1-1-2007

Adsorption and partitioning behaviour of selected trace polycyclic synthetic musks in a suspended growth aerobic activated sludge system

Vince Pileggi
Ryerson University

ADSORPTION AND PARTITIONING BEHAVIOUR OF SELECTED TRACE POLYCYCLIC SYNTHETIC MUSKS IN A SUSPENDED GROWTH AEROBIC ACTIVATED SLUDGE SYSTEM

by

VINCE PILEGGI
(B.A. Sc., University of Toronto, 1989)

A thesis
presented to Ryerson University
in partial fulfillment of the
requirements for the degree of
Master of Applied Science
in the Program of
Environmental Applied Science and Management

Toronto, Ontario, Canada, 2007

AUTHOR'S DECLARATION

Date: January, 2007

Author: Vince Pileggi

Title: Correlation of Selected Physicochemical Properties of Sludge Flocs With Partitioning And Competitive Equilibrium Adsorption-Desorption Behaviour of Selected Trace Polycyclic Synthetic Musks During Aerobic Activated Sludge Sewage Treatment Process

Degree: M.A.Sc. Convocation: Spring Year: 2007

I hereby declare that I am the sole author of this thesis. I authorize Ryerson University to lend this thesis to other institutions or individuals for the purpose of scholarly research.

I further authorize Ryerson University to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

ADSORPTION AND PARTITIONING BEHAVIOUR OF SELECTED TRACE POLYCYCLIC SYNTHETIC MUSKS IN A SUSPENDED GROWTH AEROBIC ACTIVATED SLUDGE SYSTEM

Vince Pileggi

School of Graduate Studies
Environmental Applied Science and Management, Ryerson University
January 2007

ABSTRACT

This thesis investigated the influence of sludge retention time (SRT) and temperature (T) on selected activated sludge properties and their influence on partitioning and sorption behaviour of selected trace polycyclic synthetic musks (PSMs) of environmental concern.

Suspended growth aerobic activated sludge systems under controlled temperature (10 and 20 °C) and SRTs (3.5 and 10.5 days) conditions fed by municipal sewage were investigated. The selected PSMs monitored included Cashmeran, Celestolide, Phantolide, Traseolide, Galaxolide and Tonalide.

Activated sludge floc properties including relative hydrophobicity (RH) and extracellular polymeric substances (EPS) showed significant differences which correlated well (r_p of ±0.4 to ± 0.7) to the removal and partitioning of PSMs removed from the aqueous phase and associated with activated sludge. Galaxolide and Tonalide were found to represent over 95% of the total PSMs in both the aqueous and solid phases. PSMs aqueous reduction from 62 to 80 % was observed. The total PSMs associated with sludge ranged from 15 to 27 μg/g d.m. and the lowest concentration was observed under 10.5 days SRT and 20 °C which also resulted in nitrifying conditions. SRT was the dominant operational factor, followed by SRT and TxSRT in influencing the partitioning of the PSMs and floc properties.

The Freundlich equilibrium PSMs sorption and desorption isotherms, for sludges were generated and showed significant differences in sorption behaviour.

ACKNOWLEDGEMENTS

Many people have made it possible for me to complete this work and I am grateful to all of them for their encouragement, patience and assistance. Particularly I am grateful to my supervisors, Professor Steven N. Liss and Dr. Lori Anne Lishman, P. Eng., for introducing me to the exciting challenges of non-conventional activated sludge analysis, for their direction and encouragement throughout the course of this study and in the preparation of this thesis.

I would like to express my sincere acknowledgements to the following:

- Dr. Lori Anne Lishman of the National Water Research Institute (NWRI) of Environment Canada (EC), also for handling the supervision of the operation of the sequencing batch reactors, providing focused criticism of my work and her continued friendship which helped me in the planning and execution of the many tasks that needed doing.
- Members of the reading and defense committee including Prof. Gideon Wolfaardt and Prof. Wayne J. Parker, for providing insightful conceptual and editorial comments which greatly improved the thesis manuscript.
- Dr. Peter Seto of the National Water Research Institute (NWRI) of Environment Canada (EC), for his inspiration, support, encouragement, insightful advice and friendship.
- Dr. Ramanathan Manoharan of the Ontario Ministry of the Environment for his friendship, interest, time, insightful advice and encouragement during the difficult times.
- Mr. Jason Fani for his support and friendship during my intellectual excursion into the exploration of the subtle areas of activated sludge.
- Dr. Ian G. Droppo and Christina Jaskot of the National Water Research Institute (NWRI) of Environment Canada (EC), for their assistance by conducting the plankton chamber settling analysis and sludge lyophilization.
- Members of the Ryerson University Environmental Biotechnology Laboratory and Analytical Center Dr. Jian Liu, Dennis Walmsley, Dr. Ivan Xiang, Mitzuko Katani and Declan Williams for their assistance, advice and friendship throughout the laboratory work.
- Members of the National Water Research Institute (NWRI) of Environment Canada (EC) research laboratory in Burlington, Ontario, Jian-Jun Yang, Shirley Anne Smyth, Mark Baker, the late Jim Wood, Tony Notarianni, Dr. Petra Zaplatikova and Syed Wajahat.

I dedicate this thesis to Regina and Justine for their loving support and humour.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

AS	Activated sludge
BOD_5	Biochemical oxygen demand in 5 days [mg/L]
CEPA	Canadian Environmental Protection Act
COA	Candada Ontario Agreement for Inland Waters
COD	Chemical oxygen demand
COM	Conventional optical microscopy
CSTR	Completely stirred tank reactor
d. m.	Dried matter
DNA	Deoxyribonucelic acid
DO	Dissolved oxygen (mg/L)
EDC	Endocrine disrupting compound
EDTA	ethylenediaminetetraacetate
EEM	Excitation-emission matrix (three-dimensional fluorescence spectroscopy)
EPS	Extracellular polymeric substances
ESS	Effluent suspended solids
F/M	Food (mass of COD) / Microorganisms (mass of biomass·day)
FISH	Fluorescence in situ hybridization
HS SPME	Head space solid phase microextraction
HRT	Hydraulic retention time (hours)
MATH	Microbial adherance to hydrocarbons
MLSS	Mixed liquor suspended solids (g/L)
MLVSS	Mixed liquor volatile suspended solids (g/L)

MOE	Ontario Ministry of the Environment
MWWE	Municipal wastewater effluent
MXR	Multixenobiotic resistance
PAHs	Polyaromatic hydrocarbons
PBS	Phosphate buffer saline (pH=7.4)
POP	Persistent organic pollutants
PPCPs	Pharmaceuticals and personal care products
PSM	Polycyclic synthetic musk (ng/L in aqueous phase or ng/g d.m.in solid pahse)
RH	Relative hydrophobicity (%)
SBR	Sequencing batch reactor
SC	Surface Charge (meq/g MLSS)
SCLM	Scanning confocal laser microscopy
SRT	Solids retention time (days)
STOWA	European Foundation for Applied Water Research
SVI	Sludge volume index (mL/g)
TAN	Total ammonia-nitrogen (mg/L)
tCOD	Total chemical oxygen demand (mg O_2/L)
TP	Total phosphorous (mg/L)
WFD	Wastewater Framework Initiative (Europen Union)
WWTP	Wastewater treatment plant
XOC	Xenobiotic organic chemicals

CHAPTER I

INTRODUCTION

This introductory chapter explains the motivation of the present investigation, the goals and objectives and provides an outline of the thesis.

1.1. Motivation for the Present Investigation

Municipal wastewaters and municipal wastewater effluents (MWWE) have been shown to contain a large number of anthropogenic organic contaminants, at trace concentration levels, of national and international environmental concern (Daughton *et al.*, 1999; Kanda *et al.*, 2003; Lee *et al.*, 2003; Kupper *et al.*, 2004; Osemwengie *et al.*, 2004; Lishman *et al.*, 2006; Yang and Metcalfe, 2006;). These trace organic contaminants include the active ingredients found in classes of pharmaceutical and personal care products (PPCPs) (Lishman *et al.*, 2006; Smyth *et al.*, 2006). Some of these PPCPs have been reported to be endocrine disrupting compounds (EDCs), toxic to aquatic organisms, having a tendency to bioacumulate and bioconcentrate in fish and to act as potent chemosensitizers inhibiting the multixenobiotic resistance (MXR) of some aquatic organisms (Luckenbach and Epel, 2005).

Recently numerous full scale activated sludge sewage treatment plants have been surveyed and investigated for their ability to remove environmentally significant PPCPs found in municipal wastewaters (Smyth *et al.*, 2006). Current scientific conjecture, about the removal of trace organic contaminants, suggests that nitrifying activated sludge wastewater treatment plants (WWTPs) may be more effective generally at eliminating PPCPs than non-nitrifying WWTPs. Some of the reports suggest that this removal and reduced concentrations in MWWE is compound specific, related to operating conditions, affected by floc properties and not simply related to solids retention time (SRT) or nitrifying conditions. Whether environmental factors, chemical properties, nitrifying conditions or

SRT is or are the dominant factors determining the removal of PPCPs, is an unresolved question and difficult to clearly determine from surveys of full scale facilities alone.

It is well established that sludge floc properties such as extracellular polymeric substance (EPS) play a key role in the behaviour of sludge flocs which affect the performance of the whole activated sludge process (Raszka, *et al.*, 2006). The activated sludge operating conditions, such as SRT, mixing, biomass type and reducing conditions, have also been closely linked to activated sludge floc structure and physicochemical properties which in turn could determine the effluent and sludge concentration of various contaminants. Much work has been directed at understanding how operating conditions are correlated to the structure and behaviour of activated sludge in an attempt to better control and engineer the activated sludge process. Some of the reported research spans well over 30 years (Parker *et al.*, 1970; Li and Ganczarczyk, 1986, 1987, 1989, 1989 and 1990; Li *et al.*, 1989, 1990; Decho, 1990; Leppard *et al.*, 1992, 1993 and 1995; Droppo *et al.*, 1996 and 1997; Heissenberger *et al.*, 1996; Liss *et al.*, 1996; Finlayson *et al.*, 1998; Mikkelsen *et al.*, 2002; Wilén *et al.*, 2003).

In the operation of bench scale sequencing batch reactors (SBRs) under well controlled conditions, using synthetic feed, it has been observed that an SRT of 9 to 12 days is a determining threshold which clearly distinguished certain key floc properties. The properties identified include sludge floc surface hydrophobicity (RH) and surface charge (SC) along with the ratio of proteins to polysaccharides found in EPS (Liao *et al.*, 2001). Interestingly enough this SRT threshold is also typically where the distinction between nitrifying and non-nitrifying conditions occur.

It is speculated that sludge floc surface properties like SC and RH along with EPS properties (e.g. sludge floc mean size, porosity, density and size distribution) are also significantly affected by specific operating conditions. Floc characteristics could play an important role in the partitioning, adsorption-desorption and biotransformation of selected PPCPs. The details of the mechanisms at work are complex and difficult to investigate. The typical mechanisms, that are considered important

in the fate of microcontaminants through the activated sludge process, include floc enmeshment, sorption onto solids, volatilization or stripping and biotransformation.

This study focused on six environmentally relevant polycyclic synthetic musks (PSMs) as a class of PPCPs and as potential lipophilic, low solubility model compounds. The group of PSMs were selected due to: their prevalence in municipal sewage at measurable concentrations; their current environmental classification as emerging contaminants of concern and the availability of a published analytical methods for analysis of PSMs in solid and aqueous matrices.

The approach was to evaluate, the sludge floc properties, equilibrium sorption-desorption behaviour and compare these sludge factors to the overall partitioning to the aqueous and solids of selected PSM. This was accomplished using bench-scale SBRs, with municipal sewage feed, operated under a judicious selection of SRT and T operating conditions.

1.2. Research Goals and Objectives

The central hypothesis investigated was as follows:

The operating conditions of solids retention time (SRT) and temperature (T) change key sludge floc properties sufficiently to affect the activated sludge's capacity to sorb or enmesh PSMs. The key sludge floc properties include: sludge surface charge (SC), relative hydrophobicity (RH), extracellular polymeric substances (EPS) and constituents, sludge volume index (SVI), mean particle size, size distribution, excess density and porosity.

The specific objectives consisted of investigating the following:

1. The effect of SRT and T on selected sludge floc properties.
2. The correlation of the removal of selected synthetic musks to sludge floc properties grown at different SRTs and Ts.
3. The competitive equilibrium adsorption-desorption behaviour of selected PSMs to sludge at two different SRTs.

CHAPTER II

LITERATURE REVIEW

The purpose of this review was to consider the suspended growth activated sludge (AS) treatment process, to assess the current knowledge about the interrelationships between sludge floc properties and AS treatment process operating conditions and review the current understanding related to the removal of environmentally significant microcontaminants and particularly polycyclic synthetic musks (PSMs) as potential model microcontaminants.

2.1. The Suspended Growth Activated Sludge Process

The suspended growth activated sludge (AS) process provides the conditions for flocculating consortia of microorganism to grow and flocculate, followed by a separation and recycle step with the discharge of the treated effluent to the environment. Some of the common floc forming microbial types and relative concentrations with respect to food availability and solids retention time (SRT) are shown in Figure 2-1.

The SRT is arguably the most important operating parameter and represents the average time that microorganism stay in the biological reactor (typically the aeration tank), defined in Equation 2-1 and discussed further in section 2.1.1.

$$SRT = \frac{\text{Biomass in Reactor (g)}}{\text{Biomasss Wasted (g/d)}} = \frac{V \cdot X}{Q_W X} \qquad (2\text{-}1)$$

where SRT = the average solids retention time (d); V = the total volume of the bioreactor (L); X = the biomass in the bioreactor at the time of wasting (g/L); Q_W = the volume of biomass wasted per day (L/d) (Metcalfe and Eddy, 2003).

Bacteria are the most abundant microorganisms in AS and reproduce through binary fission on average about every 30 minutes under ideal conditions. Heterotrophs utilize organic compounds

for cell synthesis and autotrophs, utilize inorganic carbon such as CO_2. In combination they form part of consortia. The typical chemical composition of prokaryotic cells are 75% water, 23% organic and 2% inorganic matter. The typical macromolecular composition is 55% proteins, 7% carbohydrates, 10% lipids, 3% DNA and 20% RNA (Rittmann and McCarty, 2001).

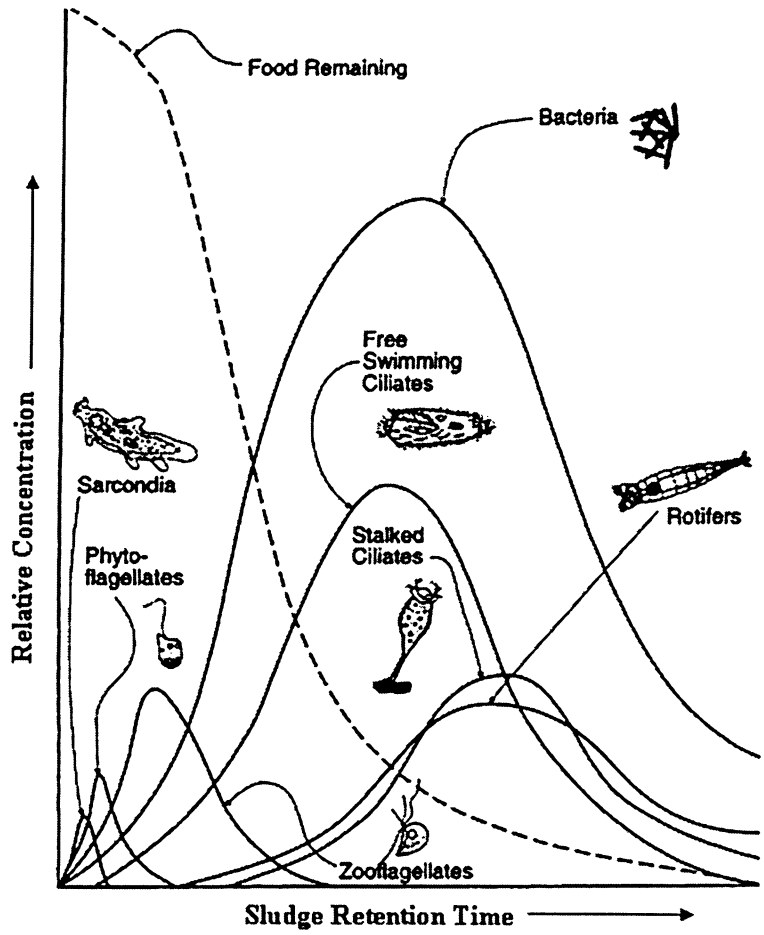


Figure 2-1. Predominance of microorganisms typically found under aerobic conditions as a function of solids retention time (SRT) (adapted from Balacko *et al.*, 1994).

The conventional configuration of the AS treatment process is shown in Figure 2-2. Separate tanks are used for the aeration and the clarification processes. The aeration reactor is designed to provide well mixed and oxygenated zones to maintain the AS flocs in suspension and biologically active. The clarifier provides quiescent conditions for AS flocs to settle and concentrate into AS.

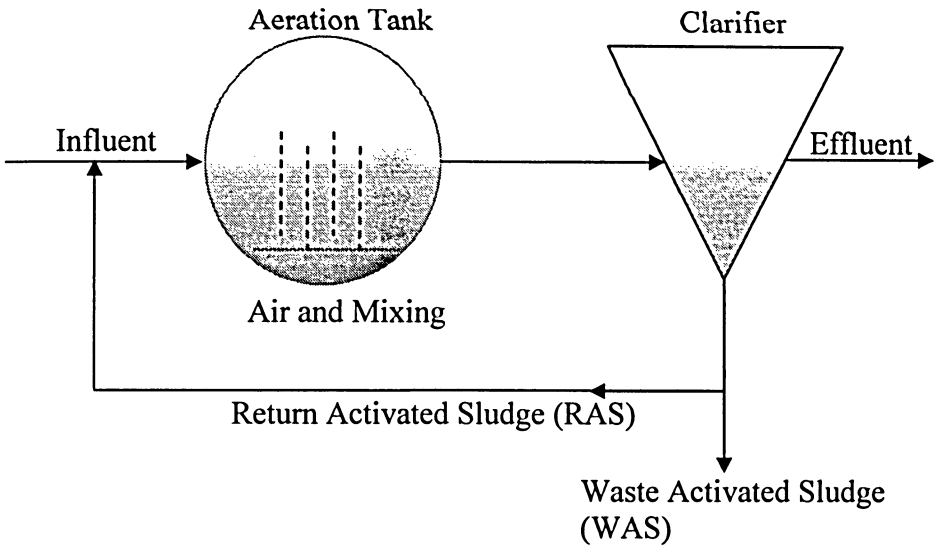


Figure 2-2. Suspended growth activated sludge treatment process schematic (adapted from Metcalfe and Eddy, 2003).

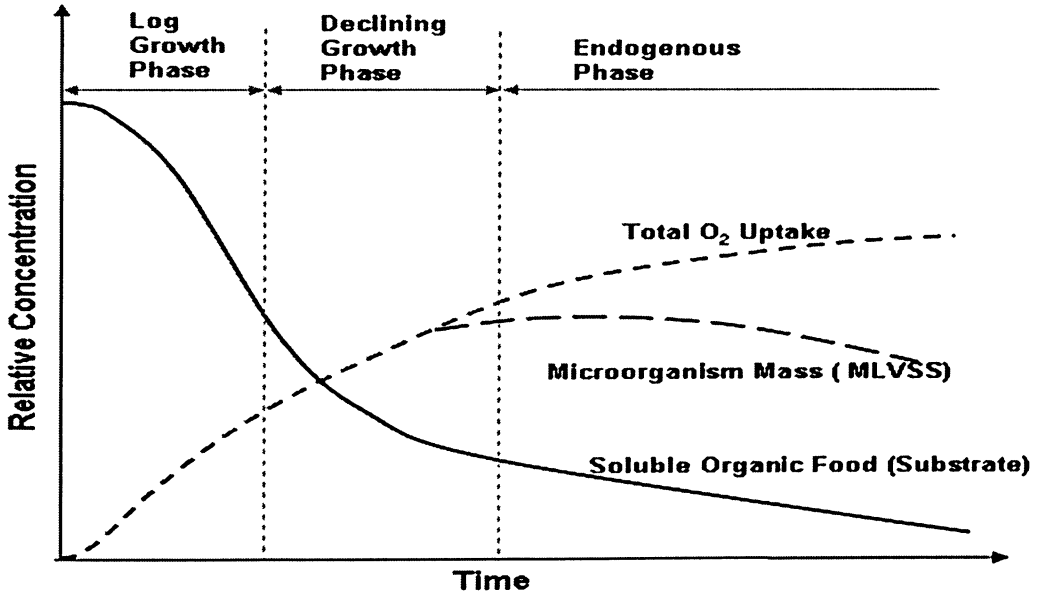


Figure 2- 3. Typical growth curve phases, the substrate uptake, microorganism growth and corresponding oxygen uptake taking place in the aeration tank (adapted from Balacko *et al.*, 1994).

Part of the AS is removed from the system (wasted) and part of it is recycled back to the aeration tank. The recycled activated sludge (RAS) acts to reseed and maintain a sufficient biomass concentration in accordance to a design food to microorganism ratio (F/M). The wasted AS is normally further treated (typically by anaerobic or aerobic biological reactors), dewatered and further processed for land utilization or disposed at municipal landfill sites.

The microorganisms that form AS combine in consortia to form flocs which operate as a complex microenvironment that extract organic matter and nutrients needed for their growth and survival and also act as an efficient sorbing media for other waste products found in sewage (Liss *et al.*, 1996; Droppo *et al.*, 1997).

The heart of the suspended activated sludge treatment process is the suspended biomass or microbial sludge flocs in the aeration tanks. A key property of the sludge flocs is their sedimentary microbial physiology or settling ability, in quiescent conditions. The ability of flocs to settle under quiescent conditions is related to the ability of microflocs to aggregate into macroflocs ("flocs") that settle under the force of gravity against the buoyant force within well designed clarifiers (Bossier *et al.*, 1996). Floc characteristics of particular importance are discussed in subsequent sections.

Good flocculating and settling flocs are critical to ensuring that sewage treatment plants can meet the conventional effluent parameters such as low effluent suspended solids (ESS), five day biochemical oxygen demand (BOD_5), total ammonia-nitrogen (TAN) and total phosphorous (TP). Typical regulatory limits to be met within the Great Lakes River Basin include BOD_5, ESS, TAN, and TP of 25, 25, 5 and 1 mg/L, respectively.

The aeration tank, in the conventional AS process, is the biological or biochemical reactor where air and adequate mixing conditions are provided to keep the biomass (primarily bacteria) in suspension and biologically active by maintaining a minimum dissolved oxygen (DO) level of 2 mg/L. The clarifier part of the AS process is a biomass (activated sludge) separation tank. Quiescent conditions are maintained in the clarifier allowing flocs time to settle out and the clarified effluent to

be discharged. The clarifier also serves the function of wasting and returning a fraction of the activated sludge back to the aeration tank (Metcalfe and Eddy, 2003).

The activated sludge process combines the physical-chemical solids and liquid separation in addition with a biological or biochemical process to reduce colloidal, suspended and dissolved nutrients found in sewage. The key mechanisms involved include biotransformation, volatilization and sorption to the activated sludge (Grady, Daigger and Lim, 1999). The typical operating approach is to optimize the wastage rate which affects the SRT.

The SRT influences the biomass available, the food to microorganism ratio (F/M) and as shown in Figure 2-3, also influences the average biological growth phase and DO uptake rate in which the bioreactor operates. Typically a minimum DO of 2 mg/L is required to prevent anoxic zones during peak loading and summer conditions particularly if nitrification occurs.

2.2. Operational, Process Considerations and Microcontaminants

The key operational and process considerations that need to be considered in the design and operation of suspended growth AS systems include: food quantity (biodegradable organic matter or substrate), DO, temperature (T), hydraulic retention time (HRT), SRT, mixing conditions, quantity of viable biomass (MLVSS), pH, toxicity and trace nutrients (Balacko *et al.*, 1994). An additional important consideration is the mixing conditions that promote a certain floc size distribution, related to shear forces and the proper distribution of organic matter and nutrients available to the biomass.

However, in the activated sludge (AS) treatment process, SRT is recognized as the most important operational parameter with the greatest impact on the process (Metcalfe and Eddy, 2003).

The sewage plant operator can control the SRT by adjusting the wastage rate. The greater the sludge wastage rate the lower the operating SRT. The SRT is also inversely related to the biomass

growth rate (μ) which as with all biological systems is proportional to temperature (T) and affected by environmental conditions in the bioreactor. Other environmental factors such as pH, DO and alkalinity being the same, a doubling of the microbial growth rate (μ) is expected for every 10 degrees increase in temperature. Thus T and SRT are coupled by the growth rate.

The SRT and T are also known to influence the type and amount of biomass present in the reactor (Figure 2-3). Figure 2-4 shows a relationship between nitrifiers and the minimum SRT and corresponding T at which the reduction of ammonia by nitrifiers (nitrification) is expected to occur. Figure 2-4 predicts the onset of nitrification, or the reduction of ammonia, at a minimum 10 and 3 days SRT at 10 and 20 °C, respectively. The coupling of T and SRT is very evident in full scale applications in WWTPs in Ontario where winter nitrification and compliance with total ammonia limits often present a challenge.

The design of the activated sludge treatment process with consideration of the removal of microcontaminants is an emerging issue and until recently all WWTPs did not consider removal of microcontaminants in their design. Generally the removal of microcontaminants in AS WWTPs is a complex process that includes various mechanisms. Some of the major processes include physical enmeshing within the floc structure, adsorption onto the surface of the solids, absorption into the cellular components, volatilization, stripping and biotransformation (Grady *et al.*, 1999).

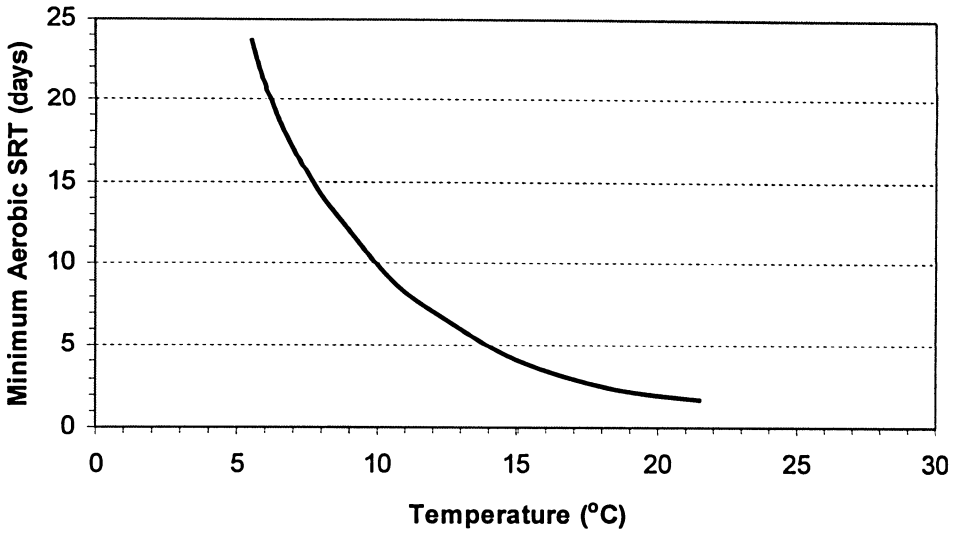


Figure 2-4. Typical expected aerobic SRT required at a given temperature for nitrification to occur (adapted from Melcer *et al.*, 2003).

Sorption is also an important process, considered dependent on SRT and T, as a priori step in the biotransformation of organic matter (substrate) which serves as food for microorganisms. Further the biomass, with its extracellular polymeric substances (EPS), serve to provide important sorption sites for xenobiotic organic chemicals (XOC) or microcontaminants which because of their recalcitrant nature or low concentration would not provide an immediate source of food for microorganisms. Microcontaminants are typically found at the ng/L concentrations and in total form only a small fraction of the available biodegradable substrate in municipal wastewater (sewage).

Another less obvious dependency of the AS process to SRT and T is the need for a sufficient length of time to allow flocculent growth of bacteria. This, as previously mentioned, is necessary so that recycle and wastage of biomass can occur effectively and the typical minimum SRT required to establish flocculent suspended growth is 1 to 3 days depending on the temperature (Metcalfe and Eddy, 2003).

2.3. Activated Sludge Floc Properties

Activated sludge flocs are intricate microstructures and their interactions within the surrounding aqueous matrix is dynamic and affected by the movement of substrate from the bulk solution into the floc interior. The list of sludge floc components and properties of importance that have been studied extensively and have been shown to vary with operational parameters such as SRT, reducing conditions, DO concentration, hydraulic retention time (HRT) and temperature (T) (Voice and Weber, 1983; Bell and Tsezos 1987; Dobbs *et al.*, 1989; Andreadakis *et al.*, 1993; Liss *et al.*, 1996; Droppo *et al.*, 1997; Bura *et al.*, 1998; Finlayson *et al.*, 1998; Wilén *et al.*, 2003a and 2003b) include:

- Extracellular polymeric substances (EPS) content
- EPS composition including protein, uronic acid, carbohydrates and DNA
- Sludge surface charge (SC)
- Sludge hydrophobicity (SH)
- Water content
- Divalent metal cations in the EPS matrix
- Microstructure (internal floc structure, density, porosity)
- Macrostructure (filament index, SVI, size distribution, fractal dimension)
- Floc stability
- Floc ecology
- Sudge organic carbon content

The activated sludge floc structure is considered an active complex microsystem within a composite aqueous matrix predominantly consisting of bacteria, exocellular polymers, multivalent cations, organic and inorganic particles (see Figure 2-5). The activated sludge floc is also considered to function autonomously and interactively between its microenvironment and the bulk phase through physical, chemical and biological interactions (Urbain *et al.*, 1993; Droppo, 2002). These floc structures are formed through dynamic interactions involving EPS originating from either (1) metabolism or lysis of microorganisms liberating proteins, DNA, polysaccharides and lipids and (2) from sewage compents such as cellulose and humic acids (Urbain *et al.*, 1993; Liao, 2000).

Floc components have been differentiated into various physical, chemical and biological dynamic phases that are intended to explain the phenomena that occur within the micro and macro structure of bioflocs shown in Figure 2-6 (Droppo *et al.*, 1997).

Table 2-1 provides some of the key biofloc parameters with the corresponding reported affected properties identifying the complex interelationships associated with biofloc structure, function and operational parameters at activated sludge sewage treatement plants (STP). The parameters and characteristics described in Table 2-1 were determined by various traditional analysis methods and by recently applied molecular analysis methodologies. Some of the important methods which have advanced our undestanding of the form and function of sludge flocs are discussed below.

