table E6 provides average ILS recoveries observed, normalized based on the average ILS signal measured for the calibration curves, for 4 PCs. All samples were spiked with 100 ng/L of ILS, with the exception of ACE which was spiked to a concentration of 10 μg/L. However, due to the analytical method used, a direct comparison of ILS signals for ACE was not available.

Table E6 – Average Recovery of ILSs from Wastewater Samples: Phase 2

Sample ID	Recovery of ILS (%)			
	CBZ	SMX	TRIM	ATEN
MSs	121	50	79	36
C Initial	38	9	12	6
A20 Initial	24	6	9	4
C Final	63	13	25	13
A20 Final	62	17	29	16

Based on the data presented in Table E6, the recovery of ILSs from the initial samples collected from both reactor A20 and C were generally between two and three times lower than their respective final samples. ILS recoveries for final samples collected from reactor A20 and reactor C were noted to be similar; suggesting that sample preparation and analysis was consistent on both days of analysis. Phase 1 final samples were noted to have achieved similar ILS recoveries to those observed for phase 1 MSs. This was not the case for phase 2 samples; final samples were noted to have achieved ILS recoveries approximately 2-3 times lower that those observed for phase 2 MSs. It is possible that the increased concentration factor utilized during phase 2 resulted in greater concentrations of co-eluting compounds and associated increases in matrix effects. Dilution of sample extracts has been utilized by researchers in the past as a method of reducing the impacts from matrix effects (Gros et al. 2006) and may partially explain the reduced ILS recoveries achieved for Phase 2 relative to Phase 1.

As discussed previously, studies have demonstrated that antibiotics, such as SMX, undergo signal suppression that has been shown to increase linearly with the organic carbon content within wastewater samples (Renew and Huang, 2004). Other research has demonstrated that the signal suppression varies between compounds, with ATEN demonstrating notably poor recovery in samples with high concentrations of co-eluting compounds (Gros et al. 2006). It was also noted that the recovery from reactor C initial samples was reduced when compared to reactor A20 initial samples by approximately 33 percent. The cause of this was not clear as both reactors received relatively consistent COD loadings as demonstrated by the conventional data collected during PC sampling. The cause of this effect may therefore be related to different primary effluent compositions during the period in which each reactor was sampled.

It was noted that all 4 PCs in both initial and final samples analyzed during phase 2 achieved recoveries of approximately one third to one quarter of the ILS recoveries observed during phase 1, with the exception of ATEN within initial samples. It is not known if the low recoveries of ILSs achieved for the 4 PCs within wastewater samples would have had an effect on the accuracy and precision achieved using the isotope dilution method. Anecdotally, SERVOS lab staff indicated that data obtained using the isotope dilution method in which recoveries of ILSs are below 30% is considered questionable. Limited information involving ILS recoveries and the impacts on accuracy and precision has been reported in the literature. However, Scheurer et al. (2010) observed recoveries of ATEN as low as 20% in wastewater samples and this was attributed primarily to ion suppression. The authors noted that the usage of ILSs improved recoveries to greater than 75%, drastically improving the analytical accuracy achieved. The ILS recoveries observed for phase 2 were less than those reported by Scheurer et al. (2010); however, based on the accuracy and precision achieved during MS analysis, it was believed that the analytical method was performing satisfactorily.

E.3. Phase 3

The PC chemical analysis for phase 3 investigations was conducted in the SERVOS lab at the University of Waterloo. Based on the improved accuracy and precision observed during Phase 2, relative to Phase 1, a concentration factor of 200 times was again used in Phase 3. A detailed assessment of the quality assurance/quality control (QA/QC) data that was collected as part of the Phase 3 analysis is presented to establish the context in which the actual test sample values were determined. QA/QC was assessed through the analysis of Matrix spikes (MSs), method blanks as well as an evaluation of ILS recoveries.

The approach utilized to assess QA/QC in Phase 3 was identical to that utilized in Phase 2. MSs, prepared in de-ionized and distilled water were used to assess the accuracy and precision achieved by the method. ILS recoveries were used to assess matrix effects associated with analysis of wastewater samples. As discussed previously in section 4.1, this was conducted by comparing the ILS signals measured for each PC, as reported by the analytical instrument during the analysis of MSs and wastewater samples, to the ILS signals measured for each PC within the calibration curve. As the MSs, the wastewater samples and the calibration curve each received an equivalent mass of ILS, the ILSs injected into each of the points on the calibration curve provide the best estimate of the "true" ILS concentration. Calibration curves were prepared in methanol, without any sample preparation, and thus provide the best estimate of the ILS concentration within all three matrices.

Sample preparation and the analytical process were considered to be achieving an acceptable level of accuracy if the average MS recovery for each PC was within ±15% of the spiked concentration. Similarly, an acceptable level of precision was considered to be achieved if the R.S.D. for the MS recoveries was below 15% for each PC. These benchmarks are commensurate with the procedures utilized in recent PC analyses (Van Nuijs et al. 2010; Tarcomnicu et al., 2011).

To ensure that the analytical method utilized achieved the highest level of accuracy and precision possible, and to recognize the limitations of data obtained during PC analysis, an estimate of the total analytical error was determined. Total analytical error was primarily attributed to analyte losses occurring during sample preparation and analysis as well as signal suppression/enhancement due to matrix effects. Additional losses associated with sample and analyte measurements, sorptive losses associated with sample contact to lab equipment and losses during sample evaporation were also expected to have occurred. However, these losses were expected to have a lesser impact on the final data than the losses associated with sample preparation and analysis (Vogeser and Seger, 2010, Hall et al. 2012). The measurement of ILSs, which were spiked into all samples following filtration, allowed for an assessment and correction of these systematic losses that occurred during sample preparation and analysis. Phase 3 analysis was carried out using the same analytical procedure as was employed during Phase 1 and 2 (isotope dilution method), as outlined in section 3.6. As a result, the MS and initial and final sample results reported reflect analyte concentrations that had undergone correction for the total analytical error via the measured ILS recovery.

To provide an assessment of the accuracy and precision of the analytical method, 16 matrix spikes (MSs) and eight method blanks were prepared and analyzed using the same method as the authentic samples collected from the reactors investigated under Phase 3. Samples from reactor C (Phase 2) were prepared and analyzed during Phase 3 and thus the QA/QC data presented in Table E7 is reflective of the accuracy and precision achieved during preparation and analysis of these samples.

The analysis of MSs provided confirmation of the analytical measurement process via quantitation of a reference standard at a known concentration. As reported in Section 3.6, all MSs were spiked with reference standards of CBZ, SMX, TRIM and ATEN to achieve a final concentration of 100 ng/L. MSs were again spiked with ACE reference standard to achieve a final concentration of 10 µg/L. If the analytical process was achieving satisfactory performance, it was expected that each of the 5 analytes would be reported at an average concentration between 85 and 115 percent of the spiked amount and would result in RSD values less than 15%. The measured MS values, expressed as a percentage of the spiked amounts (recovery), are provided in Table E7.

Table E7 - QA/QC Results - Phase 3

Sample ID	Measured Recovery (%)				
	CBZ	SMX	TRIM	ATEN	ACE
MS1	111	110	108	123	**8950**[1]
MS2	104	102	102	115	99
MS3	106	105	100	118	98
MS4	102	100	94	104	103
MS5	120	99	90	109	92
MS6	125	97	93	107	94
MS7	106	101	107	114	101
MS8	108	106	93	112	92
MS9	113	110	110	130	96
MS10	106	92	96	104	93
MS11	111	98	103	112	95
MS12	123	101	104	111	80
MS13	120	93	94	110	96
MS14	103	88	87	99	81
MS15	97	89	91	110	84
MS16	99	87	91	**216**[2]	84
AVERAGE	109.6	98.6	97.7	111.9	92.5
R.S.D	8.5	7.3	7.2	7.7	7.2

Notes:

1. Sample is believed to have been incorrectly spiked with ILS (100 ng/L), resulting in overstated concentration by a factor of 100 times. This datum was not included in the calculation of the average and R.S.D.
2. Analyte and ILS signals were unusually low, resulting in potentially skewed result. This datum was not included in the calculation of the average and R.S.D.

The results for all 5 PCs reported by the SERVOS lab met both the accuracy and precision criteria outlined above, after erroneous data was removed from the dataset. The reported value for ACE for MS1 was significantly higher (2 orders of magnitude) than all other MSs and likely resulted from an incorrect ILS volume spiked into this sample. This resulted in overcompensation by the method and an exaggerated ACE concentration. The ATEN signal for both the ILS and the unlabelled standard reported for MS16 was significantly lower relative to those observed during analysis of the 15 other MSs. It was believed that unusually poor recovery occurred for MS16 and the cause of this error is unknown. However, this error only affected ATEN as all other PCs in MS16 produced signals commensurate with the

remaining 15 MSs analyzed. On this basis, the analytical methodology used for phase 3 analysis demonstrated accuracy and precision which was commensurate with that achieved during Phase 2 and significantly improved relative to Phase 1. As a result, the analytical process was expected to provide a consistent level of accuracy and precision during the analysis of wastewater samples with those demonstrated in previous studies (Van Nuijs et al. 2010; Tarcomnicu et al., 2011).

Based on the methods employed by Matuszewski et al. (2003), the recovery of ILSs within MSs was used to provide an estimate of the losses associated with sample preparation. This was achieved through a comparison of the measured signals of ILSs for the 5 PCs within the MSs to those measured in the calibration curves. The ILS signals associated with the calibration curves were used as a baseline, representing 100% recovery of the spiked ILS concentration. However, the reported concentrations for the calibration curve were noted to be impacted by measurement errors during preparation and some minor analytical error and therefore represent a best estimate of the expected concentration only. As such, this method was noted to only provide a qualitative indication of losses and not absolute measurements. Data reported by the SERVOS lab for ILS recoveries from each of the 4 MSs as well as the initial and final wastewater samples collected from reactors K7, E, A7 and D are provided in Table E8. ACE ILS values were not reported as the methodology used did not allow for a direct assessment of the recovery of this compound.

Table E8 – Average Recovery of ILS from MS and Wastewater Samples

Sample ID	Recovery of ILS (%)			
	CBZ	SMX	TRIM	ATEN
AVERAGE MS	105	48	76	37
K7 Influent	45	9	14	7
E Influent	42	10	14	7
A7 Influent	42	10	13	6
D Influent	37	9	12	6
K7 Effluent	76	18	37	19
E Effluent	58	14	25	10
A7 Effluent	72	17	34	18
D Effluent	52	12	21	11

Based on the data presented in Table E8, the recovery of ILSs within the initial samples collected from all reactors were generally between 1.5 and 2.5 times lower than their respective final samples for all 4 PCs. Additionally, PC recoveries within final samples were noted to be between 1.5 and 3 times lower than those within MSs. This

demonstrates that matrix effects were occurring in both the initial and final wastewater samples, with the most significant impacts occurring in initial samples. This was consistent with observations made during Phase 2 in which initial sample ILS concentrations were approximately 2 to 3 times lower than those observed in final samples. Additionally, similar trends were observed during Phase 2 in which ILS recoveries of SMX and ATEN were notably reduced in comparison to CBZ and TRIM.

As discussed previously, studies have demonstrated that antibiotics, such as SMX, undergo signal suppression that has been shown to increase linearly with the organic carbon content within the wastewater sample (Renew and Huang, 2004). Other research has demonstrated that the signal suppression varies between compounds, with ATEN demonstrating poor recovery in samples with high concentrations of co-eluting compounds (Gros et al. 2006). It was noted that both control SBRs achieved recoveries of ILS within effluent which were between 30 and 40 percent lower than those measured for their respective IFAS SBBRs. This effect was not observed during phase 2. The cause of this was not clear as both reactors received relatively consistent COD loadings as demonstrated by the conventional data collected during PC sampling. The cause of this effect may therefore be related to differing levels of removal of co-eluting organic compounds which occurred during the treatment process.

It was noted that all 4 PCs in both initial and final samples analyzed during phase 3 achieved recoveries of approximately one half to one quarter of the ILS recoveries observed during phase 1, with the exception of ATEN within initial samples. As identified during Phase 2, it was not known if the low recoveries of ILSs achieved for the 4 PCs within wastewater samples would have had an effect on the accuracy and precision achieved using the isotope dilution method. The recoveries achieved were noted to be lower than those reported by researchers who conducted similar investigations into ILS recoveries (Scheurer et al. 2010). However, based on the accuracy and precision achieved through the analysis of the MSs it was believed that the analytical method was performing satisfactorily.

Appendix F

LC-MS-MS Data

	ALS				
	Influent				
Sample	CBZ	SMX	TRIM	ATEN	ACE
K1I	736	331	105	718	47300
K2I	584	285	81	551	41500
K3I	347	497	81	679	33000
AVERAGE	465.5	391	81	615	37250
STD	167.58	149.91	0.00	90.51	6010.41
Corr. STD	209.48	187.38	0.00	113.14	7513.01
RSD	45%	48%	0%	18%	20%
K4I	498	289	93	723	39200
K5I	659	297	81	660	35800
K6I	376	508	107	1340	52600
AVERAGE	437	398.5	100	1031.5	45900
STD	86.27	154.86	9.90	436.28	9475.23
Corr. STD	107.83	193.57	12.37	545.36	11844.04
RSD	25%	49%	12%	53%	26%
K7I	1030	693	156	1170	78800
K8I	473	543	88	679	36800
K9I	380	420	107	648	42200
AVERAGE	705	556.5	131.5	909	60500
STD	459.62	193.04	34.65	369.11	25880.11
Corr. STD	574.52	241.30	43.31	461.39	32350.14
RSD	81%	43%	33%	51%	53%
K10I	451	505	117	986	58200
K11I	487	498	118	862	53400
K12I	435	381	138	939	44900
AVERAGE	457.67	461.33	124.33	929.00	52166.67
STD	26.63	69.66	11.85	62.60	6735.23
Corr. STD	30.06	78.62	13.37	70.66	7601.84
RSD	7%	17%	11%	8%	15%

Sample	Influent				
B1I	535	607	224	1160	67800
B2I	556	588	197	924	54600
B3I	386	518	201	1010	58700
AVERAGE	471	553	199	967	56650
STD	120.21	49.50	2.83	60.81	2899.14
Corr. STD	150.26	61.87	3.54	76.01	3623.92
RSD	32%	11%	2%	8%	6%
B4I	511	554	202	1030	70800
B5I	610	591	189	930	56200
B6I	261	506	215	1130	74400
AVERAGE	386	530	208.5	1080	72600
STD	176.78	33.94	9.19	70.71	2545.58
Corr. STD	220.97	42.43	11.49	88.39	3181.98
RSD	57%	8%	6%	8%	4%
B7I	825	563	202	636	76800
B8I	255	480	154	632	45300
B9I	255	355	224	517	39200
AVERAGE	540	459	213	576.5	58000
STD	403.05	147.08	15.56	84.15	26587.21
Corr. STD	503.81	183.85	19.45	105.18	33234.02
RSD	93%	40%	9%	18%	57%
B10I	241	396	149	518	44500
B11I	359	565	237	1280	74800
B12I	444	556	236	1190	61900
AVERAGE	348.00	505.67	207.33	996.00	60400.00
STD	101.95	95.08	50.52	416.40	15205.59
Corr. STD	115.06	107.31	57.02	469.98	17162.07
RSD	33%	21%	28%	47%	28%

RESULTS

Sample	Effluent				
	CBZ	SMX	TRIM	ATEN	ACE
K1E	536	542	2	34	71
K2E			2	32	11
K3E	285	432	2	26	10
AVERAGE	285	432	2	29	10.5
STD				4.24	0.71
Corr. STD				5.30	0.88
RSD				18%	8%
K4E	557	210	2	29	119
K5E	543	255	2	39	10
K6E	257	415	2	20	10
AVERAGE	407	312.5	2	24.5	64.5
STD	212.13	144.96		6.36	
Corr. STD	265.17	181.20		7.95	
RSD	65%	58%		32%	
K7E	685	231	2	52	10
K8E	358	473	2	52	10
K9E	932	233	2	53	10
AVERAGE	808.5	232	2	52.5	10
STD	174.66	1.41		0.71	
Corr. STD	218.32	1.77		0.88	
RSD	27%	1%		2%	
K10E	374	463	2	62	10
K11E	364	460	2	55	10
K12E	138	287	2	60	10
	292.00	403.33	2.00	59.00	10.00
	133.46	100.76		3.61	
	150.63	113.72		4.07	
	52%	28%		7%	

Sample	Effluent				
	908	748	173	216	10
	770	555	153	150	22
	414	592	179	151	10
AVERAGE	592	573.5	166	150.5	16
STD	251.73	26.16	18.38	0.71	
Corr. STD	314.66	32.70	22.98	0.88	
RSD	53%	6%	14%	1%	
	687	604	150	162	23
	723	483	144	179	12
	448	478	143	143	19
AVERAGE	567.5	541	146.5	152.5	21
STD	169.00	89.10	4.95	13.44	2.83
Corr. STD	211.25	111.37	6.19	16.79	3.54
RSD	37%	21%	4%	11%	17%
	613	500	168	168	19
	379	566	212	28	19
	102	337	191	211	10
AVERAGE	357.5	418.5	179.5	189.5	14.5
STD	361.33	115.26	16.26	30.41	
Corr. STD	451.66	144.07	20.33	38.01	
RSD	126%	34%	11%	20%	
	314	578	170	35	10
	369	505	176	26	10
	90	481	203	38	10
AVERAGE	257.67	521.33	183.00	33.00	10.00
STD	147.78	50.52	17.58	6.24	
Corr. STD	166.80	57.02	19.84	7.05	
RSD	65%	11%	11%	21%	

	SERVOS L				
		Influent			
	Sample ID	CBZ	SMX	TRIM	ATEN
	K1I	448	<MQL	93	<MQL
	K2I	445	<MQL	83	<MQL
	K3I	Sample Destroyed			
AVERAGE					
STD					
Corr. STD					
RSD					
	K4I	445	<MQL	93	<MQL
	K5I	465	<MQL	82	<MQL
	K6I	258	<MQL	92	<MQL
AVERAGE		351		92	
STD		132.58		0.53	
Corr. STD		165.73		0.66	
RSD		47%		1%	
	K7I	465	<MQL	93	<MQL
	K8I	308	<MQL	92	<MQL
	K9I	278	<MQL	105	<MQL
AVERAGE		371		99	
STD		132.58		8.49	
Corr. STD		165.73		10.61	
RSD		45%		11%	
	K10I	308	<MQL	111	<MQL
	K11I	313	<MQL	117	<MQL
	K12I	315	<MQL	127	<MQL
AVERAGE		312		118	
STD		3.8		7.9	
Corr. STD		4.3		8.9	
RSD		1%		8%	

	Sample ID	Influent			
		CBZ	SMX	TRIM	ATEN
	B1I	480	<MQL	156	<MQL
	B2I	470	<MQL	149	<MQL
	B3I	268	<MQL	199	<MQL
AVERAGE		368.75		174.125	
STD		143.19		35.53	
Corr. STD		178.99		44.42	
RSD		49%		26%	
	B4I	Sample Destroyed			
	B5I	518	<MQL	155	<MQL
	B6I	280	<MQL	194	<MQL
AVERAGE					
STD					
Corr. STD					
RSD					
	B7I	648	<MQL	182	<MQL
	B8I	298	<MQL	181	<MQL
	B9I	278	<MQL	210	<MQL
AVERAGE		463		196	
STD		261.63		19.27	
Corr. STD		327.04		24.09	
RSD		71%		12%	
	B10I	300	<MQL	190	<MQL
	B11I	368	<MQL	185	<MQL
	B12I	268	<MQL	194	<MQL
AVERAGE		312		190	
STD		51.0		4.3	
Corr. STD		57.6		4.8	
RSD		18%		3%	

.AB DATA				
Sample ID	Effluent			
	CBZ	SMX	TRIM	ATEN
K1E	Sample Destroyed			
K2E	522.5	<MQL	<MQL	<MQL
K3E	260	<MQL	<MQL	<MQL
	391.25			
	185.62			
	232.02			
	59%			
K4E	447.5	<MQL	<MQL	<MQL
K5E	435	<MQL	<MQL	<MQL
K6E	282.5	<MQL	<MQL	<MQL
	365			
	116.67			
	145.84			
	40%			
K7E	520	<MQL	<MQL	<MQL
K8E	297.5	<MQL	<MQL	<MQL
K9E	312.5	<MQL	<MQL	<MQL
	416			
	146.72			
	183.41			
	44%			
K10E	255	<MQL	<MQL	<MQL
K11E	320	<MQL	<MQL	<MQL
K12E	305	<MQL	<MQL	<MQL
	293			
	34.0			
	38.4			
	13%			

Sample ID	Effluent			
	CBZ	SMX	TRIM	ATEN
B1E	460	778	143	343
B2E	450	<MQL	145	380
B3E	308	<MQL	152	<MQL
	378.75		148.375	
	100.76		5.13	
	125.95		6.41	
	33%		4%	
B4E	Sample Destroyed			
B5E	498	<MQL	117	370
B6E	358	<MQL	138	330
B7E	448	<MQL	141	278
B8E	330	<MQL	181	<MQL
B9E	293	<MQL	179	<MQL
	370		160	
	109.60		27.05	
	137.00		33.81	
	37%		21%	
B10E	255	<MQL	145	<MQL
B11E	Sample Destroyed			
B12E	277.5	<MQL	217	<MQL
	266		181	
	15.91		50.91	
	19.89		63.64	
	7%		35%	

SERVOS					
Sample ID	Influent				
	CBZ	SMX	TRIM	ATEN	ACE
A1	279	<MQL	158	665	42550
A2	275	<MQL	<MDL	1025	32850
A3	235	<MQL	125	695	33200
AVERAGE	263		142	795	36200
RSD	9%			25%	15%
A4	221	<MQL	134	955	42200
A5	219	<MQL	157	1415	44000
A6	243	385.5	121	1185	52500
AVERAGE	228		137	1185	46233
RSD	6%		13%	19%	12%
A7	313.5	320	138	1100	40200
A8	312	265	95	800	40150
A9	380.5	415.5	121	735	42350
AVERAGE	335	334	118	878	40900
RSD	12%	23%	18%	22%	3%
A10	254.5	478.5	250	1105	49000
A11	350	1155	266	2380	51000
A12	<MQL	<MQL	313	755	61500
AVERAGE	302	817	276	1413	53833
RSD			12%	61%	12%
A13	420.5	740	212	1550	51000
A14	302.5	<MQL	210	2215	44600
A15	334	550	156	2750	45050
AVERAGE	352	645	192	2172	46883
RSD	17%		16%	28%	8%
A16	236	<MQL	170	1190	68000
A17	282	<MQL	171	1180	70000
A18	249	<MQL	201	2345	62500
AVERAGE	255		180	1572	66833
RSD	9%		10%	43%	6%