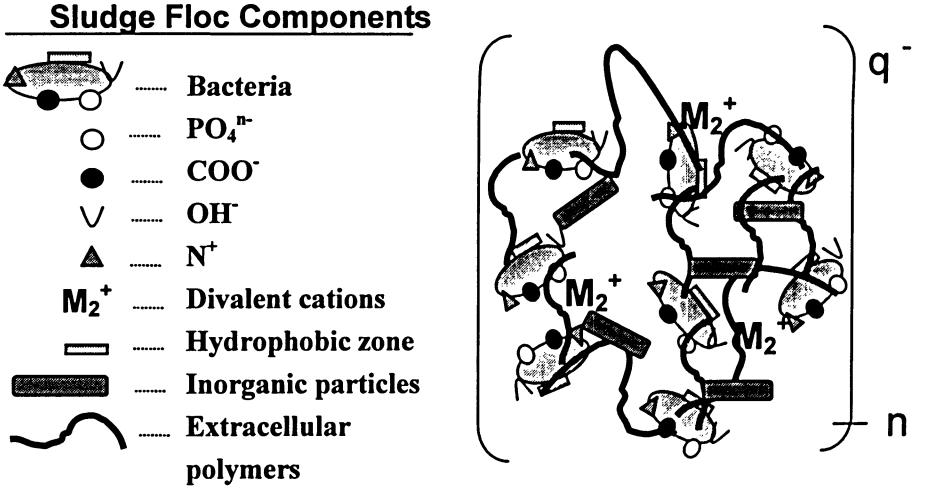


Figure 2-5. Schematic representation of a sludge floc and components within an a hydrated matrix (white space refers to the hydrated zone (adapted from Urbain *et al.*, 1993)

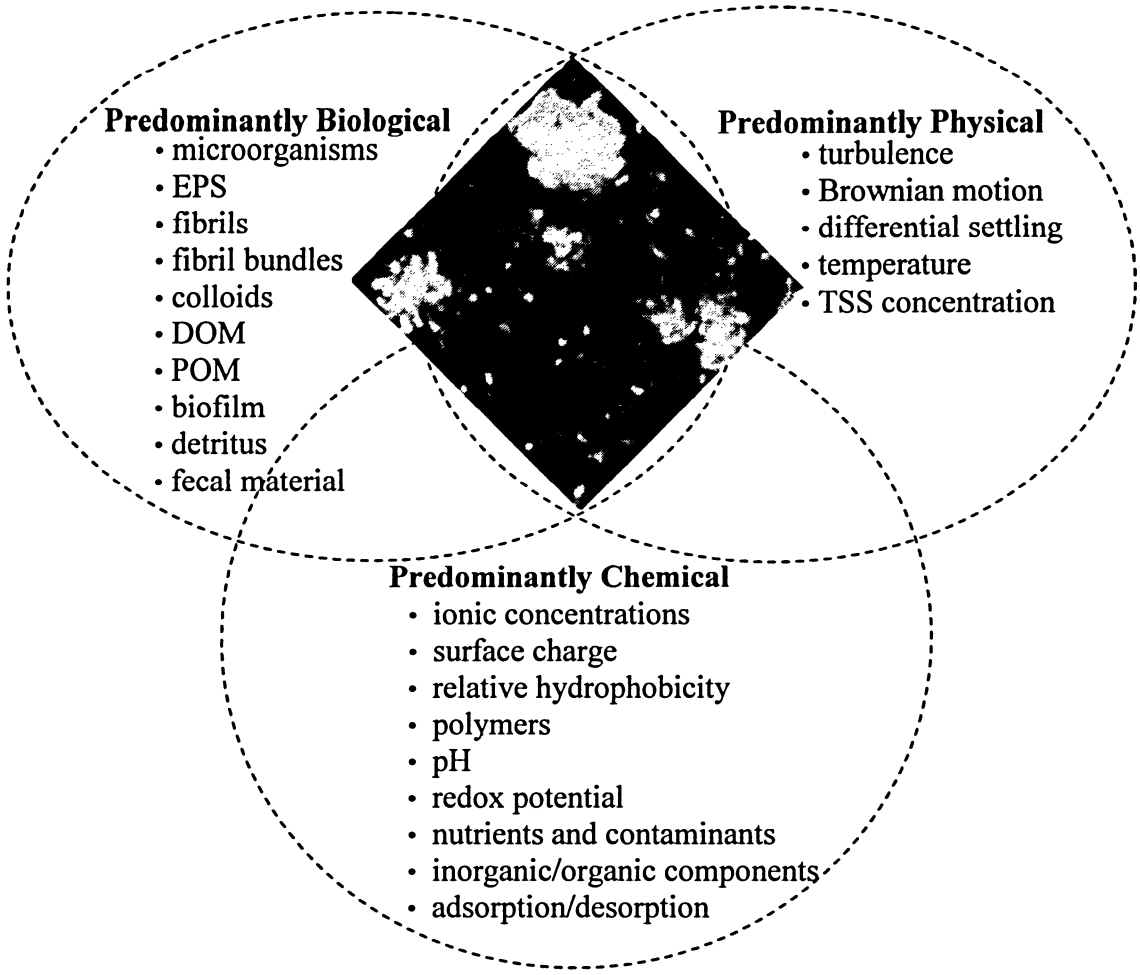


Figure 2-6. Interrelated physical, chemical and biological mediated factors influencing biofloc formation and development in a fresh water environment (adapted from Droppo *et al.*, 1997). This inserted image is a CLSM activated sludge biofloc single slice image.

Table 2- 1. Interrelationships between affected sludge floc properties with associated characteristics and references.

Affected Property	Related Sludge Floc Characteristics Or Operational Parameter	References
Floc stability	• Bimodal size distribution • Fractal dimension (D_f) • Filament index • Internal microstructure • Density • Porosity • Fibrils • Shear and erotional forces • EPS	Raska *et al.*, 2006 Wilén *et al.*, 2003 Mikkelsen *et al.*, 2003 Finlayson *et al.*, 1998 Droppo *et al.*, 1997 Liss *et al.*, 1996 Droppo *et al.*, 1997 Li and Ganczarczyk, 1989, 1990, 1989, 1986, 1987 Parker *et al.*, 1970
Bioflocculation Deflocculation Fragmentation	• EPS composition • Surface Charge • Hydrophobic zones • Divalent cationic bridging (DCB) • EPS polymer bridging • Turbulent sheer • Solids content	Liao *et al.*, 2001 Mikkelsen and Keiding, 2002 Liss *et al.*, 1996 Frølund *et al.*, 1996 Decho, 1996 Bruss *et al.*, 1992 Brown *et al.*, 1979
Settleability SVI Solids flux Zone settling velocity (ZSV)	• Size distribution • EPS content • Settling velocity • Porosity • Density	Jin *et al.*, 2003 Liao *et al.*, 2001 Liss *et al.*, 1996 Barusiński *et al.*, 1995
Relative Hydrophobicity Surface charge EPS	• SRT • Temperature • Biofloc ecology • Ionic compostion of sewage	Jin *et al.*, 2003 Liao *et al.*, 2001 Liss *et al.*, 1996
Compressibility Dewaterability	• EPS content • Bound water • Density • Porosity • Biofloc structure	Jin *et al.*, 2003 Liao *et al.*, 2001 Liss *et al.*, 1996
Flocculating ability	• SVI • Surface charge • Hydrophobicity	Jin *et al.*, 2003 Liao *et al.*, 2001 Biggs *et al.*, 2000 Liss *et al.*, 1996 Jorand *et al.*, 1994
Sludge floc viscosity	• Biofloc microstructure • EPS content • Porosity	Dental *et al.*, 2000 Abu-Orf *et al.*, 1997 Dentel *et al.*, 1997

Biofloc size distributions, density, settling velocity and porosity are generally determined using non-destructive sampling, observation and measurement of bioflocs from sludge samples within a plankton chamber, slides and conventional optical microscopy (COM) imaging analysis down to 2 μ resolution (Droppo *et al.*, 2001). Internal three-dimensional microstructure, fibrillar material, porosity, relative polysaccharide composition, fractal dimensions, internal diffusional distances can be determined using scanning confocal laser microscopy (SCLM), hydrated samples with appropriate use of specific molecular probes and analysis of stacked images using graphical statistical analysis imaging software (e.g. ISA-3D) (Liss *et al.*,1996; Lewandowski *et al.*, 1999). Enumeraton of filamentous organisms within flocs is conducted using a Filament Index in conjunction with standard COM biofloc imaging and analysis. The protocal of Jenkins *et al.* (1985) is typically used. It uses a standardized rated filament index (FI) scale for determining the concentration of filaments which ranges from 1 to 5 (1 refers to low and 5 to high number of filamentous organisms). Internal microstructure, density, porosity are more recently determined by CLSM imaging followed by image analysis.

Biofloc ecology is commonly determined by fluorescence in situ hybridization (FISH) with a judicous selection of molecular probes and stains with accomponying COM imaging and corresponding analysis. EPS extraction can be effectively accomplished by either physical shear methods using cation exchange resins or chelation with ethylenediaminetetraacetate (EDTA). Biochemical analysis for protein, carbohyudrahes, uronic acids, humic substances and DNA are described by Frølund *et al.* (1996). EPS composition such as protein, carbohydrates, uronic acid and DNA content can be determined by various standard biochemical separation techniques (Liss *et al.*, 1996). A recently developed method for EPS analysis, three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy, has revealed the component composition of EPS (Guo-Ping *et al.*, 2006).

Biofloc surface charge determination can be accomplished by colloidal titration methods (Morgan *et al.*, 1990). Stereoscopic microscopy methods with settling columns can be utilized to

determine floc settling velocities, densities and porosity distributions by image analysis using available software such as Northern Exposure™ (Droppo *et al.*, 2001). Sludge volume index (SVI), solid flux analsysis and zone settling velocity (ZSV) are normally measured using standard methods (APHA, 20th Edition, 2004).

Biofloc or sludge apparent viscosity is determined using a rotational viscocity meter. The apparent viscosity is a measure of the internal and external force interactions occurring within the bioflocs. It is a measure of the floc deformation under the influence of stress (Abu-Orf *et al.*, 1997; Dentel *et al.*, 1997; 2000).

It has been reported that when large flocs are exposed to shear and erosional forces, typical of vigorously aerated aeration basins, both erosion and fragmentation take place (Wilém *et al.*, 2003). Impacts on treatment performance related to different mixing modes of activated sludge systems may be associated with floc erosion and fragmentation which impacts the integrity of the floc macrostructure and possibly the microstructure.

Flocculation, the process of aggregation of suspended bacterial cells to form an active aggregate (Brown *et al.*, 1979), allows for physical separation of activated sludge from the secondary liquid stream and its return to the aeration basis. Flocculation plays an essential role in making the activated sludge system a viable treatment method. Bioflocculation is primarily affected by EPS constituents, surface charge (SC) and relative hydrophobicity (RH) whereas EPS content is a more important factor influencing sludge settleability (Liao *et al.*, 2001).

The floc size distribution has been reported to follow a logarithmic distribution and be strongly influenced by organic loading. Bioflocs larger than 50 μm constitute the main source of the surface area and volume of the activated sludge (Barbusiński *et al.*, 1994).

In summary the role of activated sludge floc EPS composition, hydrophobicity, surface charge and morphology, revealed by biochemical, microscopic and physical experimental methods, identified above, will need to be considered to better understand the fundamental influence of floc properties in the fate of microcontaminants through the activated sludge treatment process.

2.3.1 Extracellular Polymeric Substances (EPS)

A major component of the sludge floc is the hydrated polymeric substances consisting primarily of proteins, carbohydrates, acidic polysaccharides and DNA, which combine to form a matrix in which the microbial consortia are embedded (see Figure 2-5 and inset to Figure 2-6). The exocellular or extracellular hydrated polymeric matrix is referred to as extracellular polymeric substances (EPS) and originates from excretion and lysis of microorganisms and from wastewater components (Liss *et al.*, 1996; Guellil *et al.*, 1998; Jorand *et al.*, 1998).

The EPS has both hydrophobic and hydrophilic components. The hydrophilic parts are polar or charged while the hydrophobic components are non-polar. The presence of a bound water shell layer near the floc surface, mediated by functional groups on EPS components, are considered responsible for the hydrophobic/hydrophilic surface interactions. These surface interactions have been shown to play an important role in promoting agglomeration of hydrophobic cells and the dispersion of hydrophilic cells (Urbain et. al, 1993; Jorand *et al.*, 1994 and 1998; Liao, 2000). The EPS are typically characterized in terms of their hydrophobic/hydrophilic properties by sorption onto cationic resins (Jorand *et al.*, 1998).

The EPS matrix forms a fine porous structure, revealed by microscopic analysis (see CLSM of typical floc from SBRs, Figure 2-7), which influences the mass transport of dissolved molecules and ions within flocs and biofilms (Raszka, *et al.*, 2006). The micro and macro pores forming microchannels have fractal characteristics (pores within pores) and might be of great importance to floc behaviour. The floc structure and behaviour includes the floc density, settling characteristics, diffusional gradients, advective transport of water with

contaminants and microcontaminants, advection induced biotransformations and advective transport of floc building components (Droppo, 2001).

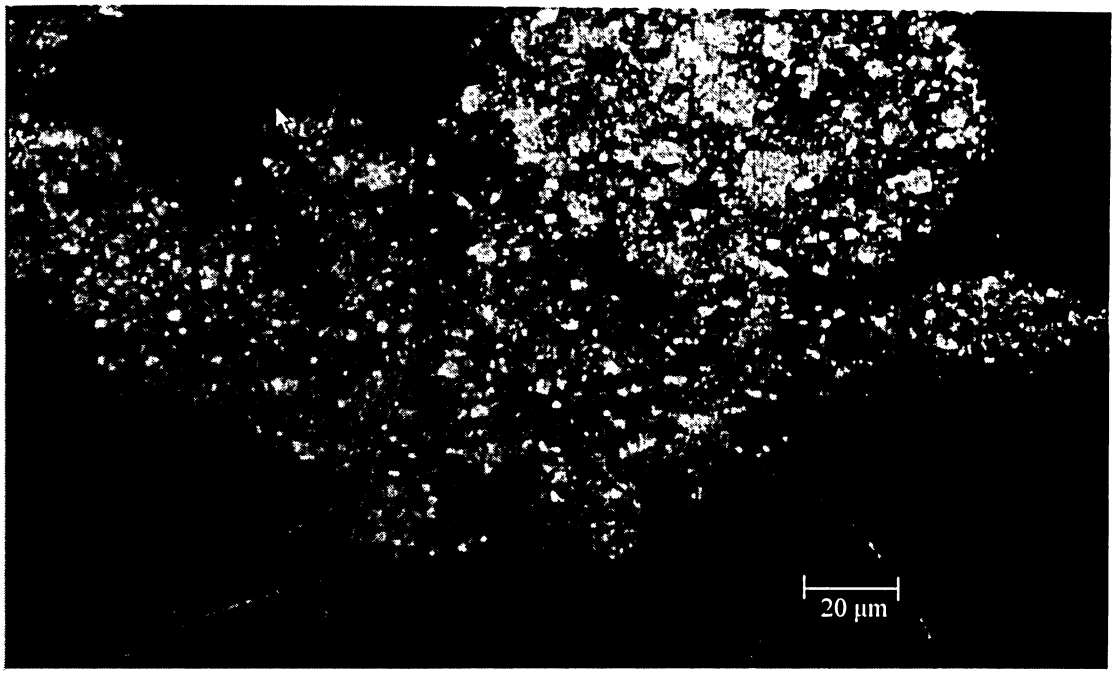


Figure 2-7. Micrograph in SCLM reflectance mode (projection depth 50 μm) showing the typical porous AS floc structure from the SBR during stable operating conditions (63x/0.9 W objective, scale bar approximately 20 μm; Ryerson Biotechnology Laboratory).

2.3.2 Surface Charge (SC)

Activated sludge flocs typically have a negative surface charge under neutral pH conditions. The negative charge is associated with the ionization of functional groups including carboxylic, sulphate and phosphate groups which are part of the EPS polymer constituents (Wilén *et al.*, 2003a and 2003b). The net negative SC is expected to increase with increasing concentration of EPS and be less negative with a higher relative hydrophobicity. The SC has been reported, when using synthetic feed, to have a weak to moderate significant correlation to: proteins, proteins to carbohydrate ratio and be inversely correlated to total carbohydrates (Liao, 200). In depth studies by Wilén, *et al.* (2003a and 2003b) using municipal sewage sources has shown a significant SC positive correlation to EPS concentration, proteins, humic substances and carbohydrates in the EPS. Reported difference in

correlation to carbohydrates and EPS, suggest differences associated with the wastewater type that influence the EPS composition.

The typical surface charge in municipal AS systems is in the range of -0.3 to -0.6 milliequivalents per gram of MLSS (meq/g MLSS) (Jin *et al.*, 2003). The floc SC is also known to be affected by pH and SRT. The AS floc isoelectric point (i.e., zero surface charge) has been reported to be at a pH of about 2.6 and the surface charge was found to vary between -0.25 to -0.45 meq/g VSS or -0.4 to -0.6 meq/g MLSS based on a VSS/MLSS ratio of 0.7 (typical of AS) (Liao, 2000).

A more negative SC on floc has been attributed to lower floc strength and poorer flocculating properties, due to increased repulsive surface interactions (Wilén *et al.*, 2003). A more negative SC has also been attributed to the conjugation with divalent metals, exopolymeric bridging and floc stability, by many references in Raszka *et al.*, (2006). Activated sludge SC is a significant floc parameter affecting the behaviour of the AS process however no direct correlation to partitioning of microcontaminants has been suggested in the referenced literature.

2.3.3 Hydrophobic properties

EPS components contain both hydrophobic and hydrophilic components with EPS-carbohydrates reported to predominantly contribute to the hydrophilic nature and proteins, humic acids and uronic acid in the EPS, primarily contributing to the relative hydrophobicity (RH). The RH was found to have a moderate negative correlation to EPS-protein and EPS-carbohydrates and a weak positive correlation to EPS-uronic acids (Wilén, *et al.*, 2003a). The EPS-protein association to RH was supported by the work of Jorand *et al.*, (1998) who found that the hydrophobic fraction of EPS was made up of only proteins (Liao, 2000).

Hydrophobic and electrostatic interactions along with polymeric entanglement are reported to promote more stable and cohesive floc structures. An increase in RH is considered to improve the flocculating ability and to be important in floc formation (Urbain *et al.*, 1993; Jorand *et al.*, 1994 and 1998). Sludge floc hydrophobicity has been reported to play an important role in bioflocculation and

floc formation with a typical reported relative hydrophobicity of AS floc in the range of 48 to 70% (Jin *et al.*, 2003). No direct specific association with microcontaminants removal or AS floc RH has been found in this literature review.

2.4. Microcontaminants of Concern

The investigations of microcontaminants of concern in our aquatic environments have identified a trail back to municipal wastewater treatment plants (WWTP) as the primary point sources. A primary non-point source of microcontaminants of concern also includes runoff from rural farmlands that receive sewage sludges and/or manures as soil amendments.

Recent WWTP survey work in Canada (Lee *et al.* 2003; Stevens *et al.* 2003; Yang and Metcalfe 2006; Lishman *et al.* 2006; Smyth *et al.* 2007 (in print); Europe (Gatermann *et al.* 1998; Rimkus 1999; Artola-Garicano *et al.* 2003; Kanda *et al.* 2003; Carballa *et al.* 2004; Joss *et al.* 2005) and United States (Simovich *et al.* 2000; Simovich *et al.* 2002; Difranchesco *et al.* 2004) have identified specific classes of compounds as environmental microcontaminants of concern. These contaminants include priority metals, pesticides, dioxins and furans, active ingredients in pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds (EDCs) and additional organic micropollutants.

Synthetic musks although not universally included in priority lists have been identified as contaminants of concern in Europe and are under extensive active research in North America. Table 1 provides a list of contaminants of concern or currently under active investigation (synthetic musks fall under this category).

2.4.1. Current Initiatives

The European Foundation for Applied Water Research, STOWA, in their recent exploratory report (STOWA 2005), identified a list of priority substances (see Table 1) and examined the need for a quaternary level of treatment to reduce contaminants in WWTPs effluents and achieve "a good

chemical status" for certain "relevant water basins" by 2021 based on the receiving water body use (STOWA 2005). The above initiative is based on the European Water Framework Directive (WFD) which became effective in December 2000. A recent and significant addition to the STOWA priority substances of concern are selected pharmaceuticals and EDCs.

A recent voluntary ban of the use of nitro musks in Europe has significantly reduced their environmental loading to the aquatic environment (Rimkus *et al.* 1999). However no ban on the use of nitro musks currently exists in North America based on survey results although the major use of synthetic musks is the polycyclic musks (Yang and Metcalfe, 2004; Lishman *et al.*, 2006; Smyth *et al.*, 2007 (in print). While bans and limitations on the use of synthetic musks and particularly nitro musks have not been legislated in North America, the Canadian Environmental Protection Act (CEPA) dictates the need for an evaluation of emerging contaminants that have demonstrated persistence, bioaccumulation, toxicity to the aquatic environment and are of an anthropogenic origin.

Environment Canada and the Ontario Ministry of the Environment have identified twenty-six Tier I/II priority contaminants (see Table 2-2) and fostered the Canada-Ontario Agreement for Inland Water (COA) whose goal is the virtual elimination of these substances.

Table 2-2. Canada Ontario Agreement for Inland Waters (COA) list of Tier I/II, Synthetic Musks and STOWA priority substances of concern

COA Tier I Substances	STOWA Priority Substances	
Aldrin	**Nutrients**	**Pesticides**
Benzo(a)pyrene	Total phosphorous	Dibutyltin compounds
Chlordane	Total nitrogen	Tributyltin compounds (TBT)
DDT and metabolites	**Biological Parameters**	Hexachlorocyclohexame / HCH / Lindane
Hexachlorobenzene	Intestinal enterococci	Pentachlorophenol (PCP)
Mercury	Escherichia coli	DRINS
Mirex (dechlorane)	Viruses	Simazine
Total PCBs	**Organic Micropollutants**	Atrazine
PCDDs (chlorinated dioxins)	4-chloro-anilin	Dichloroprop
PCDFs (chlorinated furans)	Octylphenols	MCPA
COA Tier II Substances	Nonyphenols	Mecoprop (MCPP)
Anthracene	Bis(2-eheylexyl)phthalate) (DEHP)	Diuron
Cadmium	Benzene	Chlorotoluron
1,4-Dichlorobenzene	Benzo-a-pyrene	Isoproturon
Lindane (γ-hexachlorocyclohexane)	Fluoranthene	Chlorpyrifos
Pentachlorophenol	Benzo-b-fluoranthene	Dimethoaat
Benzo(a)anthracene	Benzo-k-fluoranthene	Chlorfevinphos
7,12-Dimethylbenz(a)anthracene	Benzo-(g,h,i)perylene	Dichloorvos
Benzo(b)fluoranthene	Indeno(1,2,3-cd)pyrene	Bentazon
Benzo(g,h,i)perylene	Anthracene	Pyrazon / choridazon
Benzo(j)fluoranthene	Naphthalene	Trifluraline
Benzo(k)fluoranthene	Dichloromethane	Alachlor
Fluoranthene	Trichloromethane	Endosulfan
Indeno(1,2,3-c,d)pyrene	Tetrachloromethane	**Priority Metals and Others**
Perylene	1,2-dichloromethane	Arsene
Phenanthrene	Trichloroethene	Cadmium
Pyrene	Tetrachloroethene	Chromium
Synthetic Musks and Byproducts	Hexachlorobutadiene	Lead
Galaxolide (HHTN)	C10-13-chloroalkanes	Mercury
Tonalide (AHTN)	Trichloroethene	Nickel
Traseolide (ATII)	Hexachlorobenzene	Copper
Celestolide (ADBI)	PCB-28	Zinc
Cashmeran (DPMI)	PCB-52	**Hormone disrupters & Pharmaceuticals**
Phantolide (AHMI)	PCB-101	
Versalide (AETT)	PCB-118	17 α-ethinyloestradiol
Musk ketone (MK)	PCB-138	Biphenol A
Musk moskene (MM)	PCB-153	Oestrone
Musk ambrette (MA)	PCB-180	Ibuprofen
Musk xylene (MX)	Brominated diphenyletethers (BDPEs)	Anhydro-erythromycine
Musk tibetene (MT)		Slfamethoxazol
Amino musk ketone (2-AMK)		Carbamazepine
4-Amino musk xylene (4-AMX)		Sotalol
2-Amino musk xylene (2-AMX)		Amidotrizoic acid
Musk R1		
Musk T		
Natural Ambrette		

A large part of the current research initiatives under COA focus on the quantification of the Tier I/II contaminants, synthetic musks and the investigation of existing wastewater treatment methods to reduce the priority contaminant loadings to receiving water bodies.

It is expected that the review and analysis of the results from these and other similar studies will form the basis for future environmental risk assessments, policies or guidelines to control or minimize the discharge of priority micro contaminants from WWTPs currently approved under by the Ontario Ministry of the Environment in Ontario and national guidelines promoted through the work of Environment Canada.

2.5. Synthetic Musks in the Environment

Synthetic musks are compounds with a typical musky scent and because of their aromatic property are used extensively by the fragrance industry and are found in commercial personal care products such as cosmetics, detergents, cleansers, fabric softeners and other household products. World wide approximately 6 to 8 thousand metric tons of synthetic musks are produced annually (EHP, 2005; Rimkus, 1999; Draisci *et al.* 1998).

The synthetic musks are grouped into three major groups with similar aromatic properties but significantly different chemical structure: nitro musks, polycyclic musks and macrocyclic musks. Up to 95% of the musk market production being the polycyclic synthetic musks Galaxolide® (HHCB) and Tonalide® (AHTN) and the nitromusks (NMs) musk xylene (MX) and musk ketone (MK) (OSPAR 2004). An example of a macrocyclic musk is Thibetolide Muscone (see Table 2-1).

The synthetic musks form a subgroup of pharmaceutical and personal care products (PPCPs), and are easier to analyse for than certain pharmaceuticals ecause they are found in the high ng/L or µg/g concentration levels in municipal wastewater and municipal sludges, respectively. For this reason have been suggested as good model class of compounds for the purpose of risk assessment (Daughton and Ternes 1999). These PPCPs have been suspected of environmental aquatic sub-

chronic toxicity and endocrine disruption effects to aquatic organisms at relevant environmental concentrations (Daughtens and Terns, 1999; Bitsch *et al.*, 2002) .

The current literature on the fate and effect of PPCPs in the environment is not conclusive on environmental aquatic effects on nontarget species is considered fragmented (Daughton and Ternes, 1999) due to non-standard approaches in the sampling and analysis of these trace contaminants through the sewage treatment process (Yee *et al.* 2005). Recent significant initiatives by the European Union (STOWA), US EPA, Ontario Ministry of the Environment, Environment Canada and associated partners (Yang and Metcalfe, 2004; Lishman *et al.*, 2006; Smyth *et al.*, 2006 (in print)) have improved our understanding of the fate and treatability of selected PPCPs at full scale activated sludge WWTPs. Despite these initiatives, knowledge gaps in terms of viable monitoring strategies, removal mechanisms and effective treatment methods still exist (Strenn *et al.*, 2005).

The physicochemical musk parameters considered key to determining their ambient environmental fate are the octanol-water partition coefficient (K_{OW}), the water solubility (S_W), Henry's Law constant (H) and vapour pressure (P_L). Figures 2-1 and 2-2 provide a temperature distribution of octanol-water partition coefficient (K_{OW}) and water solubitily (S_W) for the musks of concern as a function of temperature. For the polycyclic musk of concern the Log K_{OW} range is 5 to 7 (Paasivirta *et al.*, 2002). These values suggest a high lipophilic tendency (Sawyer, McCarty and Parkin, 2003). The S_W range from 20 to 600 µg/L (Figure 2.7 (B)) suggests generally a low water solubility but a large range. The water solubility and Kow are often correlated to bioavailability for biotransformation and sorption, a step preceding biotransformation.

Henry's Law constant (H) represents the equlibrium partitioning between water and the atmosphere and for the polycyclyc musks is relatively constant at 0.0002 to 0.0003 atm/(m^3· mole). In general if H is less than 0.01 atm/(m^3· mole) the compound will not be sufficiently removed from water by air stripping in an engineered reactor (Sawyer, McCarry and Parkin, 2003). H is strongly dependant on temperature as shown in Figure 2-8 (A).

The vapour pressure constant (P_L) in conjunction with H, gives a direct measure of the compound's tendency to volatilize. The higher the P_L the greater the tendency for the compound to volatilize. The polycyclic synthetic musks (PSMs) have a very low vapour pressures and therefore a low tendency to volatilize. Similar to S_W and H, P_L is strongly temperature dependent (see Figure 2-8 (B)).

In trying to understand the fate of PSMs through the activated sludge process the S_W , K_{OW} and H are the most important physicochemcal properties that need to be considered under normal environmental conditions. The P_L and H of the PSMs under consideration suggest that volatilization or stripping, during the suspended growth activated sludge process, would play a minor role.

The temperature comparison at 10 and 20 °C of S_W, log Kow and H is provided in Table 2-3 (values taken from Figures 2-8 and 2-9). The comparison shows that Kow does not change from 10 to 20 °C and that S_W and H increase with T by a factor of 1.1 up to 1.7.