LAB DATA

Sample ID	Effluent				
	CBZ	SMX	TRIM	ATEN	ACE
A1	287	356.5	101	324	<MDL
A2	230.5	320.5	96.5	351	<MDL
A3	229	<MQL	91.5	301	<MDL
	249	339	96	325	
	13%	8%	5%	8%	
A4					
A5	211	407	91.5	335	<MDL
A6	260	510	105.5	495.5	<MDL
	236	459	99	415	
A7	256	<MQL	90	375	<MDL
A8	267.5	<MQL	128	358.5	<MDL
A9	270	<MQL	93	353.5	<MDL
	265		104	362	
	3%		20%	3%	
A10	227.5	510	111.5	464	<MDL
A11	223.5	489.5	112.5	357	<MDL
A12	239.5	487.5	109	396.5	<MDL
	230	496	111	406	
	4%	3%	2%	13%	
A13	236.5	384	96	291.5	<MDL
A14	261.5	<MQL	113.5	399	<MDL
A15	228	427	101.5	358.5	<MDL
	242	406	104	350	
	7%		9%	16%	
A16	247	437	100	438	<MDL
A17	248	510	88.5	397.5	<MDL
A18	229	471.5	105.5	464.5	<MDL
	241	473	98	433	
	4%	8%	9%	8%	

SERVOS LAB D					
Sample ID	Influent				
	CBZ	SMX	TRIM	ATEN	ACE
A7-1	No Samples				
A7-2	No Samples				
A7-3	No Samples				
A7-7	146	<MQL	<MQL	735	35150
A7-8	148	<MQL	<MQL	735	34650
A7-9	162	<MQL	<MQL	910	35950
AVG	152			793	35250
RSD	6%			13%	2%
A7-13	185	<MQL	93.5	625	33900
A7-14	185	<MQL	92	655	36450
A7-15	219	<MQL	<MQL	670	34200
AVG	196		93	650	34850
RSD	10%		1%	4%	4%
A7-19	173	<MQL	124.5	1200	44650
A7-20	174	<MQL	116	910	44200
A7-21	187.5	<MQL	108	955	38300
AVG	178		116	1022	42383
RSD	5%		7%	15%	8%
A7-25	229	<MQL	98	1060	35350
A7-26	268.5	<MQL	118.5	NO IS	50000
A7-27	227	<MQL	94	950	31600
AVG	242		104	1005	38983
RSD	10%		13%		25%
A7-31	189	<MQL	118.5	1325	38800
A7-32	168.5	<MQL	140	765	34600
A7-33	228	<MQL	96	820	37350
AVG	195		118	970	36917
RSD	15%		19%	32%	6%
K7-1	No Sample				
K7-2	No Sample				
K7-3	126	<MDL	<MDL	499.5	22050
AVG	126			500	22050
RSD					
K7-7	150.5	<MDL	<MDL	725	37150
K7-8	153.5	<MDL	<MDL	685	36300
K7-9	177	<MDL	<MDL	850	35950
AVG	160			753	36467
RSD	9%			11%	2%
K7-13	201	<MDL	<MDL	610	35700
K7-14	186	<MDL	<MDL	610	37650
K7-15	202	<MDL	<MDL	<MDL	36200
AVG	196			610	36517
RSD	5%				3%

K7-19	183	<MDL	112.5	990	48550
K7-20	185	<MDL	103.5	835	47000
K7-21	202.5	<MDL	91.5	750	43750
AVG	190		103	858	46433
RSD	6%		10%	14%	5%
K7-25	219.5	<MDL	<MDL	850	40850
K7-26	222.5	<MDL	<MDL	580	39850
K7-27	191.5	<MDL	91	805	31100
AVG	211		91	745	37267
RSD	8%			19%	14%
K7-31	173	<MDL	85.5	695	36400
K7-32	167.5	<MDL	110.5	1335	34550
K7-33	174	<MDL	99	660	39100
AVG	172		98	897	36683
RSD	2%		13%	42%	6%

C1	116	<MDL	100	705	27550
C2	113.5	<MDL	105	870	25350
C3	112	<MDL	90.5	730	22700
AVG	114		99	768	25200
RSD	2%		7%	12%	10%
C7	148.5	<MDL	128	1390	47050
C8	160.5	<MDL	136	835	49350
C9	218.5	<MDL	131	945	46250
AVG	176		132	1057	47550
RSD	21%		3%	28%	3%
C13	207.5	<MDL	114	1070	47850
C14	209	<MDL	160.5	930	46050
C15	204.5	<MDL	115	870	47600
AVG	207		130	957	47167
RSD	1%		20%	11%	2%
C19	186.5	<MDL	170.5	1360	60000
C20	176.5	<MDL	151	1455	56500
C21	182	<MDL	143	1070	59000
AVG	182		155	1295	58500
RSD	3%		9%	15%	3%
C25	231.5	<MDL	150.5	1235	57000
C26	237	<MDL	172	1200	52000
C27	214	<MDL	121.5	1145	45450
AVG	228		148	1193	51483
RSD	5%		17%	4%	11%
C31	158.5	<MDL	154.5	1275	48450
C32	155.5	<MDL	140	900	50500
C33	242.5	<MDL	165.5	1315	55000
AVG	186		153	1163	51317
RSD	27%		8%	20%	7%

D1	120	<MDL	112.5	975	26500
D2	134	<MDL	130	1375	27850
D3	114	<MDL	108	1145	23750
AVG	123		117	1165	26033
RSD	8%		10%	17%	8%
D7	149	<MDL	127	865	48200
D8	152.5	<MDL	147	1210	50500
D9	172	<MDL	113	1135	47100
AVG	158		129	1070	48600
RSD	8%		13%	17%	4%
D13	209	<MDL	135	1005	45150
D14	202.5	<MDL	128	1160	45050
D15	200.5	<MDL	105.5	1155	42650
AVG	204		123	1107	44283
RSD	2%		13%	8%	3%
D19	166.5	<MDL	166	1595	56500
D20	184	<MDL	183.5	1580	59000
D21	174.5	<MDL	151	NO IS	55000
AVG	175		167	1588	56833
RSD	5%		10%	1%	4%
D25	227.5	<MDL	179.5	1085	47750
D26	202	<MDL	161.5	1390	40150
D27	202.5	<MDL	157	1555	39950
AVG	211		166	1343	42617
RSD	7%		7%	18%	10%
D31	300.5	<MDL	157.5	1030	48250
D32	261	<MDL	162	1370	43250
D33	2295	<MDL	126.5	1500	45650
AVG	952		149	1300	45717
RSD	122%		13%	19%	5%

E1	103	<MDL	113.5	670	27150
E2	106.5	<MDL	102	560	25050
E3	98.5	<MDL	94	830	23400
AVG	103		103	687	25200
RSD	4%		10%	20%	7%
E7	128	<MDL	111	920	45700
E8	126	<MDL	154.5	840	47400
E9	164	<MDL	128.5	1140	48050
AVG	139		131	967	47050
RSD	15%		17%	16%	3%
E13	225	<MDL	159	1460	55000
E14	183	<MDL	112.5	925	43800
E15	199	<MDL	114.5	655	45000
AVG	202		129	1013	47933
RSD	10%		20%	40%	13%
E19	176	<MDL	140	930	63000
E20	169.5	<MDL	199	1085	64000
E21	172	<MDL	137	1040	60000
AVG	173		159	1018	62333
RSD	2%		22%	8%	3%
E25	236.5	<MDL	167	1095	52500
E26	223	<MDL	141	NO IS	50500
E27	216.5	<MDL	136.5	815	49250
AVG	225		148	955	50750
RSD	5%		11%		3%
E31	187.5	<MDL	153	990	53500
E32	174	<MDL	146	975	57000
E33	585	<MDL	141	1010	52000
AVG	316		147	992	54167
RSD	74%		4%	2%	5%

ATA - Phase 3

Sample ID	Effluent				
	CBZ	SMX	TRIM	ATEN	ACE
A7-4	127	<MQL	51.5	186	<MDL
A7-5	132	<MQL	51.5	186	<MDL
A7-6	137	<MQL	51.5	228	<MDL
AVG	132		52	200	
RSD	4%		0%	12%	
A7-10	173	303	63	229	<MDL
A7-11	135	276	48	214	<MDL
A7-12	186	<MQL	60	212	<MDL
AVG	165	289	57	218	
RSD	16%		14%	4%	
A7-16	182	<MQL	62	226	<MDL
A7-17	186	307	58	240	<MDL
A7-18	203	<MQL	57	196	<MDL
AVG	190		59	220	
RSD	6%		5%	10%	
A7-22	186	500	68	210	<MDL
A7-23	184	505	75	256	<MDL
A7-24	213	411	62.5	261	<MDL
AVG	194	472	68	242	
RSD	8%	11%	9%	12%	
A7-28	257.5	370	<MQL	<MQL	<MDL
A7-29	204	377	<MQL	<MQL	<MDL
A7-30	223.5	<MQL	<MQL	<MQL	<MDL
AVG	228	374			
RSD	12%				
A7-34	173.5	342	71	208.5	<MDL
A7-35	171.5	379.5	71	253.5	<MDL
A7-36	327.5	<MQL	70.5	234	<MDL
AVG	224	361	71	232	
RSD	40%		0%	10%	
		361			
K7-4	133	<MDL	<MDL	<MDL	<MDL
K7-5	137	<MDL	<MDL	<MDL	<MDL
K7-6	130	<MDL	<MDL	<MDL	<MDL
AVG	133				
RSD	3%				
K7-10	166	<MDL	<MDL	<MDL	<MDL
K7-11	143	<MDL	<MDL	<MDL	<MDL
K7-12	185	<MDL	<MDL	<MDL	<MDL
AVG	165				
RSD	13%				
K7-16	189	<MDL	<MDL	<MDL	<MDL
K7-17	192	<MDL	<MDL	<MDL	<MDL
K7-18	195	<MDL	<MDL	<MDL	<MDL
AVG	192				
RSD	2%				

K7-22	215	<MDL	<MDL	<MDL	<MDL
K7-23	203	<MDL	<MDL	<MDL	<MDL
K7-24	195	426	<MDL	<MDL	<MDL
AVG	204				
RSD	5%				
K7-28	204	<MDL	65	247	<MDL
K7-29	202	<MDL	68	227	<MDL
K7-30	190	<MDL	67	181	<MDL
AVG	199		66	218	
RSD	4%		2%	15%	
K7-34	196	395	<MDL	<MDL	<MDL
K7-35	199	<MDL	<MDL	<MDL	<MDL
K7-36	288	<MDL	<MDL	<MDL	<MDL
AVG	227				
RSD	23%				

		410			
C4	110	<MDL	88.5	407.5	<MDL
C5	143	<MDL	95	479.5	<MDL
C6	117	<MDL	91.5	530	<MDL
AVG	123		92	472	
RSD	14%		4%	13%	
C10	151	<MDL	110	650	<MDL
C11	154	<MDL	119	580	<MDL
C12	184.5	<MDL	100	665	<MDL
AVG	163		110	632	
RSD	11%		9%	7%	
C16	209	<MDL	143	620	<MDL
C17	203.5	<MDL	114	705	<MDL
C18	201.5	<MDL	118	655	<MDL
AVG	205		125	660	
RSD	2%		13%	6%	
C22	213.5	<MDL	158.5	980	<MDL
C23	174.5	<MDL	121.5	710	<MDL
C24	193	<MDL	136	715	<MDL
AVG	194		139	802	
RSD	10%		13%	19%	
C28	221.5	<MDL	139	670	<MDL
C29	215.5	<MDL	118.5	600	<MDL
C30	216.5	<MDL	143.5	645	<MDL
AVG	218		134	638	
RSD	1%		10%	6%	
C34	278	<MDL	133	745	<MDL
C35	314.5	<MDL	140.5	655	<MDL
C36	670	<MDL	143.5	670	<MDL
AVG	421		139	690	
RSD	51%		4%	7%	

D4	117	<MDL	94	935	<MDL
D5	125	<MDL	108	955	<MDL
D6	115	<MDL	86	720	<MDL
AVG	119		96	870	
RSD	4%		12%	15%	
D10	153	<MDL	125	890	<MDL
D11	171	<MDL	154	945	<MDL
D12	180	<MDL	132	950	<MDL
AVG	168		137	928	
RSD	8%		11%	4%	
D16	222	<MDL	123	1115	<MDL
D17	219	<MDL	152	1045	<MDL
D18	212	<MDL	103	1020	<MDL
AVG	217		126	1060	
RSD	2%		20%	5%	
D22	175	630	157	1270	<MDL
D23	164	<MDL	140	1175	<MDL
D24	179	444	139	1270	<MDL
AVG	172	537	145	1238	
RSD	4%		7%	4%	
D28	217	<MDL	150	1280	<MDL
D29	227	<MDL	170	980	<MDL
D30	227	<MDL	137	1300	<MDL
AVG	223		152	1187	
RSD	3%		11%	15%	
D34	179	<MDL	138	1190	<MDL
D35	163	<MDL	145	1115	<MDL
D36	775	<MDL	161	1015	<MDL
AVG	372		148	1107	
RSD	94%		8%	8%	

		537			
E4	122.5	<MDL	92.5	261	<MDL
E5	128	<MDL	101.5	246	<MDL
E6	124.5	<MDL	100.5	222.5	<MDL
AVG	125		98	243	
RSD	2%		5%	8%	
E10	159	364	115	357.5	<MDL
E11	145	<MDL	111.5	210	<MDL
E12	188	<MDL	101.5	260.5	<MDL
AVG	164		109	276	
RSD	13%		6%	27%	
E16	215	<MDL	127.5	322.5	<MDL
E17	219	<MDL	115	266	<MDL
E18	205	<MDL	104	348.5	<MDL
AVG	213		116	312	
RSD	3%		10%	14%	
E22	167.5	570	140	265	<MDL
E23	168	565	168.5	279	<MDL
E24	173	491.5	139	307.5	<MDL
AVG	170	542	149	284	
RSD	2%	8%	11%	8%	
E28	219	<MDL	145	369.5	<MDL
E29	212.5	468	109.5	382	<MDL
E30	216	<MDL	131	463	<MDL
AVG	216		129	405	
RSD	2%		14%	13%	
E34	177.5	463.5	133.5	294	<MDL
E35	176	422	137	267.5	<MDL
E36	407.5	452.5	129	330.5	<MDL
AVG	254	446	133	297	
RSD	53%	5%	3%	11%	

Appendix G

Statistical Analysis Data

Table H1 – Summary of General Linear Model ANOVA– CBZ, TRIM and ATEN											
ANOVA Summary – Carbamazepine				**ANOVA Summary - Trimethoprim**				**ANOVA Summary - Atenolol**			
Source	DF	F	P	Source	DF	F	P	Source	DF	F	P
Main Effects	3	0.92	0.442	**Main Effects**	3	65.47	0.000	**Main Effects**	3	137.19	0.000
SRT	1	0.33	0.571	**SRT**	**1**	**7.15**	**0.011**	**SRT**	**1**	**38.88**	**0.000**
Temp	1	0.63	0.434	**Temp**	**1**	**4.78**	**0.035**	**Temp**	**1**	**237.08**	**0.000**
IFAS	1	1.79	0.188	**IFAS**	**1**	**169.34**	**0.000**	**IFAS**	**1**	**150.02**	**0.000**
2-Way Interactions	3	1.39	0.258	**2-Way Interactions**	3	5.12	0.005	**2-Way Interactions**	3	25.48	0.000
SRT*Temperature	1	0.67	0.419	**SRT*Temperature**	**1**	**10.10**	**0.003**	SRT*Temperature	1	3.29	0.077
SRT*IFAS	1	2.66	0.111	SRT*IFAS	1	1.39	0.246	**SRT*IFAS**	**1**	**16.38**	**0.000**
Temperature*IFAS	1	0.83	0.368	Temperature*IFAS	1	2.06	0.160	**Temperature*IFAS**	**1**	**57.87**	**0.000**
3-Way Interactions	1	1.81	0.187	**3-Way Interactions**	1	5.05	0.031	**3-Way Interactions**	1	17.32	0.000
SRT*Temperature*IFAS	1	1.81	0.187	**SRT*Temperature*IFAS**	**1**	**5.05**	**0.031**	**SRT*Temperature*IFAS**	**1**	**17.32**	**0.000**
R^2	**18%**			**R^2**	**87%**			**R^2**	**93%**		
Notes: Factors with P values less than 0.05 are considered significant and are highlighted in green and red text.											

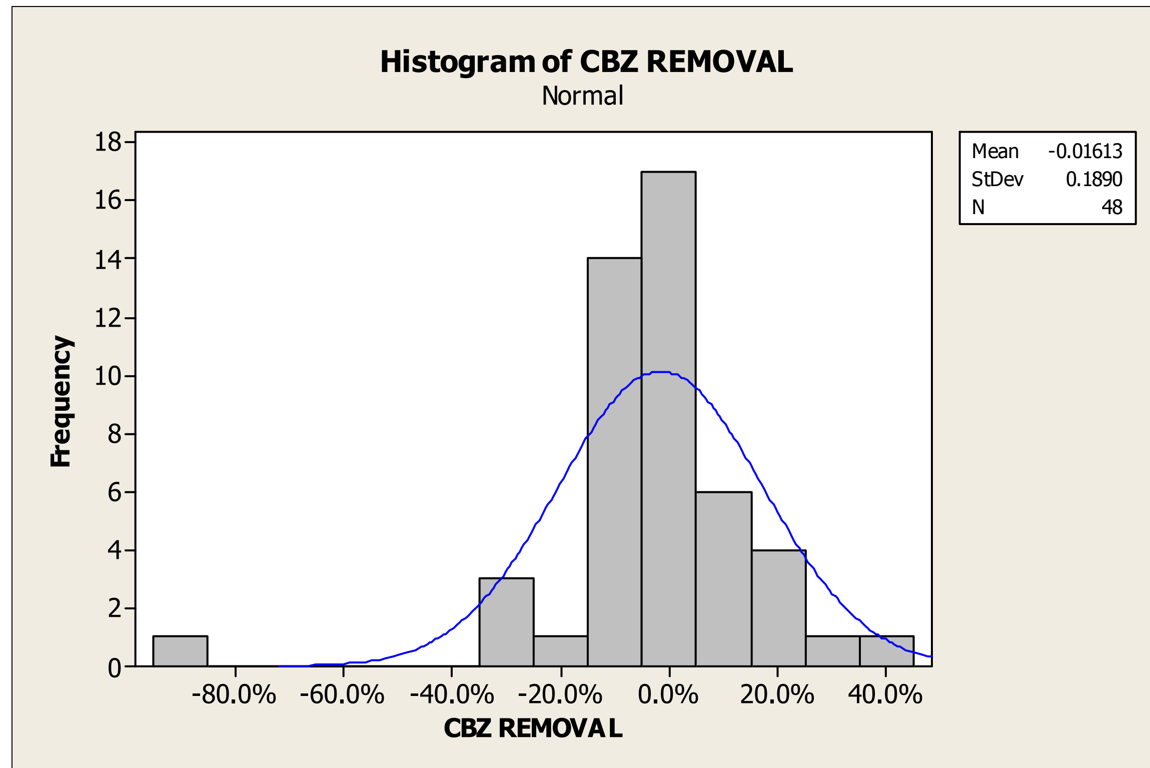


Figure H1 – Histogram demonstrating observed CBZ transformation efficiencies

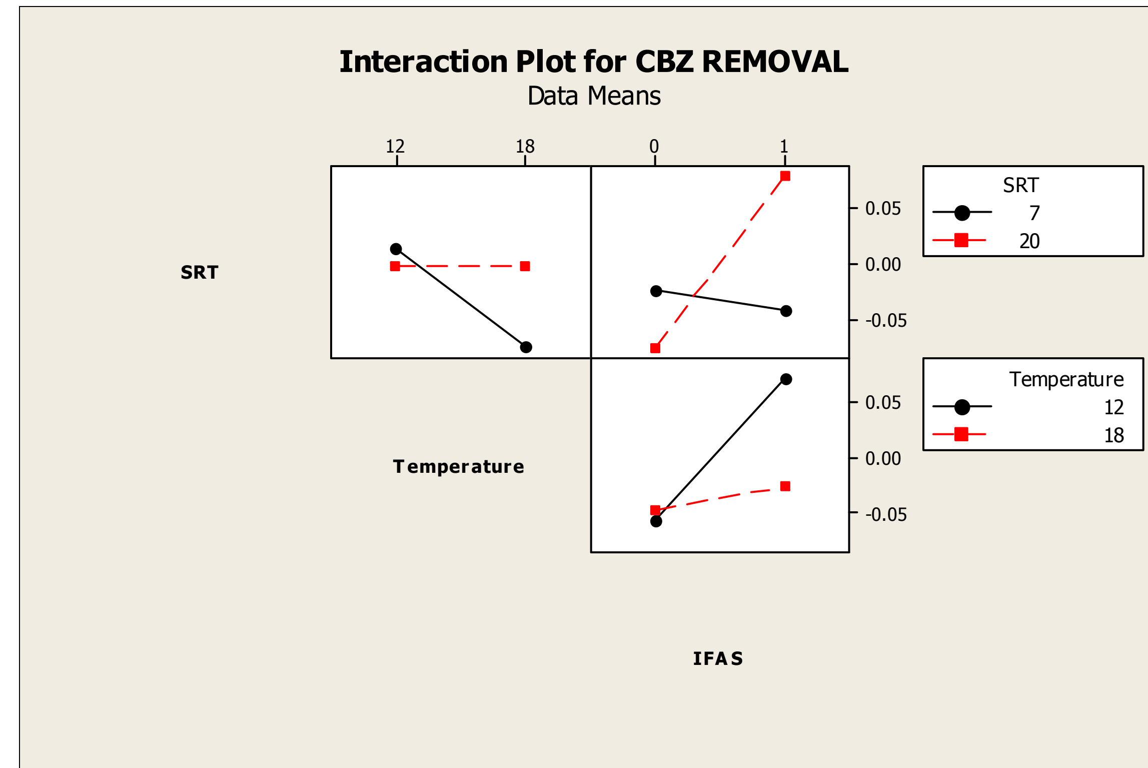


Figure H2 –Interaction plot for SRT, Temperature and IFAS for CBZ transformation efficiency