Table 2-3. Comparison of S_W, log K_{OW} and H at 10 and 20 °C of the PSMs investigated[1]

Polycyclic Synthetic Musks	Water Solubility Sw (µg/L)			Log Octanol-water Partition Coefficient (Log Kow)			Henry's Constant (dimensionless) (H)		
	10 °C	20 °C	Sw (20 °C) / Sw (10 °C)	10 °C	20 °C	Kow (20 °C) / Kow (10 °C)	10 °C	20 °C	H (20 °C) / H (10 °C)
Cashmeran	114	175	1.5	4.902	4.902	1.0	0.004	0.003	1.4
Celestolide	10	15	1.5	6.636	6.637	1.0	0.074	0.058	1.3
Phantolide	20	26	1.3	6.676	6.676	1.0	0.027	0.018	1.5
Traseolide	74	85	1.1	8.116	8.116	1.0	0.035	0.020	1.7
Galaxolide	128	168	1.3	7.271	7.272	1.0	0.018	0.013	1.4
Tonalide	463	632	1.4	7.278	7.278	1.0	0.409	0.278	1.5

1. Temperature dependent physical-chemical characteristics adapted from Paasivirta *et al.* (2002).

Table 2-4. Environmentally imporatant synthetic nitromusks, polycyclic musks and an example of a macrocyclic musk (not enviornmentally important) with estimated or measured properties at 20 °C[1]

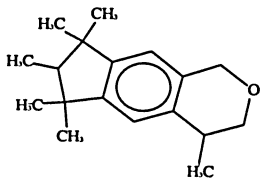
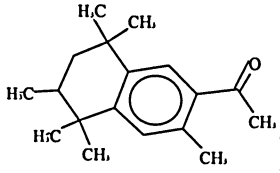
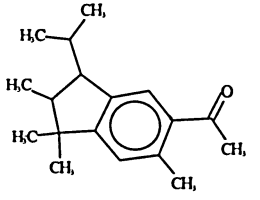
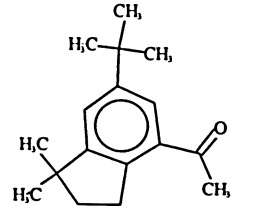
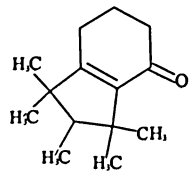
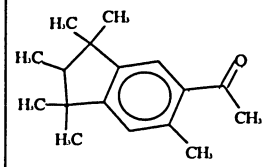
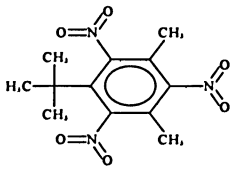
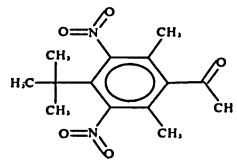
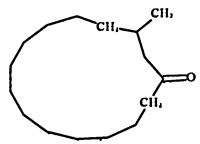
Trade name, CSA number, CSA name, Molecular Weight and Formula	Chemical Structure	LogK_{OW}	LogK_{OC}	LogP_{BW}	H (atm-m^3/mole)	H/K_{OW}	S_W (mg/L)	V_P (mm Hg)	14D F LC_{50} (µg/L)	Log*BCF*
· Galaxolide® · 1222-0505 · 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylchlopenta-[g-2benzopyran (HHCB) · 258.4 · $C_{18}H_{26}O$		7.2 5.9[2] 5.9[3]	4.0 4.86[3]	6.0	0.0003	$7 \cdot 10^{-9}$	0. 1.75[2] 1.75[1]	$9 \cdot 10^{-5}$	70	4.0
· Tonalide® · 1506-02-01 · 1-(5,6,7, 8-tetrahydro-3,5,5, 6,8,8-hexamethyl-2-naphthlenyl-ethanone (AHTN) · 258.4 · $C_{18}H_{26}O$		7.2 5.75[2] 5.7[3]	4.0 4.80[3]	6.0	0.0002	$4 \cdot 10^{-9}$	0.6 1.25[2] 1.25[3]	$6 \cdot 10^{-5}$	60	3.0
· Traseolide® · 68140-48-7 · 1-[2,3-dihydro-1,1,2,6-tetreamethyl-3-(1-methyl-ethyl)-1H-inden-5-y1]-ethanone (ATII) · 258.4 · $C_{18}H_{26}O$		6.6 6.3[2]	4.0	5.6	0.0002	$5 \cdot 10^{-9}$	0.09 0.09[2]	$7 \cdot 10^{-5}$	60	3.3
· Celestolide® · 13171-00-1 · 1-[6-(1,1-dimethyethyl)-2,3-dihydro-1,1-methyl-1H-inden-4-y1]-ethanone (ADBI) · 244.38 · $C_{17}H_{24}O$		6.6 5.4[2]	3.7	5.2	0.0002	$1 \cdot 10^{-8}$	0.02 0.22[2]	$1 \cdot 10^{-4}$	120	3.0

Table 2-4. Environmentally imporatant synthetic nitromusks, polycyclic musks and an example of a macrocyclic musk (not enviornmentally important) with estimated or measured properties[1] **(continued)**

Trade name, CSA number, CSA name, Molecular Weight and Formula	Chemical Structure	LogK_{OW}	LogK_{OC}	LogP_{BW}	H	H/K_{OW}	S_W (mg/L)	V_P (mm Hg)	14 Day Fish LC_{50} (µg/L)	Log*BCF*
· Cashmeran® · 33704-61-9 · 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4H-inden-4-one (DPMI) · 206.32 · $C_{14}H_{22}O$		4.9 4.5[2]	3.0	4.0	0.0002	$2 \cdot 10^{-7}$	0.2	0.004	2000	2.8
· Phantolide® · 153233-35-0 · 1-(2,3-dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl)-ethanone (AHMI) · 244.38 · $C_{17}H_{24}O$		6.7 5.8[2]	3.7	5	0.0002	$1 \cdot 10^{-8}$	0.03	$2 \cdot 10^{-4}$	145	3.0
· Musk Xylene® · 81-15-2 · 1-(1,1-dimethyllethyl)-3,5-dimethyl-2,4,6-trinitro-benzene · 297.27 · $C_{12}H_{15}N_3O_6$		4.5	4.4	3.8	0.00001	$4 \cdot 10^{-10}$	0.53	$6 \cdot 10^{-7}$	2900	2.8
· Musk Ketone® · 81-14-1 · 1-*tert*-butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene · 294.31 · $C_{14}H_{18}N_2O_5$		4.3	3.1	3.6	0.00001	$6 \cdot 10^{-10}$	1.1	$3 \cdot 10^{-7}$	3800	1.8
· Thibetolide Muscone · 41-91-3 · 3-methyl-cyclopentadecanone · 238.42 · $C_{13}H_{30}O$		6.0	3.8	2.3	0.03	$3 \cdot 10^{-8}$	0.2	$5 \cdot 10^{-4}$	114	3.9

1. Physical-chemical properties of the synthetic musks were determined from the US EPA EPI Suite V 3.1.2 (2005) screening level tool and adapted from Paasivirta *et al.* (2002). 2. T. Herber *et. al.*, (2002) 3. Balk *et al.*, (1999).

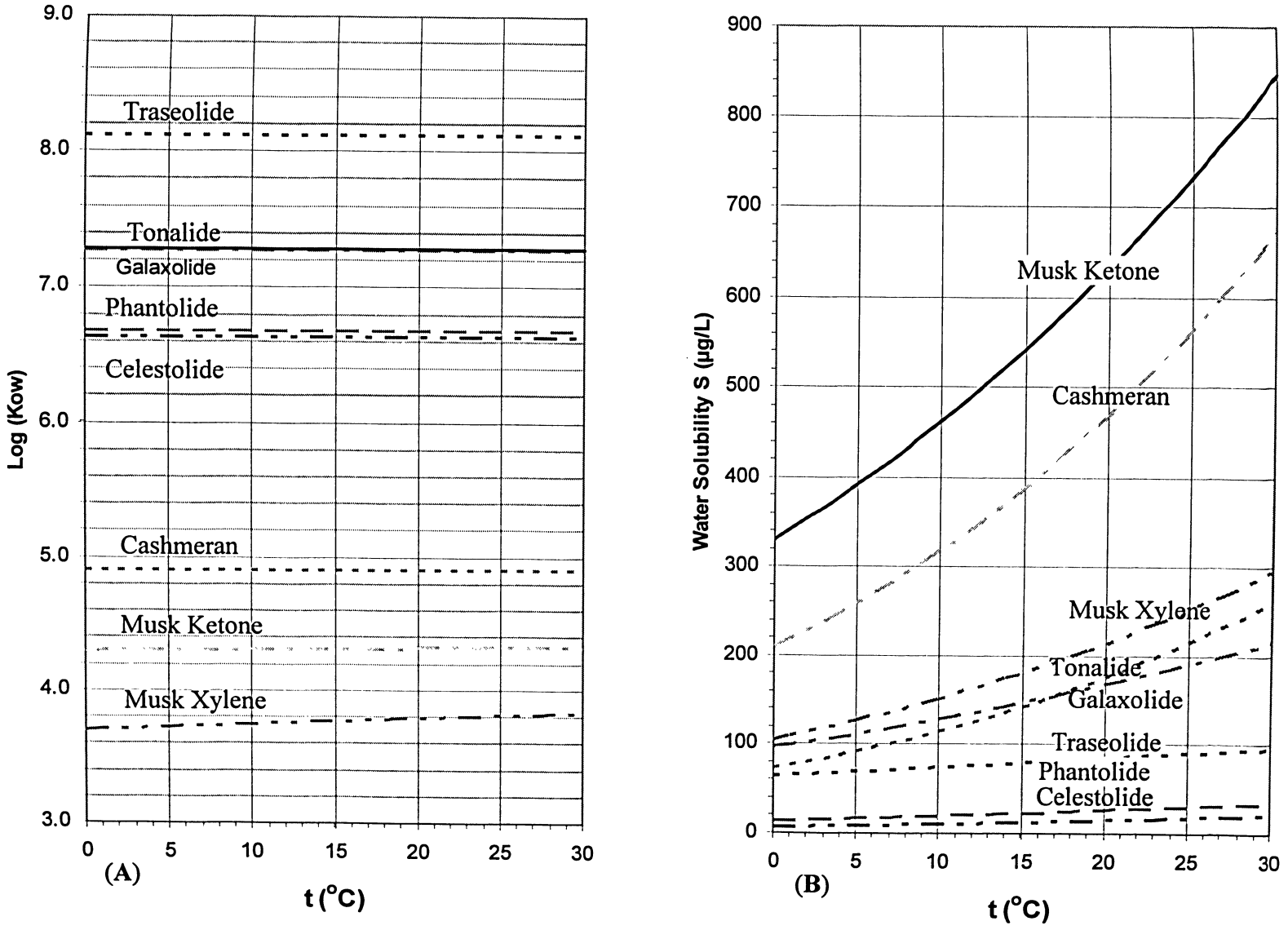


Figure 2-8. Log Kow (A) and water solubility Sw (µg/L) (B) of environmentally significant synthetic musks over an environmental temperature range (adapted from Paasivirta *et al.*, 2002).

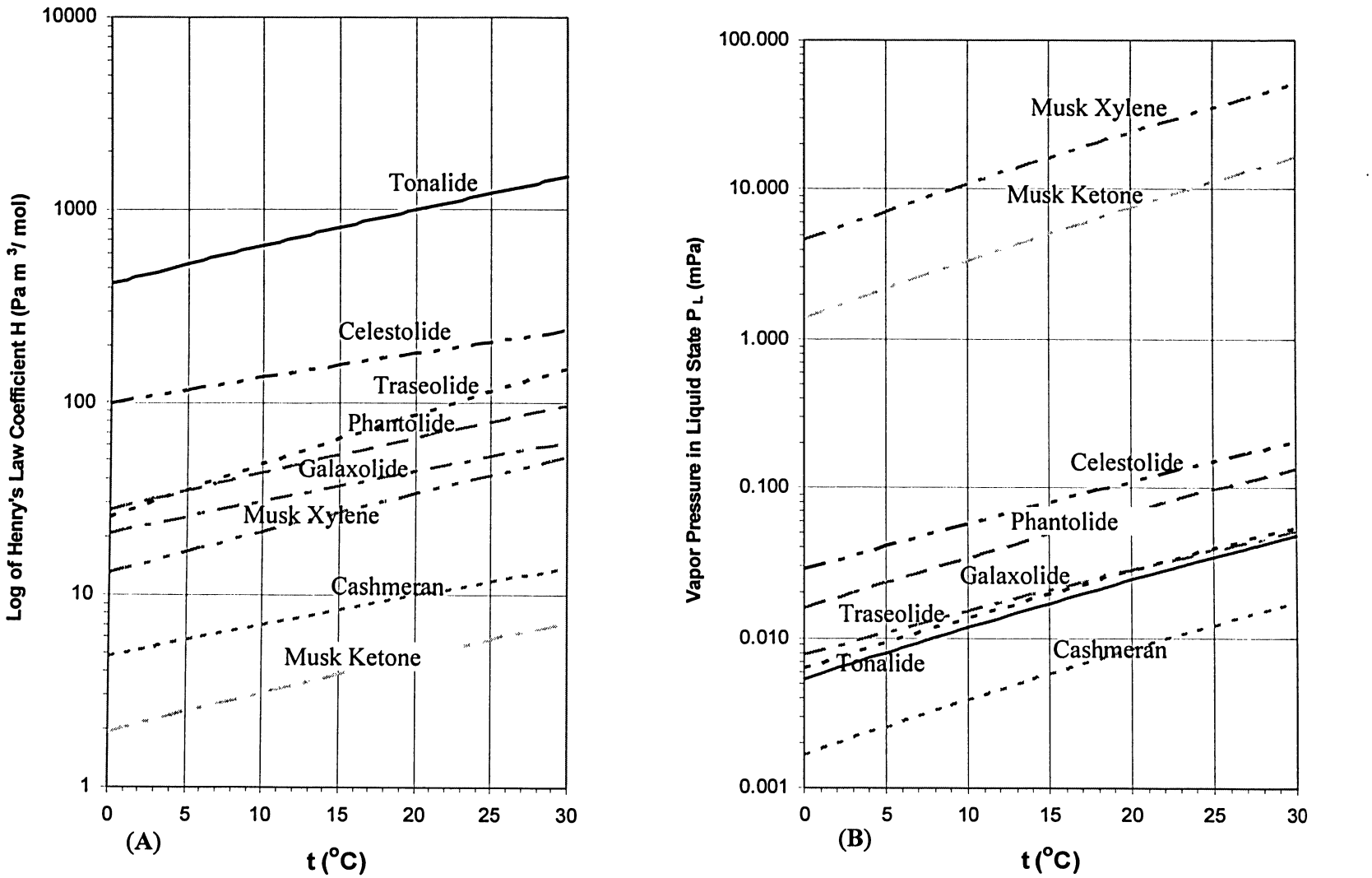


Figure 2-9. Log of Henry's law coefficient H (A) and vapor pressure in the liquid state P_L (B) of selected environmentally significant synthetic musks. (derived from equations in Paasivirta *et al.*, 2002).

2.6. Musks through the Sewage Treatment Process

Polycyclic musks most prevalent species found in municipal sewage are Galaxolide (HHCB) and Tonalide (AHTN). Because of their high volume of use and their lipophilic tendency concerns about their safety provoked investigations on polycyclic synthetic musks. Both HHCB and AHTN are chiral compounds and enantiomeric species of HHCB and ATTN have been found in aquatic organisms. However no correlation was found between lipid levels, enrichment, and enantioselective biotransformation of HHCB or AHTN. It was found that the biotransformation was selective and depended on the compound along with the species involved (Franke *et al.* 1999).

Detailed investigation of parent musks and its main metabolites are scarce. One investigation at a secondary sewage treatment plant has shown that the mean percent removal was between 60% for AHDI and 90% for HHCB resulting in mean effluent concentrations below 860 ng/L however at the same time HHCB-lactone (a major transformation product of HHCB) was observed to increase from 400 ng/L to 900 ng/L. The removal ratios for HHCB, AHDI and particularly ATII was determined and reported to indicate a stereospecific removal process (Berset *et al.* 2004).

The most important removal process of the environmentally relevant polycyclic synthetic musks due to their lipophilic properties (LogK_{OW} values from 5.4 to 8 , Table 2-2) is sorption on activated sludge (Osemwengie and Steinberg, 2001). Biotransformation may also contribute to the removal however it is not clear whether if it is a biologically or abiotically mitigated process (Simonich *et al.* 2002).

The high volume of use of selected synthetic musks, their lipophilic nature, tendency for bioconcentration and the relative ease of analysis has resulted in synthetic musks to being found in many environmental compartments including sewage effluents, sludges, rivers, lakes, oceans, fish, sediments and other biota in the ppb range (Bester *et al.* 1998; Winkler *et al.* 1998; Gatermann *et al.* 1999; Heberer *et al.* 1999; Rimkus 1999; Fromme *et al.* 2001; Dsikowitzky *et al.* 2002). Additionally, transformation products of HHCB and AHTN have been described in biota samples (Franke *et al.*,

1999; Gatermann *et al.*, 2002b) and HHCB-lactone has recently been quantified in sewage sludge (Kupper *et al.*, 2004).

2.6.1. Sorption and desorption process

The fate of many organic priority pollutants have been found to be associated with waste activated sludge solids and sorption has been proposed as the primary mechanism responsible for this phenomena. The final sorbed concentration of contaminants on sludges is regarded as an equilbrium process between sorption and desorption (Bell *et al.*, 1987). However sorption, in activated sluge processes, is a complex phenomena that involves phase partitioning, to the organic matter in the sludge, as well as adsorption onto the lipid fraction of the biomass and absorption into the biomass. It was reported that polynecleur aromatic hydrocarbons (PAHs) can be well described by equilibrium distribution coefficients which relate the solubility of PAHs in the sludge organic fraction to the solubility of the PAH in the aqueous phase (Moretti and Neufeld, 1989). This suggests that exopolymers or EPS may play an important role in the sorption process with the activated sludge system.

Both the Langmuir and Freundlich isotherm models have been used to correlate the equilibrium aqueous and solids concentration of toxic organic compounds in activated sludge and sediments. No single model has been universally adopted however the Freundlich equation has been widely used and it has been found that the empirical isotherm data are generally better described by the Freundlich power experession (Dobbs, Wang and Govind, 1993):

$$C_s^{sor}(eq) = K_F^{sor} \cdot C_{aq}^{sor}(eq)^{1/n} \qquad (2\text{-}2)$$

where: $C_s^{sor}(eq)$ = concentration sorbed onto sludge at sorption equilibrium (μg/g), K_F^{sor} = Freundlich sorption coefficient ($\mu g^{1-1/n} \cdot (mL)^{1/n} \cdot g^{-1}$), $C_{aq}^{sor}(eq)$ = concentration in solution at sorption equilibrium (μg/L) and n = regression constant.

The K_F can be understood as a relative indicator of sorption capacity and *1/n* as the intensity of the sorption reaction (Weber, 1972). The Freundlich model was physically justified, under dilute solute conditions, to Gibbs monolayer coverage of a surface model and its applicability to dilute solutions is generally recommended (Voice and Weber, 1983). The batch equilibrium method (OECD 106, 2000) recommends the use of the Freundlich equation in the modeling of sorption and desorption under dilute equilibrium conditions. When dealing with trace (ng/L) environmental contaminants, the dilute solutions assumption is generally met.

The OECD 106 (2000), equilibrium batch method, also recommends the use of inactivated biomass to elimate the difficulties associated with the accurate measurement of solids in sludge slurries and potential biotransformation interference due to viable biomass. Kördel *et al.*, (1997) have reported that the equilibrium sorptive capacities of live and inactivated activated sludges are similar for various substances.

Knowing the equlibrium adsorption and desorption behaviour of a contaminant can assist researchers in predicting the fate of important environmental contaminants in the natural environment.

2.7. Environmental Fate and Effects of Synthetic Musks

Active ingredients in pharmaceuticals and personal care products (PPCPs) (e.g. synthetic nitro musks and polycyclic musks) are ubiquitous in wastewaters, sludges and the natural aquatic environments of large cities throughout the world at the micro (μg/L) and trace (ng/L) concentration levels. Their prevalence in the natural environment is due to their large production, extensive use by the general population, disposal through municipal wastewater, generally inert and nonbiodegradable physical-chemical properties and associated difficulties with the virtual elimination or removal to below trace levels at typical wastewater treatment plants (WWTPs). The concentrations of PPCPs found in the natural environment are generally the same as some persistant organic pollutants (POPs) including pesticides, polychlorinated byphenyles (PCBs), PAHs, polybrominated diphenyl ethers

(PBDEs) some of which have a signinificant effect on the human endocrine system (act as endocrine disrupting chemicals (EDCs)) and some which act as potent chemosensitizers inhibiting the multixenobiotic resistance (MXR) to aquatic organisms.

Currently even the most sophisticated and advanced WWTPs are not designed to remove PPCPs or POPs from microconcentration levels in raw sewage down to below trace levels in the final effluent or in the biosolids. Typically these microcontaminants are found at concentrations ranging from 10 to 2000 ng/L in sewage and in the range of 1 to 20 μg/g in sewage sludges (Lee *et al.*, 2003; Bester, 2004; Lishman *et al.*, 2006; Yang *et al.*, 2006).

Due to recent published results regarding PPCPs ability to bioconcentrate and bioaccumulate in biota, persistence in aquatic environmets, potential impacts on environmetnal ecology, rise in antibiotic resistence of bacteria and potential impact on source water supplies from sewage effluents, regulators have been actively investigating these emerging issues since the early 1990s (Daughton *et al.*, 1999) with focus on existing WWTP.

The reported removal rates of selected pharmaceuticals and synthetic musks at various WWTPs has been found to be highly variable and removal rates range from negative values to between 50 to 100 percent. Negative removal rates have been attributed to the reconjugation of the deconjugated parent compound at an interstage treatment process between the initial and final sampling points or due to sampling errors (Yang and Metcalfe, 2004; Lishman *et al.*, 2006; Smyth *et al.*, preview of manuscript).

To achieve virtual elimination of selected priority pollutants (see Table 1) from WWTP effluents is the goal of the Canada-Ontario Agreement (Enviornment Canada, COA, 1994). Further to eliminate the risk concerns from selected organic micro contaminants, from sewage effluents entering source water supplies, it has been suggested that engineered quaternary level of treatment is required of the WWTP effluents. An estimated average cost from $\$20/m^3$ to $\$45/m^3$ (in Canadian dollars) for facilities serving from 20 000 to 100 000 population equivalent (P. E.), respectively, has been given

for conditions found in the European Union (STOWA, 2005). These conditions would apply to similar site specific areas in North Amaerica.

The above costs include additional treatment of municipal wastewater effluents (MWWE) by applicable treatment works normally reserved for water treatment which include: (1) coagulation with metal salts, addition of a carbon source, biological floc filtration and activated carbon filtration; (2) addition of a carbon source, biofiltration, addition of metal salts and powdered activated carbon with flocculation/coagulation followed by filtration; and (3) the use of coagulation with metal salts, addition of a carbon source, biological floc filtration and oxidation by a chemical or ultraviolet light.

To date the fate of microcontaminants by chemical class or individual compounds and particularly the mechanisms of removal, through the activated sludge sewage treatment process, is an emerging area of research and thus poorly understood. Some of the analytical challenges are related to the low concentration levels (10 to 1000 ng/L range) and the difficult to analyse sewage and sludge matrix. The analytical methodologies available are only now beginning to be standardized, the cost associated with analysis are high and currently there are no regulations in North America or Europe requiring such monitoring by operators of STPs.

Most of the current research has focused on full scale activated sludge sewage treatment plants (AS STPs) surveys. Although this is a necessary and important first step to quantify microcontaminants, it has made it difficult to arrive at any fundamental conclusions as to the determining factors that influence the fate of microcontaminants. The reason is primarly related to the complexity of AS STPs and due to the lack of adequate experimental controls. It has been well documented that PSMs tend to partition preferentially to biomass, however the conditions that promote this phenomena within the AS treatment process or whether partitioning to biomass is a reversible process, has not been definitively established. Still more controlled fundamental experimental work is required to understand the fate of PSMs though the AS treatment process.

CHAPTER III

EXPERIMENTAL MATERIALS AND METHODS

3.1. Experimental Methodology

A summary schematic of the proposed analysis is provided in Figure 3-1. This schematic includes analysis for conventional performance, musk analysis and sludge floc characterization. The conventional performance is important since it is a method to establish regulatory compliance and environmental impacts related to nutrients, TSS and BOD loadings to receiving water bodies. The polycyclic synthetic musks (PSMs) represent our selected active agents in PPCPs and are current microcontaminants of concern. The floc characterization is divided into surface analysis, general floc internal structural or bulk performance characteristics and microstructure (see Figure 3-1).

The target operating conditions for the sequencing bench reactors are provided in Table 3-1. Differences in operational conditions were expected to result in significant differences in sludge floc properties and conventional performance. It was hypothesised that differences in sludge floc properties would significantly impact the removal or partitioning of the PSMs as our model microcontaminants of concern.

A bench scale SBR system was fed primary effluent generated from municipal sewage and operated at the Wastewater Technology Centre of Environment Canada (EC) in Burlington. The system consisted of two SBRs operated in parallel each operated at different sludge retention time (SRT) and temperature (T) conditions (Table 3.1). Four unique operating conditions were investigated both in terms of conventional performance (e.g. COD, ESS, TAN) and non-conventional WAS characterization (e.g. surface charge, hydrophobicity, EPS). Further six selected polycyclic synthetic musks (PSMs) were analysed for in the settled and screened influent, final effluent and WAS.

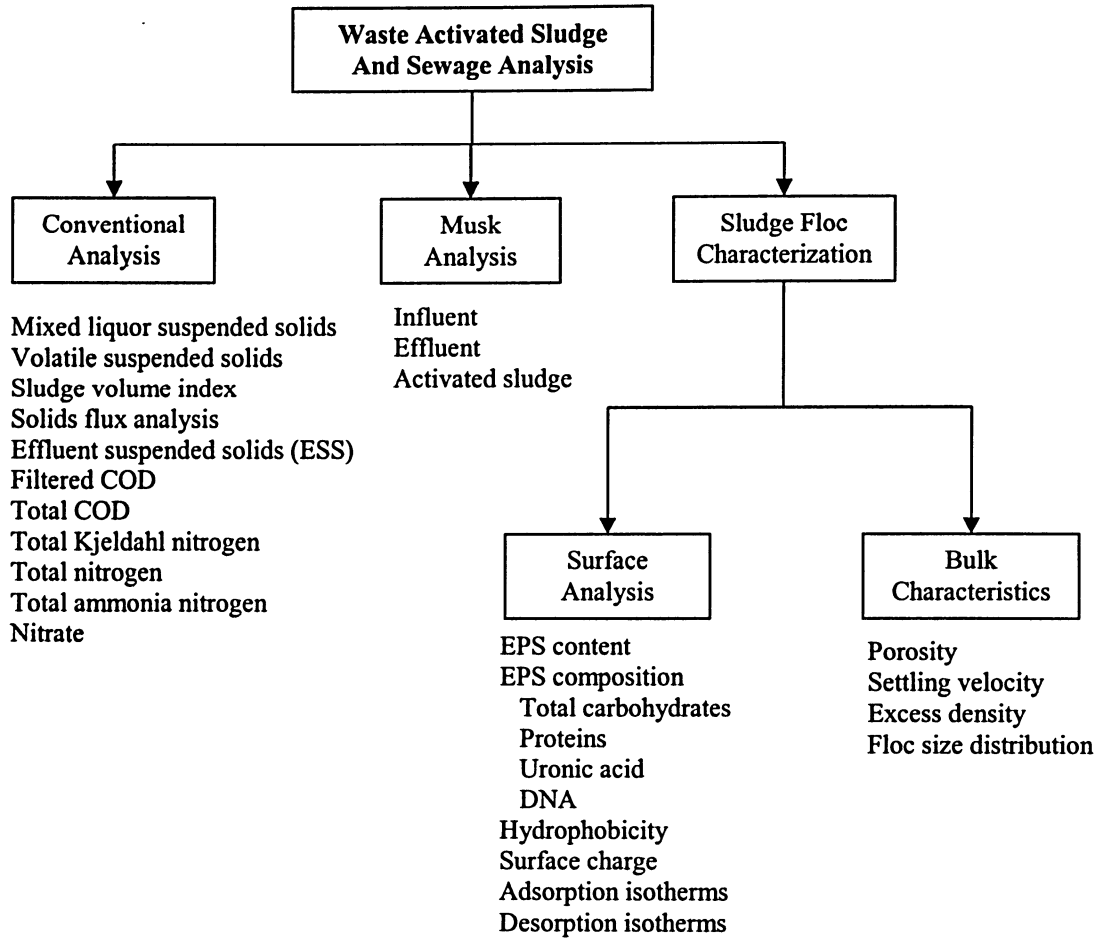


Figure 3-1. Grouping of analyses conducted on sewage and waste activated sludge floc.

Table 3-1. Sequencing batch reactors (SBRs) target design operating conditions.

Reactor Designation	Target SRT (days)	Target Temperature (°C)	Target DO (mg/L)
SBR 1-1	4	10	> 2
SBR 1-2	12	10	> 2
SBR 2-1	4	20	> 2
SBR 2-2	12	20	> 2

This experimental system provided well controlled SRT and T conditions using controlled wastage rates and water jacketed reactors for temperature control. The direct feed provided raw sewage which represents expected variable quality feed conditions experienced at full scale

wastewater sewage treatment plants (WWTPs). Typical feed variability, in terms of conventional parameters and six polycyclic synthetic musks is provided in Table 3.2.

Table 3-2 Variability of conventional parameters and selected synthetic polycyclic musks monitored in the common sewage feed to the SBRs during stable operating conditions.

Sewage Feed Parameter (24 hour Composites except pH)	Average ± Standard Deviation
TSS (mg/L) (n=33)	200 ± 98
sCOD (mg/L) (n=10)	158 ± 84
tCOD (mg/L) (n=10)	363 ± 125
TAN (mg/L) (n=10)	29 ± 3
TKN (mg/L) (n =10)	40 ± 9
NO_3 (mg/L) (n=10)	0.5 ± 0.1
TN (mg/L) (n=10)	41 ± 9
pH (n=12)	7.4 ± 0.5
Cashmeran (ng/L) (n=5)	28 ± 5
Celestolide (ng/L) (n=5)	88 ± 20
Phantolide (ng/L) (n=5)	62 ± 10
Traseolide (ng/L) (n=5)	246 ± 51
Galaxolide (ng/L) (n=5)	7952 ± 1000
Tonalide (ng/L) (n=5)	1794 ± 280

The SRT was controlled by wastage of mixed liquor at the end of the react phase of each cycle. The SRT was calculated by taking into account the effluent suspended solids (ESS) that were lost in the effluent during the draw phase of the cycle using Equation 3-1.

$$SRT = \frac{\text{Sludge in Reactor (g VSS)}}{\text{Sludge Wasted (g/d)}} = \frac{V \cdot X}{(Q_W X + Q_E X_E)} \quad (3\text{-}1)$$

where: SRT = the average solids retention time (d), V = the total volume of the SBR (L), X = the MLSS in the SBR at the time of wasting (g/L), Q_W = the volume of MLSS wasted per day (L/d), Q_E = the volume of final effluent per day (L/d), X_E = the effluent suspended solids (g/L).

The mixed liquor suspended solids (MLSS) and ESS were monitored on a daily to weekly basis. The measurement provided a means to correct the wastage rate to maintain the appropriate SRT.

The SBRs were inoculated with sludge from the Hamilton Woodworth WWTP which is a conventional non-nitrifying activated sludge WWTP. The SBRs were initially operated at a target SRT of 4 days. The MLSS of the reactors along with the SVI, ESS and effluent COD were periodically monitored to determine if stable operating conditions were achieved. Following approximately 16 days of operation (equivalent to approximately 4 SRTs) stable operating conditions were achieved based on the consistency of the MLSS level, the ESS and effluent COD values. The operation of the SBRs was switched over to an SRT of 12 days following completion of the first phase of the study with a similar methodology towards achieving stable operating conditions. Phase 1 was subsequently rerun to continue with missed WAS time sensitive analysis. Details of the parameter values are provided in Chapter IV Experimental Results.

In addition to the conventional parameters selected synthetic polycyclic musk concentrations were determined in the influent, effluent and WAS.

3.2. SBR Bench Scale System

The SBR system is pictured in Figure 3-1 and 3-2 and the operating phases and target operating conditions are provided in Table 3-3. The SBR system consisted of two glass 20 L reactors operated in parallel with primary effluent generated from a municipal sewage treatment plant. The raw sewage takeoff was downstream of the ferric salt additions used for phosphorous control. Temperature control was maintained by circulating temperature controlled water at about 8 and 18 °C from chilled reservoirs to water jackets around the SBRs. All experimentation was done with plexiglass water sleeves filled with temperature controlled water from the chillers (see Figure 3-2 (A).

Mechanical mixers and humidified fine bubble diffusers were used and controlled by timers which controlled the cyclic operation of each SBR. The peristaltic pumps controlled the feed, wastage and effluent draw by on-and-off timers (see Figure 3-1).

A general description of the laboratory-scale SBR system follows:

Sewage Feed: The sewage feed was taken downstream of the ferric salt addition in a municipal treatment plant. The sewage was screened and settled prior to being fed in parallel to the SBRs. A custom designed primary clarifier followed by screens to remove any floatables we used as part of the primary treatment before feeding the reactors.

Temperature Control Units: Two cooling system consisted of two plexiglass holding tanks with approximately a 100 L capacity, two chilling units and associated tubing to and from the water jackets surrounding the SBRs.

Sequencing Batch Reactors: The SBRs were made of 13 mm thick transparent glass (300 mm I.D. x 450 mm height, 15 L operating capacity). The SBRs were enclosed by water jackets made of 13 mm transparent plexiglass (500 mm x 400 mm x 400 mm) used for temperature control. Sewage feed, ESS and WAS ports were preset at about the 3 L, 5 L and 13.5 L marks, from the bottom of the reactors, designed for the sewage feed, collection of effluent and WAS, respectively. The sewage was fed over a period of about 2.7 hours, WAS was collected at the end of the react cycle over a 10 minute period and the effluent was collected after the settle cycle over a 20 minute period. The effluent and WAS was collected four times over the day every 6 hours and stored in refrigerated samplers for future analysis. The top of each reactor was covered with a plexiglass lid with predrilled circular holes for the influent, effluent, WAS and air tubing along with, the mixer shaft.

The mixed liquor in the SBRs was stirred by a mechanical mixer and diffused humidified air provided through stone fine bubble air diffuser. The level of the mixer and stone level corresponded to about the 2 L mark from the bottom of the SBRs, offset horizontally.

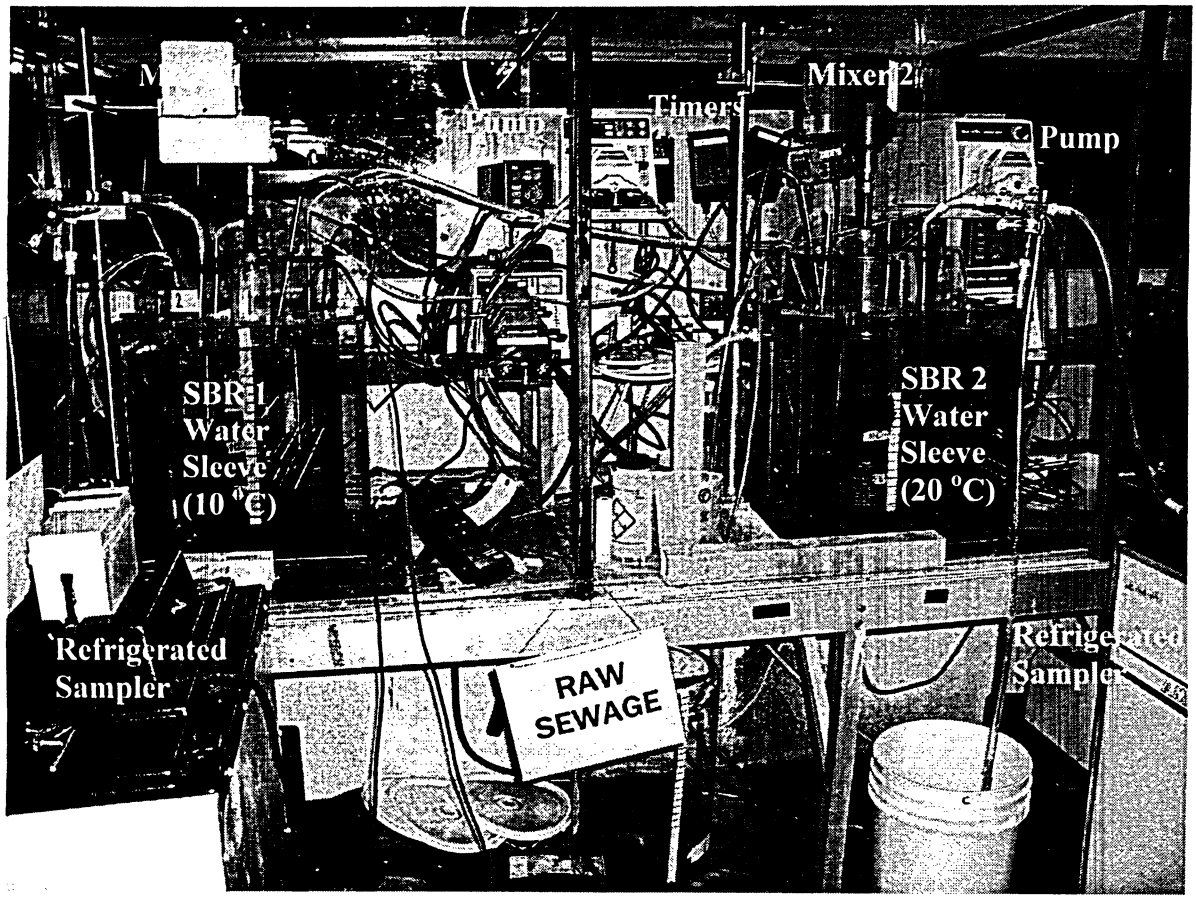


Figure 3-2. Laboratory-scale sequencing batch reactor (SBR) setup showing the 20 L SBRs (SBR 1 and 2), mixers, Masterflex pumps, timers, feed, effluent and WAS lines (the setup was at the Water Technology Centre in Burlington, Ontario).

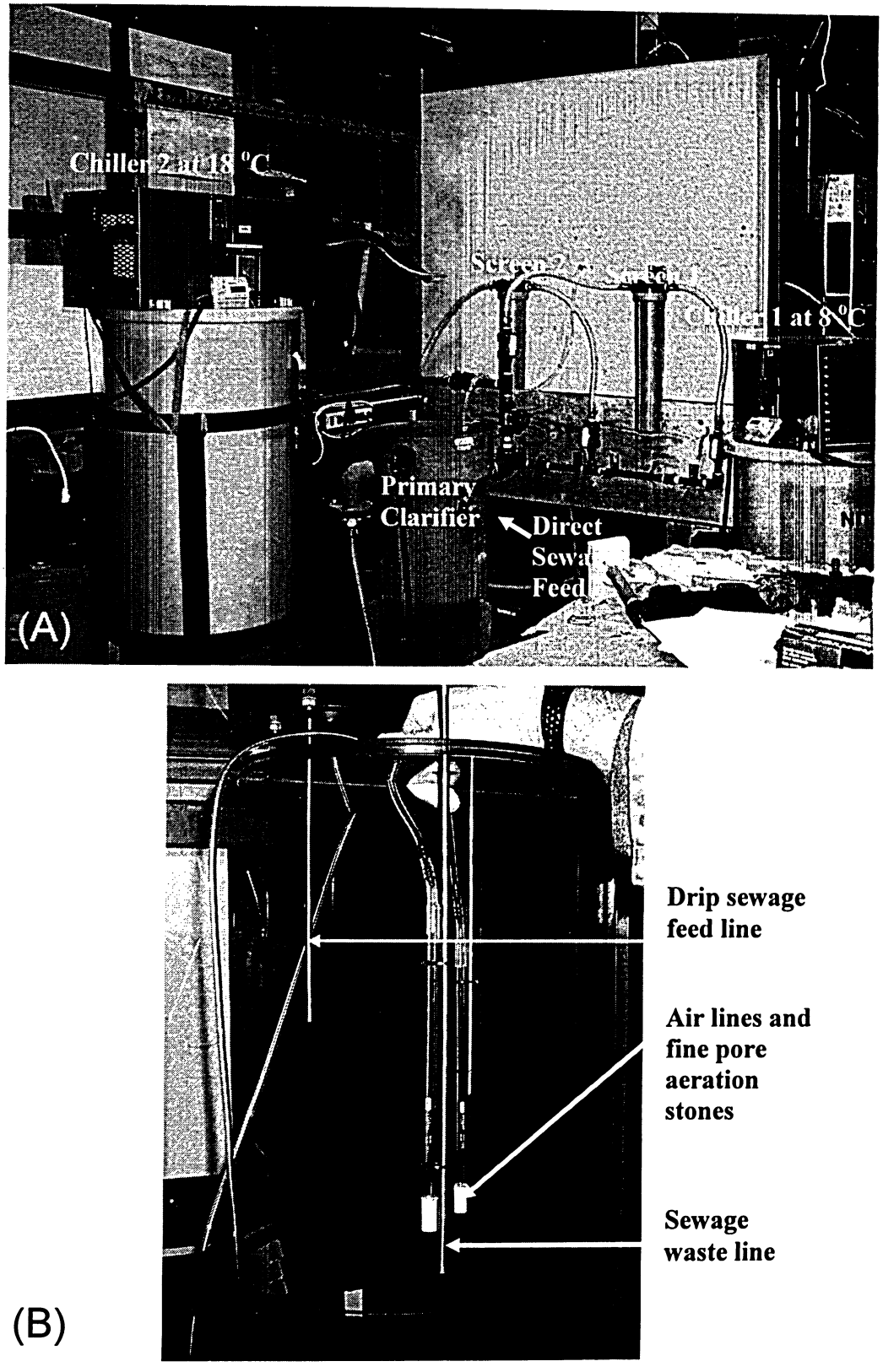
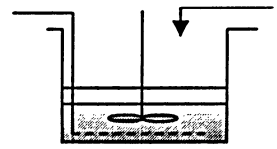
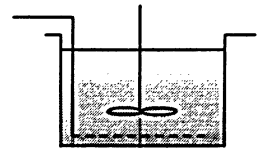
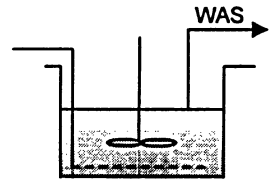
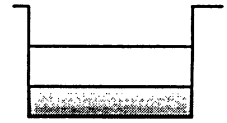
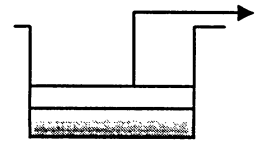


Figure 3- 3. Above photograph (A) shows the chillers, preliminary and primary treatment provided by the screens and clarifiers and (B) a close-up of the empty 20 L glass SBR

Table 3-3. Process flow timed sequence operation of the SBR with a theoretical 6.4 h HRT

Sequence Mode	SBR	Air /Mixing	Duration (hours)
Fill		On / On	2.7
React		On / On	2.5
Waste		On / On	0.1
Settle		Off / Off	0.5
Decant		Off / Off	0.2

The hydraulic retention time (HRT) of a SBR can be computed by considering the incremental residence time of the settled and fill volume fractions. The total cycle time (T_C) which includes the fill to decant sequence (see Table 3-3) was selected to be 6 hours resulting in 4 cycles per day with a fill time (T_F) of 2.7 hours. The settled volume (V_S), to ensure a minimal loss of solids after decant, was set at 4 L and resulted in a settled fraction of 0.22 (V_S/V_T = 4 L/ 18 L) and a fill fraction of 0.78 (V_F/V_T = 14 L/18 L) (Metcalfe and Eddy, 2003). An expression for the HRT can then be determined by considering that:

$$\text{HRT} = \text{Setted Volume HRT} + \text{Fill Volume HRT} + \text{React to Decant HRT}$$

$$= \frac{V_S}{V_T}(HRT + T_F) + \frac{V_F}{V_T} \cdot \frac{\int_0^{T_F} Q \cdot t \cdot dt}{\int_0^{T_F} Q \cdot dt} + (T_C - T_F) \tag{3-2}$$

$$= \frac{V_S}{V_T}(HRT + T_F) + \frac{V_F}{V_T} \cdot \frac{T_F}{2} + (T_C - T_F)$$

Upon rearrangement of Equation 3-2 and taking into account that:

$$\frac{V_S}{V_T} + \frac{V_F}{V_T} = 1 \tag{3-3}$$

the HRT expression simplifies to:

$$HRT = \frac{V_T}{V_F} \cdot T_C - \frac{T_F}{2} \tag{3-4}$$

Upon inserting our experimental values of 18 L, 14 L, 6 h and 2.7 h for V_T, V_F, T_C and T_F, respectively, the computed HRT given by Equation 3-4 is 6.4 hours which agrees with Equation 3-2. This HRT result applies equally to both Phase I and II, of the different SRT operation since the wastage volume occurs during complete mixed conditions and is assumed to affect equally the settled and fill volume residence time (derived with the assistance from L. A. Lishman).

Temperature Control: The temperature of the SBRs was maintained at 10 and 20 °C by circulating 8 and 18 °C water through the water jackets. The chilled water was circulated using variable speed peristaltic pumps driving multiple pump heads (Masterflex) with 75 mm O.D. polyvinyl tubing.

DO Control: Dissolved oxygen target levels of 2 mg/L were maintained through the use of a on-and-off aeration which was connected to a DO controller lead situated at approximately the 2 L level from the bottom of the SBR.

Timers: The SBRs cycles were controlled by the use of four on-and-off timers. The four programmable timers were preconfigured to turn pumps on and off at appropriate times in accordance with the timed operating cycle provided in Table 3-3.

All pumps used were Masterflex Standard Pump Drive and all tubing for sewage, effluent and WAS was 50 mm O.D. polyvinyl.

3.3. Conventional Monitoring of the SBR Performance

The general conventional characterization of sewage, effluent and WAS followed standard methods described by American Public Health Association (APHA, 2004).

Mixed Liquor and Volatile Suspended Solids: Mixed liquor and volatile suspended solids (MLSS and VSS) were sampled at the end of the react phase and representative samples analysed for in accordance with Standard Methods (APHA, 2004).

Chemical Oxygen Demand: The closed reflux colorimetric method (Section 5220D, APHA, 2004) was used to determine the chemical oxygen demand (COD) of the screened and clarified sewage feed and treated effluent. The treated effluent was filtered though a 0.45 μm pore size filter paper prior to COD measurement. Culture tubes with effluent aliquots (2.5 mL), with digestion solution ($K_2Cr_2O_7$ + HgSO4 + H_2SO_4), and reagents (Ag_2SO_4 + SO_4) were heated in a Hach COD reactor (Model 45600-00, Hach Co., Loveland, CO, USA) for 2 hours at 150 °C. The cooled samples were then measured spectrophotometrically (Bausch & Lomb Spectronic 20D with Hach 19230-00 Adapter) at 600 nm. Potassium hydrogen phthalate (KHP) was used as a COD standard. All chemical reagents used for the COD measurement were of analytical grade.

Dissolved Oxygen: The DO levels were periodically monitored and full cycle DO profiles were determined at selected periods using a DO meter. The DO levels were maintained between 2 and 6 mg/L during the react phase of each SBR.

pH: The pH levels in the SBR were periodically monitored and full cycle pH profiles were determined at selected periods using a pH meter. The pH range through out the SBR cycle was from 7.2 to 7.8.

Effluent Suspended Solids: The effluent suspended solids (ESS) were frequently monitored (APHA, 2004). The ESS was used in the calculation of the SRT (see Equation 3-1). The ESS was sampled following 30 minutes of settling of the MLSS.

Sludge Volume Index: The sludge general settleability was determined using the sludge volume index (SVI). The SVI is defined as the sludge volume occupied by one gram of MLSS after 30 minutes of settling and was calculated using Equation 3-2.

$$SVI = \frac{\text{Volume of MLSS after 30 minute settling (mL/L)}}{\text{MLSS (g/L)}} \qquad (3\text{-}5)$$

In measuring the SVI representative well mixed samples of WAS were transferred to 250 mL to 1 L graduated cylinders. Aliquots from the WAS samples were used to determine the MLSS. The MLSS concentration was typically in the range of 2000 ± 300 mg/L, where the effect of settling errors are not typically a concern for the SVI measurement and the measurement of a diluted SVI (DSVI) was not recommended (Metcalfe and Eddy, 2003).

3.4. Non-conventional Analysis of WAS

The non-conventional analysis of the WAS included the extraction and analysis of extracellular polymeric substances (EPS) for total polysaccharides, proteins, acidic polysaccharides and DNA. The WAS biomass was analysed for relative hydrophobicity (RH) and surface charge (SC). Plankton chamber analysis of WAS further characterized the biomass porosity, excess density, floc size (average diameter and volume) distribution.

Sorption and desorption tests on lyophilized WAS were conducted to determine the sorption and desorption isotherms for six selected synthetic polycyclic musks (PSMs) at environmental

concentration levels for Galaxolide and Tonalide and above the typical environmental range for Cashmeran, Celestolide, Phantolide and Traseolide. The PSMs were also monitored at selected times in the influent, effluent and WAS of the SBRs. The subsequent sections provide the details of these non-conventional analyses.

3.5. Extracellular polymeric substances

Extracellular polymeric substances (EPS) were extracted from WAS using a cation exchange resin (CER) method described by Frølund et al. (1996). The WAS was concentrated from 2 to 10 g/L by settling in 250 mL graduated cylinders. Approximately 66 mL aliquots of the concentrated MLSS were washed twice with a pH balanced extraction buffer solution. After each washing the WAS was separated by centrifugation at 5000 x g at 4 °C for 5 minutes. The pH balanced solution consisted of a pH balanced aqueous solution of 2mN Na_3PO_4; 4 mN NaH_2PO_4; 9 mN NaCl and 1 mN KCl. The MLSS of the washed WAS was determined.and the amount of DOWEX® HCR-W2 Cation Exchange Resin (CER) was added based on 80 g of CER per g of MLSS. The CER was washed in PBS solution until the solution ran clear and then the CER was added to a 250 mL beaker with the washed MLSS. Two to four beakers were attached to the extraction apparatus. The beaker assembly was kept at 4 °C in an ice bath and stirred at 600 rpm for 2 hours (Liao, 2000; Whittaker, 2002; Kraemer, 2002). The samples were then decanted into 50 mL high speed centrifuge tubes and centrifuged at 10,000 x g at 4 °C for 15 minutes. The supernatant was further decanted into clean centrifuge tubes and further centrifuged to produce a clear solution. It was stored at -20 °C for later analysis of proteins, carbohydrates, acidic polysaccharides and DNA.

The extracted EPS was analysed for proteins, total polysaccharides, acidic polysaccharides and DNA as follows:

Proteins: The concentration of proteins in the EPS was determined by the colorimetric method using the Folin reaction (Lowry et al., 1951; Liao et al., 2001; Whittaker 2002; Kraemer 2002). A standard curve with a range of 20 to 160 mg/L using Bovine serum albumin (BSA) was used as a standard.

Aliquots of 1 mL of standard, blank and sample, in triplicates, were added to separate HACH 10 mL test tubes. Prepared reagent (20 g Na_2CO_3 in 1L of 0.1 N NaOH, combined with 0.25 g $CuSO_4 \cdot 5H_2O$ dissolved in 50 mL of 1% (w/v) aqueous solution of sodium tartrate, in a ratio of 25:1) was added to each test tube, then each tube was vortexed for 15 seconds and allowed to stand for 10 minutes at room temperature. A 0.5 mL aliquot of 1:1 distilled and deionized water (ddH_2O) diluted Folin reagent was added and vortexed on a mixer for 15 seconds. The mixture was allowed to stand for 30 minutes to allow for complete reaction which resulted in a colour change. The absorbance of the solutions were measured at 750 nm using a spectrophotometer (Spectronic 20, Spectronic Instruments, Rochester, NY, USA) and compared to a standard curve. The measured values were converted to mg (BSA equivalent) per g MLSS.

Acidic Polysaccharides: The acidic polysaccharides in the extracted EPS were determined using the D-glucuronic acid method according to Filisetti-Cozzi and Carpita (1991). A stock standard solution of D-glucuronic acid was used and sequentially diluted to prepare a standard calibration curve with a range of 2 to 32 mg/L. Aliquots of 0.8 mL blank, standard and sample solutions, in triplicates, were pipetted to 10 mL HACH test tubes. 80 µL of 4 M sufamic acid-potassium sulfamate (pH 1.6 adjusted with saturated KOH at 4 °C) was added to each test tube and mixed with a vortex mixer for 20 seconds. At 60 second intervals a 4.8 mL aliquot of H_2SO_4 96.4 % analytical grade containing 75 mM sodium tetraborate was added to each test tube, vortexed for 30 seconds and placed in boiling water for 20 minutes to allow the reaction to proceed to completion. Each test tube was sequentially chilled in an ice bath and brought to room temperature. A 160 µL aliquot of 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH was added and vortexed for 15 seconds. The sample test tubes were allowed to stand for 10 minutes to complete the reaction as evidenced by a pink-reddish colour. The absorbance was measured at 525 nm and compared to the D-glucuronic acid concentration standard curve and expressed as mg (D-glucuronic acid equivalent) per g MLSS.

Total Polysaccharides: The Anthrone method (Gaudy, 1962) was used to determine the total polysaccharides concentration in the WAS extracted EPS. A standard calibration curve of D-glucose

in the range of 5 to 120 mg/L was prepared by sequential dilution from a stock standard solution and this standard curve was used as comparative standard. The stock solution was refrigerated at 4 °C for less than 7 days. A solution of anthrone was prepared weekly by dissolving 0.2 g of anthrone reagent in 100 mL of 95% H_2SO_4 solution. Triplicate samples extracted EPS (2.0 mL) with 4 mL of D-glucose standard were added to the 10 mL HACH test tubes. To each test tube 5 mL of cold Anthrone reagent was added at 60 second intervals. The test tubes were vortexed for 30 seconds and placed in a boiling water bath for 15 min to complete the polysaccharides digestion reaction. Following the digestion step the samples were cooled sequentially in an ice bath and at room temperature. The absorbance of each sample at 625 nm was measured (Spectronic 20, Spectronic Instruments, Rochester, NY, USA) and compared to the standard. The measured values were converted to total polysaccharide concentration mg/L (glucose equivalent) present in the extracted EPS from the standard curve and then converted to mg/g MLSS, based on the original mass of sludge where the EPS was extracted.

DNA Quantification: The DNA present in the EPS extracted from the WAS was quantified using the DAPI (4,6-diaminodino-2-phenylindole) method (Brunk et al., 1979) which used salmon testes DNA (Sigma) in salt form as a standard. Aliquots of standard, blank and sample each 200 µL were added to 10 mL HACH test tubes and to each test tube 5 mL of DAPI reagent (0.2 mg/L DAPI in 100 mM NaCl, 10 mM EDTA, 10 mM Tris solution all at pH of 7.0) was added and vortexed vigorously for 30 seconds. The mixture was allowed to stand at room temperature for 10 minutes to complete the reaction and develop fluorescence. An aliquot of the samples was transferred to cuvettes in the fluorimeter for fluorescence measurement, using a 360 nm and a 460 nm, excitation and emission filter, respectively. The measured fluorescence was compared to the standard curve and converted to mg/mg MLSS.

Total EPS : The total EPS was calculated as the sum of the above four components since proteins, polysaccharides and DNA are considered the dominant components of EPS (Forster, 1976 and 1985; Frølund et al., 1996; Bura et al., 1998).

3.6. Sludge floc surface charge and relative hydrophobicity

The surface charge and relative hydrophobicity of the microbial bioflocs were measured on fresh WAS using colloidal titration and the microbial adherence to hydrocarbons (MATH) methods, respectively.

Surface Charge: The surface charge of microbial bioflocs was determined using the colloidal titration method (Morgan et al., 1990). The measurement process involves back titration of excess positive charge introduced into a known amount of AS and comparing the excess volume of titrant added to a blank sample.

Samples of AS were transferred to 50 mL high speed centrifuge test tubes and washed once in Millipore water then centrifuged at 3000 g for 5 minutes at 4 °C. A second washing in pH-balanced ddH_2O (pH=7.0) was completed following centrifugation at 3000 g for 5 minutes at 4 °C. The MLSS of the washed AS samples was quantified and the AS samples were diluted down to 2000 mg/L. Blanks, duplicates and samples, all in triplicates (2 mL), were mixed with 43 mL of pH-balanced ddH2O with 1 mL of polybrene. The 1 mL polybrene represented an excess of positively charged polymer. A 0.001 N solution of polyanetholesulfonic (PAS) acid solution was used to titrate the excess polybrene using toluidine blue as an indicator in both the blank and sample solution. The blank solution consisted of 2.0 mL of ddH_2O rather than the 2 mL of AS. The surface charge of the samples was calculated using the following formula:

$$q = \frac{-(V - V_o) \cdot N \cdot 10^3}{2 \cdot \mathrm{MLSS\,(g/L)}} \qquad (3\text{-}6)$$

where: q = the surface charge (meq/g MLSS), V = the titrant volume added to the sample (mL) t o reach the endpoint, V_o = the titrant volume added to the blank (mL) to reach the endpoint, N=the normality of the titrant PAS solution (0.001 N).

Relative Hydrophobicity: The microbial adherence to hydrocarbons (MATH) method was used to determine the relative hydrophobicity of microbial bioflocs. The MATH method is based on hydrophobics in the microbial sludge suspension adhering to the hexadecane (a hydrocarbon) at the hydrocarbon-aqueous interface. Following separation of the aqueous phase, the absorbance is measured to estimate the average relative hydrophobicity of the microbial sludge suspension or cell hydrophobicity index (A%= percentage of adhesion) (Rosenberg et al., 1980; Guellil et al., 1998).

Samples of fresh AS from each SBR was sampled and analyzed within 12 hours of sampling. The AS sludge samples were transferred in 50 mL high speed centrifuge test tubes, washed twice with ddH_2O and centrifuged at 3000 g for 5 minutes at 4 °C after each washing. The initial absorbance of the dispersed suspension (I_o) was adjusted to 1.5 ± 0.2 at 400 nm, using ddH_2O for dilution. A 10 mL aliquot of the adjusted WAS suspension was mixed with 1 mL of hexadecane using a vortex mixer for a period of 2 minutes. The phases were transferred to a separatory funnel and allowed to separate for 10 minutes. The aqueous phase was collected and the absorbance (I) at 400 nm was measured using a Spectronic 20 (Spectronic Instruments, Rochester, NY, USA). The relative hydrophobicity was calculated using the following:

$$RH = \frac{(I_o - I)}{I_o} \cdot 100 \qquad (3\text{-}7)$$

where: RH = the percent relative hydrophobicity (%), I_o = the initial absorbance of the dispersed, suspension (adjusted to 1.5 ± 0.1) and I = the absorbance of the aqueous phase following separation.

3.7. Sludge Floc Settling Velocity, Porosity, Excess Density and Size Distribution

Sludge floc settling velocity, porosity, excess density and size distribution of sludge flocs were determined with a plankton chamber and microscopic video taping followed by the use of imaging analysis with Northern Exposure™ (Empix Imaging Inc.) (Droppo et al., 1997).

The porosity of the sludge flocs was calculated based on the measured density from measured floc settling velocity using the modified Stokes Law (Li and Ganczarczyk, 1987).

$$\varpi = \frac{1}{18} D^2 (\rho_f - \rho_w) \frac{g}{\mu} \tag{3-8}$$

where: ϖ = settling velocity, D = floc diameter, ρ_f = wet density of the floc, ρ_w = density of water, and μ = dynamic viscosity of water.

The plankton chamber and imaging analysis derives the ϖ and D. The ρ_w and μ are constant for a given water temperature from which the wet density of the floc(ρ_f) is calculated. The densities in this type of analysis are expressed as excess density (ρ_f-1) (Droppo et al., 1997). The floc porosity is calculated (Equation 3-6) based on a mass balance analysis by assuming a typical density of dried silt and clay of 1.65 g/cm^3.

$$\varepsilon = \frac{\rho_w - \rho_f}{\rho_s - \rho_w} \tag{3-9}$$

Where: ε = floc porosity, and ρ_s = density of the dried solid material.

3.8. Analysis of Synthetic Polycyclic Musks

Two different methods were used in the analysis of PSMs: (1) head space solid phase micro-extraction (HS SPME) and (2) microwave assisted solvent extraction (MASE). Both methods relied on gas chromatography mass spectrometry analysis (GC MS) for musk quantification.

The HS SPME GC MS followed the optimized method by Llompart et al. (2003). The method was verified at Ryerson University Analytical Centre (RUAC). This method was primarily used to determine the presence of PSMs in the aqueous phase in the sorption-desorption study (see section 3.7).

The MASE GC MS was optimized at EC WTC analytical laboratory in Burlington and this method was utilized to determine the concentration of PSMs in the influent, effluent and solids from the SBRs (Svoboda et al., EC internal manuscript, 2006).

The HS SPME GC MS method was previously optimized by Llombart et al. (2003) using an ion trap GC-MS operated in selected ion monitoring (SIM) in the electron ionization (EI) mode. Instrumentation and equipment used at RUAC (see Table 3-3) were not identical to conditions used by Llompart et al. (2003) and some variations, primarily in retention times and sensitivity were identified.

The reduced sensitivity of the PSM detection is related to the inherent differences between the ion trap MS (used in Llompart et al.'s study) versus the Quadrupole MS used in our study. However it is also known that improved peak resolution can be achieved using a Quadrupole MS over a ion trap MS..

The method involves the use of selective 65 μm polydimethylsiloxane-di-vinylbenzene (PDMS-DVB) solid fibers that are injected into the headspace of a heated 22 mL vial where the sludge slurry or aqueous solution containing the PSMs is stirred by a microflee at about 600 rpm (see Figure 3-3). The vial is heated in a water bath at 100 °C and allowed the PSMs in a 3 mL WAS slurry or sewage solution to volatilize and adsorb onto the solid fiber. The solid fiber was subsequently desorbed into the GC-MS column for analysis. Table 3-4 provides the instrumentation and equipment conditions used in this analysis and Table 3-5 provides the retention times, identification and quantification ions for the selected synthetic musks and internal standards.

The quantification is based on the development of standard curves at known applicable PSM concentrations and correlated to quantification EI ions and retention times (see Table 3-6 for calibration curves).This method once verified at RUAC was later transferred to EC WTC where it was adapted and later applied to assist with the hundreds of manual injection analyses required for the adsorption-desorption study described in section 3.7.