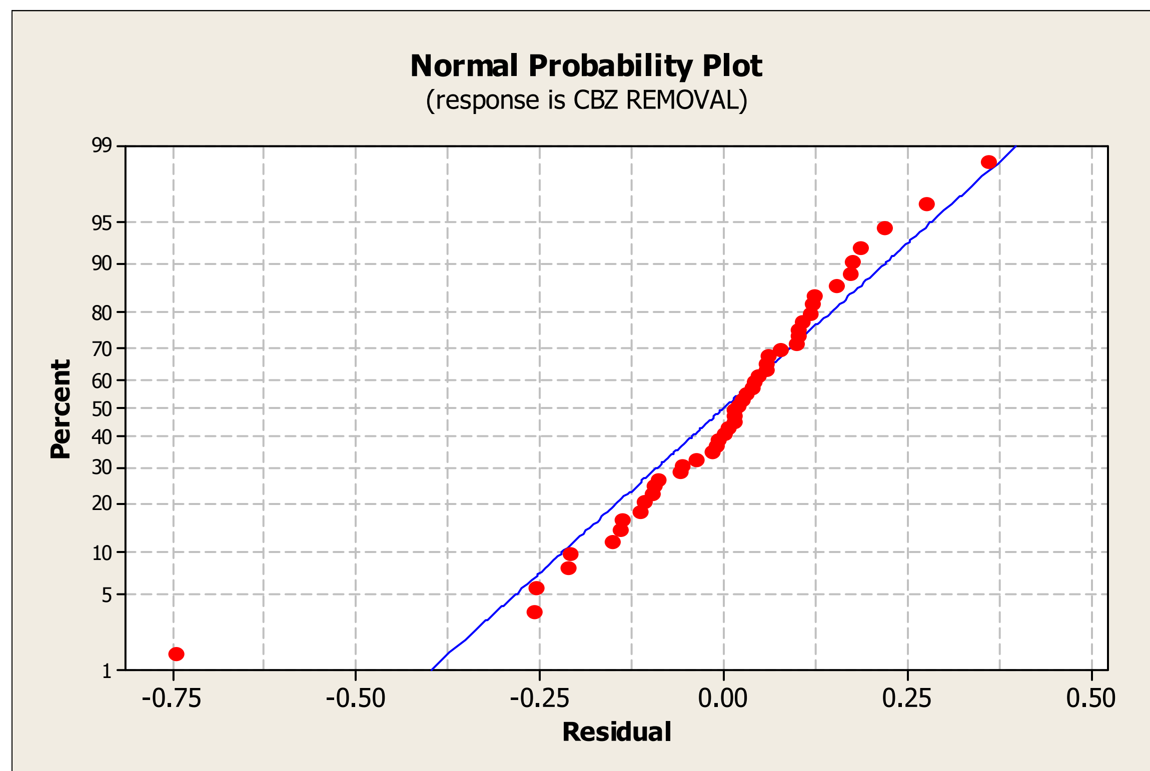


Figure H3 - Normal Probability Plot for Linear Regression based on CBZ Transformation Efficiency

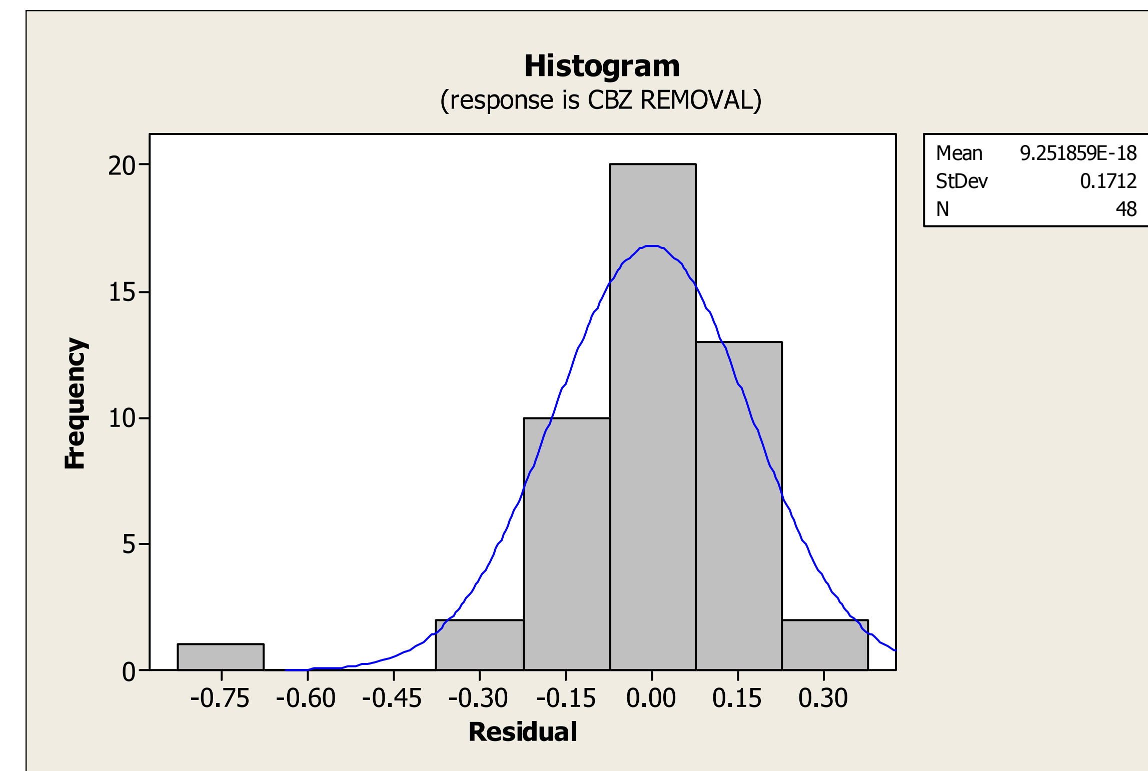


Figure H4 – Historgram of Residuals associated with Linear Regression for CBZ Transformation Efficiency

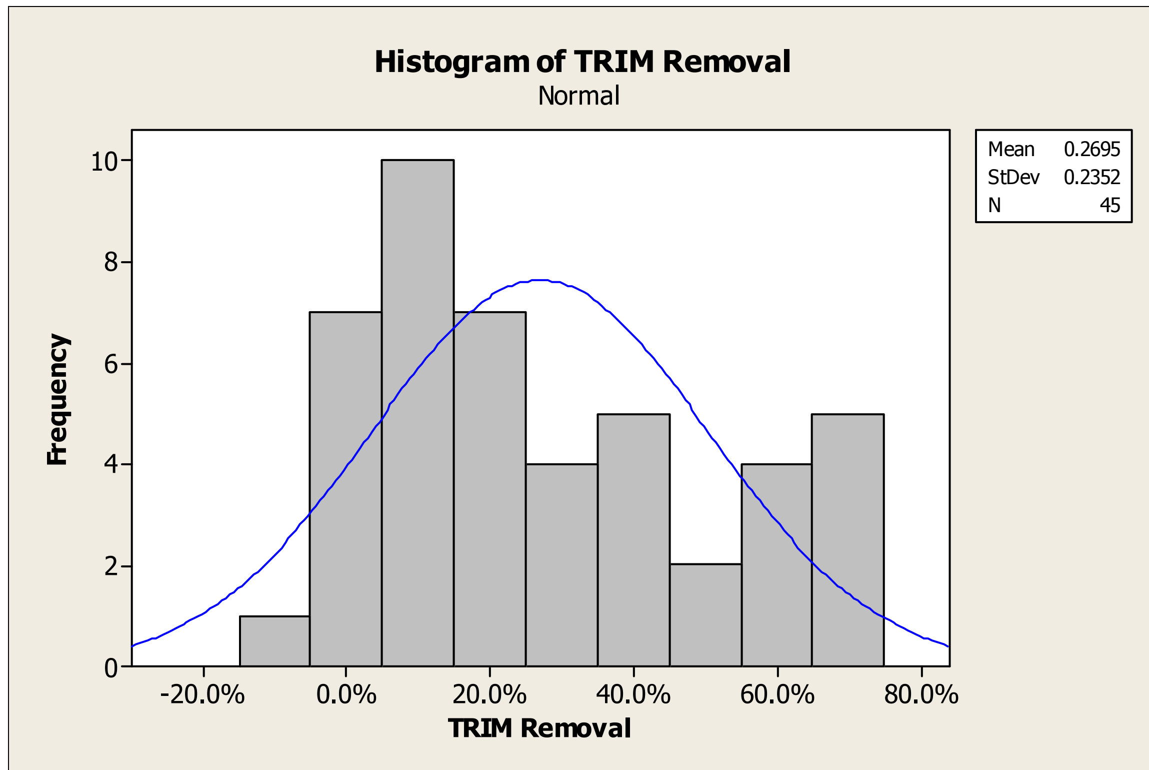


Figure H5 – Histogram demonstrating observed TRIM transformation efficiencies

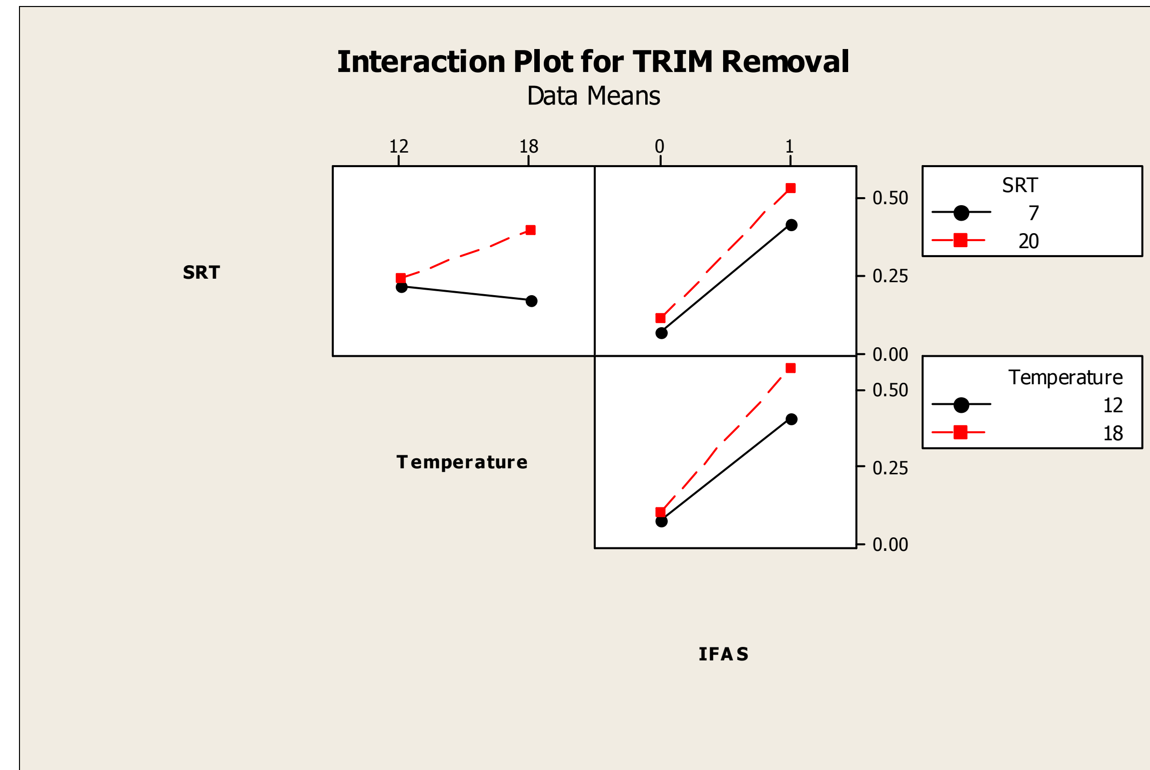


Figure H6 –Interaction plot for SRT, Temperature and IFAS for TRIM transformation efficiency

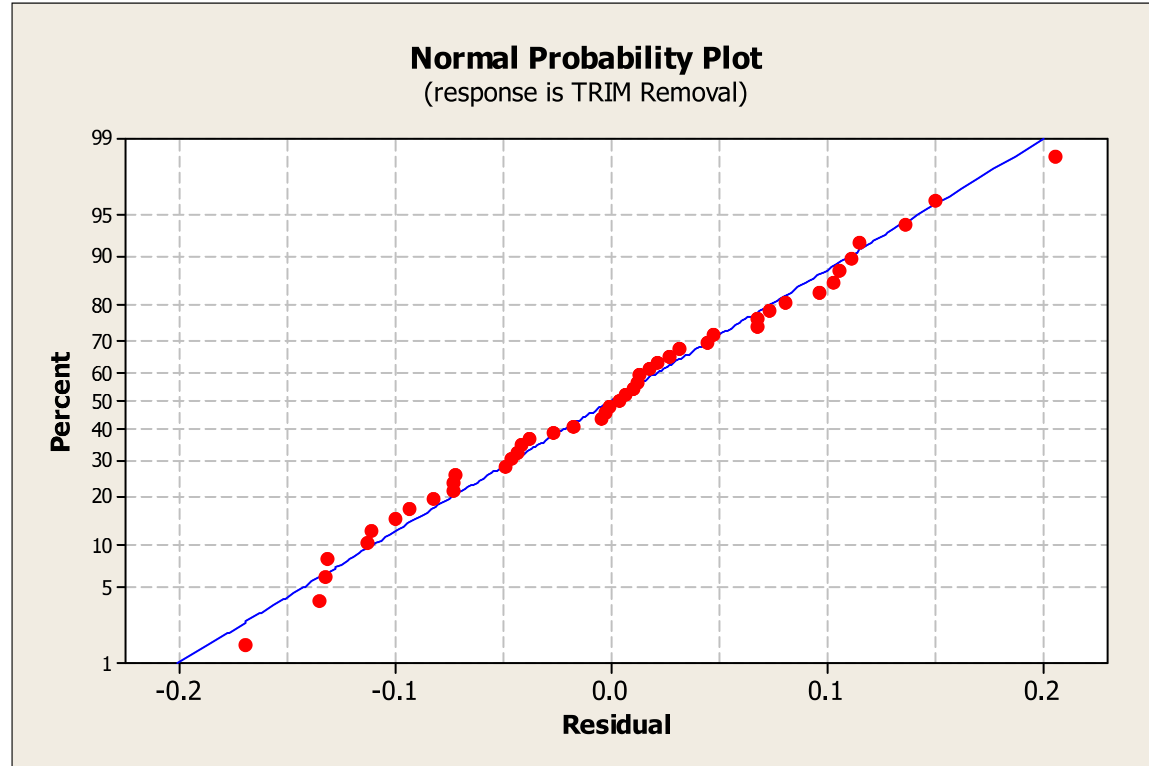


Figure H7 - Normal Probability Plot for Linear Regression based on TRIM Transformation Efficiency

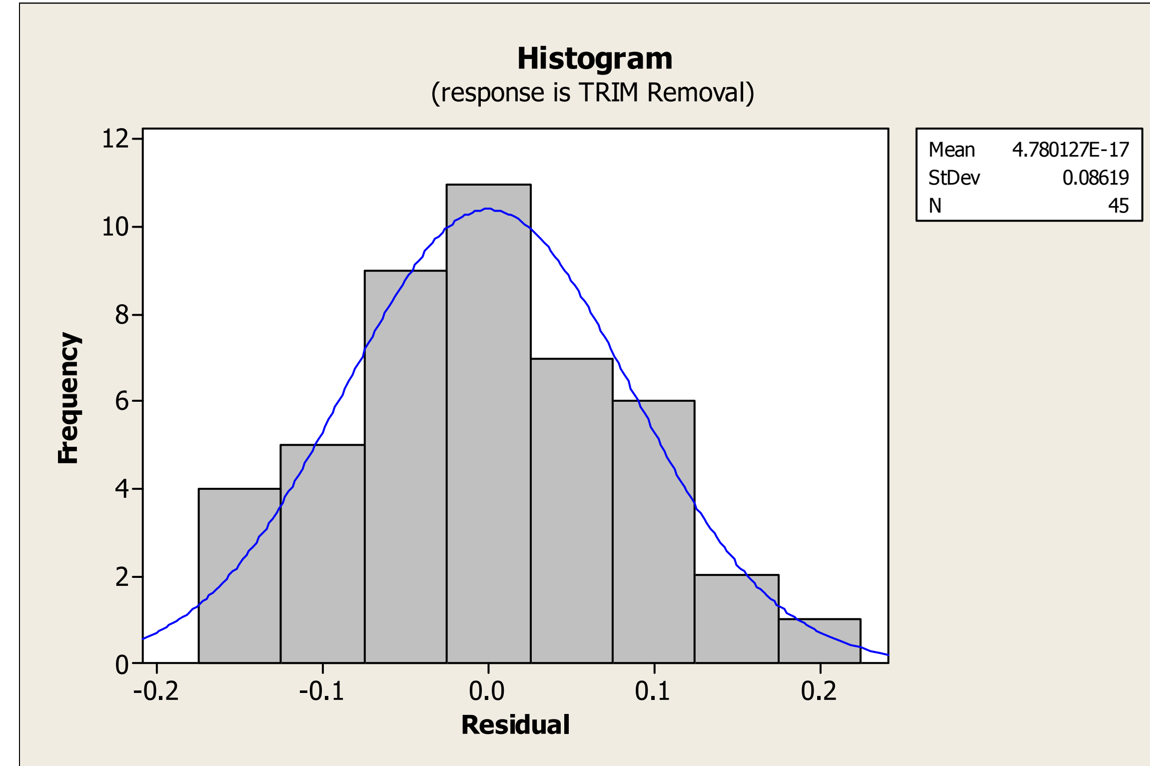


Figure H8 – Historgram of Residuals associated with Linear Regression for TRIM Transformation Efficiency

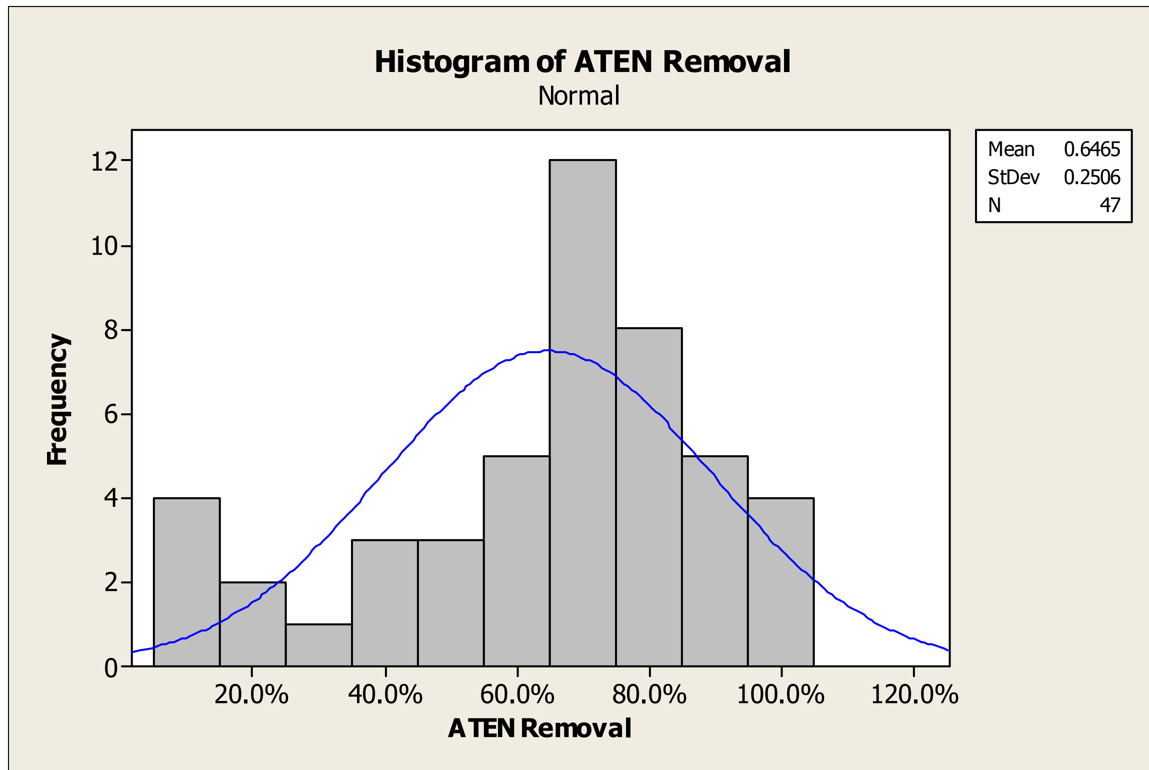


Figure H9 – Histogram demonstrating observed ATEN transformation efficiencies

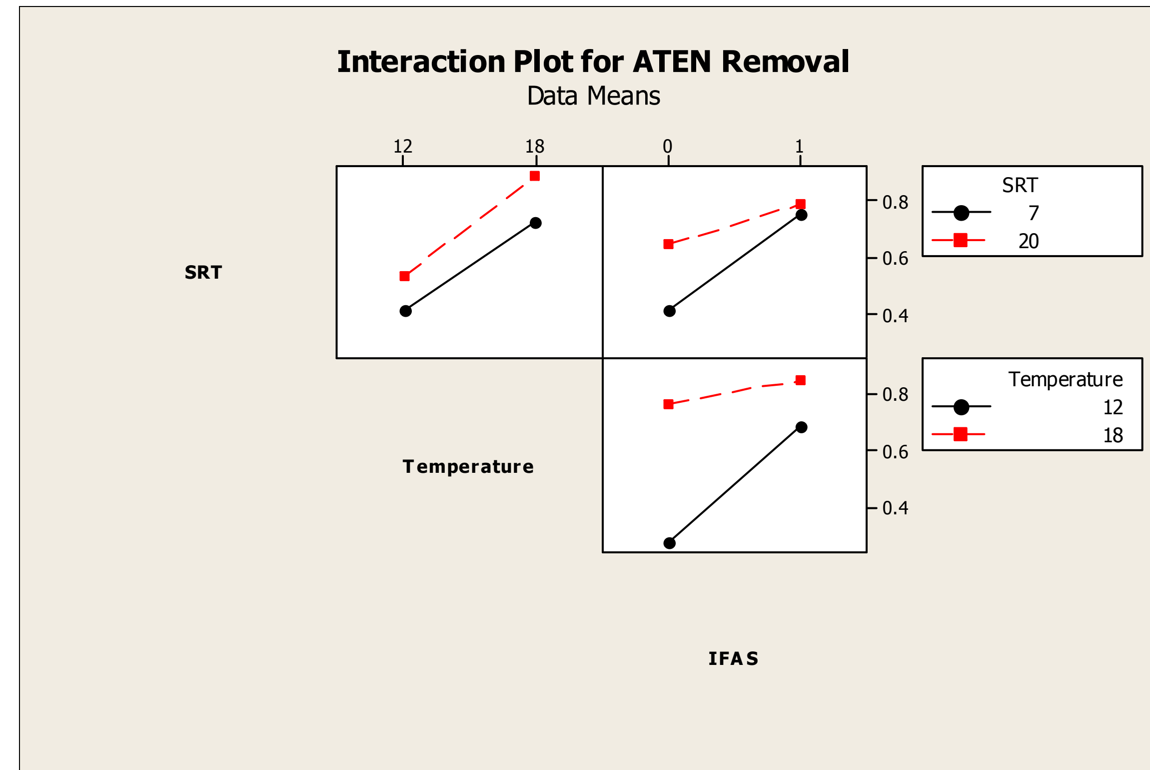


Figure H10 –Interaction plot for SRT, Temperature and IFAS for ATEN transformation efficiency