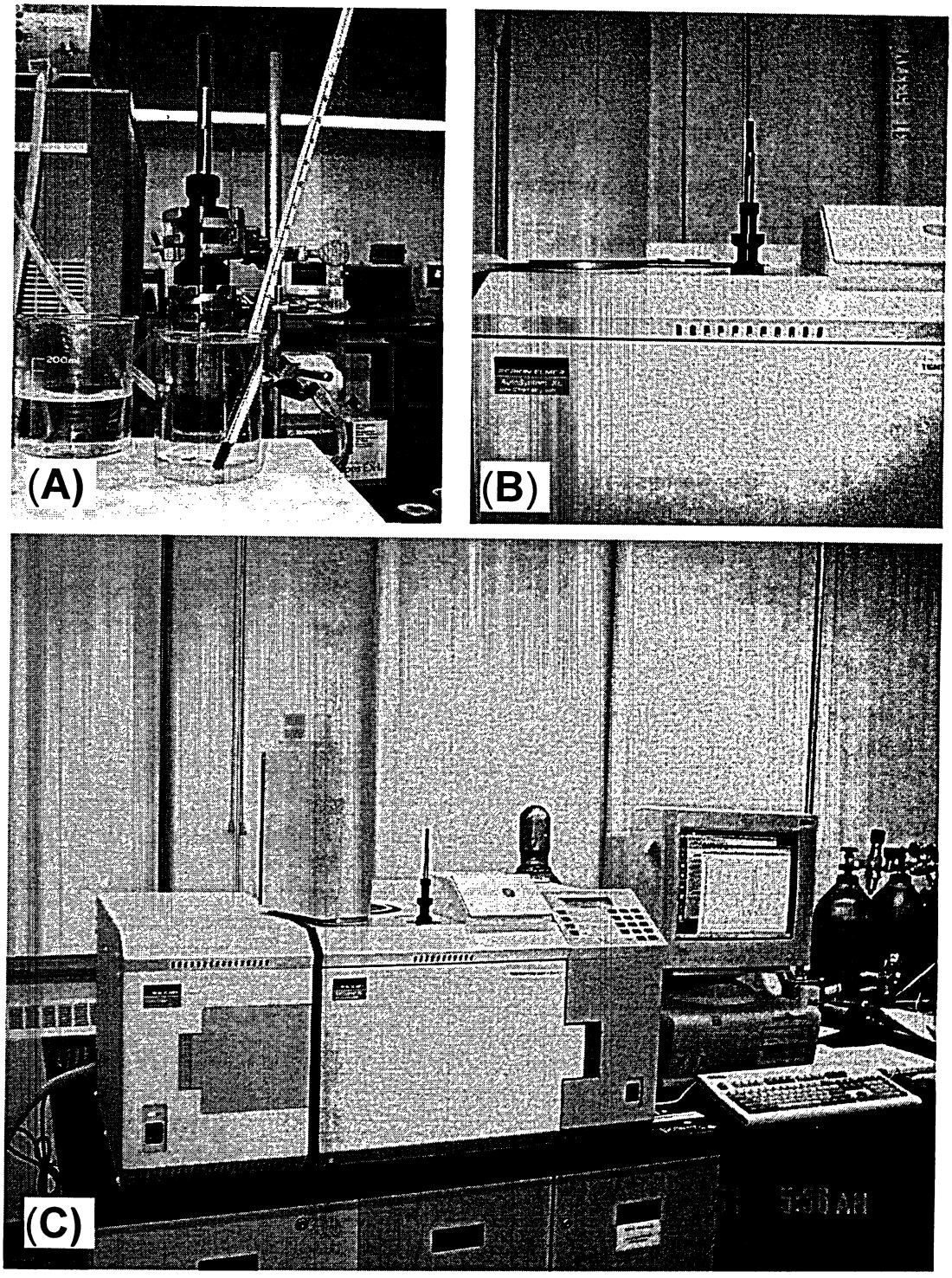


Figure 3- 4. Photograph (A) showing the sorption phase using the solid fibre injected into the headspace vile using a syringe; (B) close up of the desorption phase and (C) the GS-MS with computerized control during the desorption phase located at the Ryerson University Analytical Centre (RUAC).

Table 3-4. The GC-MS and HS SPME fiber specifications and equipment operating conditions.

HS SPME	65 μm polydimethylsiloxane-di-vinylbenzene (PDMS-DVB) coated fiber 22 mL headspace vial 3 mL aqueous sample and 19 mL headspace volume in 22 mL vial 100 °C and agitated at about 600 rpm using a micro-flea stirrer for 5 minute equilibration time 100 °C and agitated at about 600 rpm using a micro-flee stirrer for 15 minute extraction time manual injection in GC port for 5 minutes extraction period
Injection	Auto sampler of 2 μL was used for musk standards in methanol and manual injection of PMDS-DVB fibre was used. Manual injection of fibre was used for injection during aqueous samples and sludge samples .
Gas Chromatogram and Column Oven program	Injection: 2.0 μL splitless for 2 min followed by 50:1 split Injector Temperature: 250 °C Column: 30m x 0.2mm I.D., 0.25 μm film thickness. MBM-5S (5% phenyl, 95% methyl poly-siloxane) Carrier Gas : He, 1mL/min Column Oven: 60 °C hold for 2 min; 60-220 °C at 10 °C/minute; 220-325 °C at 30 °C/minute; Total Cycle Time: 21 minutes (includes 4 minutes GC-MS setup time and 17 min run time)
Mass Spectrogram (PE Quadrupole, 1997)	Mass Range: 50 to 300u; Scan Rate: 0.5 sec/scan Retention Window (min) : 0.000 to 20.650; Electron energy: 65 volts; Ionization mode: EI+

Table 3-5. Retention times, identification and quantification ions for the selected synthetic musks and internal standards

Polycyclic Synthetic Musks (PSMs)	Retention time (min)	Identification Molecular Ions (m/z)	Quantification EI Ions (m/z) for SIM
Cashmeran	13.1 ± 0.1	191, 206	191
Celestolide	15.6 ± 0.1	244, 173	229
Phantolide	16.1 ± 0.1	187, 244	229
Traseolide	17.1 ± 0.1	173, 258	215
Galaxolide	17.1 ± 0.1	213, 258	243
Tonalide	17.2 ± 0.1	187, 159	243
Anthracene-d10[1]	16.5 ± 0.1	160, 94, 80	188
Phenanthrene-d10[1]	16.6 ± 0.1	160, 94, 80	188

1. Anthracene-d10 and Phenanthrene-d10 were used as internal standards, each at a concentrations of 0.5 ng/μL, with full spectrum identification possible based on library reference.

Table 3-6. Calibration curves of PSMs in the aqueous phase

PSMs (µg/L)	Ratio of Chromatograph Peak Intensities (As/Ais)					
	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
0	0	0	0	0	0	0
17	0.5	1.5	1.9	1.1	1.4	1.7
33	0.8	3.0	3.9	2.5	2.9	3.4
67	1.2	6.6	8.2	5.9	6.6	7.5
133	2.4	14.8	19.1	14.1	15.0	17.8
233	3.9	26.0	32.7	24.8	26.2	30.5
333	4.6	34.0	42.9	33.0	34.9	40.8
Slope	0.015	0.11	0.13	0.10	0.11	0.13
R^2	0.971	0.996	0.996	0.996	0.997	0.996

3.9. Adsorption and Desorption Study

Competitive adsorption-desorption of selected polycyclic synthetic musks using lyophilized sludge was conducted in accordance with OECD Method 106 (OECD, 2001). The method is a batch equilibrium test usually applied to soils and it was necessary to make adjustments based on the work by Kördel et al. (1997) and following preliminary tests for verification. The following modifications were made:

1. Use 0.1 g of dried sludge for 50 mL test solution setting the ratio of sludge/test solution to 1/500;
2. The aqueous solution used was PBS at a pH of 7.4 consistent with typical sewage; and
3. The agitation time was set to 90 minutes because the sorption plateau was reached within 30 minutes.

It has been previously demonstrated (Kördel et al., 1997) that sorption capacities of fresh and lyophilized sludges, once rehydrated, are comparable and therefore lyophilized sludges were used for convenience. A recommended freeze drying process followed by inactivation (Kerr et al., 2000) has been followed to eliminate biological activity while not altering the original structure or surface properties of the sludge. The recommended process was followed which included the following steps:

1. Washing (thrice) and centrifuging sample activated sludge;

2. Freezing at –40 °C using dry ice and lyophilization in a shelf freeze dryer and passing through a 4-mm sieve;
3. Heating the solids to 103 °C for 3 hours for inactivation prior to storage at 4 °C.

All the sorption-desorption tests were conducted at room temperature (about 25 ± 2 °C) and at pH of 7.4 using a phosphate buffer saline (PBS) solution. The use of lyophilized sludge is reported to have the same sorption-desorption characteristics and makes the experimental work more accurate since one can use measure solids gravimetrically to a greater degree of accuracy.

An option of the parallel and serial method is available in OECD Method 106 and the parallel method was selected. The parallel method was used which allowed separate shaker flasks for each PSM concentration that was utilized as opposed to the serial method because the sample size necessary (15 mL) was greater than the serial method could provide based on 50 mL aqueous samples.

Five separate concentration levels of PSM were tested with a target aqueous PSM spike from 10 μg/L up to 300 μg/L and the OECD Method followed as outlined in Figure 3-5. Preliminary tests were conducted to determine: (1) the time of equilibrium between the sludge solids; (2) the ideal sludge/solution ratio and (3) the verification that the PSMs are stable, and not significantly lost by sorption on the test vessels (250 mL glass Erlenmeyer Flasks) or 50 mL polyethylene (PE) centrifuge test tubes during the separation step.

Two different lyophilized sludges (SRT of 3.5 and 10.5 days) were tested at various PSMs concentration levels within the reported environmental range and in the case of the SRT of 10.5 days sludge well beyond this range. Both adsorption and desorption equilibrium Freundlich isotherms were determined.

The background PSMs associated with the lyophilized sludge used in the equilibrium batch tests was determined using the standard addition method (SAM) which involves the sequential addition of known concentration to the original sample shown in Table 3.7 and the resulting SAM curves given in Table 3-8 and the derived initial PSM concentrations.

Appropriate duplicate use of controls and blanks were integrated in the final determination of the concentration of PSMs in the aqueous phase and the determination of PSMs by difference using the indirect method (OECD 106, 2001).

Table 3-7. Calibration curves in solid phase[1, 2]

PSMs (ng/g)	Ratio of Chromatograph Peak Intensities Times Concentration of Internal Standard Concentration added (AsCis/Ais)					
	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
2400	52	116	143	44	72	73
4800	122	284	346	109	167	173
9600	471	706	830	248	364	384
19200	392	1150	1450	530	690	783
24000	792	1470	1860	659	832	968
38400	1322	2649	3327	1176	1465	1714
Slope	0.0325	0.0659	0.0827	0.0292	0.037	0.0428
R^2	0.9358	0.9909	0.9923	0.9923	0.9963	0.9945

1. Internal standard used was Phenanthrene-d10 at a concentration of 0.1 µg/mL (100 ppb) volume of 50 µL to 3 mL of PBS with 0.1 g of dried solids from SBR 1-1. The mixture was equilibrated overnight in 22 mL headspace vials with different musk standards additions and mixed with a micro flea mixers and frozen at -22 °C until ready for analyzes using HS SPME in the RUAC.
2. Adjusted values for the sorbent control are tabulated.

Table 3-8. Concentration of PSMs in lyophilized and unspiked sludge using SAM[1]

PSMs	Initial PSMs in Lyophilized Sludge Co (µg/g)[2]	SAM Linear Equation ($Cs = m \cdot Co + B$)[3] (duplicates of n=7 points)	r^2
Cashmeran	0.04	Cs = 0.033Co – 11.95	0.936
Celestolide	0.47	Cs = 0.067Co – 31.41	0.992
Phantolide	0.63	Cs = 0.085Co – 53.69	0.994
Traseolide	0.87	Cs = 0.0305Co – 30.32	0.996
Galaxolide	1.8	Cs = 0.0376Co – 67.01	0.997
Tonalide	0.35	Cs = 0.0443Co – 15.59	0.996

1. SAM means standard addition method and is used in the absence of a blank matrix. We do not have a blank matrix to work with in our case.
2. Co (µg/g) represents the PSM concentration associated with the unspiked and lyophilized sludge and calculated from the SAM curve by –B/m.
3. Cs (µg/g) is the added PSM musk spike to the solids (0.1g) and PBS mixture (3 mL). The Cs values are given in Table J-6. The SAM linear curves are based on the linear plot of data in Table J-6.

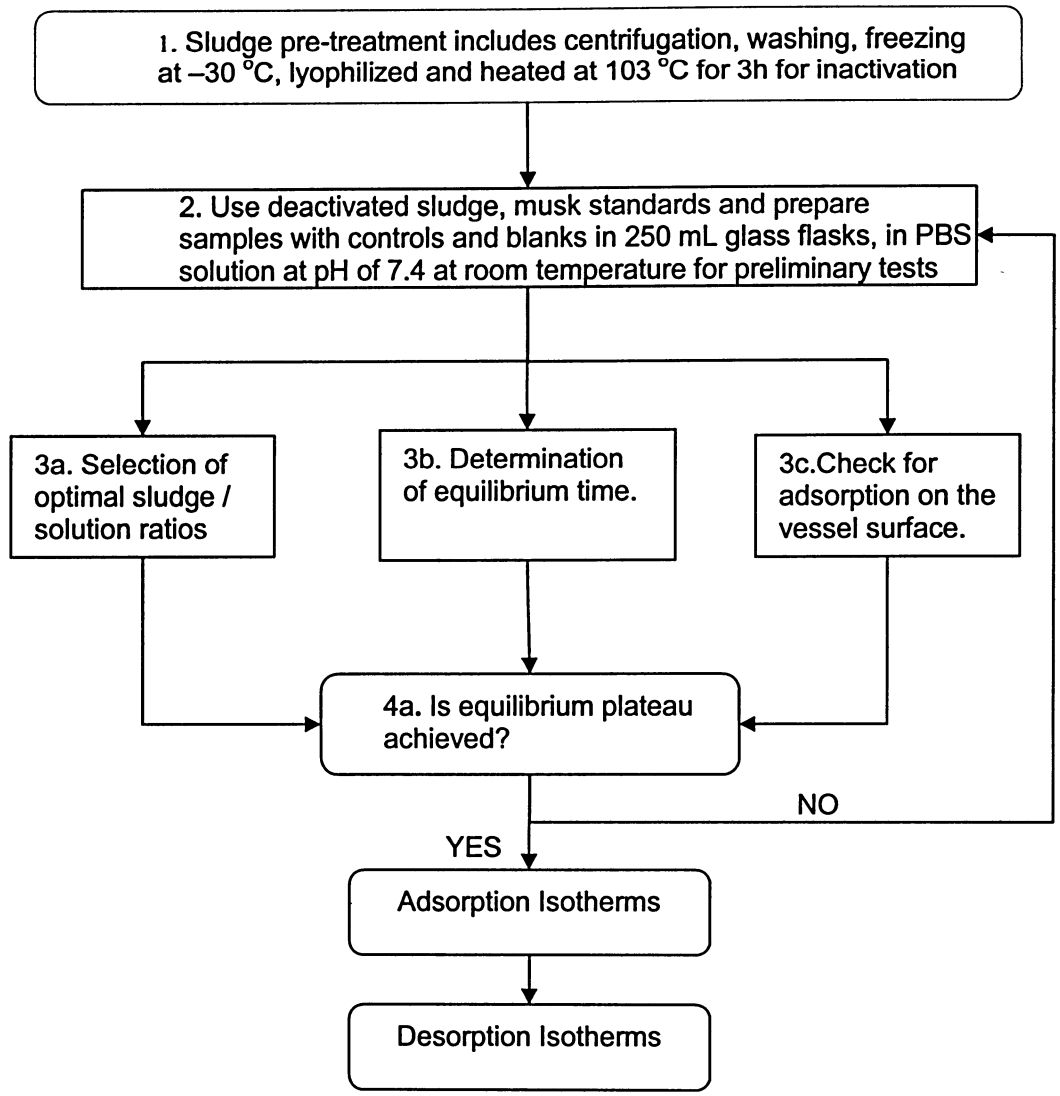


Figure 3- 5. Overall steps for the sorption and desorption study (adapted from OECD 106, 2001)

3.10. Statistical Analysis

The statistical analysis generally involved a two step process: (1) determination of the dataset underlying distribution; and (2) applying the appropriate parametric or non-parametric test to evaluate the null hypothesis (H_o) for the mean difference in the SBR response. Pearson's linear correlation analyses were conducted to assess if a linear correlation was present between certain sludge characteristics and PSM removal in the aqueous matrix and partitioning to the solids.

Typically conventional environmental effluent and sludge contaminants from WWTPs are lognormally distributed except when assessing trace contaminant concentrations which tend to be non-parametric. The non-parametric distributions are generally due to limitations in the instrumentation method detection limits (MDLs) which are typically close to the environmental concentration levels in the order of 10 ng/L or 10 ng/g d.m and effectively truncate the lower values below the MDLs. This truncation of data results in positively biased sensored datasets which require non-parametric analysis procedures to evaluate (U.S. EPA, 2001).

The general parametric descriptive statistics included the mean and standard deviation and the application of the Student's t-test or the two-factor analysis of variance (ANOVA) on the mean for AS characteristics. This method of analysis was applied to the AS characterization for mean response comparisons to Normally distributed datasets (Zar, 1996).

For the PSMs effluent and sludge comparisons analyses, if the datasets were lognormally distributed, appropriate normalization preceded the application of Student's t-test or ANOVA tests. For non-lognormally (assumed non-parametric) distributed datasets, the Kruskal-Wallis test without the Bonferroni correction or Mann-Whitman test was used to compare the mean response. The 95% confidence level (α =0.05) was assumed as applicable to establish significant differences in all comparisons (Zar, 1996).

The comparison analyses were all conducted using XLSTAT (Addinsoft®, 2006), a statistical add on software package to MS Excel®.

CHAPTER IV

EXPERIMENTAL RESULTS AND DISCUSSION

This chapter presents and discusses the results related to the operation and performance of the sequencing batch reactors (SBR) under selected sludge retention time (SRT) and temperature (T) operating conditions.

The target SRT (4 and 12 days) and T (10 and 20 °C) operating conditions were selected to effect different sludge characteristics and to reflect typical winter and summer operating conditions found in southern Ontario, respectively. By operating under these well defined conditions it was expected to observe significant differences in the performance of the SBR related to sludge floc properties and reflected in the partitioning of environmentally significant polycyclic synthetic musks (PSM).

The results in this chapter are ordered as follows: conventional performance of the SBR; sludge floc properties and characteristics; the concentration of environmentally significant PSM found in the influent, effluent and sludge and the PSM equilibrium sorption-desorption isotherms at the two different SRT conditions.

4.1. Operational and Conventional Performance of the SBRs

The conventional performance parameters monitored included the total chemical oxygen demand (COD), the total ammonia-nitrogen (TAN) and the effluent suspended solids (ESS). These three parameters represent conventional non-specific (except for TAN) regulated parameters that reflect the general conventional performance of sewage treatment plants (STPs). Biochemical oxygen demand (BOD) is commonly used in Ontario as a regulatory parameter however COD is preferred as a comparative parameter since it is better conserved through the system and is a much faster test (2 hours) versus five days for the 5-day BOD test. The operational parameters included the mixed liquor

suspended solids (MLSS), the mixed liquor volatile suspended solids (MLVSS), the reactor dissolved oxygen (DO), pH and sludge volume index (SVI).

Table 4-1 summarizes the average stable operating characteristics and Table 4-2 provides the conventional performance. Aerobic conditions during the react-cycle with average DO above 2 mg/L were maintained along with a hydraulic retention cycle time of 6 hours for all the SBRs throughout the operational period. Figures E-1 to E-4 (see Appendix E) show the variation in conventional parameters assessed during the period towards stable operating conditions of the reactors indicated by low variability. A two week intensive 24-hour composite sampling regiment for parameters of interest was conducted once stable operating conditions had been achieved.

The SBRs were monitored for MLSS, MLVSS, SRT and ESS to determine when stable operating conditions had been established and thereafter (see Figure E-1 to E-4, Appendix E) for the full duration of operation of about 62 days at SRT of 3.5 days and about 85 days at the SRT of 11 days. The SBR 1-1 and 2-1, operated at 3.5 days SRT, experienced fluctuation of ESS values ranging from 10 to 30 mg/L and on some days about 50 mg/L up to day 40. From day 40 to 62, ESS stabilized to an average of 22 mg/L. The SBRs had taken about 10 times the SRT duration to achieve stable operating conditions. Typically 3 SRTs are adequate to achieve stable operating conditions however during the first 40 days power outages and loss of sewage flow to the reactors occurred which lengthened the time to achieve stable operating conditions

The SBR 1-2 and 2-2, operated at abut 11 days SRT, were meeting about 5 mg/L ESS by the 30^{th} day of operation. SBR 1-2 and SBR 2-2 also experienced power-out conditions with a loss of feed sporadically from about the 35^{th} to 50^{th} day of operation. During this 15 day period the reactors experienced a higher than usual ESS exceeding 25 mg/L.

Table 4-1. The average operating conditions during stable operating conditions of the SBRs[1]

SBR Phase	Target SRT (d)	Actual SRT (d)	pH	T (°C)	DO (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	F/M (COD/ MLVSS/d)	SVI (mL/g)
SBR 1-1	4	3.4 ± 0.2	7.6 ± 0.2	12 ± 1	5 ± 3	1266 ± 177	985 ± 143	0.31± 0.05	82 ± 6
SBR 1-2	12	11 ± 1	7.4 ± 0.5	10 ± 1	5 ± 2	2932 ± 316	2137 ± 233	0.11± 0.02	71 ± 8
SBR 2-1	4	3.5 ± 0.2	7.3 ± 0.3	20 ± 1	6 ± 2	946 ± 195	749 ± 152	0.41± 0.08	73 ± 6
SBR 2-2	12	10 ± 1	7.5 ± 0.5	20 ± 1	4 ± 3	2663 ± 405	1830 ± 303	0.13 ± 0.03	66 ± 25

1. The number of samples varied from daily over a 2 week to 3; see Appendix A and B for details.

Table 4-2. Conventional performance of SBRs under the four unique operating conditions[1]

SBR Phase	Influent[2] (Average ± Standard Deviation)			Effluent (Average ± Standard Deviation)		
	Total COD (mg/L)	TAN (mg/L)	TSS (mg/L)	Total COD (mg/L)	TAN (mg/L)	TSS (mg/L0
SBR 1-1	304 ± 54	28 ± 3	174 ± 75	55 ± 18	24 ± 2	22 ± 8
SBR 1-2	243 ± 64	22 ± 4	128 ± 38	15 ± 15	12 ± 4	6 ± 3
SBR 2-1	304 ± 54	28 ± 3	174 ± 75	54 ± 38	22 ± 2	11 ± 4
SBR 2-2	243 ± 64	22 ± 4	128 ± 38	42 ± 17	0.2 ± 0.4	13 ± 9

1. Number of samples varied from 3 to 15 depending on the parameter (see Appendix A and B).
2. Two reactors were operated at the same SRT and at different temperatures (10 and 20 °C) in parallel at any one time and shared a common feed.

From the 50th day onward the TSS had come down again to about 10 mg/L on average. A TSS level less than 15 to 25 mg/L monthly average is considered a well operated activated sludge sewage treatment plant (STP) (Metcalfe and Eddy, 2003).

Stable operating conditions were achieved after approximately 40 days. The stable operation was evident based on low fluctuations in MLSS, (11 to 25 %), MLVSS (11 to 20%) and low ESS (6 to 22 mg/L) which were also maintained thereafter. In addition, SBR 2-2 (SRT = 10 days; T=20 °C),

achieved full nitrifying conditions with an average effluent TAN of 0.2 ± 0.4 mg/L. Reactors SBR 1-1 and SBR 2-1 did not nitrify, however the total COD reduction was consistently above 82%, indicative of expected carbonaceous oxygen demand reduction at a low SRT. Stable operating conditions were achieved within 3 to 4 SRTs (see Appendix E).

Nitrification is indicated by a TAN of less than 5 mg/L in the effluent. The onset of nitrification in SBR 2-2 indicated a fundamental shift in the operational conditions indicating that sufficient slower growing nitrifiers were present in the aerobic SBR. Figure 4-1 shows the common nitrification threshold line in relation to SRT and T conditions of an aerobic biological reactor. The operation of the four SBRs is superimposed and it is evident from the performance data (see Table 4-2) that SBR 2-2 did nitrify (mean TAN of 0.2 ± 0.4 mg/L) as predicted. The nitrification curve, in Figure 4-1, is based on the maximum suggested temperature dependency coefficient of 1.13 which has a typical range of 1.08 to 1.13. The higher dependency coefficient implies a larger temperature dependency of nitrifier growth which has a significant impact on the required SRT for full scale STPs operated during winter (Melcer et al., 2003).

Another commonly used indicator that measures the settling characteristics of activated sludge is the sludge volume index (SVI) which is a sludge settling and an indirect indicator of sludge compressibility.

The SVI is the volume occupied by 1 g of sludge after 30 minutes of settling. A good settling sludge should have a value below 100 mL/g and not above 150 mL/g (Metcalfe and Eddy, 2003). All the SBRs averaged an SVI below 100 mL/g, indicative of good settling sludge (see Table 4-1).

The SVI is an empirical test and has been found to be subject to significant error with sludges of high concentration in the 10 g/L range. A diluted SVI (DSVI) has been adopted in cases where MLSS concentrations are high (Metcalfe and Eddy, 2003). In our SBRs, the use of the DSVI, was not necessary because the MLSS were regularly below 2500 and 3500 mg/L in SBRs operated at 11 and 3.5 days SRT respectively.

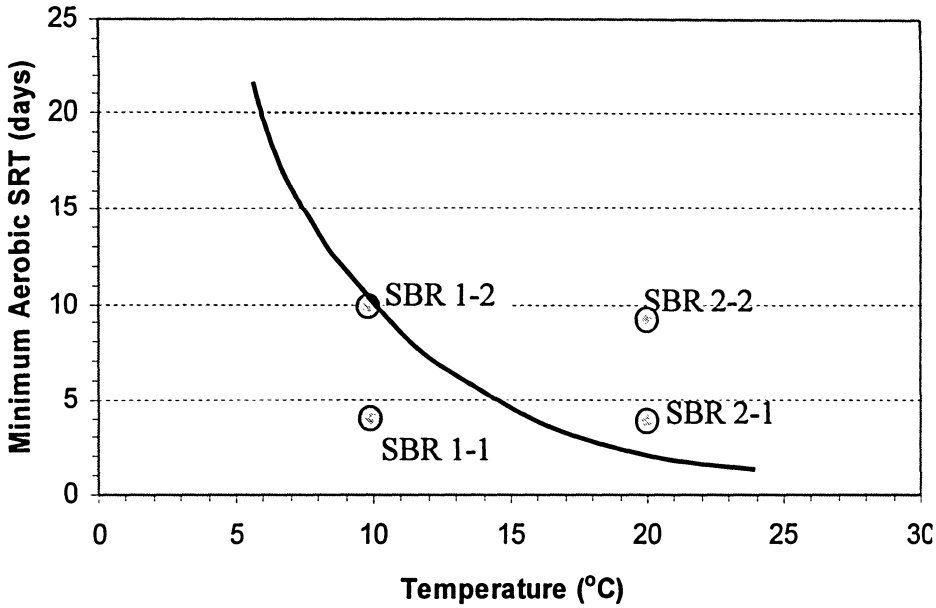


Figure 4-1. Minimum expected SRT required at a given temperature for nitrification to occur with the operating zone of the SBRs superimposed (adapted from Melcer et al., 2003).

The average results of Table 4-2 show that SBR 1-1 and SBR 2-1 maintained at 10 and 20 °C, respectively, operated under non-nitrifying conditions at effectively the same SRT of 3.5 days. Similarly SBR1-2 only partially nitrified (approximately 45 %) when operated at 10 °C and an SRT of 11 days. SBR 2-2 operated in fully nitrifying conditions when operated at an SRT of 10 days and at a temperature of 20 °C. Reactor SBR 2-2 was operated well above the minimum nitrification curve threshold and complete nitrification was observed. Reactor SBR 1-1 showed effectively no nitrification, SBR 2-1 limited nitrification and SBR 2-2 provided partial nitrification.

4.1.1. The Organic Loading

The SBRs were drip-fed at a rate of 5 L/h over 2.7 hours of the overall 6 hour cycle time to mimic completely stirred tank reactors (CSTR). This mode of operation allowed the food to microorganism ratio (F/M) to be kept low. The F/M ratio is the process loading rate and is calculated as the COD received by the reactor per day in kg COD divided by the kg MLVSS (total biomass) in

the reactor. The F/M ratio is also generally referred to as the organic matter available for microorganism growth. A low, medium and high F/M ratio has been typically classified as 0.1 to 0.4, 0.4 to 0.8 and 0.8 to 3.0 kg COD/(d · kg MLVSS), respectively (Balacko et al., 1994).

The F/M ratio was calculated based on the total daily feed (Q (L/d)·COD (mg/L)) divided by the total average MLVSS in the SBR (MLVSS (mg/L)·V(L)). The F/M ratio averaged 0.36 and 0.12 kg COD/(d · kg MLVSS) for the SBRs operated at 3.5 days and 10.5 days SRT respectively, which fall within the low F/M ratio classification. The main reason for the difference was the higher biomass, by a factor of 2, in the reactors operated at the higher SRT. By keeping the reactors at a low F/M ratio and completely mixed allowed the influent substrate to be readily distributed and the reactors to operate at a declining growth phase or starvation phase rather than feed and starve phase (see Figure 2-3). Some potential benefits of operating at low F/M ratio include the ability to control surges in organic loading with little changes in effluent quality, a reduction of biomass to be wasted (see Figure 2-3) and the corresponding lower oxygen demand allowing the DO to be kept low.

Maintaining a low F/M ratio is a common strategy in the operation of conventional, complete mix and extended aeration AS WWTPs since providing air and handling the waste activated sludge are the two largest operational cost factors. However two common problems of low F/M and low DO is foaming and bulking caused by the growth of filamentous microorganisms such as *H. hydrossis* and *M. parvicella*, respectively (Grady et al., 1999). Foaming was not observed during the operation of the SBRs however some limited bulking evidenced by high SVI was observed. Figure 4-4 (A) shows a case of excessive filamentous growth suspected to be M. *parvicella* which caused a high SVI or poorly settling sludge and (B) a case of ideal settling sludge with a proper distribution of filamentous and floc forming constituents.