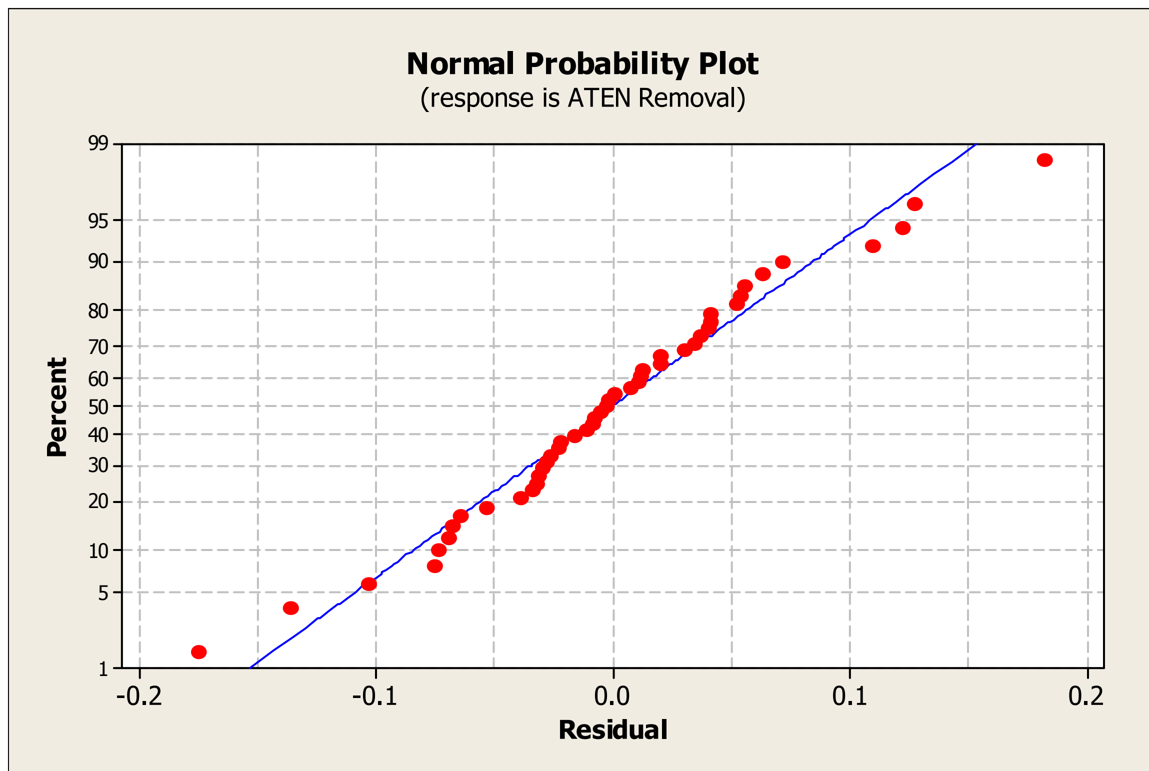


Figure H11 - Normal Probability Plot for Linear Regression based on ATEN Transformation Efficiency

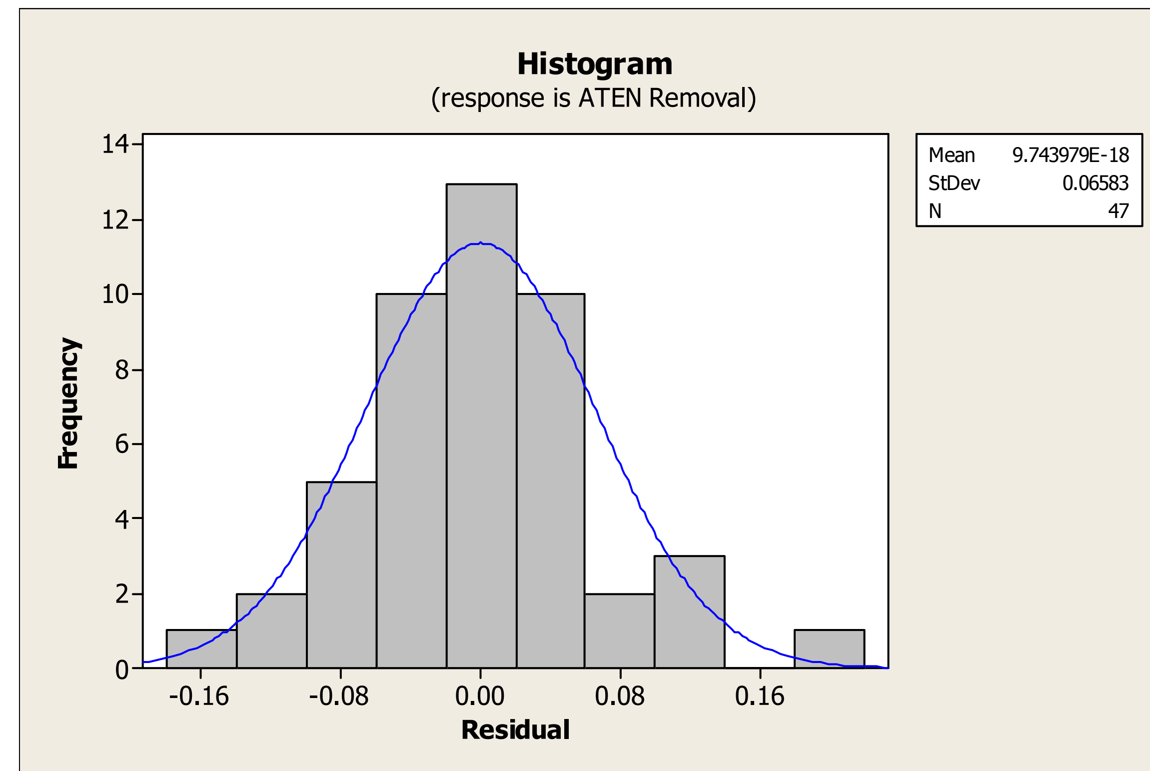


Figure H12 – Historgram of Residuals associated with Linear Regression for ATEN Transformation Efficiency

Appendix H

Microscope Images

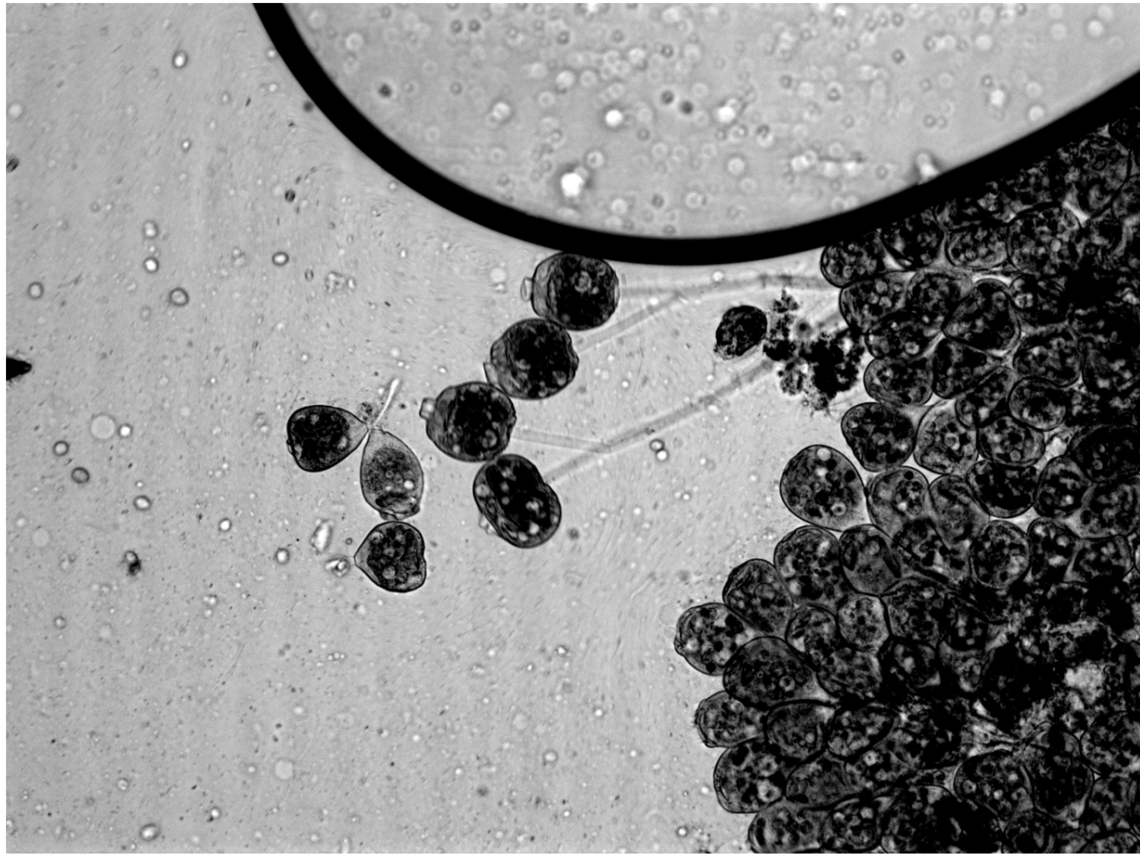
Microscopic analysis was performed on December 4 and 5, 2012 to determine if a filamentous issue was the cause of poor performance within reactor C. WAS samples were collected from Reactor C, B, K and E on December 4, 2012 and investigated. WAS samples from reactors B and E were investigated for comparison as these processes were performing well. Microscope images captured during the investigation of Reactor C WAS are provided in **Appendix G**. This investigation confirmed that filamentous organisms were present which were causing biomass settlement issues and the associated loss of nitrification performance.

A summary of the observations for each reactor mixed liquor sample are provided below:

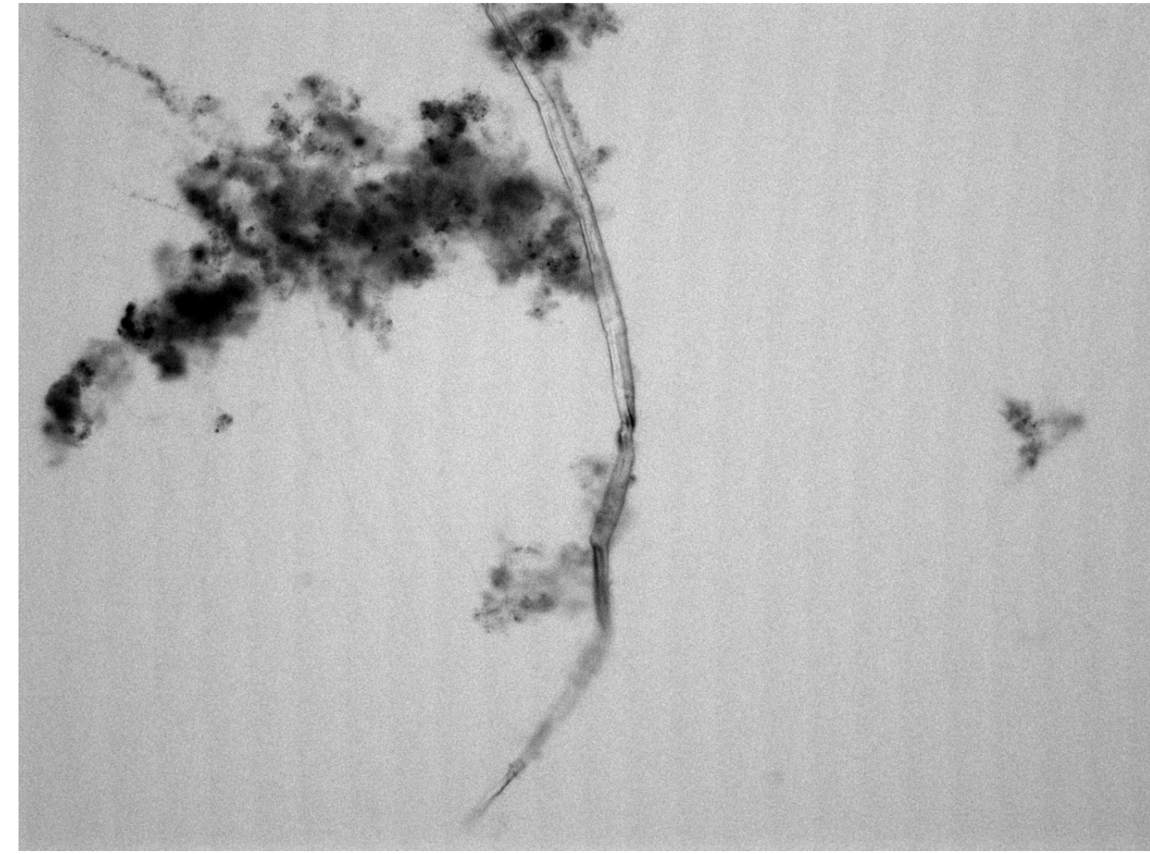
- Reactor C demonstrated significant filamentous organisms, but also contained stalked ciliates and rotifiers, which are typically found in reactors with a long sludge age. It was therefore believe that reactor C, despite operating at a significantly reduced SRT throughout the monitoring period, was retaining bacteria reseeded from reactor B.
- The flocs from reactor B contained an abundance of stalked ciliates as well as rotifiers Euchlanis and Rotaria, suggesting a long SRT was being achieved. Reactor B biomass had a dark colour, another indicator of a long sludge age. Filamentous organisms were rarely observed.
- Reactor E contained lighter coloured flocs, which were noted to be smaller and more dispersed. However, stalked ciliates, free swimming ciliates, rotifiers and a nematode were also observed. More filamentous organisms and fibers were noted in comparison to Reactor B.

Based on the observations conducted on the above reactors, it would appear that all reactors contained microorganisms indicative of a long sludge age. Reactor C mixed liquor was supplemented with hydrogen peroxide to obtain a concentration of 30 mg/L in an attempt to eradicate all filamentous organisms based on operational suggestions found within Jenkins et al (2004). Reactor C was then reseeded with WAS from reactor B for several weeks prior to the Christmas Holiday period. Despite the loss of biomass, Reactor C appeared to provide some nitrification as well as COD removal during the monitoring period. Effluent samples collected on January 4, 2013 demonstrate that the reactor had regained the ability to nitrify; producing an effluent with a TAN concentration of 0.42 mg/L. Elevated effluent TSS was still occurring and WAS rates were adjusted to compensate for these solids losses.

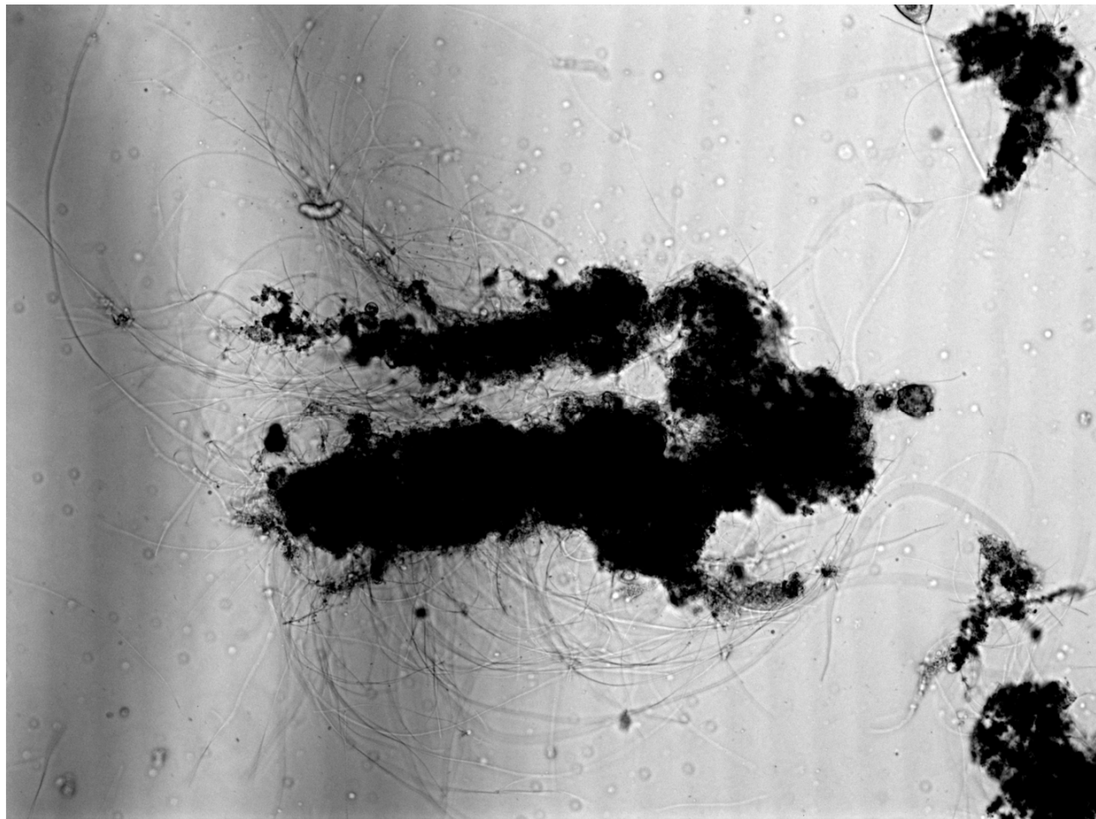
Reactor C



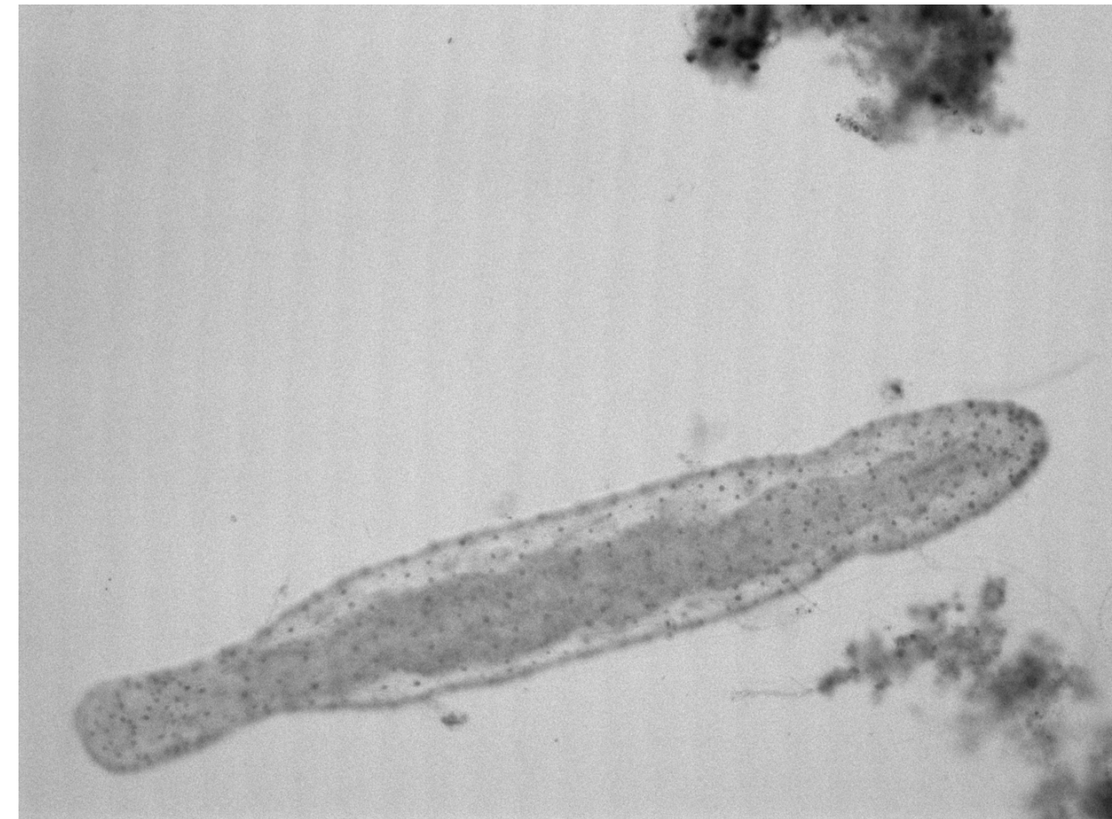
20 – 40 x stalked cil



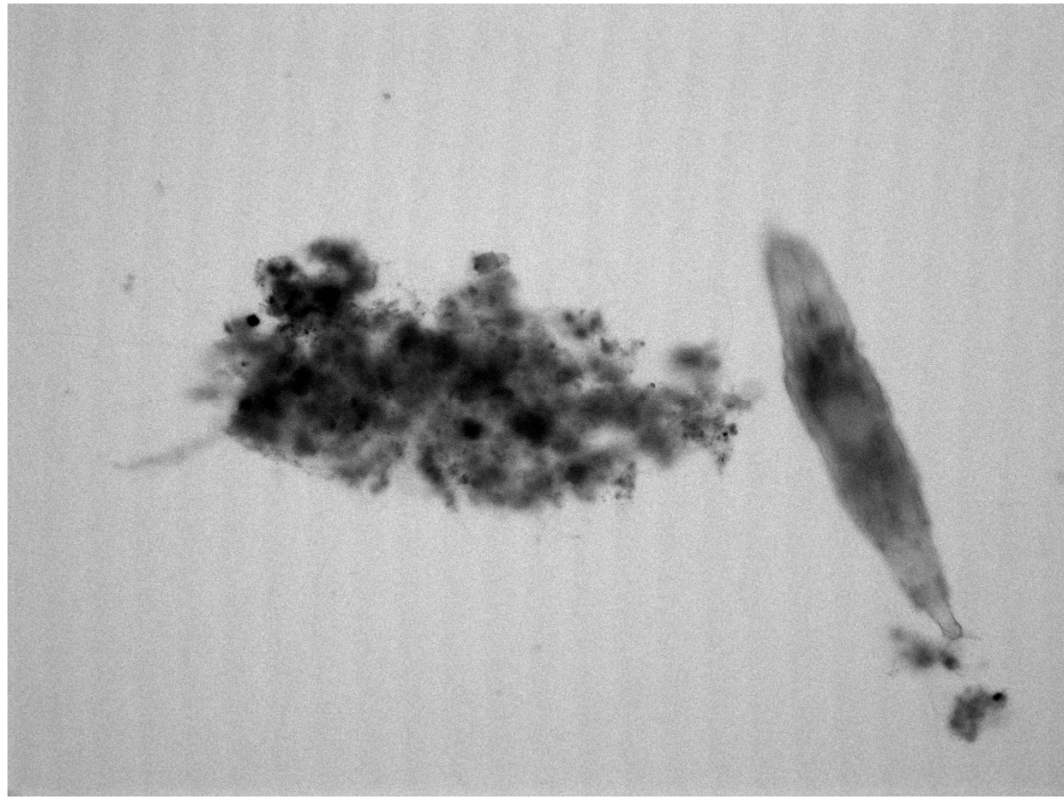
10x



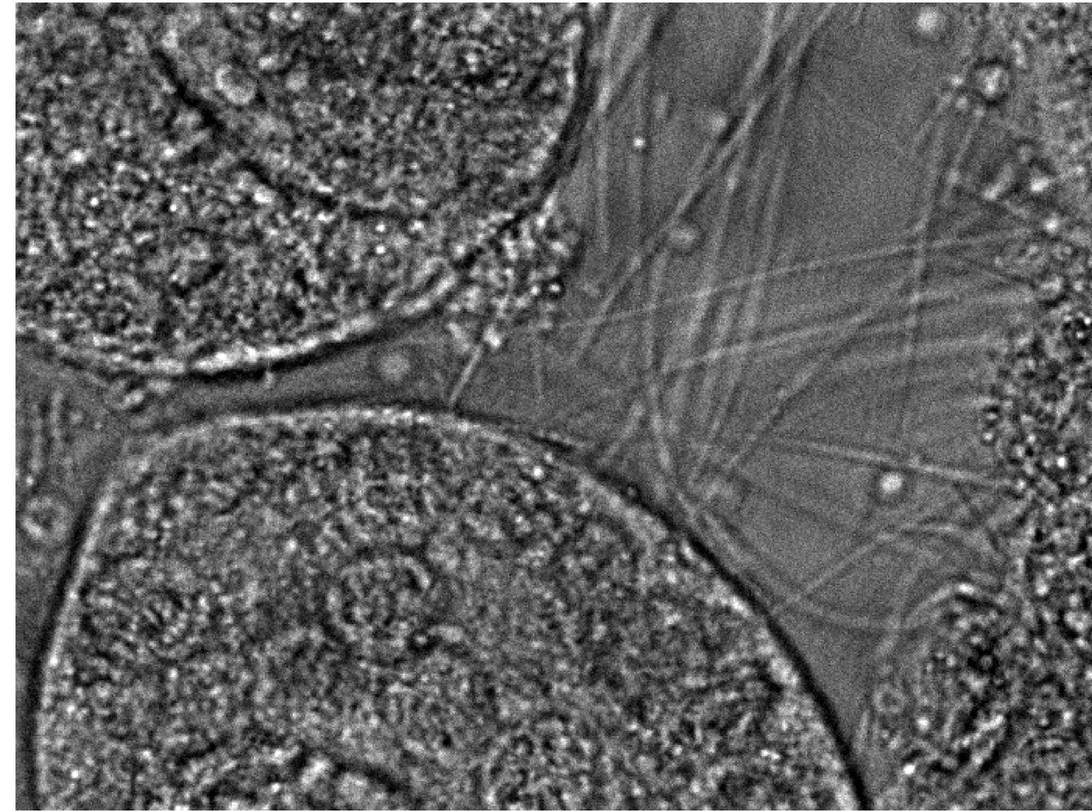
40x showing Filamentous



10 x Worm



Rotifer - 10 x



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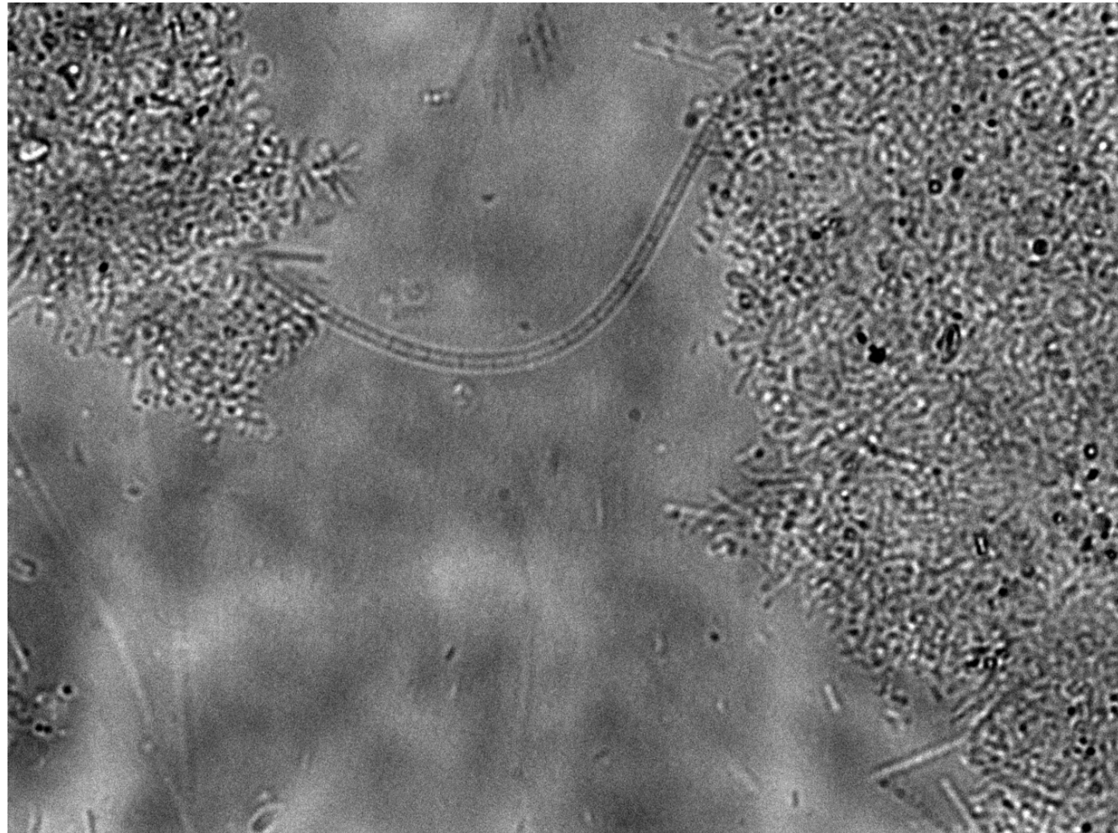

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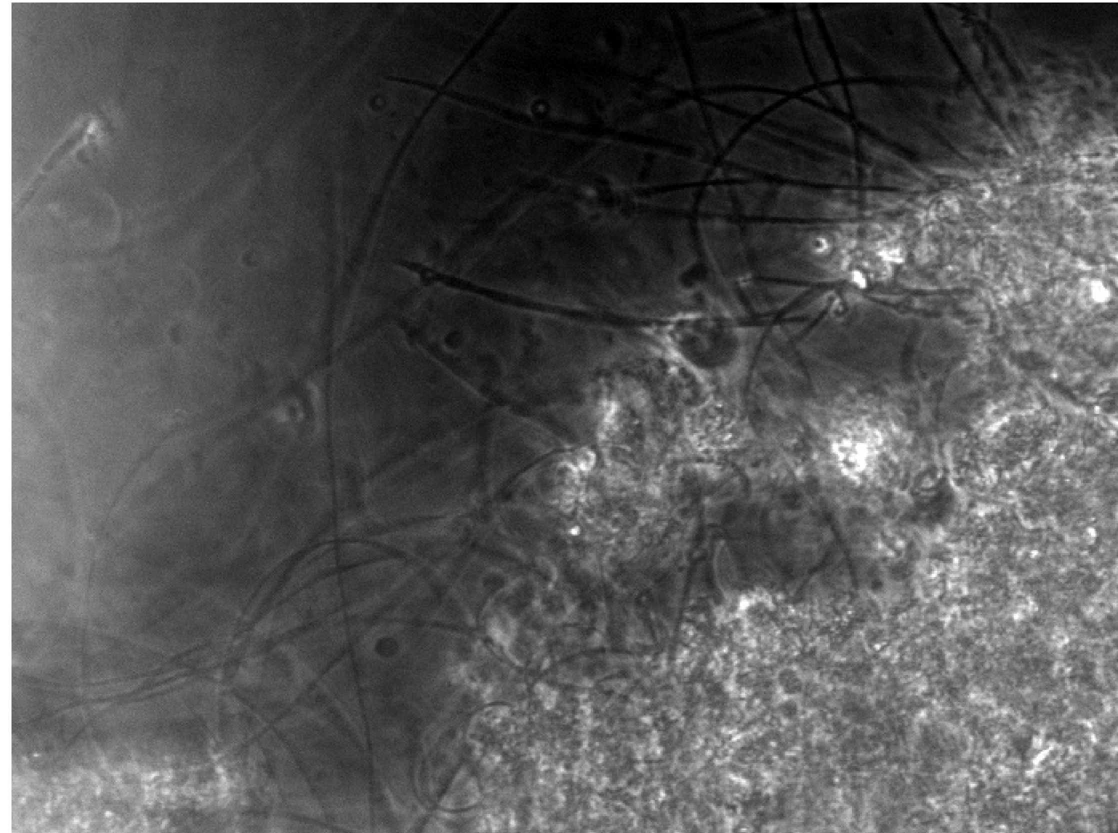

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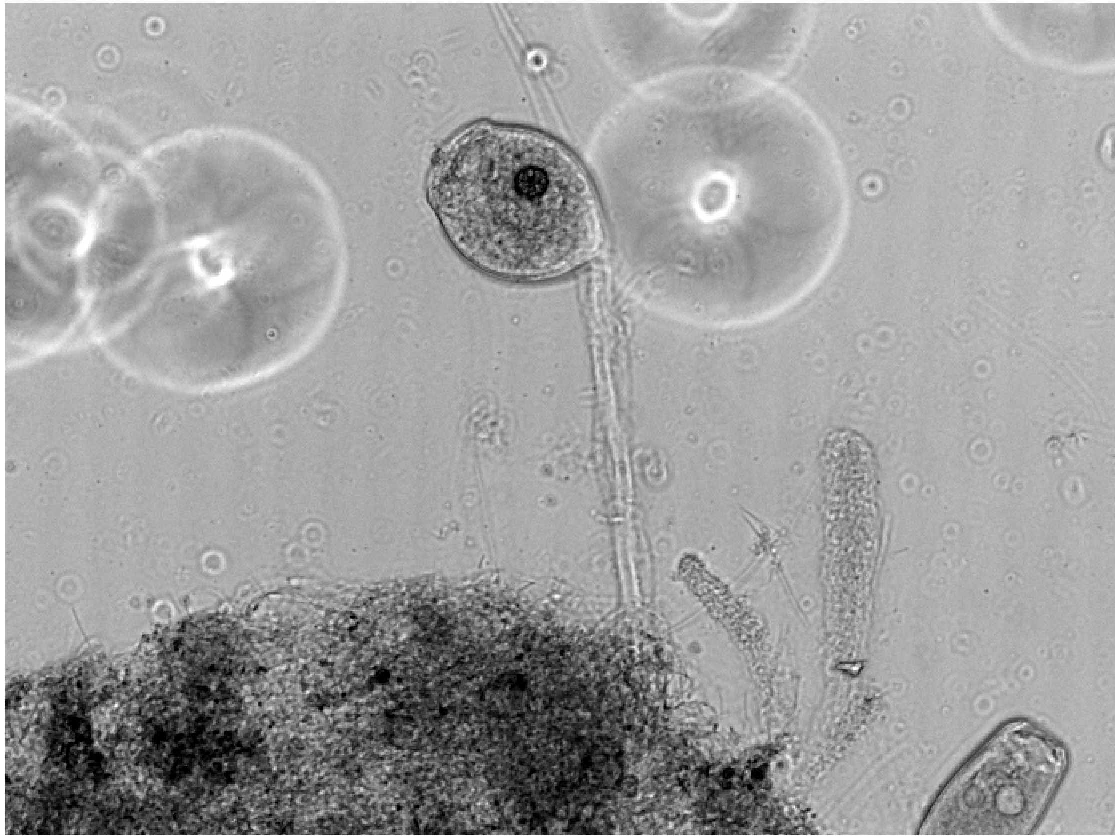
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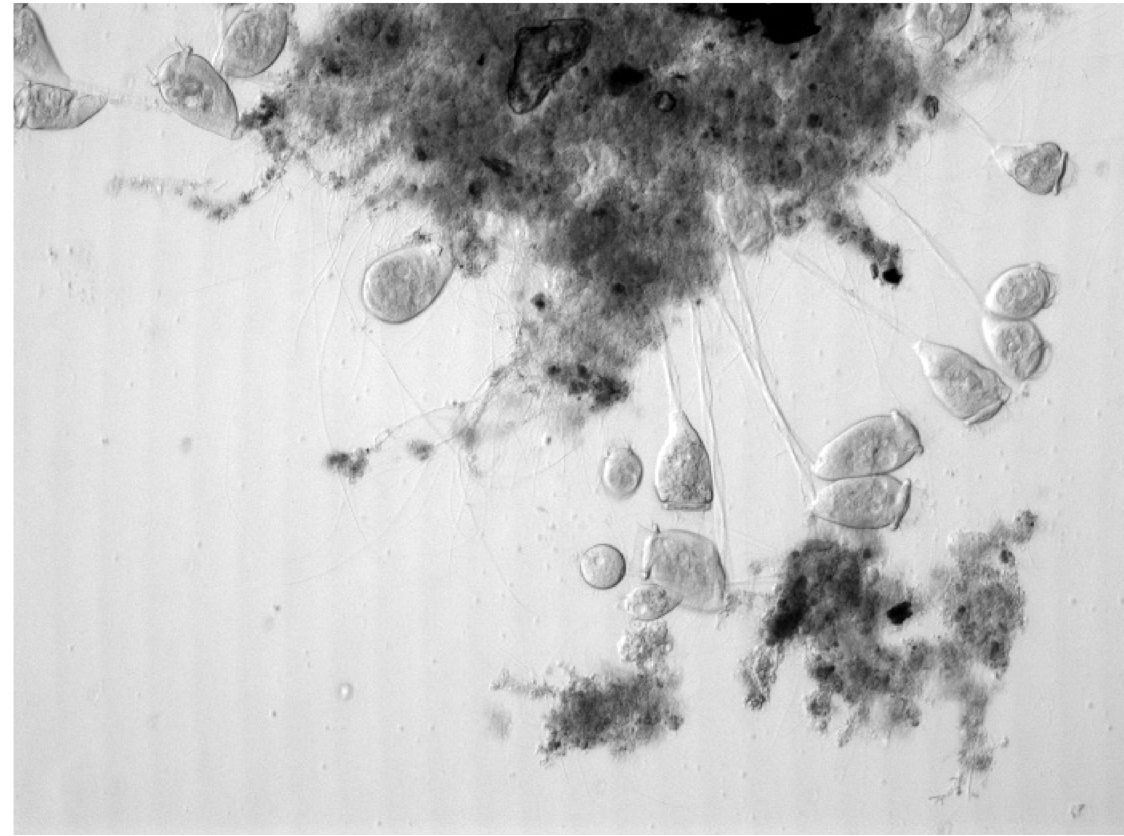
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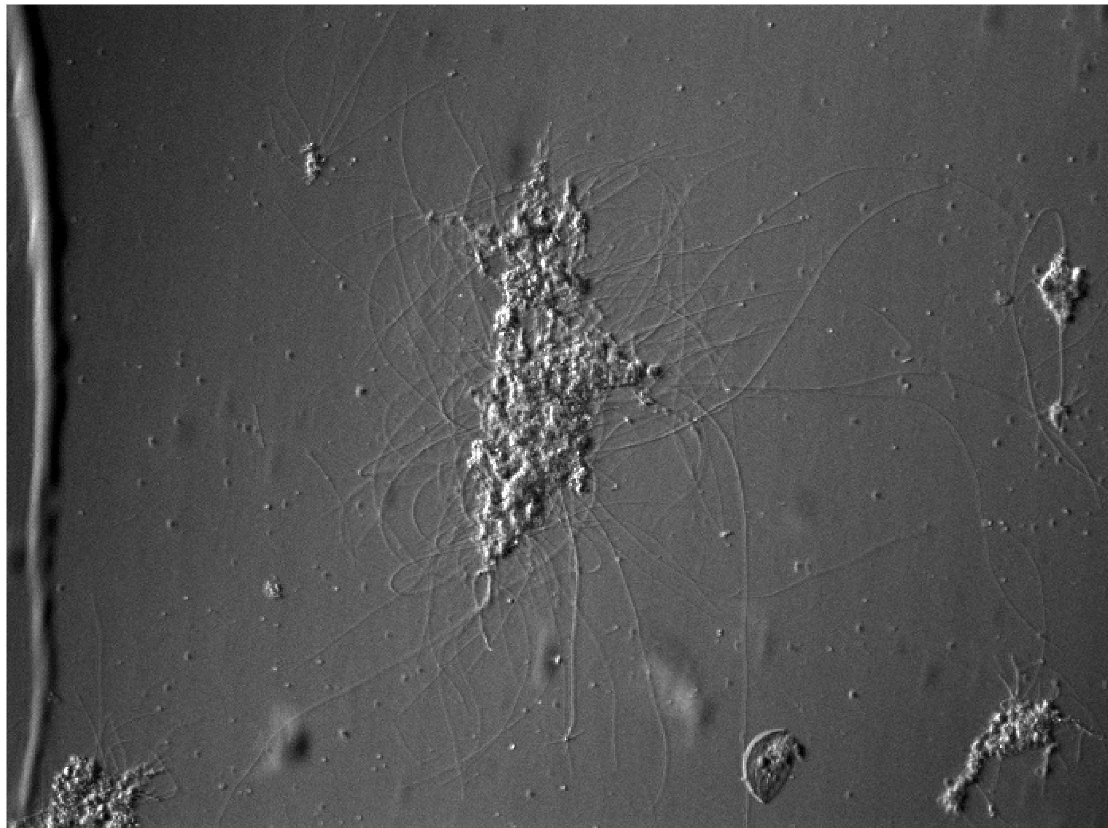
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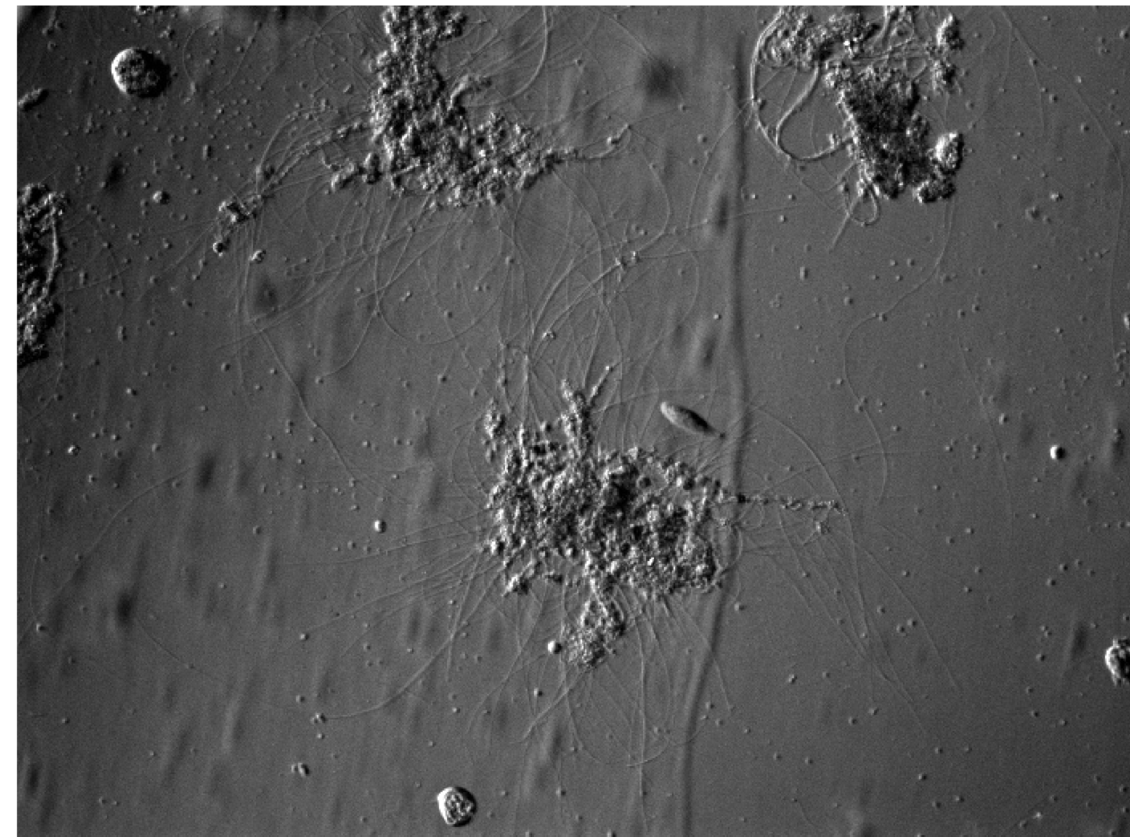
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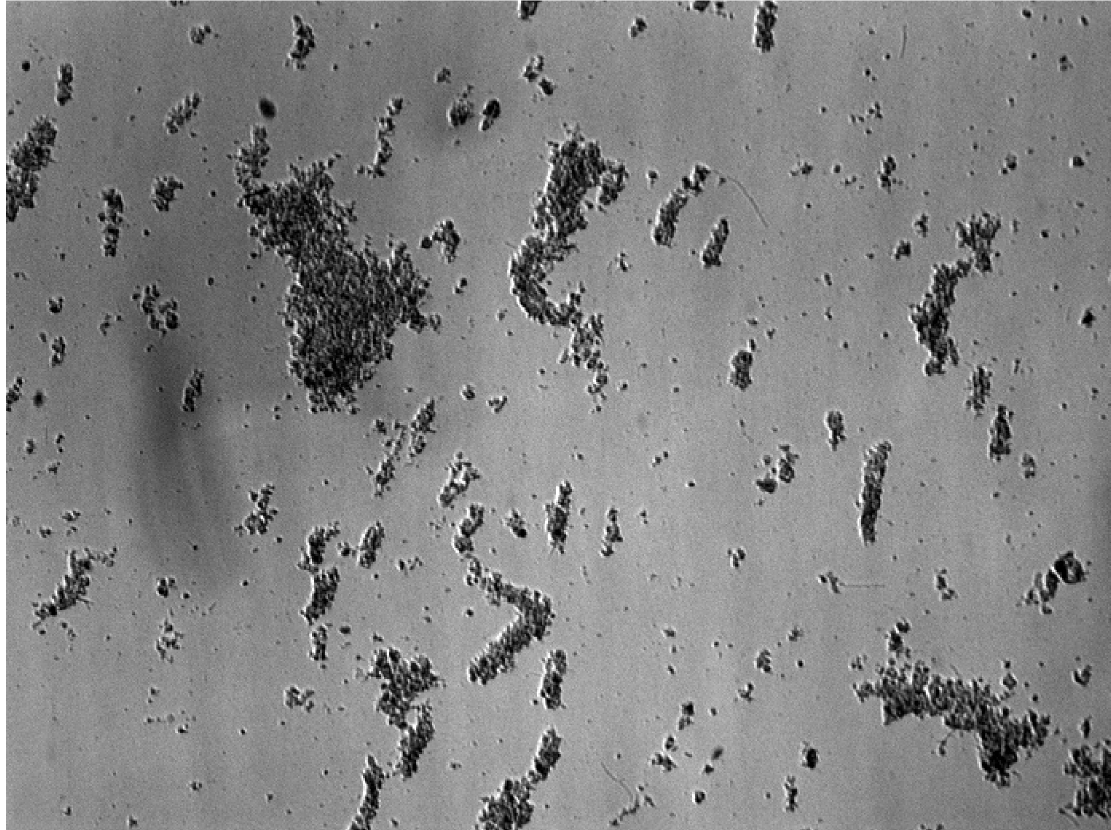
Reactor K