4.1.2. The dissolved oxygen, pH and temperature

The bulk dissolved oxygen (DO) concentration operating set point was set at the recommended minimum Ontario Ministry of the Environment (MOE) design level of 2 mg/L in all the SBRs. It is well established that low DO concentration reduces the nitrification rate however there are cases with poor mixing and large floc sizes where nitrification reduction is observed despite the high bulk DO concentration. One possible cause is diffusional limitations at the floc level which may reduce the nitrification rate (Melcer et al., 20003).

The conditions in the SBRs were well mixed provided by a separate mechanical mixer and the DO concentrations were maintained by on-off timer. The DO levels averaged above the recommended minimum of 2 mg/L during the important react mode. A sample DO average profile is shown in Figure 4-2 for SBR 1-2 and 2-2 operated at the high SRT and 10 and 20 °C respectively. Figure 4-2 shows the complete four 6 hour cycles through the full day for SBR 1-2 and 2-2. The DO cycling is evident in Figures 4-2 and 4-3 (all SBRs) and corresponds to low points (< 0.5 mg/L) at the beginning of the Fill Cycle, medium level (2-4 mg/L) during the React Cycle and high points (> 4 mg/L) at the end of the React Cycle in each of the 6 hour cycle time.

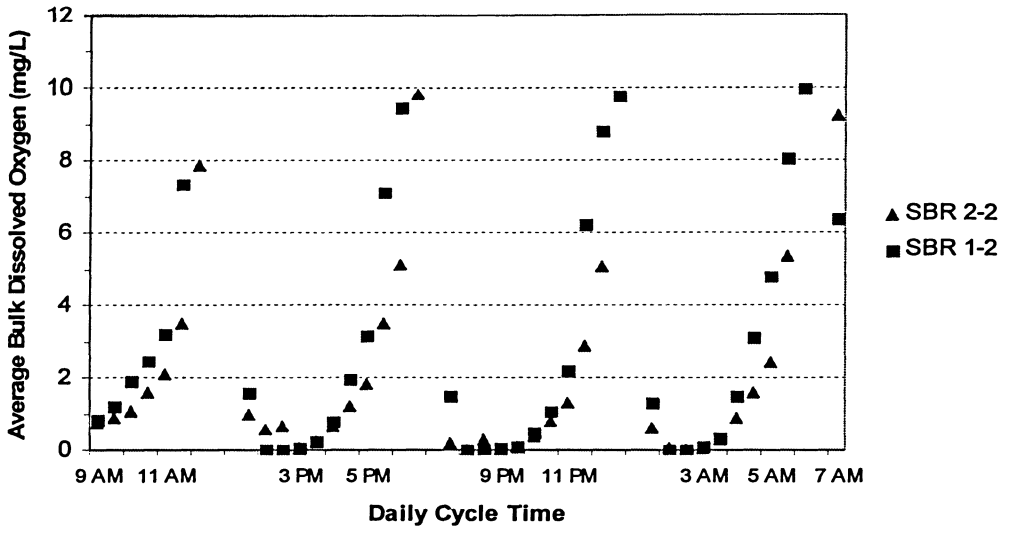


Figure 4-2. Average bulk dissolved oxygen profiles for the full day cycle in SBR 1-2 and 2-2 operated at 11 and 10 days SRT.

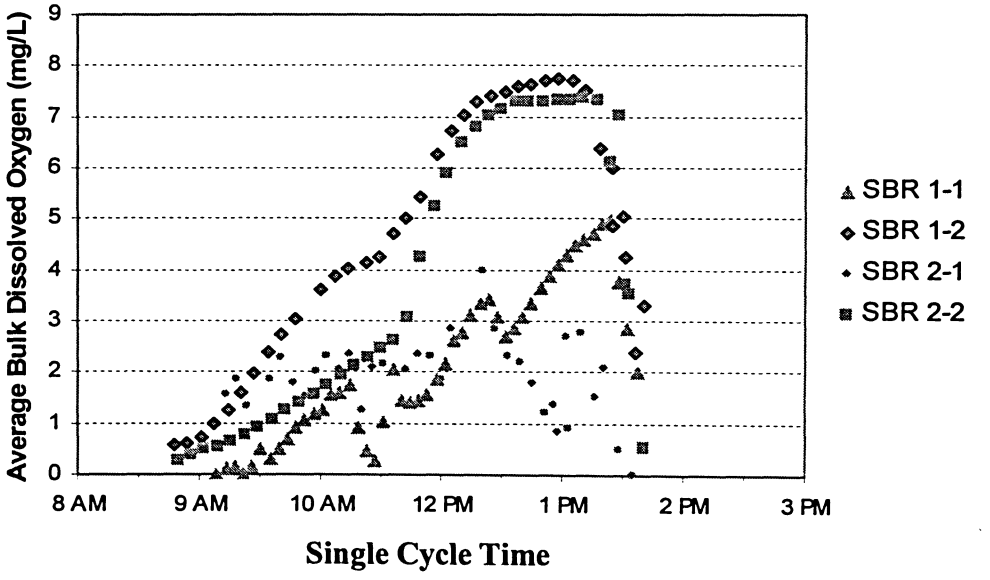


Figure 4-3. A single 6-hour cycle comparison among the SBRs of the average bulk dissolved oxygen profile.

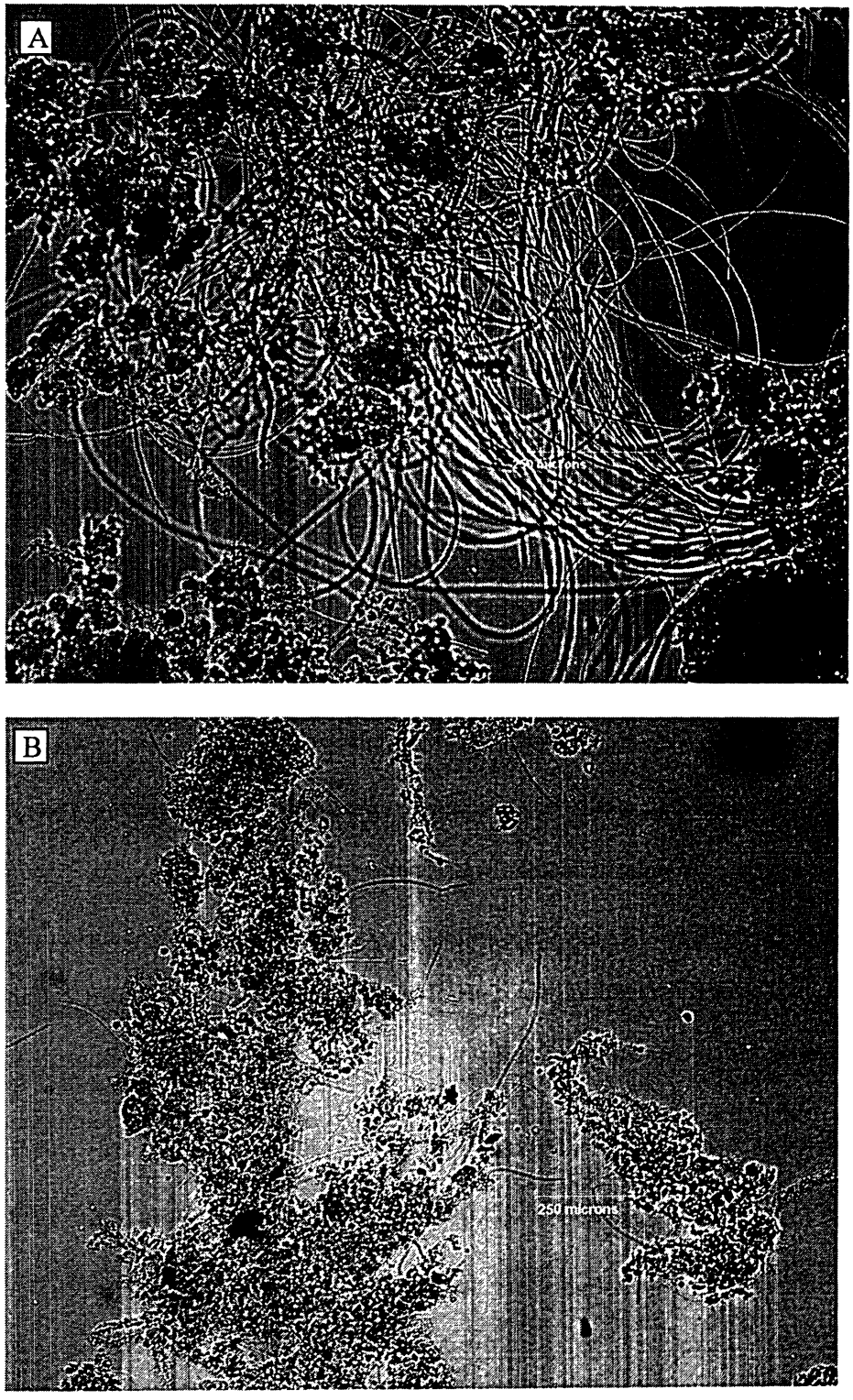


Figure 4-4. Sample photomicrograph of activated sludge flocs from SBR operated at 11 days SRT and 10 °C: (A) with excessive filaments when showing poor settling characteristics; (B) good settling biomass (COM 400x, DIC, DICII 2.3V).

Table 3-3 shows the air/mixing operation for the SBR and the low DO was expected since the air was turned off during the Settle and Draw Cycle, just before the Fill Cycle. The DO profiles were equivalent in each SBR and were typical of this mode of bench scale SBR operations.

Figure 4-5 shows reactor profiles for pH and T and Figure 4-6 shows daily pH and T readings taken once daily during the operation of the reactors. The pH profiles were relatively constant which indicates that there was adequate buffering provided by carbonates within the sewage. Typically sewage alkalinity is in the range of 60 to 120 mg/L as $CaCO_3$ (Metcalfe and Eddy, 2003) and the MOE recommends a minimum value of 50 mg/L as $CaCO_3$ to ensure pH remains in the recommended 6.5 to 8.5 range. Although carbonates were not monitored the fact that pH remained within the recommended MOE range, particularly during nitrification in SBR 2-2, suggests that alkalinity was not adversely depleted. Water jackets were designed and utilized for the reactors to allow temperature controlled conditions by circulating water at 8 and 18 °C around the reactors to maintain the required 10 and 20 °C operating temperatures within the SBRs. The reactor temperature profiles in Figure 4-6 indicate that the water jackets were effective.

The daily profiles in Figure 4-6, indicate very stable pH and T readings throughout the reactor operations. The average readings were pH of 7.8 ± 0.4 and 7.7 ± 0.4 for SBR 1-1 and 2-1, respectively, which are within biological tolerance limits. The temperature profile shows an average of 11 ± 1 and 19 ± 1 °C for SBR 1-1 and 2-1, respectively, which matched our operating set points.

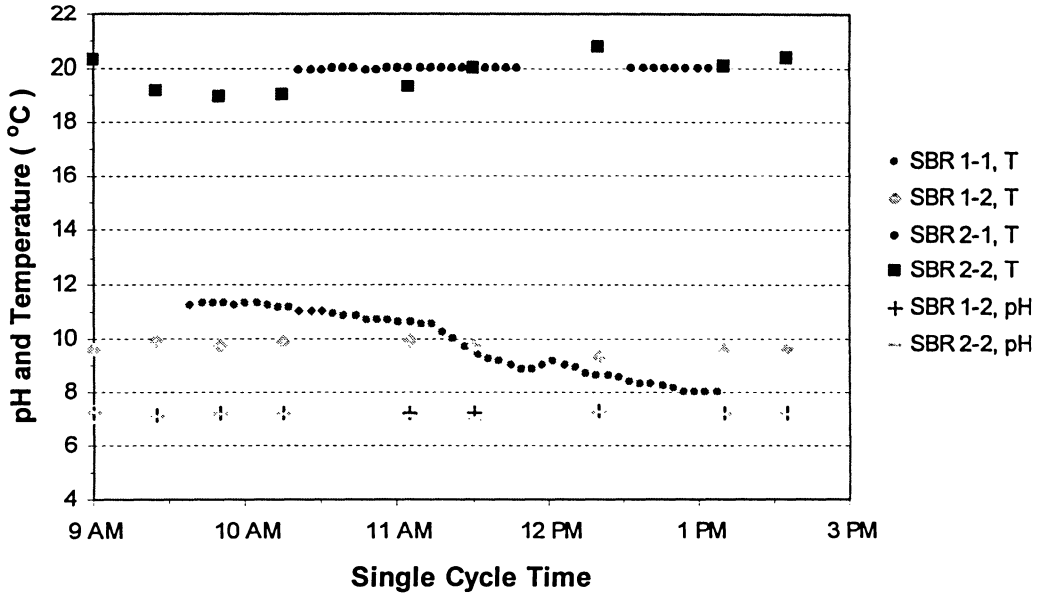


Figure 4-5. Sample temperature and pH profile comparison for first 6-hour cycle in SBR reactors.

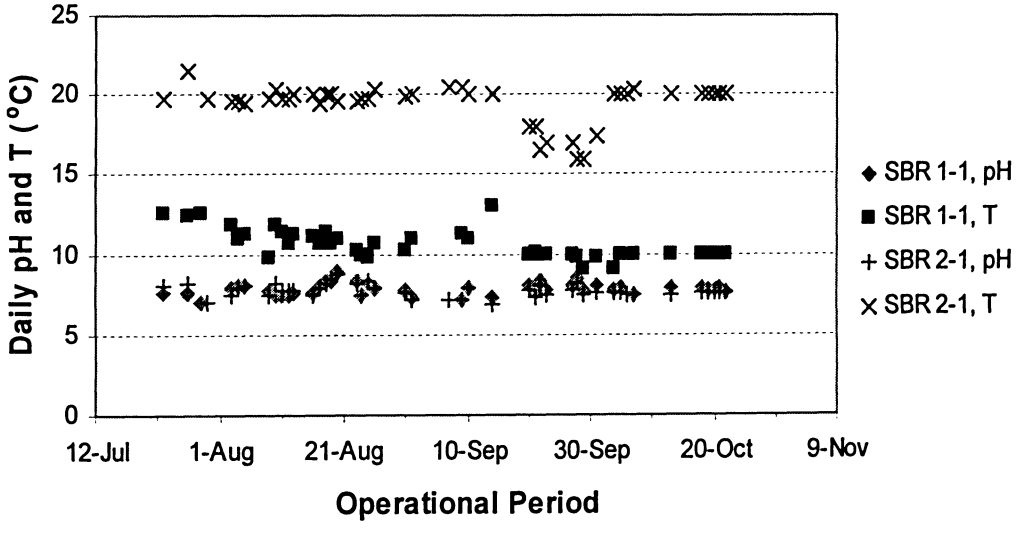


Figure 4-6. Daily temperature and pH readings from SBR 1-1 and 2-1 during Phase I (July to November 2004) of the operational period of the SBRs.

4.2. Activated Sludge Floc Morphological Characteristics

Certain activated sludge (AS) floc properties were considered indicative of possible removal of microcontaminants. The AS floc properties included the sludge floc size distribution, settling velocity, porosity, excess density, the relative hydrophobicity (RH), the surface charge (SC), the extracellular polymeric substances (EPS) content and EPS components. The EPS components included total proteins, total carbohydrates, total acidic polysaccharides and total deoxyribonucleic acid (DNA).

The following subsections compare the floc physicochemical sampled results under the different operating conditions.

4.2.1 Size distribution of activated sludge flocs

The sludge floc nominal diameter distributions were determined from the analysis of 24-hour composite sludge samples using the plankton chamber analysis (Li and Ganczarczyk, 1987, 1990; Droppo et al., 1997). More than 45000 AS flocs, in total, from three reactor conditions were analysed from 24-hour composite samples. The 50 (d_{50}) and 90 percentile (d_{90}) floc diameters were computed and used for comparison between the SBRs.

Figure 4-7 shows the cumulative distribution of AS floc versus their nominal length. The activated sludge samples from the reactors operated at 10 and 11 days SRT were combined and compared to the AS samples from the reactors at 3.4 and 3.5 days SRT. Each curve in Figure 4-7 represents a total of about 5000 flocs (see Appendix H).

The d_{50} values at 3.5 and 10.5 days SRT were 13 ± 2 and 10.5 ± 0.2 μm, respectively. The d_{90}, at 3.5 and 10.5 days SRT were 51 ± 15 and 32 ± 7 μm, respectively. When compared the 50 and 90 percentile floc diameter in the higher SRT reactors were significantly (Z-test, $p < 0.05$) smaller by $20 \pm 2\%$ and $38 \pm 15\%$, respectively.

The floc distributions at the lower SRT of 3.5 days at 10 and 20 °C, both at the 50 and 90 percentile level, did not show any significant (t-test, $p > 0.5$) difference. The lack of T influence on the 50 and 90 percentile floc diameters at the lower SRT, suggests that T changes even may not influence floc diameter changes at the 10.5 days SRT. This assumption unfortunately could not be verified due to sample corruption.

The floc distribution at different SRTs is consistent with the results of Andreadakis et al. (1993), which indicate a tendency to smaller flocs at the higher SRT operating conditions.

The distribution of the floc nominal size at the higher SRT also exhibited a smaller range in size. Approximately 60 % of the flocs at the 10.5 days SRT were between the range of 10-32 μm while the range was 10-51 μm at the lower SRT of 3.5 days.

The larger number of smaller flocs at the higher SRT may increase the available surface area within a given volume and increase the available sorption sites for microcontaminants. A direct relationship between diameter to surface area is however complicated by the known porous and fractal nature of flocs (Li and Ganczarczyk, 1987; Andreadakis, 1993). The specific surface area of flocs was examined by Andreadakis et al., (1993) by a dye adsorption technique and found to be in the range of 100-200 m^2/g dry matter.

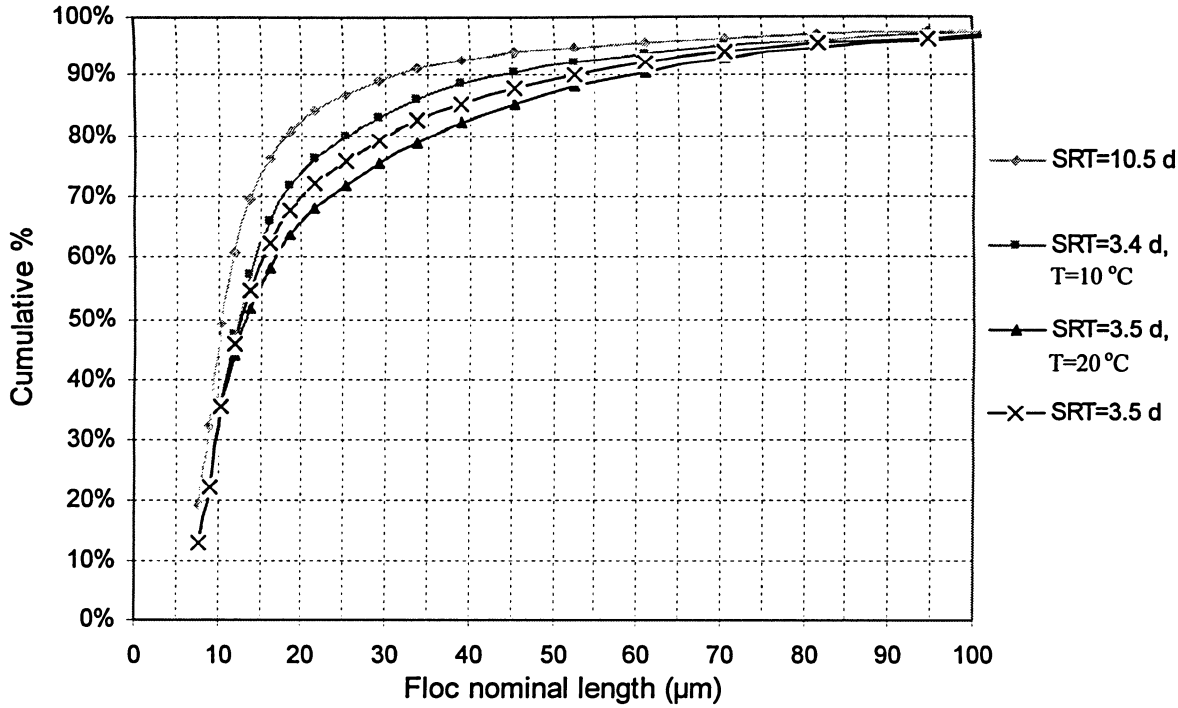


Figure 4-7. Floc size distribution from 5 to 100 µm at different sludge ages and temperatures.

Table 4-3. Mean sludge floc size comparison based on Student's t-test analysis of about 5000 flocs per sample with a total of 9 daily 24-hour composite samples.

SBR Phase and p-value Identifier	Operating SRT (d)	T (°C)	Floc Diameter (µm)		Mean Floc Percent Difference	
			d_{50}	d_{90}	d_{50} (% difference)	d_{90} (% difference)
SBR 1-1 (n=3)	3.4 ± 0.2	10 ± 1	13 ± 3	43 ± 19	4 ± 2 %	27 ± 19
SBR 2-1 (n=3)	3.5 ± 0.2	20 ± 1	13 ± 1	59 ± 5		
p-value	-	-	0.69	0.20		
Low SRT[1] (n=6)	3.45 ± 0.2	-	13 ± 2	51 ± 15	20 ± 2 %	38 ± 15 %
High SRT[2] (n=3)	10.5 ± 1	-	10.5 ± 0.4	32 ± 7		
p-value	-	-	**0.0001**	**0.01**		

1. Sequencing batch reactors SBR 2-1 and SBR 1-1 were combined to form the Low SRT group.
2. Sequencing batch reactors SBR 1-2 and SBR 2-2 were combined to form the High SRT group.
3. d_{50} and d_{90} refers to the 50 and 90 percentile floc nominal length of in µm; on average 50% and 90%, respectively, of all the AS flocs are below these nominal lengths.

4.2.2 Settling Velocity, Porosity and Excess Density of Sludge Flocs

Typical observed distribution graphs of sludge floc nominal linear length with floc settling velocity (v), porosity (ε) and excess density (ρ_e) are shown in Figure 4-8 and 4-9. The mean v, ε and ρ_e of the sludge floc distributions were assumed to reflect the mean floc characteristics under the operating conditions within each SBR and compared. The results of the comparison are provided in Table 4-4.

The results suggest that T did not influence the v, ε and ρ_e significantly at the 3.5 days SRT operating conditions. When the data from the low SRT was grouped together and compared to the high SRT, the mean v, ε and ρ_e, a significant (t-test, $p < 0.05$) difference was evident.

The mean settling flocs at the 10.5 days SRT were significantly (t-test, $p < 0.05$) less porous by about 6 %, more dense by 55 %, smaller by 26 % and had a higher settling velocity by 36% then the mean settling flocs at the 3.5 days SRT (see Table 4-4).

Table 4-4. Mean sludge floc settling velocity, porosity and excess density comparison based on analysis of about 100 flocs per sample with a total of nine 24-hour composite samples.

SBR Phase and p-value Identifier	Operating SRT (d)	T (°C)	Mean Floc Diameter d_{50} (μm)	Mean Floc Settling Velocity ω (mm/s)	Mean Floc Porosity ε (%)	Mean Floc Excess Density ρ_e (g/mL)
SBR 1-1 (n=3)	3.4 ± 0.2	10 ± 1	291 ± 45	1.0 ± 0.4	94 ± 2	0.04 ± 0.01
SBR 2-1 (n=3)	3.5 ± 0.2	20 ± 1	338 ± 83	0.8 ± 0.3	96 ± 1	0.02 ± 0.01
p-value	-	-	0.40	0.42	0.210	0.210
Low SRT[1]	3.45 ± 0.2	-	315 ± 65	0.9 ± 0.4	95 ± 2	0.03 ± 0.01
High SRT[2]	10.5 ± 1	-	225 ± 75	1.4 ± 0.1	89 ± 2	0.07 ± 0.02
p-value	-	-	**0.001**	**0.01**	-	-

1. Sequencing batch reactors SBR 2-1 and SBR 1-1 (n=6) results were combined to form the Low SRT group.
2. Sequencing batch reactors SBR 1-2 and SBR 2-2 (n=3) results were combined to form the High SRT group.

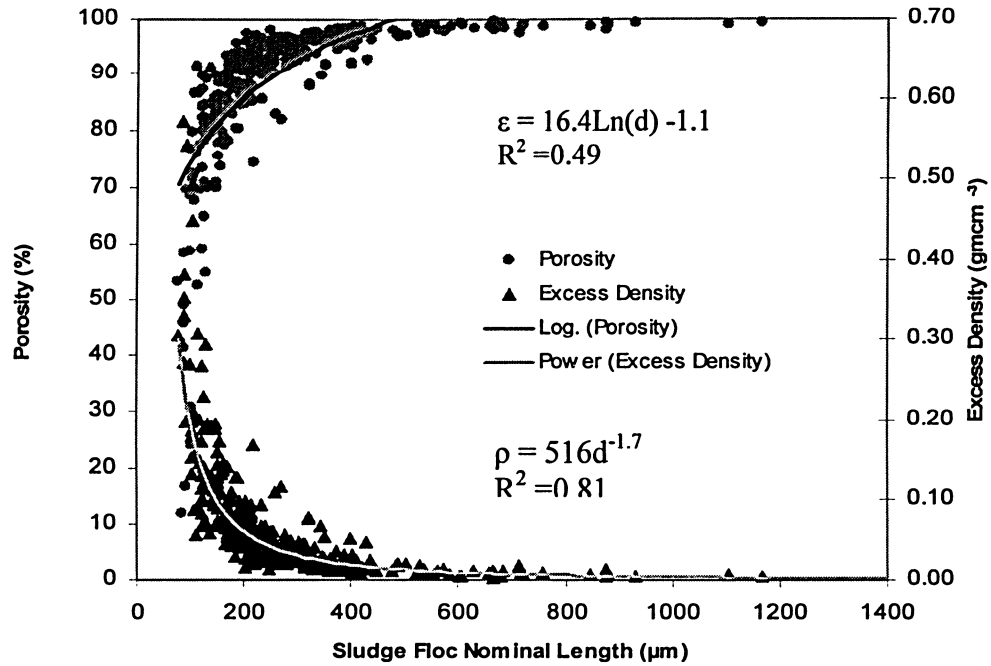


Figure 4-8. Typical floc size distribution and equations (319 flocs) with respect to percent porosity and excess density for the combined sludge from SBRs operated at 10 and 11 days SRT.

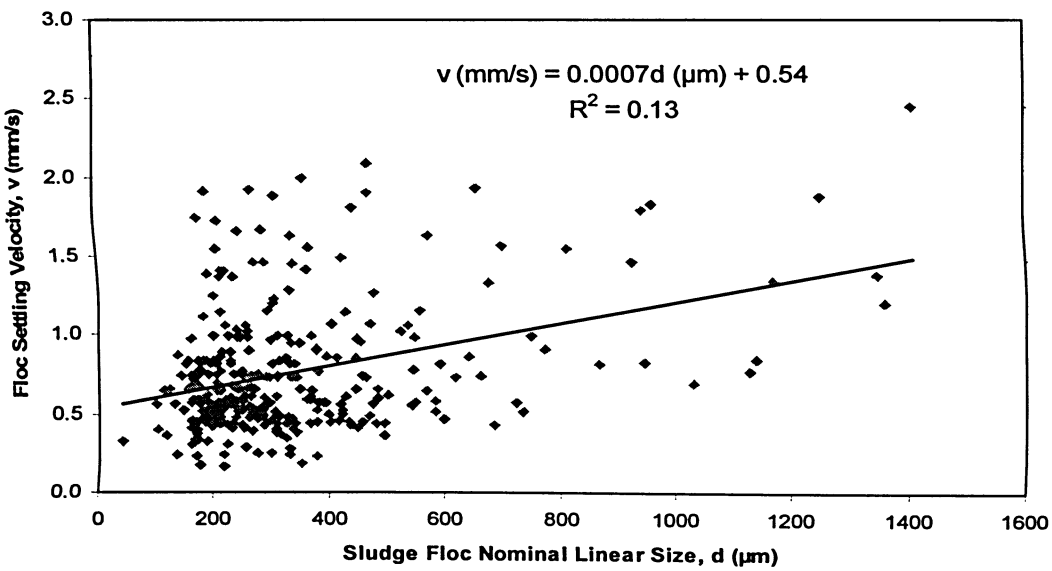


Figure 4-9. Typical floc size versus settling velocity distribution and equation (319 total flocs) from SBR 2-1 operated at 3.5 days SRT and 20 °C.

4.3. Sludge Extracellular Polymeric Substances

In addition to microorganisms and other particles, activated sludge flocs consist of a heterogeneous polymeric matrix, which includes neutral and acidic polysaccharides, lipopolysaccharides, proteins, nucleic acids and humic acids, generally referred to as extracellular polymeric substances (EPS) (Bura et al., 1998). In this study the most abundant components of EPS which include proteins, carbohydrates, acidic polysaccharides and DNA, were analysed. The total EPS, based on the sum of the components, was determined and compared.

Figure 4-10 provides the concentration of EPS sludge components under stable operating conditions, at the selected T and SRT values. In all cases the protein fraction (79 to 167 mg/g MLSS), followed by carbohydrates (13 to 66 mg/g MLSS) and acidic polysaccharides (2 to 7 mg/g MLSS) were the largest components of EPS with DNA (0.6 to 6 mg/g MLSS) being consistently the least part. This EPS component distribution is in agreement with previous reports where real sewage was used (Frølund et al., 1994; Bura et al 1998). In cases where a synthetic feed was used acidic polysaccharides were low or not measured (Liao et al., 2001) however the other EPS component distributions were in agreement.

The data generated under the four different operating conditions (see Figure 4-10) was regrouped and considered under six different conditions:

(1) Low SRT (3.5 days, SBR 1-1 and 2-1, n=8);
(2) High SRT (10.5 days, SBR 1-2, 2-2, n=8);
(3) Low T (10 °C, SBR 1-1 and 1-2, n=8);
(4) High T (20 °C, SBR 2-1 and 2-2, n=8);
(5) Nitrifying (SRT of 10 days and 20 °C, SBR 2-2, n=4); and
(6) Non-nitrifying condition (SRT of 3.5 days, 10 and 20 °C, SBR 1-1 and 1-2 n=8).

Three comparisons were conducted for significant differences between the Low and High T and SRT conditions as well as the nitrifying and non-nitrifying conditions (see Table 4-5).

Among the six groups there were no overlaps between reactors. Of the six groupings however group (3) Low T and group (6) Non-nitrifying, turned out to be the same grouping of SBRs. The separation of T and SRT operating parameters and grouping of SBRs was justified by the ANOVA interaction term (TxSRT) analysis which revealed that there is no significant interaction term that affects EPS components under our experimental conditions (see Table 4-5). Further the separation of variables is supported by the previous results in section 4.2 which showed that the temperature difference was not a significant factor in influencing the morphological characteristics tested (see Table 4-3 and 4-4).