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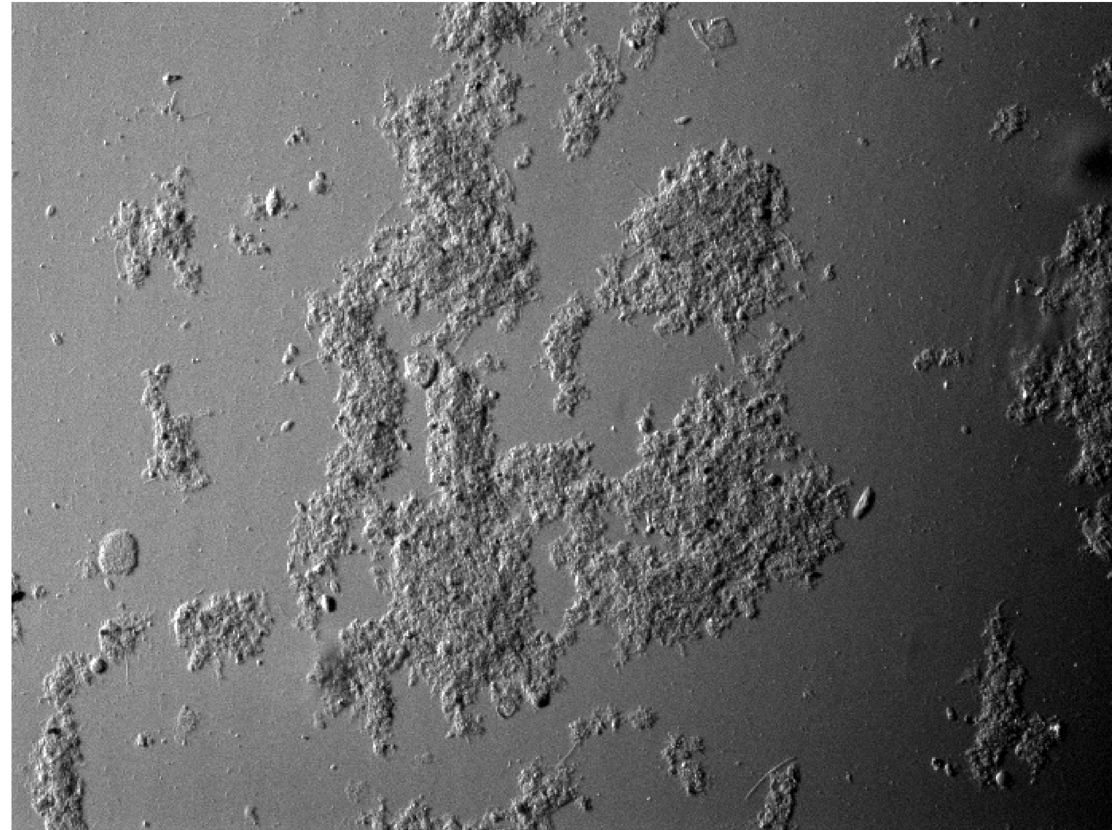
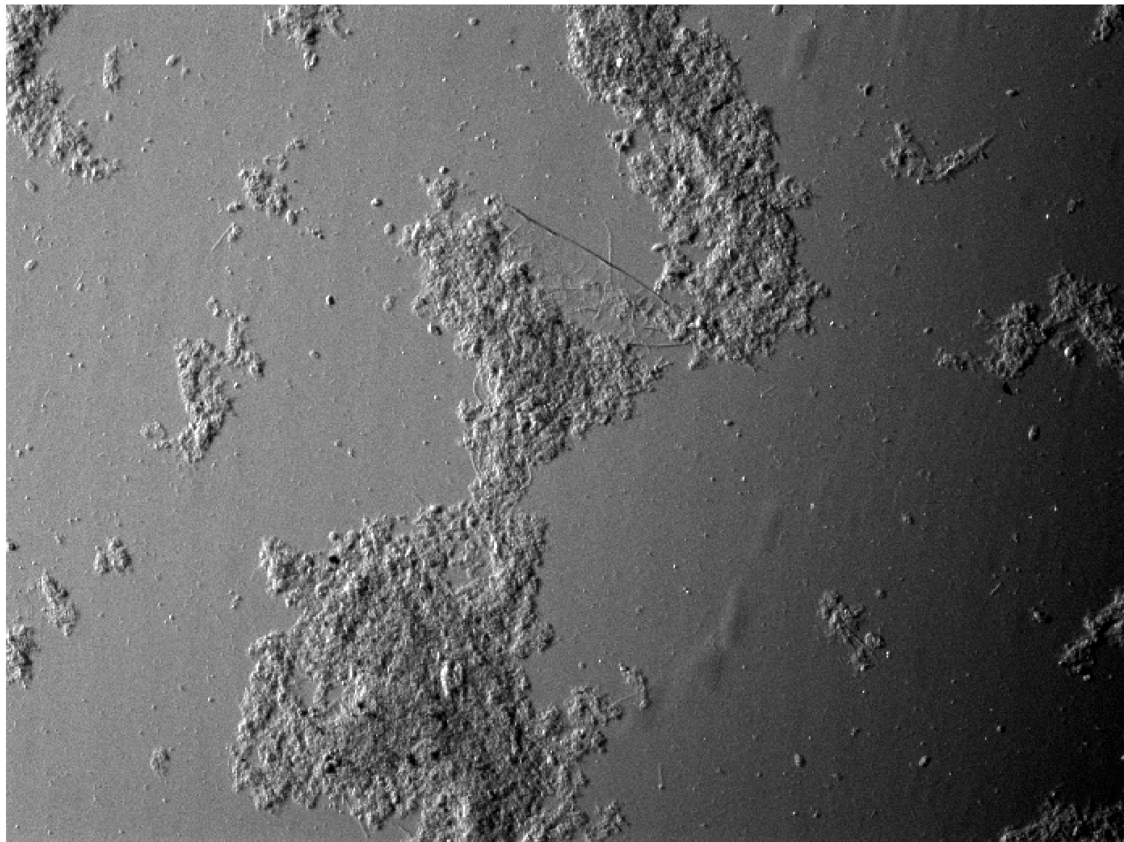
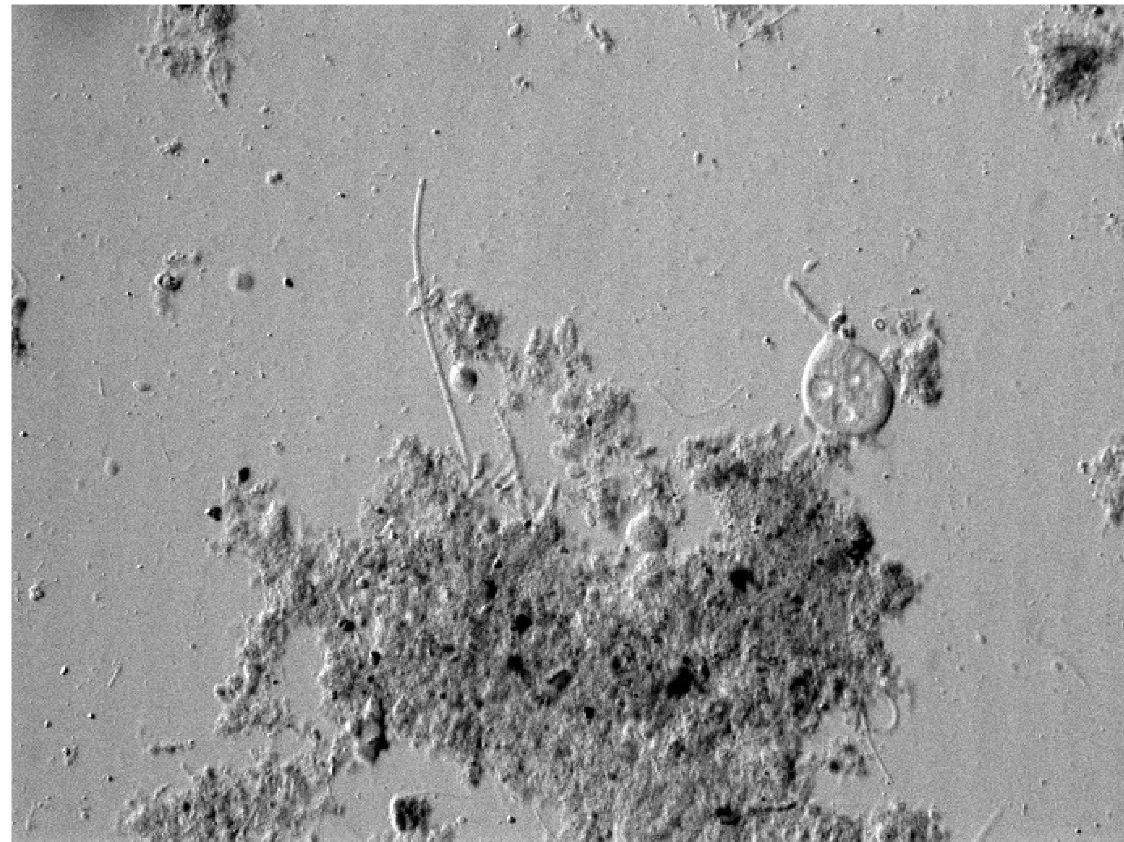


10 x mag



some fil, 10x



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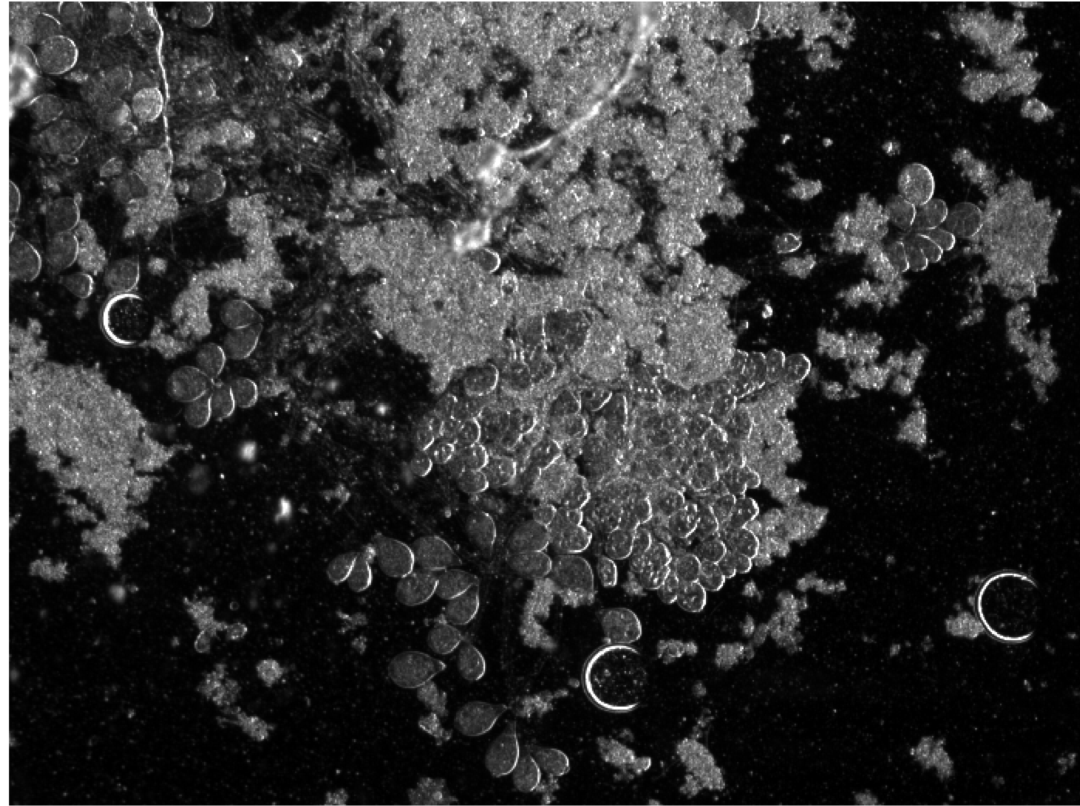
Reactor B



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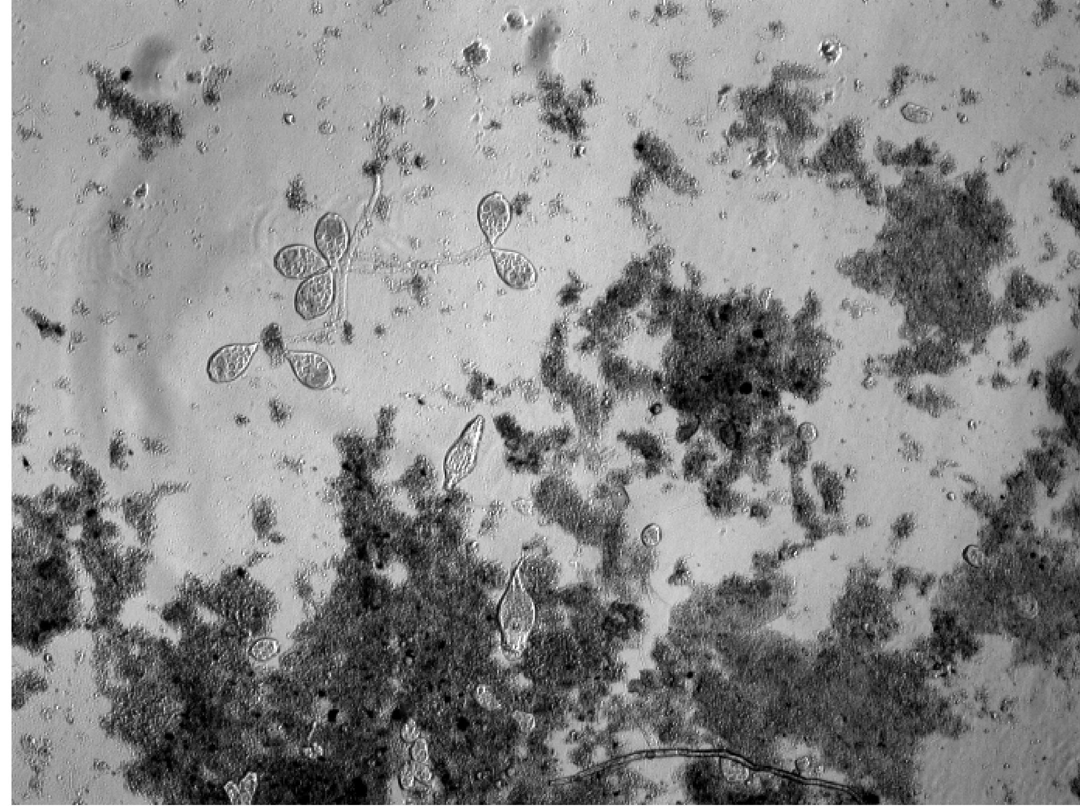

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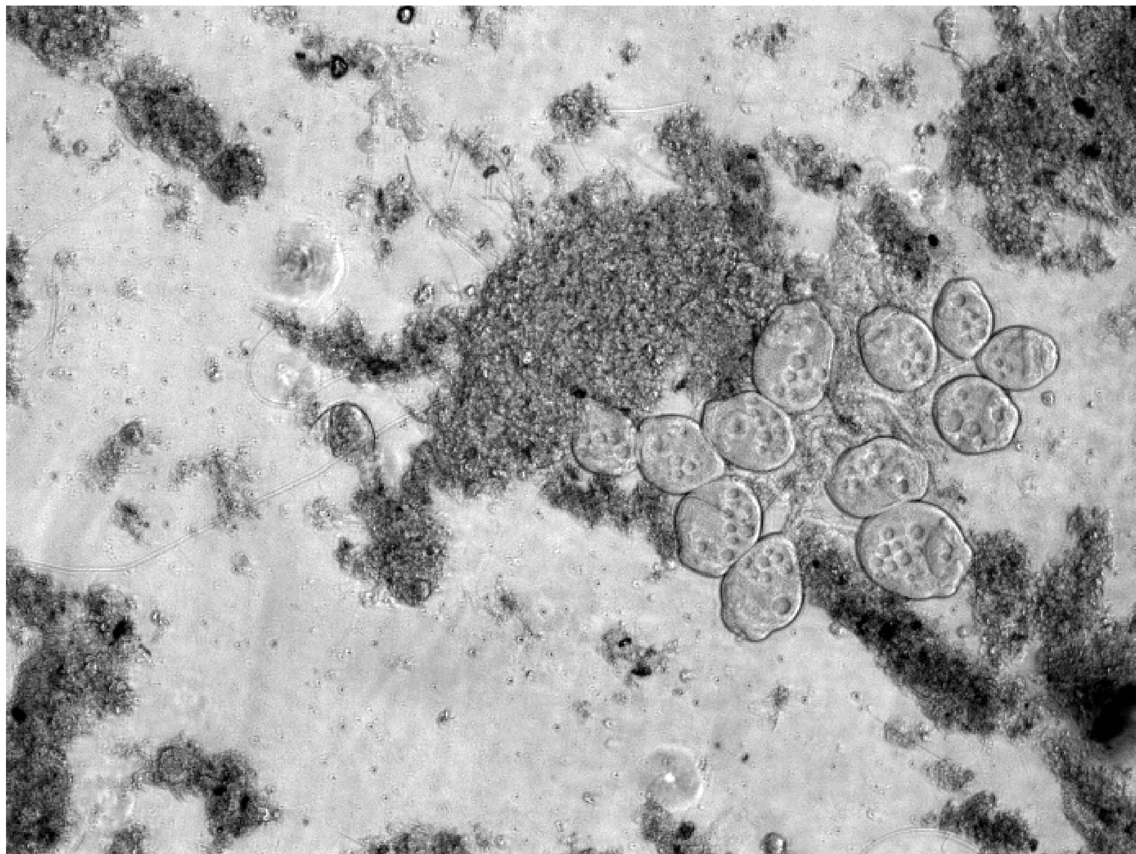
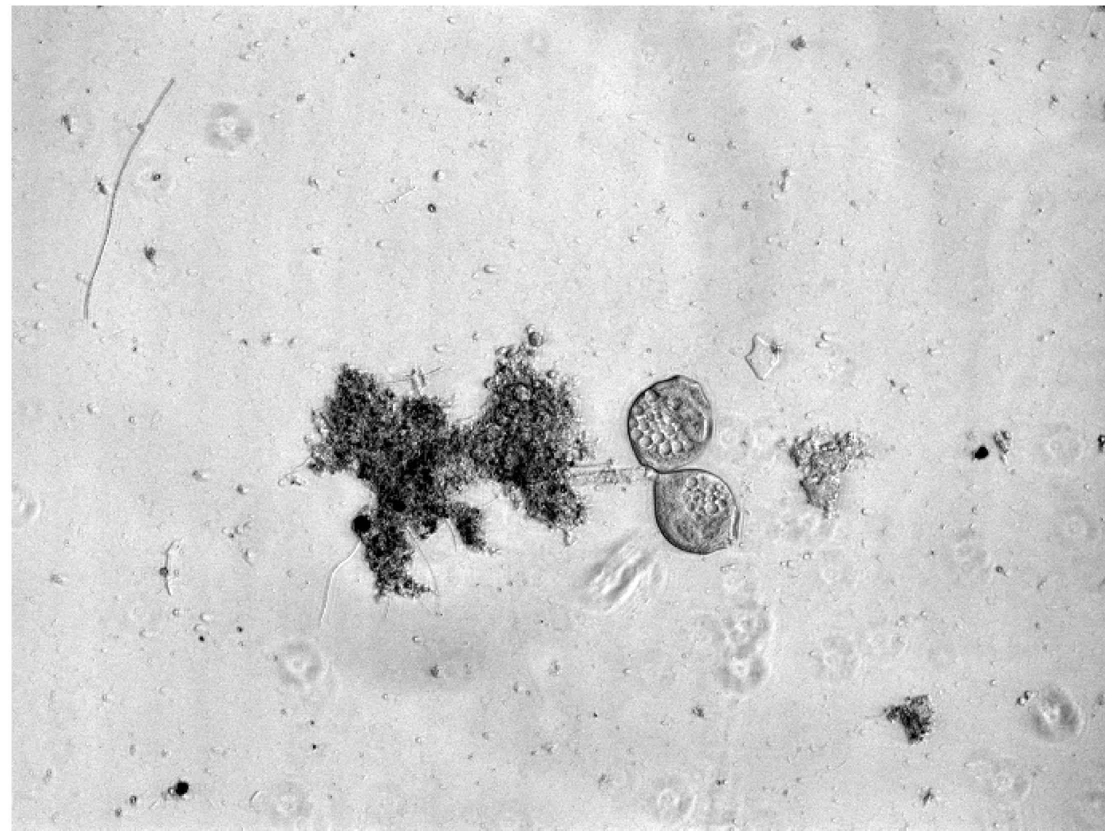


Some fil 10x



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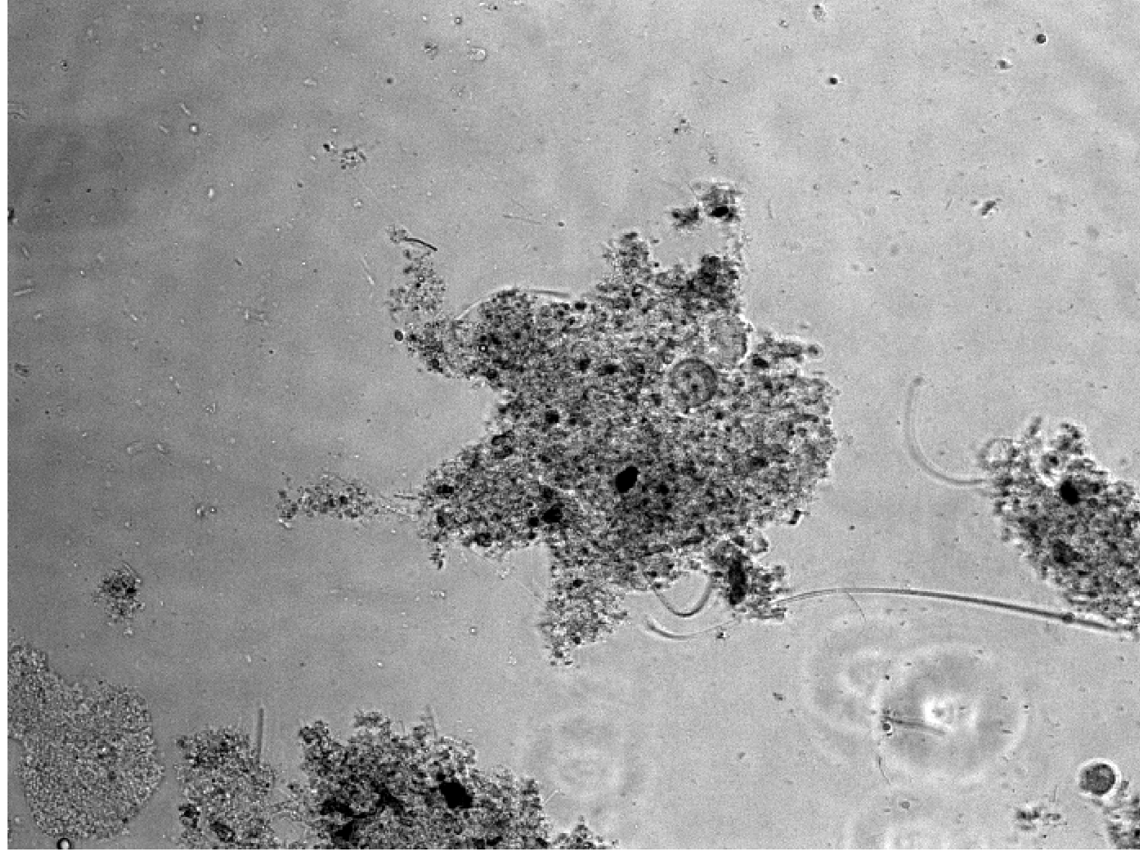
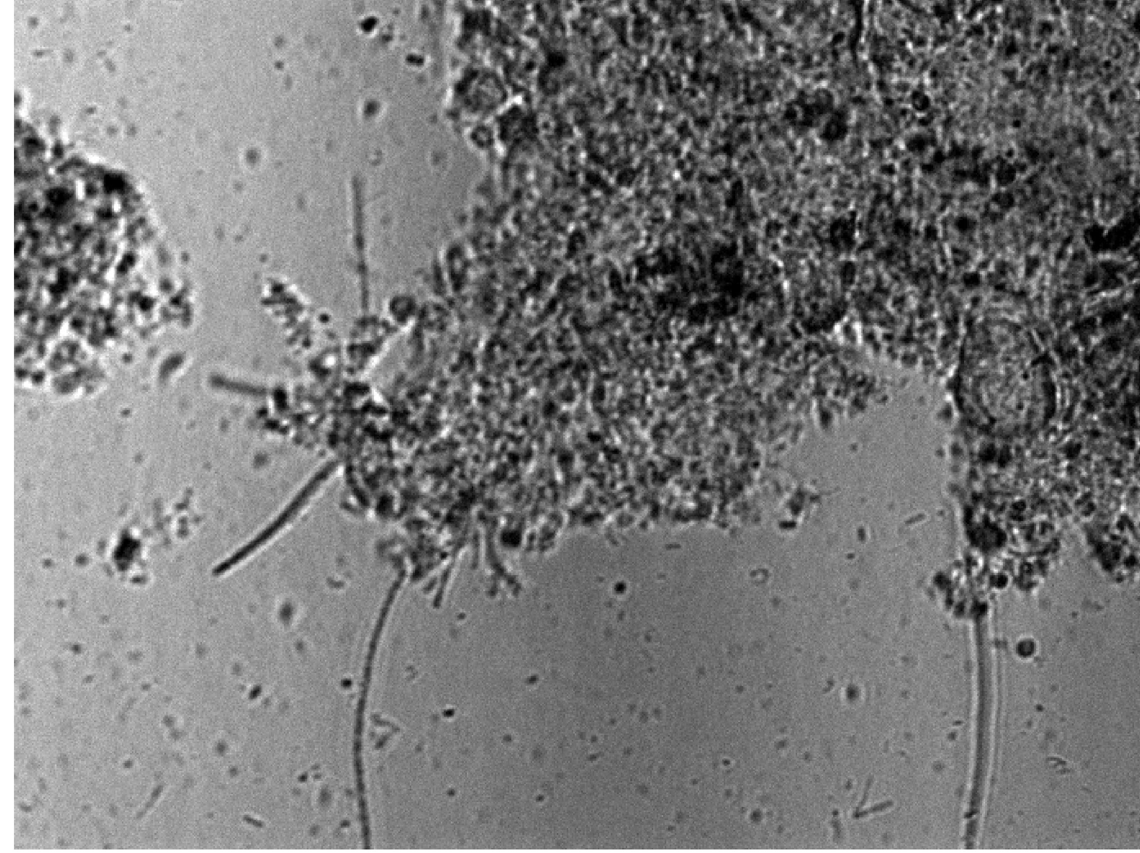
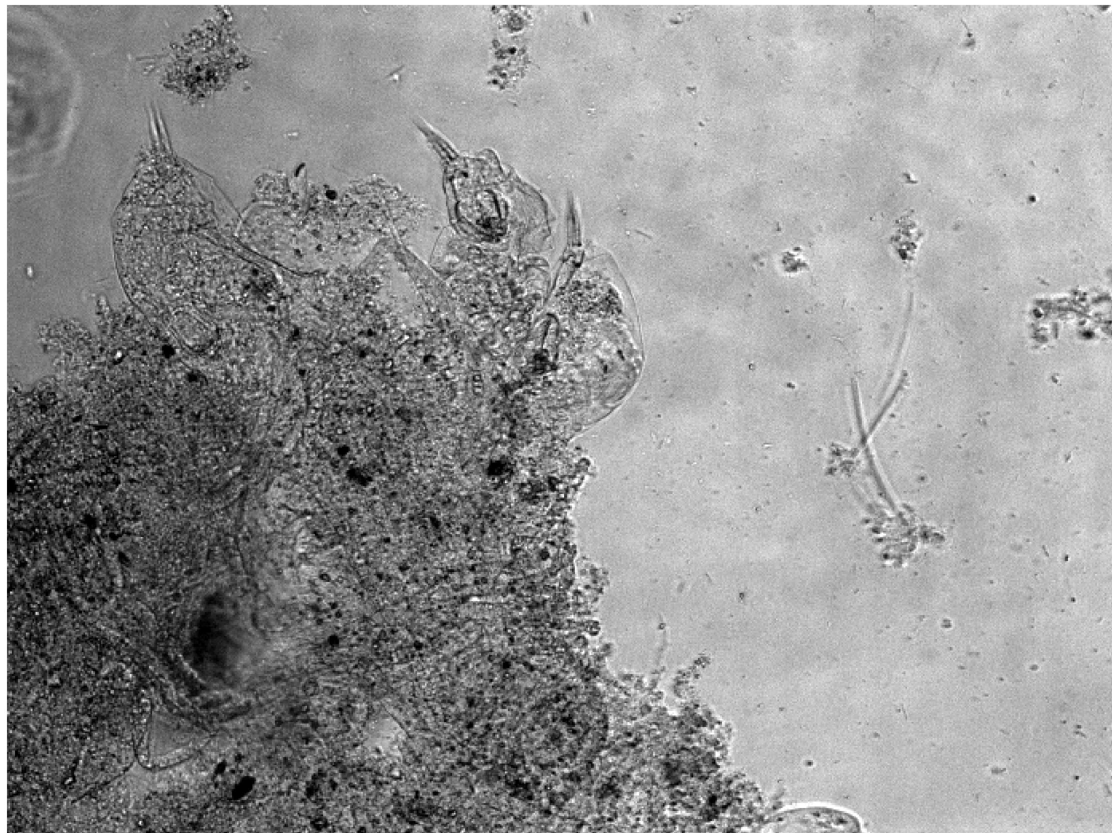