The mean EPS protein to carbohydrate ratio was found to vary from 4 to 8 (see Figure 4-11) consistent with previously reported values (Jorand et al., 1995 and 1998; Frølund et al., 1996; Liao et al., 2001) and was not significantly different under the different SRT, T or nitrifying conditions evaluated (see Table 4-6). With the use of synthetic feed, the ratio of proteins to carbohydrates has been reported to increase from about 2 to 5 with increasing SRT from 4 to 12 days, respectively, and found to level off at 5 at higher SRTs (Liao et al., 2001). The ratio range observed of 3 to 8, was however not significantly different at the operating SRTs of 3.5 and 10.5 days.

The individual EPS constituents were observed to change significantly with changes in SRT (see Table 4-5). The EPS protein, carbohydrates and total EPS concentration increased significantly (ANOVA, $p < 0.05$) with increasing SRT from 3.5 to 10.5 days; the EPS DNA and acidic polysaccharides decreased significantly (ANOVA, $p < 0.5$) with similar changes in SRT. No significant (ANOVA, $p > 0.05$) temperature effects on the EPS components were observed between 10 and 20 °C. The results suggest that activated sludge at a higher SRT consists of higher protein and carbohydrates but less DNA and acidic polysaccharides. Protein and carbohydrate changes in sludge flocs EPS are expected to influence the physicochemical sludge floc surface properties such as hydrophobicity which are known to influence the surface interactions of sludge flocs such as flocculation and adhesion (Hsu et al., 2002).

Under nitrification (NI) significant (ANOVA, $p < 0.5$) increases in EPS proteins, carbohydrates and total EPS were observed and a corresponding significant (ANOVA, $p < 0.5$) decrease in EPS DNA and polysaccharides were found. The changes in EPS found under NI versus non-NI conditions were similar to the High SRT versus the Low SRT operating conditions, respectively.

The negative correlation between protein and DNA content in the EPS, under all operating conditions, is consistent with work by Sponza et al., (2003), which showed a high correlation associated with a high protein content and low DNA in activated sludge flocs from municipal, pulp-paper, petrochemical and winery wastewater.

There was no significant (ANOVA, $p > 0.5$) change in EPS protein to carbohydrates ratio under the different SRT, T and NI conditions investigated. The ratio of protein to carbohydrates has been reported to vary depending on wastewater feed nutrient composition from 1 to as high as 13 in full scale municipal activated sludge STP (Bura et al., 1998; Liao et al., 2001; Jin et al., 2003).

No significant (ANOVA, $p > 0.5$) differences in the mean EPS constituents due to T or interactions, TxSRT factor, were observed under experimental conditions. No other studies could be found which considered temperature effects on EPS constituents in the range of 10 and 20 °C.

The observed changes in EPS characteristics with changes in SRT and nitrifying conditions suggest changes in the surface properties of sludge flocs, such as hydrophobicity and surface charge and may in turn impact how microcontaminants interact with the different sludge flocs. Work by Jorand et al. (2003) and a recent review article, by Raszka et al. (2006), identify EPS constituents as being of primary importance for the structural and functional integrity of flocs and further to influence the physicochemical and biological properties such as sorption of exogeneous organic compounds.

Acidic polysaccharides with their abundance of hydroxyl groups (Flemming et al., 1996) are considered to play an important role in maintaining the EPS highly hydrated by attracting water molecules.

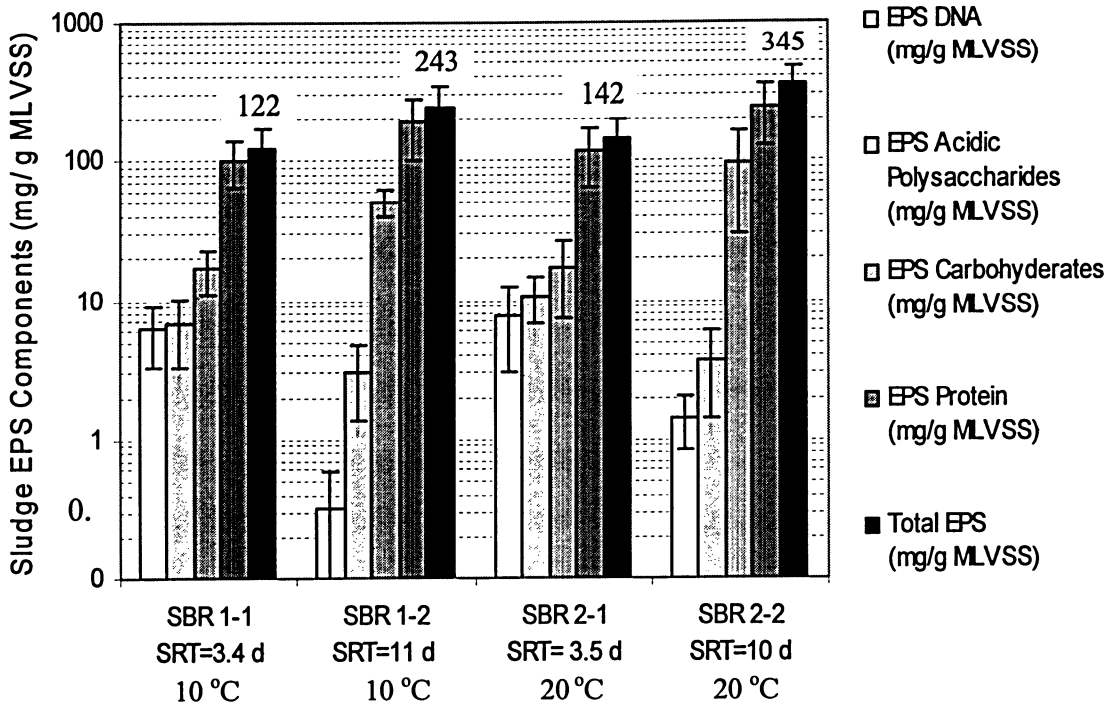


Figure 4-10. Comparison of the average EPS sludge components under the four different operating conditions expressed as the mean ± one standard deviation. The bar labels refer to the total EPS in mg/g MLVSS.

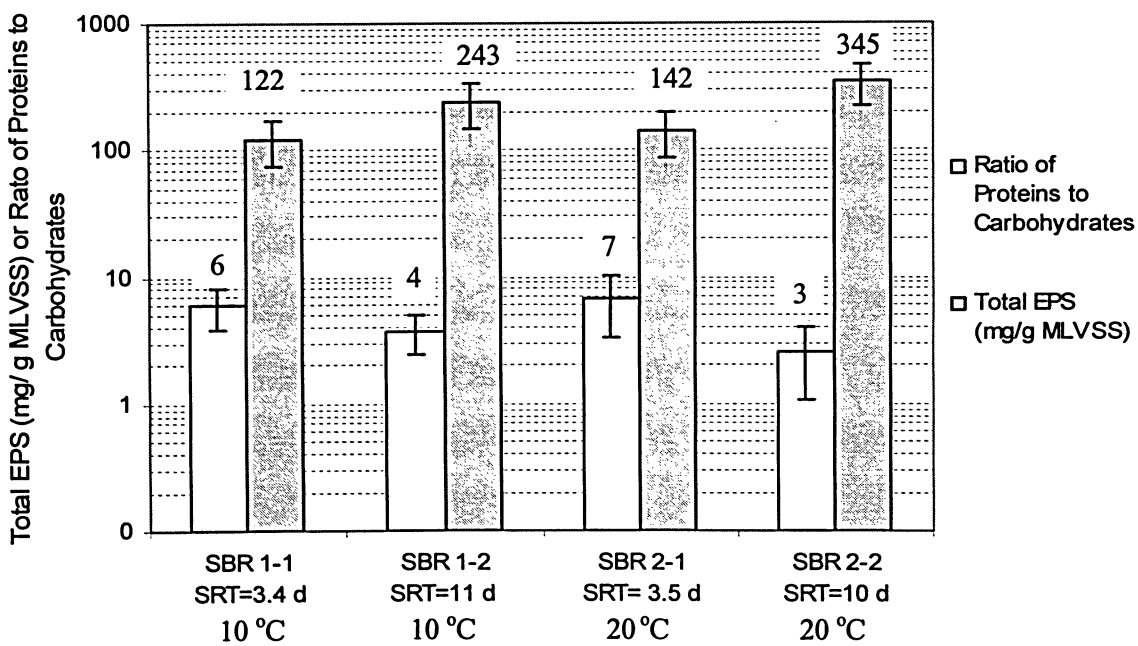


Figure 4-11. Effect of operating conditions on the average (n=4) total EPS and ratio of EPS proteins to carbohydrates expressed as mean ± one standard deviation. The lower bar labels refer to the ratio and the upper bar labels refer to the total EPS.

Table 4-5. ANOVA and Student's t-test comparisons of EPS components under different operating conditions with p-value at the 95% confidence level

Operational Conditions and p-value	Total EPS and EPS Components (mg/g MLVSS)					
	EPS DNA	EPS Acidic Polysac-charides	EPS Carbohy-drates	EPS Protein	EPS Proteins to Carbohy-drates Ratio	Total EPS
Low SRT (n=8)	7 ± 4	9 ± 4	17 ± 8	110 ± 46	6 ± 3	132 ± 52
High SRT (n=8)	0.9 ± 0.5	3 ± 2	74 ± 34	216 ± 100	3 ± 1	294 ± 108
p-value	**0.001**	**0.001**	**0.008**	**0.03**	0.6	**0.02**
Low T (n=8)	3 ± 2	5 ± 3	34 ± 10	146 ± 66	5 ± 2	183 ± 71
High T (n=8)	5 ± 2	7 ± 4	57 ± 36	180 ± 83	5 ± 3	243 ± 90
p-value	0.86	0.38	0.24	0.50	0.50	0.33
Nitrification (n=4)	1.4 ± 0.6	4 ± 2	97 ± 67	243 ± 114	3 ± 2	345 ± 119
Non-Nitrification (n=8)	7 ± 4	9 ± 4	17 ± 8	110 ± 46	6 ± 3	132 ± 52
p-value	**0.001**	**0.01**	**0.006**	**0.02**	0.93	**0.006**

Further as polymers, polysaccharides and proteins, have been identified as promoting bioflocculation which assists in aggregation and improving the settleability of sludge flocs and ensuring that the AS process can achieve low ESS in the final effluent (Raszka et al., 2006).

4.4. Influence of T and SRT on Sludge Hydrophobicity (RH) and Surface Charge (SC)

Sludge relative hydrophobicity (RH) and surface charge (SC) are dominant surface properties which have been reported to have a strong negative correlation, be influenced by EPS constituents, by wastewater substrate type, operational conditions (i.e., SRT and F/M ratio) and in turn have a strong influence on floc behaviour such as flocculating ability, settleability, compressibility, dewaterability and floc stability (Liao et al., 2001, 2002 and 2006; Jin et al., 2003; Sponza et al., 2002 and 2003; Wilén et al., 2003; Jin et al., 2004).

The mean sludge relative hydrophobicity (RH) of 83% at the 10.5 days SRT was calculated to be significantly (ANOVA, $p < 0.5$) more hydrophobic than the sludge at 3.5 days SRT with a 73% RH at an average pH of about 7.4 ± 0.5 (see Figure 4-12 and Table 4-7). In an evaluation of five full scale AS STPs operated at 4 to 35 days SRT, Jin et al. (2003), found the hydrophobicity to be

between 60 to 70 % with a RH of 68% at 4 days SRT and 60% at 12 days SRT. This is opposite to our finding.

The RH was also reported to be pH dependent, to increase with increasing SRT and increase with lower F/M ratio (Allison et al., 1990; Pere et al., 1990; Frølund et al., 1994 and 1996; Liao et al., 2001). The RH was found to increase with SRT up to 9 days and then found to plateau at an SRT of 16 to 20 days, during bench scale studies (Liao et al., 2001). The cumulative findings suggest SRT to be a dominant influence on RH which generally increases with increasing SRT.

The corresponding measured mean SCs were -0.53 and -0.63 meq/g MLSS, respectively, at 10.5 and 3.5 days SRTs. These values are on the high end of reported range of SCs of -0.3 to -0.6 meq/g MLSS. Generally a higher RH has been found to be associated with the less negative SC. Sponza et al. (2003) and Bura et al. (1998) found a strong negative correlation between SC and RH. Typically more hydrophobic flocs with less negatively charged surfaces correspond to a higher SVI and poorer settling sludge (Sponza et al., 2003).

The overall combined SC in this study was -0.6 ± 0.2 meq/g MLSS at about pH of 7.4 ± 0.5. in good agreement with previous reported SC -0.67 meq/g MLSS at pH of about 7.2 (Liao et al., 2001).

Table 4-6 provides results of the ANOVA and Student t-tests analysis and shows that SRT generally has a more dominant and significant influence on RH with T and the interaction term SRTxT playing a significant but secondary influence (ANOVA, $p<0.05$, F of 70 to 10). The surface charge was not found to be significantly (ANOVA, $p < 0.5$) different under the experimental conditions.

The analysis results, in Table 4-6, suggest that SC is not significantly influenced by SRT, T or SRTxT at the range between 3.5 and 11 days SRT or at the temperature range of 10 to 20 °C (ANOVA, $p>0.05$) operating conditions. The results of this study are consistent with the reported similar SC in the range of -0.5 to -0.4 meq/g MLSS observed in the range of 4 to 16 days SRT, respectively (Liao et al., 2001).

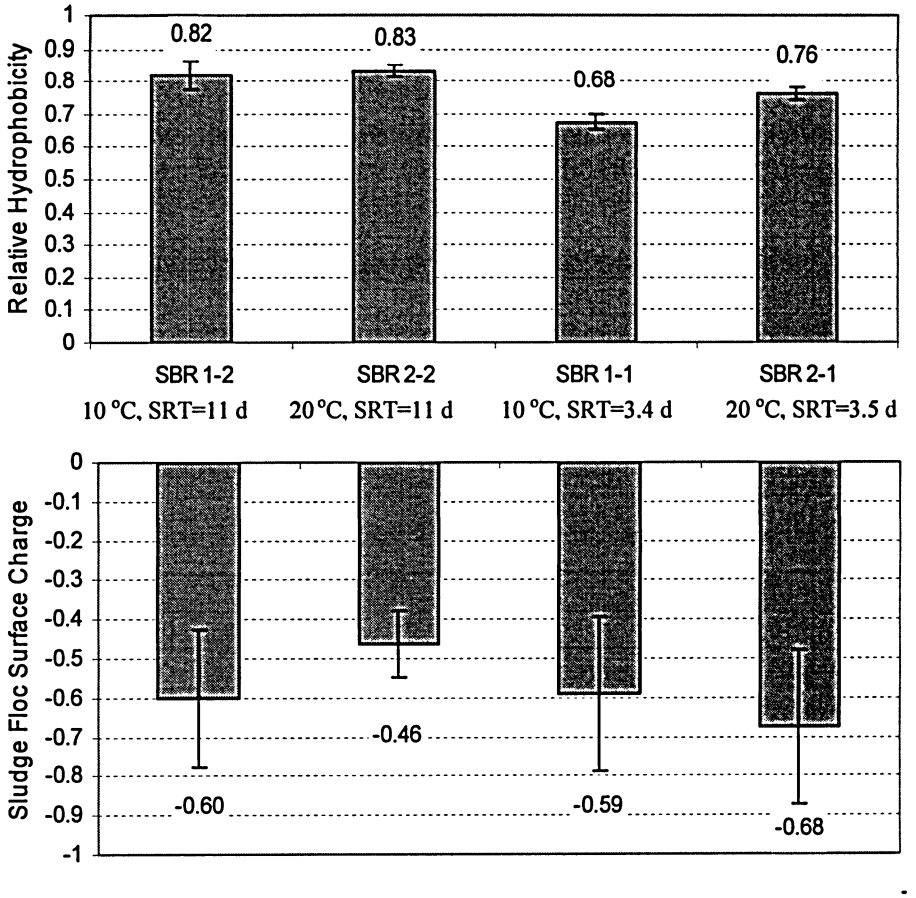


Figure 4-12. Sludge mean (n=5) relative hydrophobicity and corresponding floc surface charge under the four different operating conditions. The error bars refer to a standard deviation from the mean.

The negative surface charge attributed to sludge is considered to be primarily from the ionization of functional groups such as carboxylic, sulphate and phosphate associated with the polymers in the EPS (Wilén et al., 2003). A significant difference in EPS constituents were found at the different SRTs (see Table 4-6) however the SC only showed a marginal insignificant difference in our study. This alludes to the complex nature of the role of EPS constituents in determining sludge floc charge properties and the difficult task in resolving this phenomenon (Jin et al., 2003).

Table 4-6. ANOVA and Student's t-test analysis results of surface charge (SC) and relative hydrophobicity (RH) comparisons between individual SBRs and SBRs combined at equal SRT and T, at the 95% confidence level

SBR Phase and p-value Identifier	Operating SRT(d)	Temperature (°C)	Mean Surface Charge SC (meq/ g MLSS)	Mean % Sludge Relative Hydrophobicity (SH)
SBR 1-1 (n=5)	3.4 ± 0.2	10 ± 1	-0.6 ± 0.2	68 ± 4 %
SBR 2-1 (n=5)	3.5 ± 0.2	20 ± 1	-0.7 ± 0.1	76 ± 2 %
p-value	-	-	0.15	0.46
SBR 1-2 (n=5)	11 ± 1	10 ± 1	-0.6 ± 0.2	82 ± 4 %
SBR 2-2 (n=5)	10 ± 1	20 ± 1	-0.5 ± 0.1	83 ± 2 %
p-value	-	-	0.15	0.46
High SRT (n=10)	10.5	-	-0.5 ± 0.1	83 ± 3 %
Low SRT (n=10)	3.45	-	-0.6 ± 0.2	72 ± 5 %
p-value	-	-	0.23	**0.0002**
Low T (n=10)	-	10 ± 1	-0.6 ± 0.2	76 ± 8 %
High T (n=10)	-	20 ± 1	-0.6 ± 0.2	80 ± 4 %
p-value	-	-	0.76	0.16
Nitrification (n=5)	10 ± 1	20 ± 1	-0.46 ± 0.08	83 ± 2 %
Non-Nitrification (n=10)	3.5 ± 0.2	10± 1, 20 ± 1	-0.6 ± 0.2	72 ± 5 %
p-value	-	-	0.053	**0.004**

ANOVA Results for RH			**ANOVA RH Model Analysis (Type I, SS)**		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	70	**0.001**	0.85	31	**0.001**
T	13	**0.002**			
TxSRT	10	**0.006**			

ANOVA Results for SC			**ANOVA SC Model Analysis (Type I, SS)**		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	1.7	0.2	0.2	1.4	0.2
T	0.1	0.7			
TxSRT	2.2	0.2			

4.5. Musks in the Aqueous and Solids Matrix of the SBRs

One of the objectives of this study was to evaluate the removal of selected polycyclic synthetic musks (PSMs) under the different SRT and T operating conditions and to relate the results back to sludge floc properties. The average concentrations of the PSMs associated with the effluent and solids is shown in Figures 4-13 and 4-16.

The PSMs in the effluent were compared to the influent and 62 to 80% removals based on influent minus effluent concentrations, were observed (see Figure 4-14). Generally the reactors operated at higher T and higher SRT provided a significantly ($p< 0.05$ or $K > 7.33$) reduced effluent PSMs concentration particularly for Galaxolide, Tonalide and total PSMs. Galaxolide and Tonalide together represented more than 95% of the total PSMs. For this reason the total PSMs, combined index of PSMs which excludes Cashmeran, responds similarly to Galaxolide and Tonalide. The results in Figure 4-13 and 4-14 represent a five day composite sampling period and the standard deviations were based on paired comparisons.

The difference dataset was analysed for underlying distribution for each PSM and found that all the difference concentrations with the exception of Phantolide, followed a Normal distribution. Normality was confirmed at the 95% confidence level based on the combined agreement of the Chi-square and Kolmogorov-Smirnov tests (see Table K-2).

The aqueous difference in PSM concentration between the influent and effluent was calculated (see Table K-1, Appendix K) and the dataset distribution was determined to be normal for all PSMs except Phantolide (see Table K-2, Appendix K). An ANOVA analysis on the full dataset was conducted and the results summarized in Table 4-7 indicated that SRT is the dominant determining factor followed by T and the interaction term TxSRT.

The total concentration of PSMs in the solids was in the range of 17 to 24 μg/g d.m.. This represents approximately a 3 to 4 order of magnitude (on a weight basis) increase of PSMs associated with solids as compared to the concentration of PSMs in the aqueous phase. This is consistent with

the high K_{OW} values (Log Kow of 4 to 6.3, see Table 2-7(A)) associated with PSMs which promote partitioning to solids.

When comparing the PSMs associated with solids (see Table 4-8) the largest significant difference (K values from 16 to 25) was between nitrifying and non-nitrifying conditions, followed by high T versus low T (K values from 10 to 18) and then the high SRT versus the low SRT (K values of 7 to 12) operated at 10 °C. There are significant differences observed under various combinations of T and SRT conditions which suggest that there is also a significant interaction, TxSRT, effect at work.

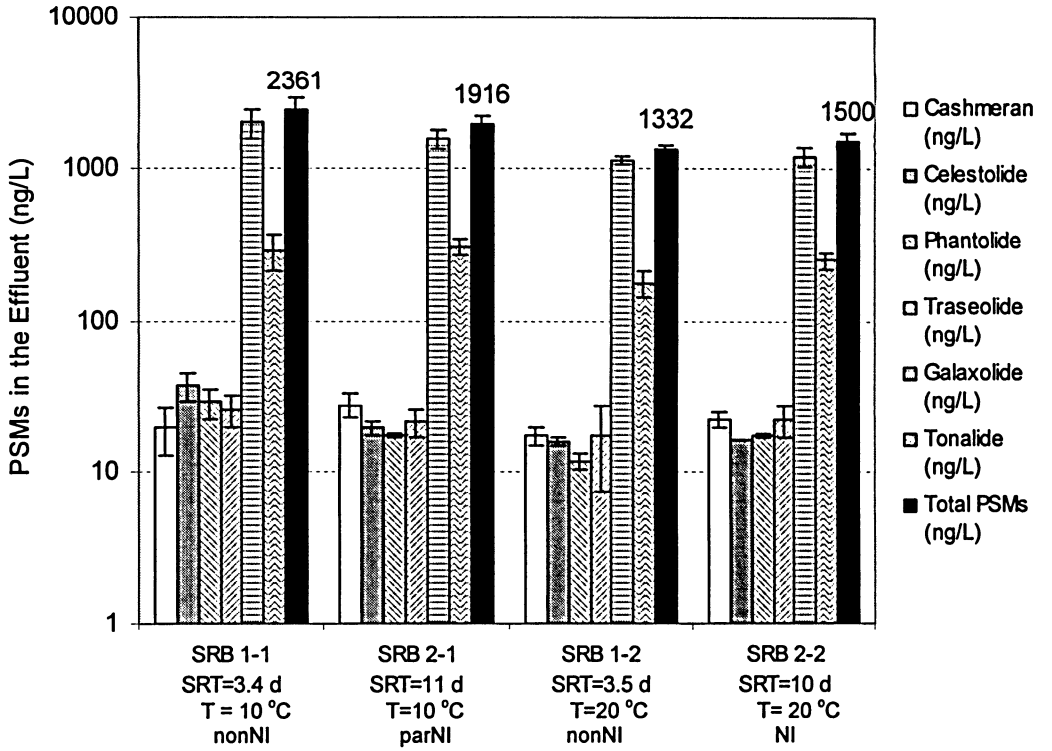


Figure 4-13. Mean (n=5) effluent musk concentrations under the four different operating conditions. Bar labels refer to the total PSMs in the effluent.

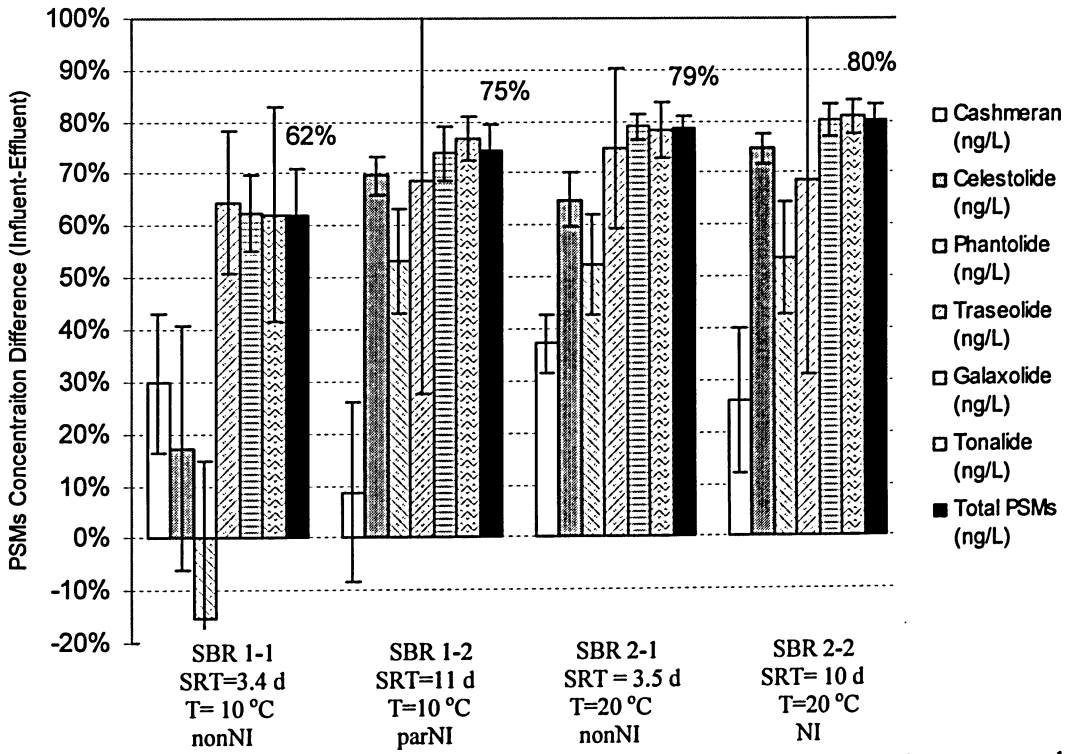


Figure 4-14. Comparison (n=5) of the mean percent total PSMs removed from the aqueous phase (Effluent –Influent) from each SBR. The labels refer to the total PSMs percent removal.

Table 4-7. ANOVA analyses of differences on influent and effluent aqueous PSM concentrations[1]

ANOVA Results for tPSM Aqueous Difference			**ANOVA Model Analysis (Type I, SS)**			
Parameters	F-value	p-value	SS	MS	Fisher Value	p-value
T	3.056	0.100	13628830	4542943	5.325	0.010
SRT	**12.367**	**0.003**	13651239	853202		
T*SRT	0.551	0.469	27280069			
ANOVA Cashmeran Difference			**ANOVA SC Model Analysis (Type I, SS)**			
Parameters	F-value	p-value	SS	MS	Fisher Value	p-value
T	4.204	0.057	139.430	46.477	2.517	0.095
SRT	2.738	0.117	295.440	18.465		
T*SRT	0.609	0.446	434.870			
ANOVA Celestolide Difference			**ANOVA SC Model Analysis (Type I, SS)**			
Parameters	F-value	p-value	SS	MS	Fisher Value	p-value
T	**11.349**	**0.004**	5055	1685	25.325	< 0.0001
SRT	**58.444**	**< 0.0001**	1064	66		
T*SRT	**6.181**	**0.024**	6120			
ANOVA Traseolide Difference			**ANOVA SC Model Analysis (Type I, SS)**			
Parameters	F-value	p-value	SS	MS	Fisher Value	p-value
T	0.027	0.871	22091	7363	2.565	0.091
SRT	**7.630**	**0.014**	45932	2870		
T*SRT	0.038	0.848	68024			
ANOVA Galaxolide Difference			**ANOVA SC Model Analysis (Type I, SS)**			
Parameters	F-value	p-value	SS	MS	Fisher Value	p-value
T	3.256	0.090	6308846	2102949	3.623	0.036
SRT	**7.053**	**0.017**	9286043	580378		
T*SRT	0.561	0.465	15594889			
ANOVA Tonalide Difference			**ANOVA SC Model Analysis (Type I, SS)**			
Parameters	F-value	p-value	SS	MS	Fisher Value	p-value
T	0.644	0.434	995297	331766	6.276	0.005
SRT	**18.105**	**0.001**	845773	52861		
T*SRT	0.080	0.781	1841070			

1. See Table K-6 for analyses of data distributions. The PSM aqueous concentration difference between the influent and effluent was considered normal for all the PSMs based on the dataset distribution analysis (see Table .