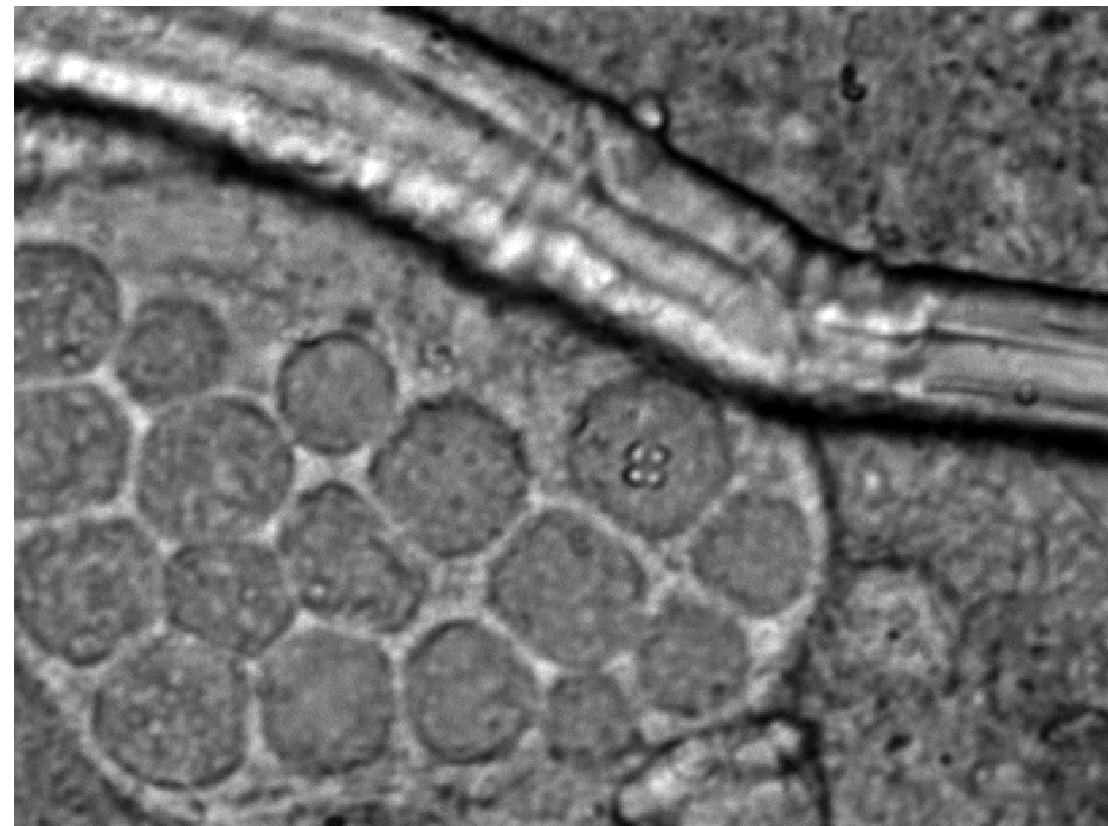
20x



40x

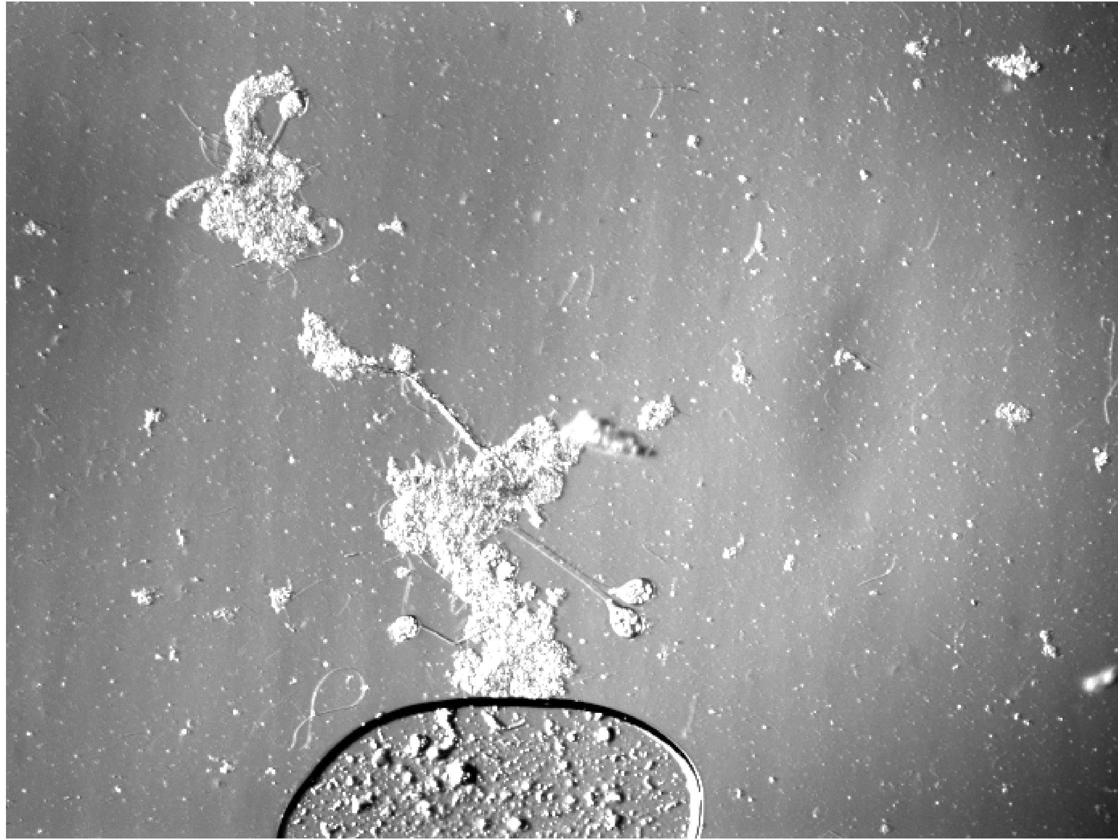


20x

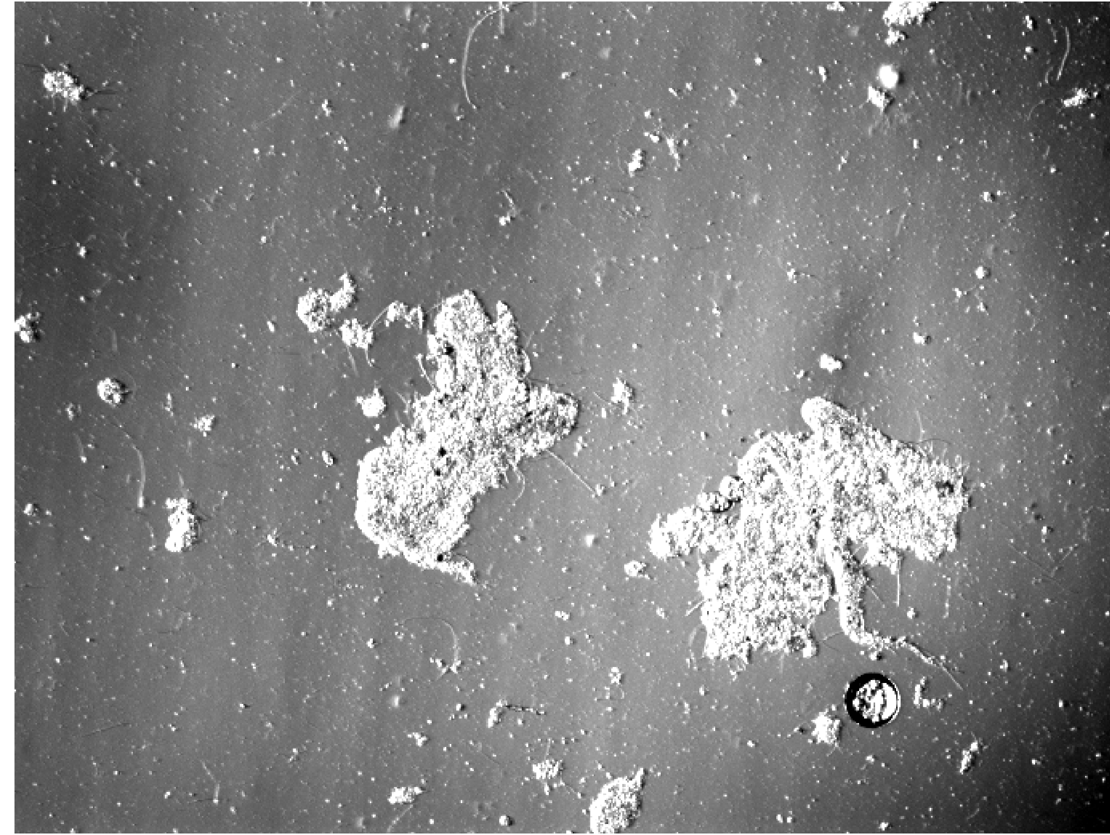


100x

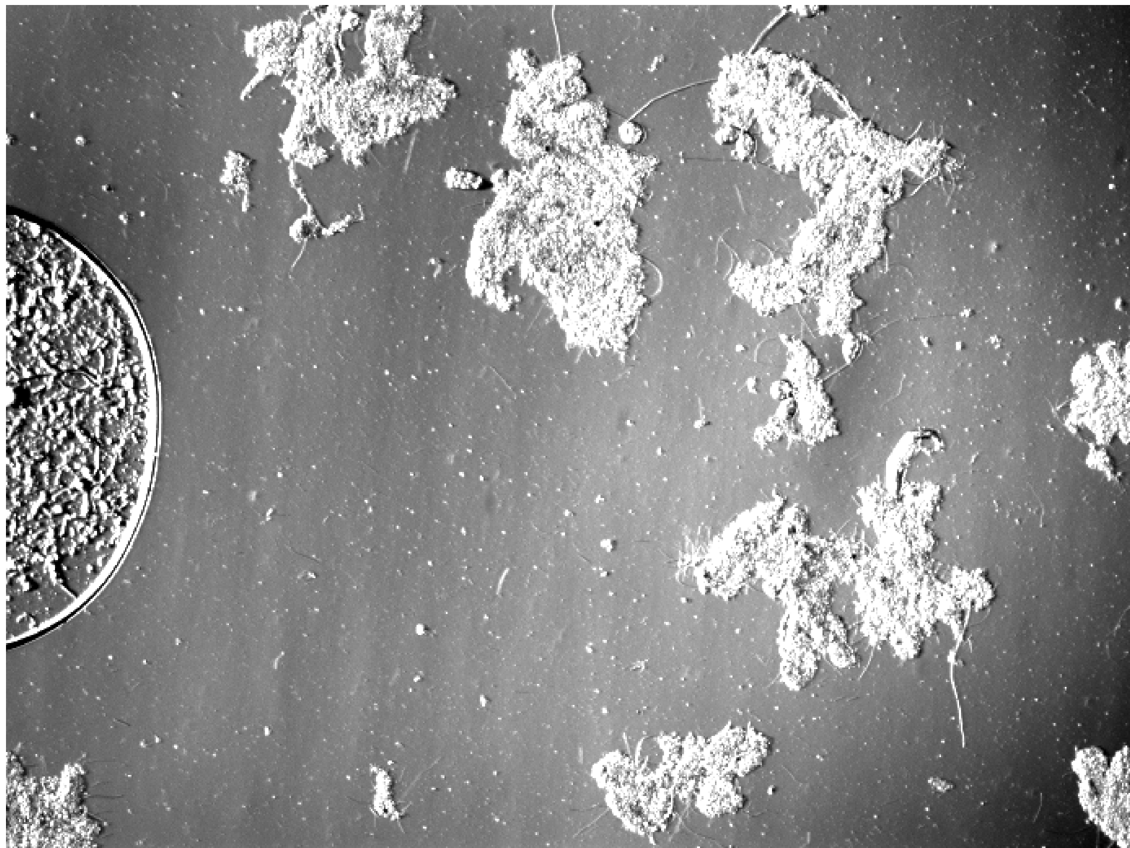
Reactor E



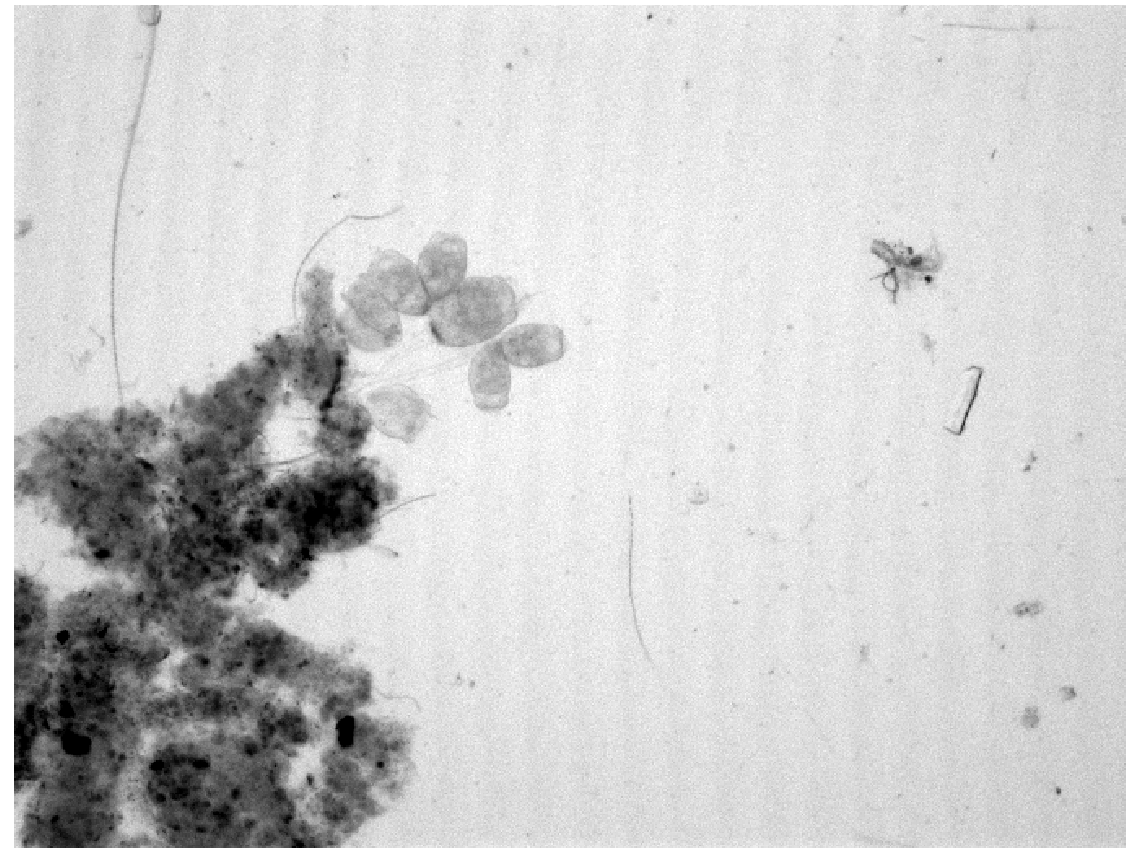
4x



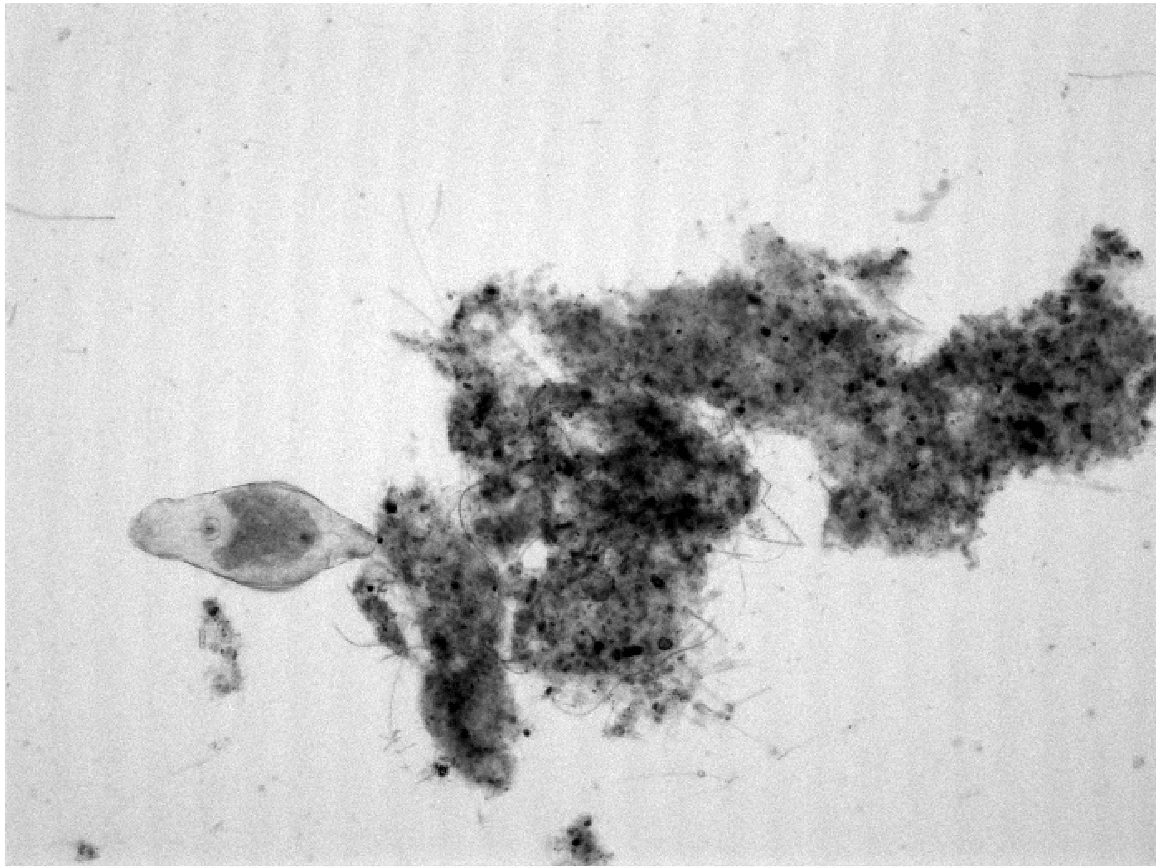
4x - Generally smaller flocs, more dispersed



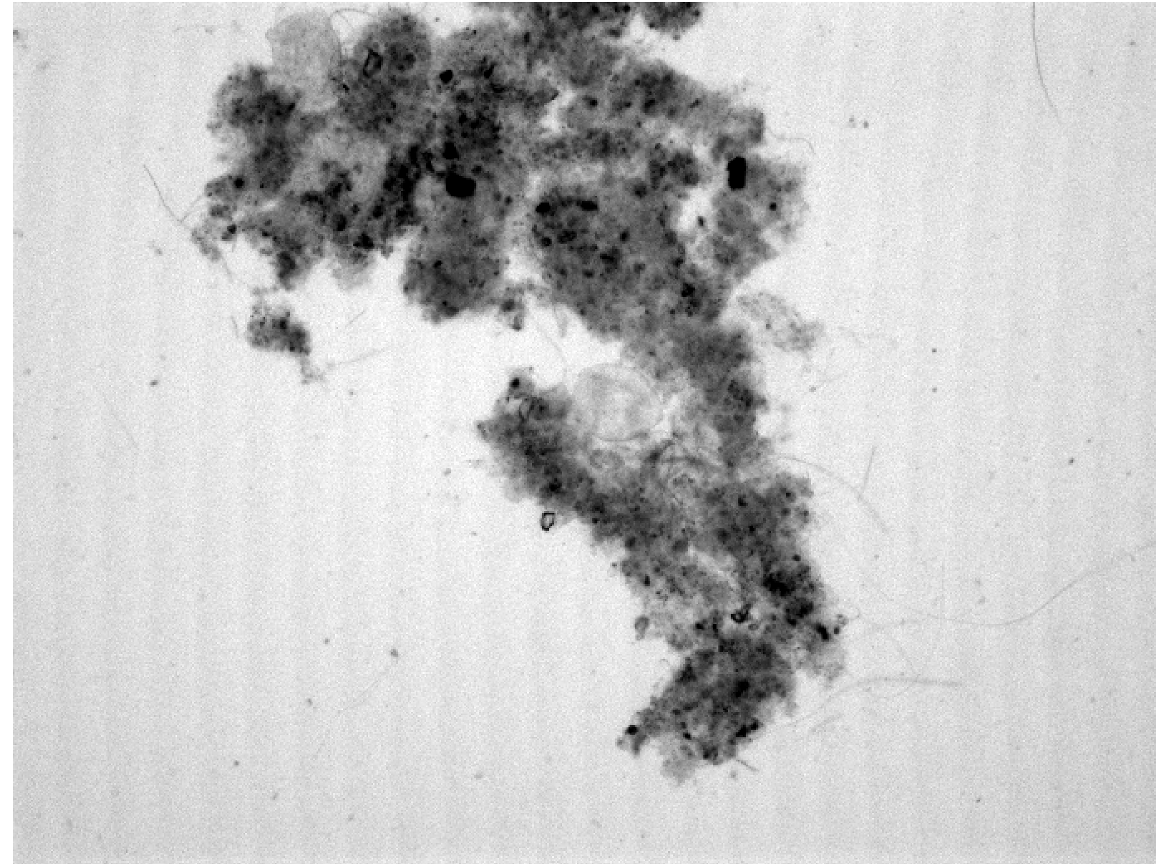
4x note higher organism



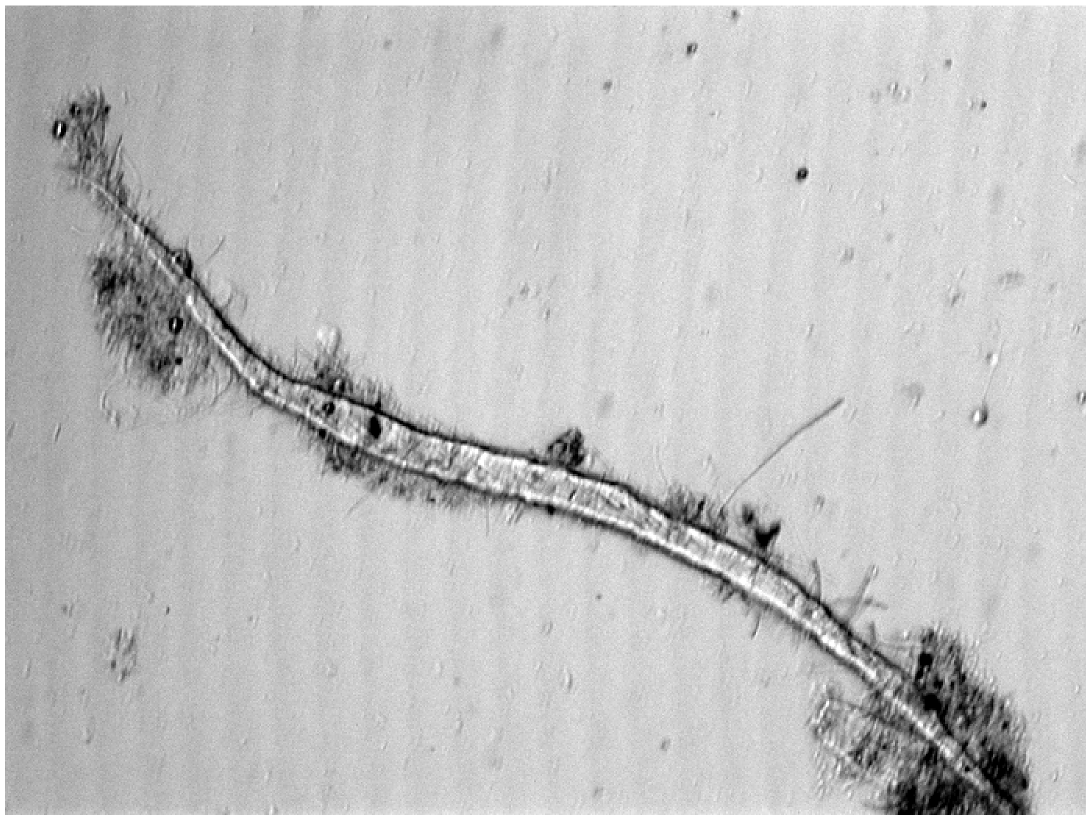
10 x



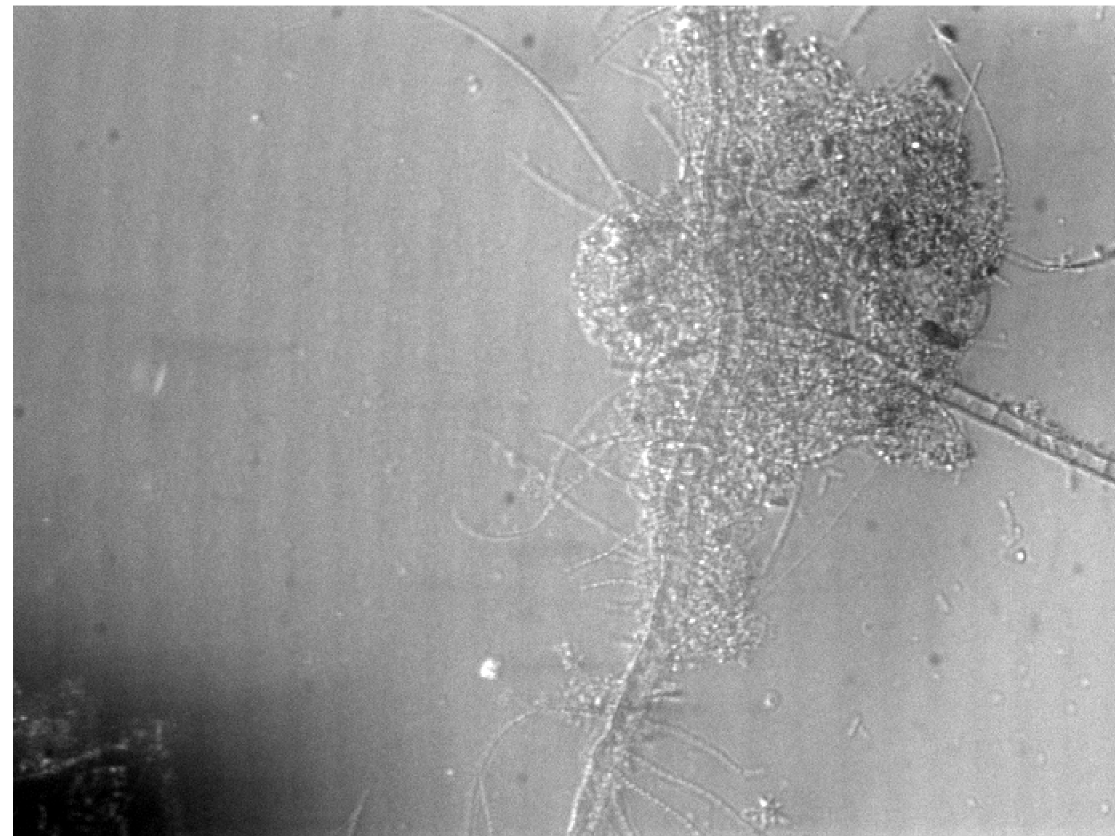
10x, fil and rotifer



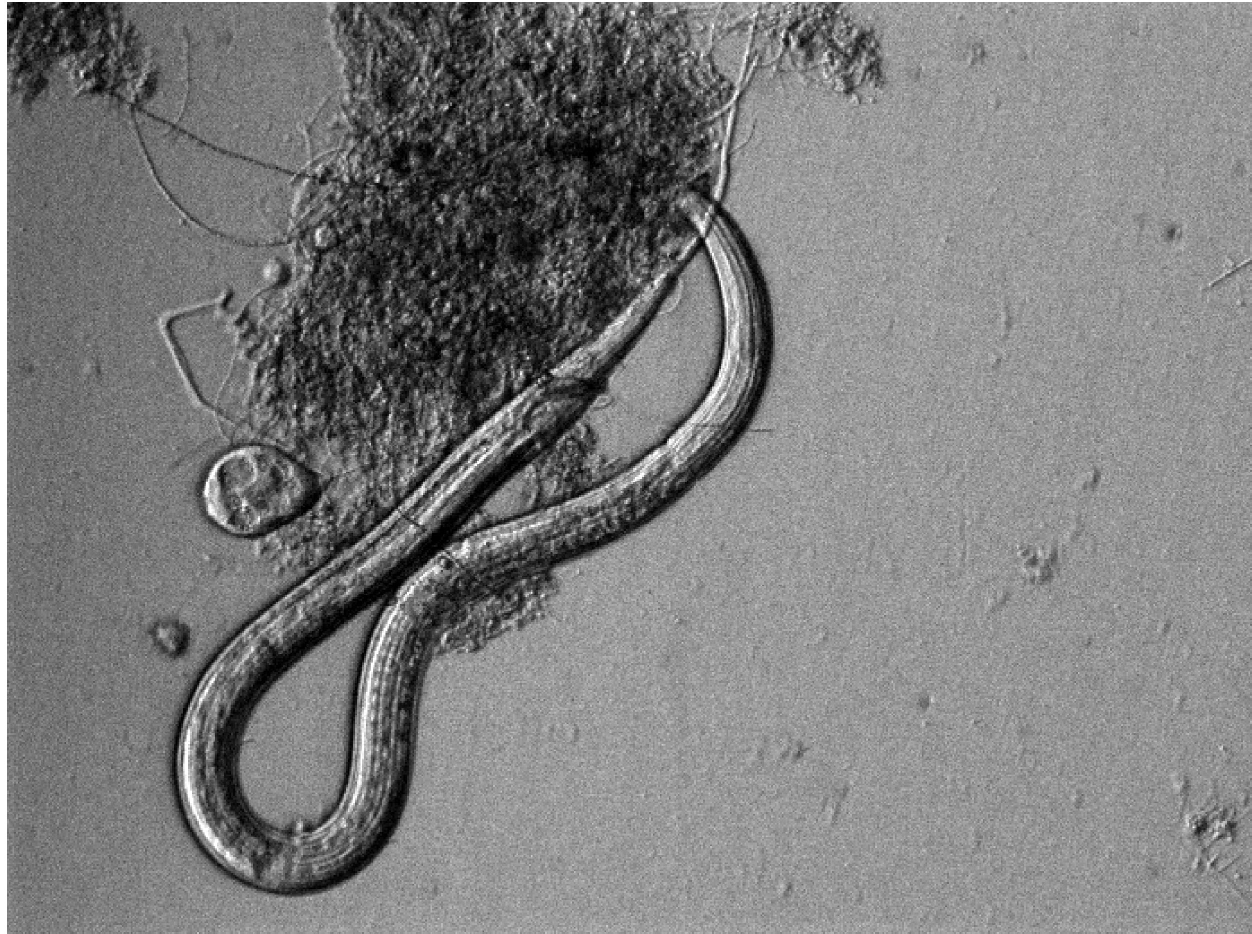
10x



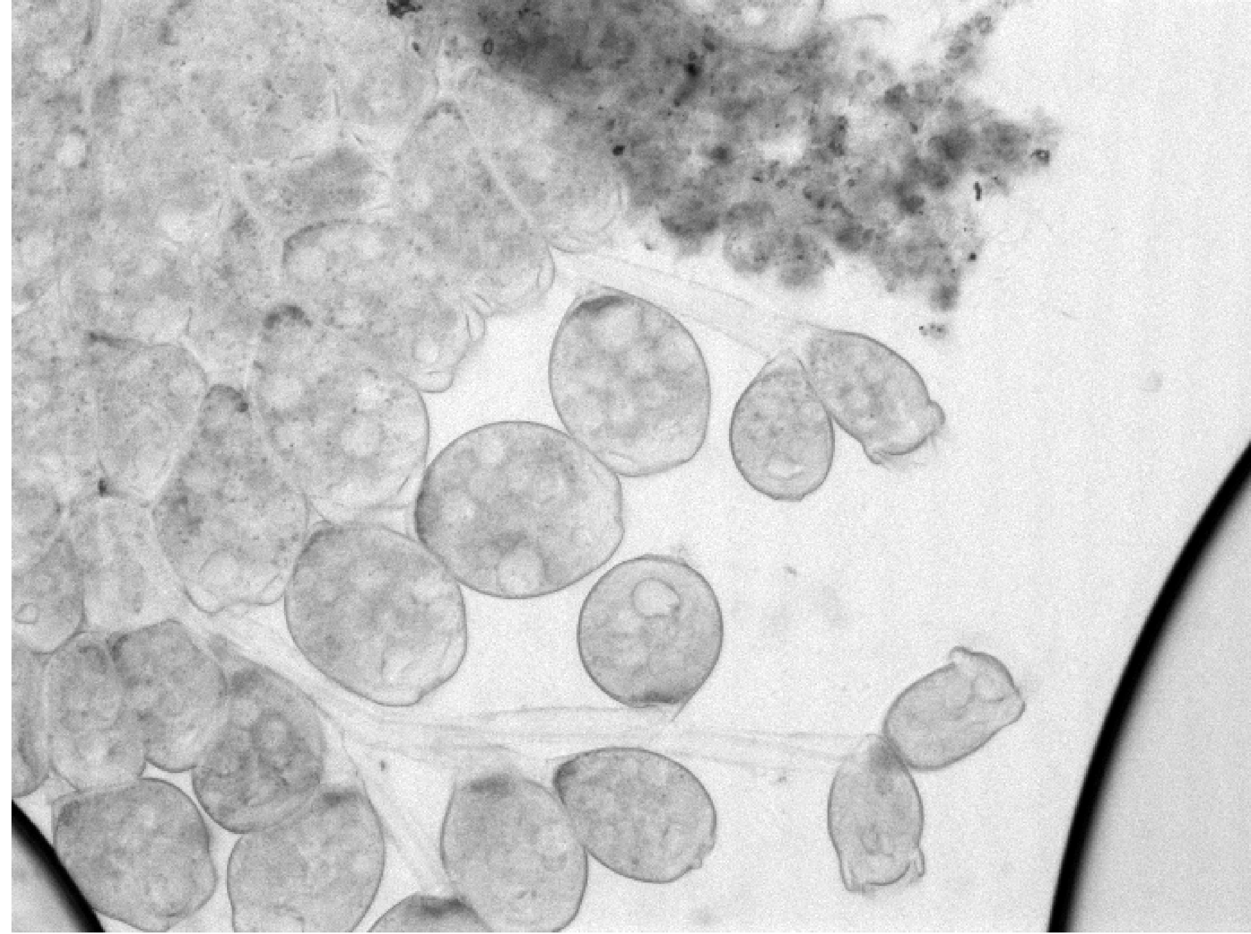
20x flocs attached to this



40x



20x worm



20x Stalked Cil