Table 4-8. Comparison of PSMs median concentrations in the sludge of the SBRs[1, 2, 3]

SBRs	Cashmeran (MDL 27 ng/L)	Celestolide (MDL 36 ng/L)	Phantolide (MDL 27 ng/L)	Traseolide (MDL 39 ng/L)	Galaxolide (MDL of 41 ng/L)	Tonalide (MDL of 32 ng/L)	Total PSMs (MDL of 41 ng/L)
SBR 1-1 (n=5)	29 ± 1	224 ± 76	179 ± 73	178 ± 49	16890 ± 5454	3832 ± 1399	21302 ± 6945
SBR 1-2 (n=5)	<MDL	207 ± 40	151 ± 30	97 ± 30	21215 ± 3596	5620 ± 864	27291 ± 4489
K-value, 3 DF	-	**11.60**	**7.60**	4.80	**10.60**	7.20	**12.40**
SBR 1-1 (n=5)	29 ± 1	224 ± 76	179 ± 73	178 ± 49	16890 ± 5454	3832 ± 1399	21302 ± 6945
SBR 2-1(n=5)	29 ± 3	155 ± 50	112 ± 70	465 ± 511	14296 ± 4196	3551 ± 1129	18580 ± 5317
K-value, 3DF	0.8	0.60	1.20	**8.80**	7.20	0.20	5.0
SBR 1-1 (n=5)	29 ± 1	224 ± 76	179 ± 73	178 ± 49	16890 ± 5454	3832 ± 1399	21302 ± 6945
SBR 2-2 (n=5)	<MDL	100 ± 10	103 ± 12	143 ± 17	11599 ±1296	3014 ± 329	14952 ± 1807
K-value, 3 DF	-	5.80	5.20	**12.40**	**8.00**	3.40	**7.80**
SBR 1-2 (n=5)	<MDL	207 ± 40	151 ± 30	97 ± 30	21215 ± 3596	5620 ± 864	27291 ± 4489
SBR 2-1(n=5)	29 ± 3	155 ± 50	112 ± 70	465 ± 511	14296 ± 4196	3551 ± 1129	18580 ± 5317
K-value, 3 DF	-	**11.00**	**8.80**	4.00	**7.60**	**7.40**	**7.40**
SBR 1-2 (n=5)	<MDL	207 ± 40	151 ± 30	97 ± 30	21215 ± 3596	5620 ± 864	27291 ± 4489
SBR 2-2 (n=5)	<MDL	100 ± 10	103 ± 12	143 ± 17	11599 ±1296	3014 ± 329	14952 ± 1807
K-value, 3 DF	-	5.80	2.40	7.60	3.20	**10.60**	4.60
SBR 2-1 (n=5)	29 ± 3	155 ± 50	112 ± 70	465 ± 511	14296 ± 4196	3551 ± 1129	18580 ± 5317
SBR 2-2 (n=5)	<MDL	100 ± 10	103 ± 12	143 ± 17	11599 ±1296	3014 ± 329	14952 ± 1807
K-value, 4 DF	-	5.20	6.40	3.60	0.20	0.40	2.80
Low T (n=10)	< MDL	215 ± 58	165 ± 55	137 ± 57	19052 ± 4915	4726 ± 1446	24296 ± 6352
High T (n=10)	<MDL	128 ± 45	108 ± 48	302 ± 381	12948 ± 3281	3280 ± 841	16766 ± 4204
K-value, 4 DF	-	**18.25**	**15.85**	**9.65**	**17.35**	**12.05**	**16.95**
Low SRT (n=10)	29 ± 2	190 ± 71	146 ± 76	321 ± 374	15593 ± 4787	3692 ± 1207	19941 ± 6005
High SRT (n=10)	<MDL	154 ± 63	127 ± 34	118 ± 32	16407 ± 5687	4315 ± 1512	21121 ± 7259
K-value, 4 DF	-	6.25	4.25	**19.15**	0.15	4.35	0.15
Nitrification (n=5)	<MDL	100 ± 12	103 ± 13	140 ± 17	11599 ±1435	3009 ± 365	14952 ± 1807
Non-nitrification (n=10)	29 ± 2	190 ± 71	146 ± 76	321 ± 374	15593 ± 4787	3692 ± 1207	19941 ± 6005
K-value, 4 DF	-	**25.10**	**18.50**	1.00	**22.40**	**15.70**	**22.20**

1. The analytical method used was microwave assisted extraction (MAE) by J.J. Yang at Environment Canada (EC), Burlington (Svoboda et al., EC internal manuscript, 2006).
2. The total PSMs was calculated by combining Celestolide, Phantolide, Traseolide, Galaxolide and Tonalide, concentration levels. Cashmeran was intentionally not included in the total PSMs.
3. Kruskal-Wallis non-parametric test was applied at the 95% confidence level with a critical K-value of 9.49 at 4 degrees of freedom (DF) and 7.33 at 3 DF. Significant differences are bolded.

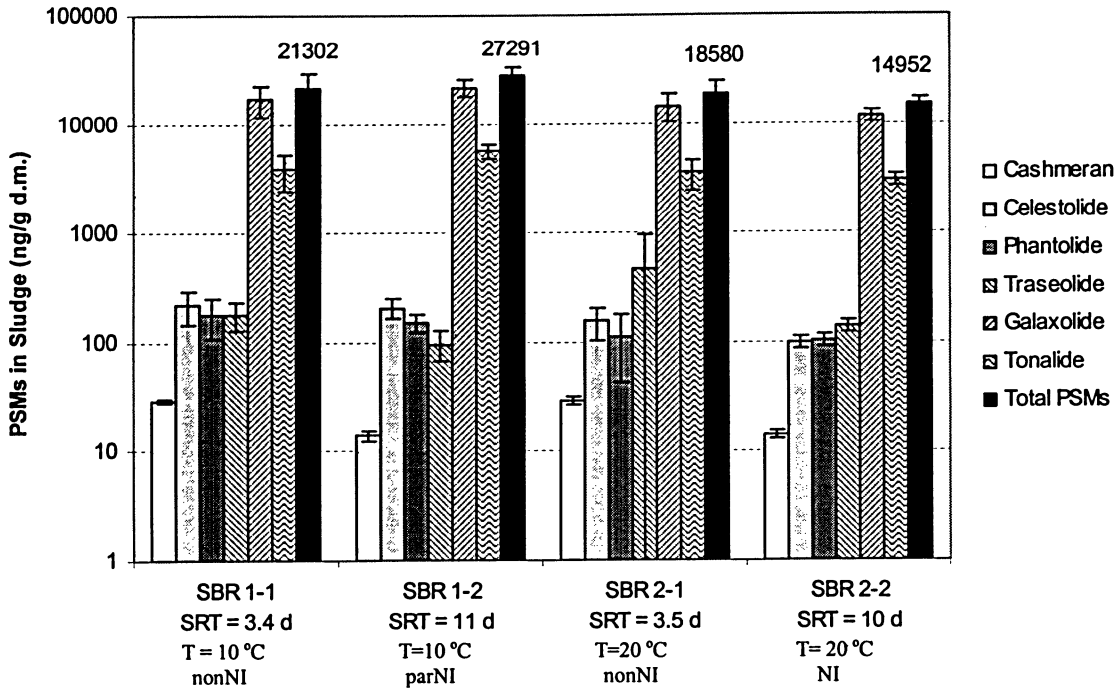


Figure 4-15. Mean (n=5) PSMs concentrations in sludge under the four different operating conditions. The black bar labels refer to the Total PSMs concentration.

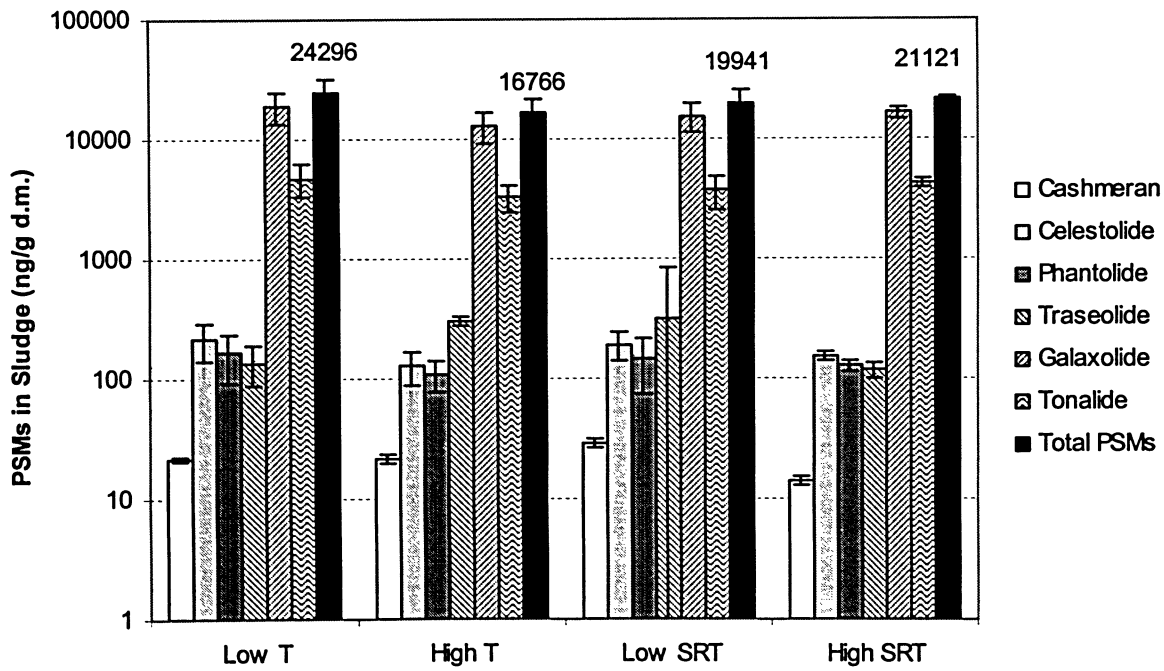


Figure 4-16. Mean (n=5) PSMs concentrations in sludge under operating Low (10 °C) and High (20 °C) temperatures and Low (3.5 days) and High (10.5 days) SRT conditions. The black bar labels refer to the Total PSMs concentration.

4.6. Pearson Correlation Analysis

A key part of the hypothesis was the correlation of sludge properties to the removal of PSMs from the aqueous matrix and PSMs partitioning to sludge. A Pearson correlation analysis was conducted to assess if a linear correlation exists between some of the sludge properties and partitioning of PSMs. Results of the analysis are shown in Table 4-9 and sample scatter plots are provided in Figures 4-17 and 4-18. The results indicate that: (1) relative hydrophobicity and surface charge are significantly and positively linearly correlated to the aqueous removal of PSMs and (2) significantly and negatively correlated to the partitioning between the solids and aqueous matrix (K_P).

Table 4-9. Pearson correlation analysis results of sludge and EPS parameters with total PSMs aqueous removals and partitioning as approximated by K_P

Sludge and EPS Parameters	Pearson's Linear Correlation Analyses [1]					
	Total PSM Removed From the Aqueous Steam (Influent-Effluent) Concentration			Total PSM K_P (C_S/C_L) (L/g)		
	p	r_p	s_r	p	r_p	s_r
EPS Proteins (n=16)	0.003	**0.7**	0.2	0.14	-0.38	0.2
EPS Carbohydrates (n=14)	0.021	**0.6**	0.2	0.20	-0.35	0.3
EPS DNA (n=16)	0.005	**-0.7**	0.2	0.07	0.44	0.2
Acidic Polysaccharides (n=16)	0.137	-0.3	0.3	0.89	0.04	0.3
Total EPS (n=16)	0.001	**0.8**	0.2	0.10	-0.40	0.2
Surface Charge (n=20)	0.021	**0.5**	0.2	0.05	**-0.44**	0.2
Relative Hydrophobicity (n=20)	0.0002	**0.7**	0.2	0.02	**-0.53**	0.2

1. Bolded Pearson's coefficients (r_p) are significant at the 95% confidence level and s_r provides the error associated with r_p (Zar, 1996).

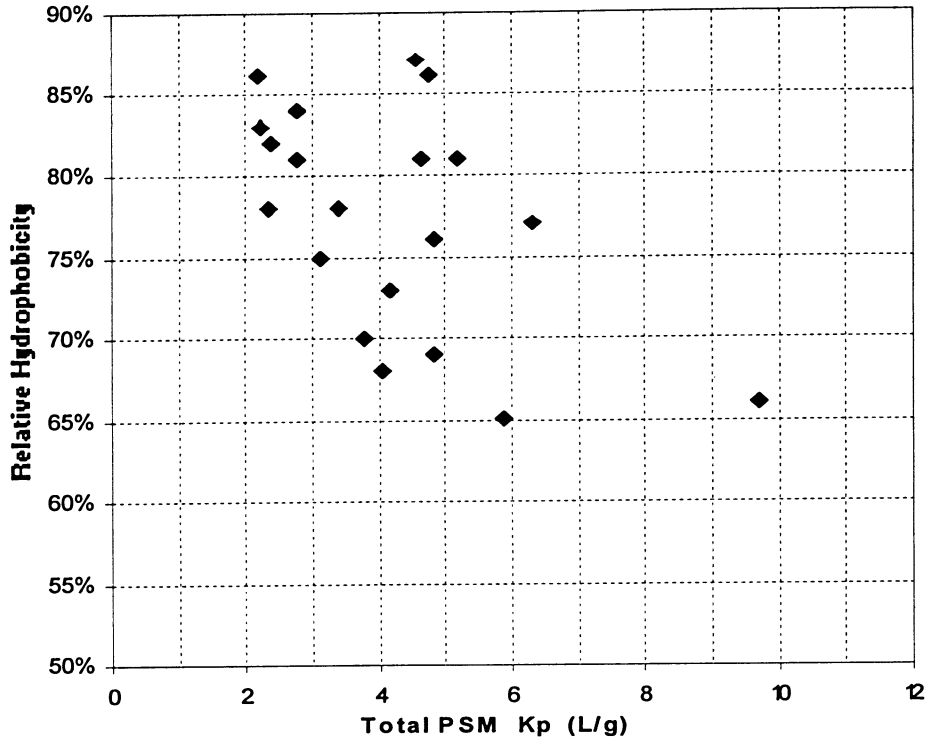


Figure 4-17. Scatter plot of relative hydrophobicity versus the total PSMs K_P (L/g) in the reactors.

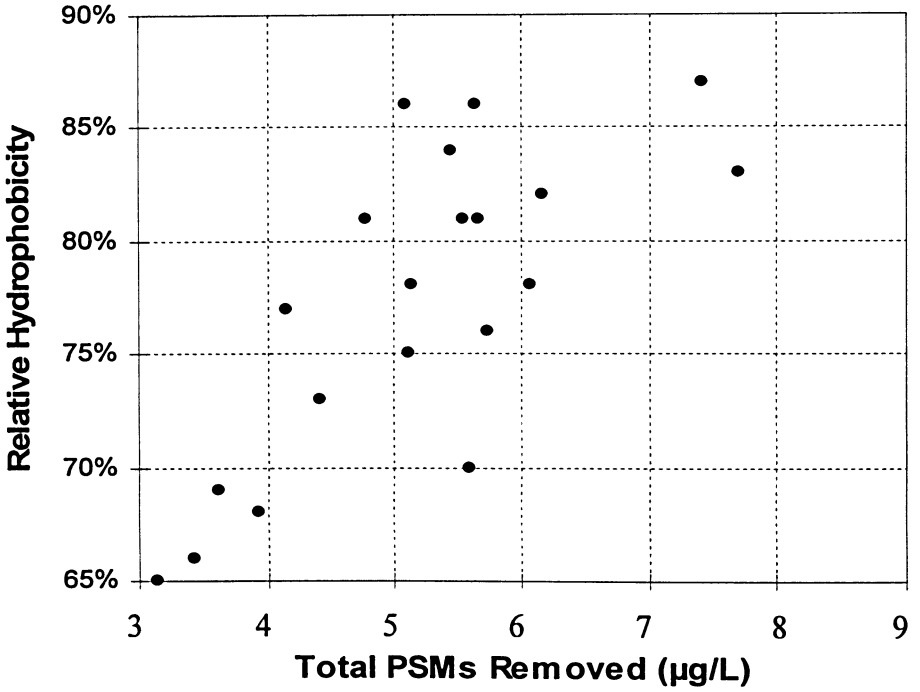


Figure 4-18. Scatter plot of relative hydrophobicity versus the total PSMs removed from the aqueous phase (influent – effluent concentration).

A significant positive linear correlation is observed between the PSMs removed from the aqueous stream (influent minus effluent PSM concentration) and the EPS proteins, carbohydrates and total EPS. A significant negative linear correlation is observed with EPS DNA. A positive and negative linear correlation between K_P and EPS DNA and total EPS, respectively, both significant at the 90% level is also observed.

No literature references were found that correlate EPS constituents or sludge surface properties (SC and RH) in the removal of trace PSMs from AS sewage treatment. However it is speculated that, due to the hydrophobic nature of PSMs, and the correlation results, that the sludge RH is the most important sludge characteristic that influences the partitioning of PSMs from the aqueous to the solids matrix. EPS constituents and SC, based on the correlation analysis (see Table 4-9), are also considered important but to a lesser degree.

4.7. Sorption and Desorption PSM Isotherms

An evaluation of the equilibrium sorption and desorption Freundlich isotherms on lyophilized sludge (biologically inactivated) was conducted and considered to approximate the behaviour of fresh sludge for the purpose of sorption and desorption (Kerr et al., 2000). The Freundlich equation was recommended (OECD 106, 2000) based on previous empirical evidence which demonstrated that the power expression (Equation 4-1 and 4-2) provides the best linear fit to sorption data and this has been widely used (see discussion in section 2.7.1).

The sludge from the SBRs operated at a SRT of 3.5 at the two different temperatures was combined. Similarly the sludge from the SBRs operated at 10.5 days was combined. A priori decision was made due to time and resource limitations and it was decided to combine the sludge from the two different operating temperatures.

The general Freundlich sorption and desorption equations are similar and given as:

$$C_s^{sor}(eq) = K_F^{sor} \cdot C_{aq}^{sor}(eq)^{1/n} \quad (4\text{-}1)$$

and

$$C_s^{des}(eq) = K_F^{des} \cdot C_{aq}^{des}(eq)^{1/n} \quad (4\text{-}2)$$

Where: $C_s^{sor}(eq)$ = concentration of PSM sorbed onto sludge at sorption equilibrium (μg/g), K_F^{sor} = Freundlich sorption coefficient ($\mu g^{1-1/n} \cdot (mL)^{1/n} \cdot g^{-1}$), $C_{aq}^{sor}(eq)$ = concentration of PSM in solution at sorption equilibrium (μg/L), n = regression constant, $C_s^{des}(eq)$ = concentration of PSM sorbed onto sludge at desorption equilibrium (μg/g), K_F^{des} = Freundlich desorption coefficient ($\mu g^{1-1/n} \cdot (mL)^{1/n} \cdot g^{-1}$), $C_{aq}^{des}(eq)$ = concentration of PSM in solution at desorption equilibrium (μg/L).

The Freundlich sorption isotherms were used in this study to predict the equilibrium concentrations of PSMs sorbed onto sludge knowing the equilibrium aqueous concentration. The Freundlich desorption isotherms can be used to predict the equilibrium concentrations of PSMs desorbed from sludge with a known concentration of sorbed PSM. In subsequent sections appropriate calculations are shown that demonstrate the usefulness of the derived PSM isotherms.

4.7.1 Sorption equilibration time

Preliminary tests were conducted to establish the sorption equilibrium time as well as to determine optimum spiking concentration ranges. Figure 4-17 shows that a stable aqueous equilibrium concentration plateau is reached within about 30 minutes except for Cashmeran which did not show a significant plateau although the concentration level was relatively steady at about 350 μg/L (see Appendix J).

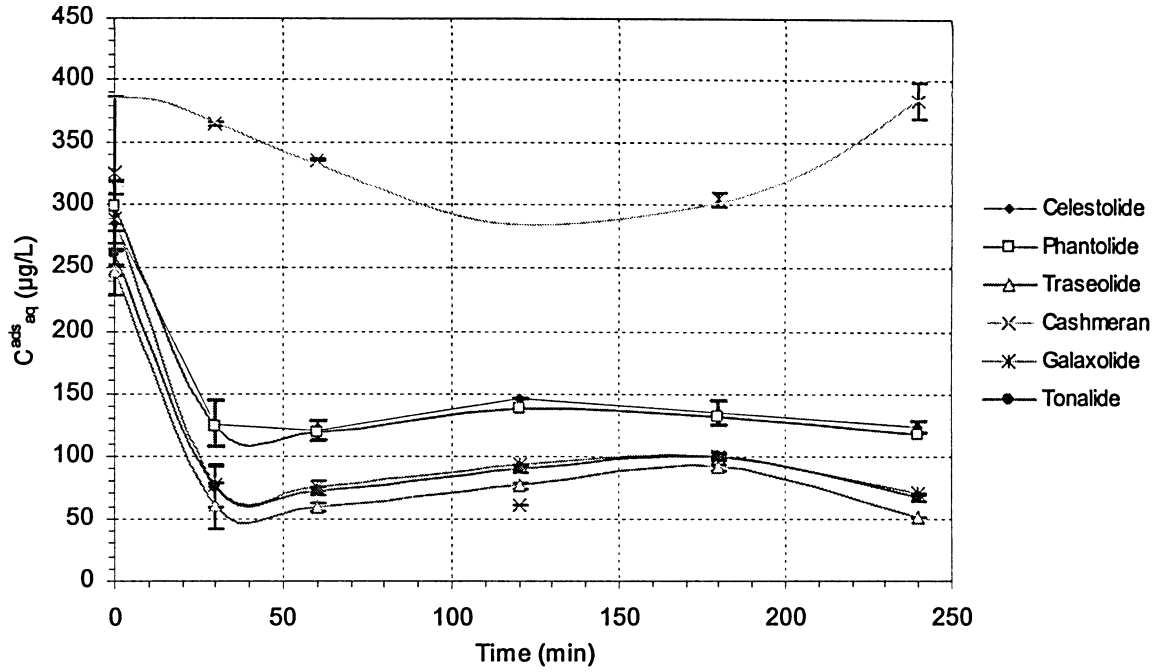


Figure 4-19. Aqueous PSMs concentration (n=3), at T=25 °C at pH =7.4, at different equilibration times using 0.05g lyophilized sludge in 50 mL of PBS.

Of all the PSMs Cashmeran has the highest water solubility (*Sw*) at about 0.5 mg/L at 20 °C and the lowest *Kow* of about 4.9 which is much closer to the physicochemical properties of the nitro musk Musk Ketone (see Table 2-2). The other PSMs have *Sw* and *Kow* values in the range of 0.06 to 0.25 mg/L and 6.5 to 8, respectively.

Based on the above equilibrium plateau time of 30 minutes, a study at 90 minute equilibrium adsorption and desorption experimental plan was established for the remaining study. The equilibration time is consistent with similar studies that show equilibrium plateaus to be established within 60 minutes using PAHs (Moretti and Neufeld, 1989; Kördel et al., 1997).

The use of 0.1 g of lyophilized activated sludge (LAS) was considered more appropriate than 0.05 g due to improved accuracy and a resulting solids concentration (2 g/L) closer to what was SBRs were operated at. The LAS was added to 50 mL of PBS (pH of 7.4)

resulting in a 1 to 500 sludge to solution ratio at a constant pH. The indirect method, of measuring the depletion of PSMs in the solution, was adopted using HS SPME GC-MS in accordance with Method 106 OECD (2001) (see section 3-8).

4.7.2 Sorption and Desorption Results at SRT of 3.5 and 10.5 Days

Figures 4-20, 4-21and 4-22 provide the logarithm linear plots from which regression linear analyses were conducted to generate the regression coefficients and constants given in Tables 4-10 for SRT of 3.5 days. The equilibrium isotherm regression constants for the PSMs were between 1.0 and 1.3 indicating slightly nonlinear sorption behaviour. The Freundlich adsorption coefficients range from 0.3 to 26 $(\mu g/g)/(\mu g/L)^{1/n}$ range (L/g for Cashmeran since n=1) (OECD, 2000) and are assumed to be constant over the aqueous concentration range of 10 to 300 µg/L of PSMs.

The concentration aqueous range used was based an assumed 90 % adsorption of PSMs to the solids and ensuring that we remained approximately 10 times above the analytical method detection limits (MDL) as recommended by the batch equilibrium method used (OECD/OCDE 106, 2000). The highest MDL for PSMs in aqueous media was 21 ng/L and a minimum 200 ng/L aqueous concentration was required. Based on a 90 % adsorption to solids a 2 µg/L PSMs spike was required to correspond to a 200 ng/L aqueous concentration. Since there was uncertainty about what the actual adsorption percentage between the two sludge would be a 10 µg/L minimum PSM spike was used.

The sorption-desorption batch tests were conducted at 25 °C rather than 20 °C due to equipment failure and timing issues. The sorption-desorption behaviour is expected to be correlated predominantly to K_{OW} values and *Sw* values which are not significantly influenced by changes in T from 20 to 25 °C and it is assumed that the sorption-desorption results apply at 20 °C without any significant T error.

Table 4-10. Freundlich equilibrium sorption and desorption coefficients K_{sor}, K_{des}, regression constant n and linear correlation coefficient r^2 at SRT of 3.5 days, pH=7.4 and T=25 °C

Polycyclic Synthetic Musks (Kow)	K_{sor}	n	r^2	K_{dess}	n	r^2
Cashmeran (4.9)	0.3	1.0	0.92	---	---	---
Celestolide (6.6)	8	1.2	0.96	7	1.0	0.80
Phantolide (6.7)	10	1.3	0.95	10	1.1	0.77
Traseolide (8.1)	26	1.3	0.95	26	1.1	0.84
Galaxolide (7.2)	15	1.3	0.95	11	1.0	0.83
Tonalide (7.2)	20	1.3	0.96	19	1.2	0.82

The observed equilibrium partitioning of the PSMs is similar to that reported by Moretti and Neufeld (1997) for PAHs, where the sorption coefficient was determined to represent the sludge lipid-wastewater distribution closely represented by the *Kow* of the PAHs. The *Kow* is often correlated to the lipid content and used to predict the partitioning of chemicals between the aqueous and organic components of environmental compartments. When the *Kow* (in Figure 2.8 (A) and given in Table 4-10) are compared, to the experimental K_F values, there is direct correlation evident. This observation suggests that partitioning mechanism of PSMs in the activated sludge process may be occurring by similar mechanisms as PAHs. The *Kow* of the referred to PAHs ranged from 4.2 to 5.9, similar to some of the PSMs (Moretti and Neufeld, 1997). Section 4.7 also presents and discusses the correlation of the partition coefficient K_P with K_{OW}.

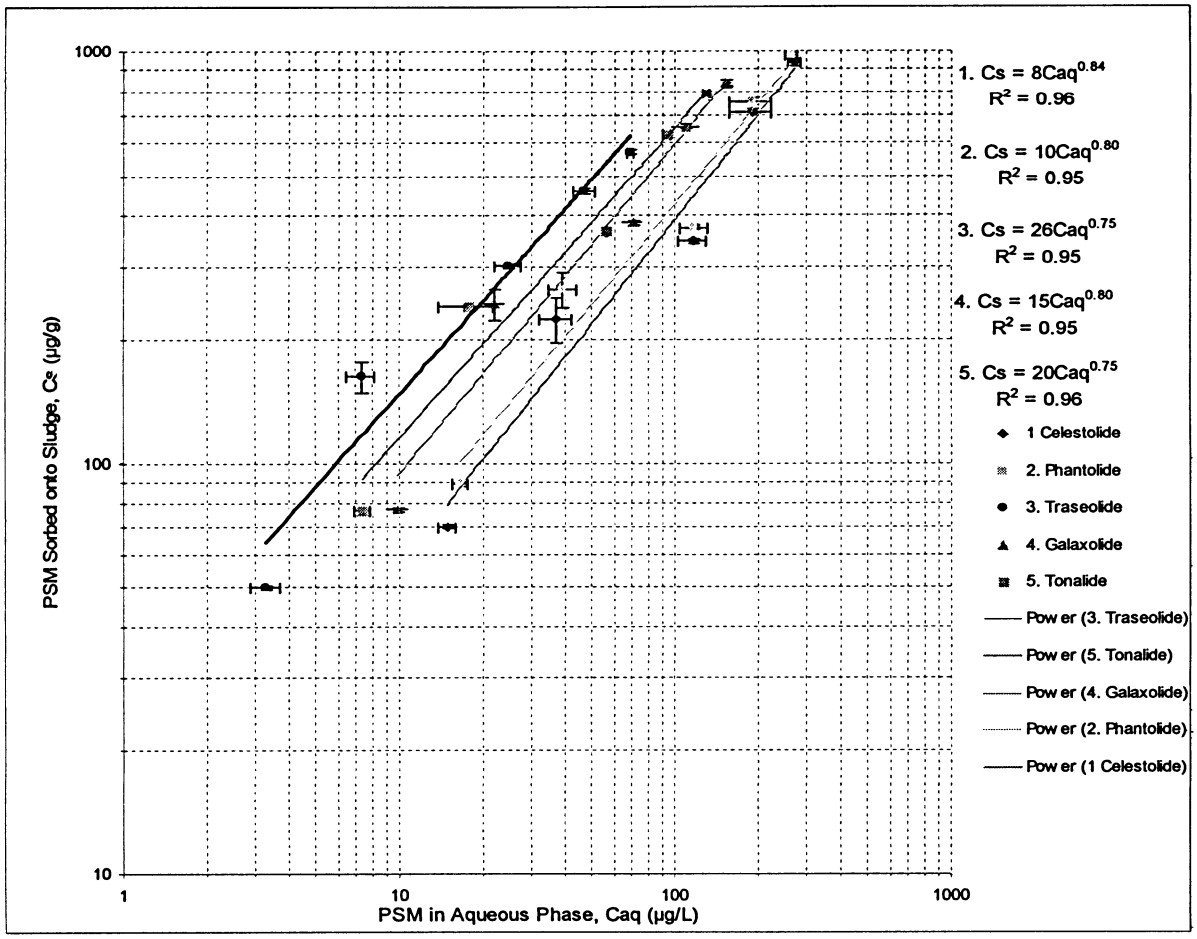


Figure 4-20. Freundlich equilibrium sorption isotherms (n=5), at T=25 °C at pH =7.4, of selected PSM using lyophilized sludge from the SBRs operated at the SRT of 3.5 days.

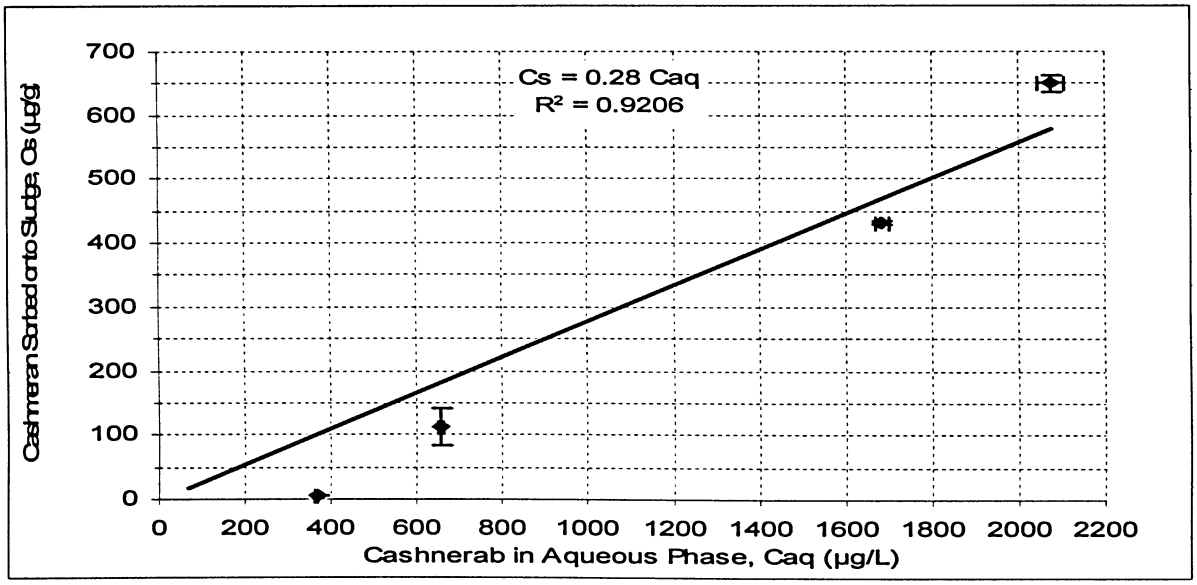


Figure 4-21. Freundlich adsorption isotherm of Cashmeran (n=4) onto lyophilized sludge from the SBR operated at the SRT of 3.5 days (T=25 °C, pH =7.4).

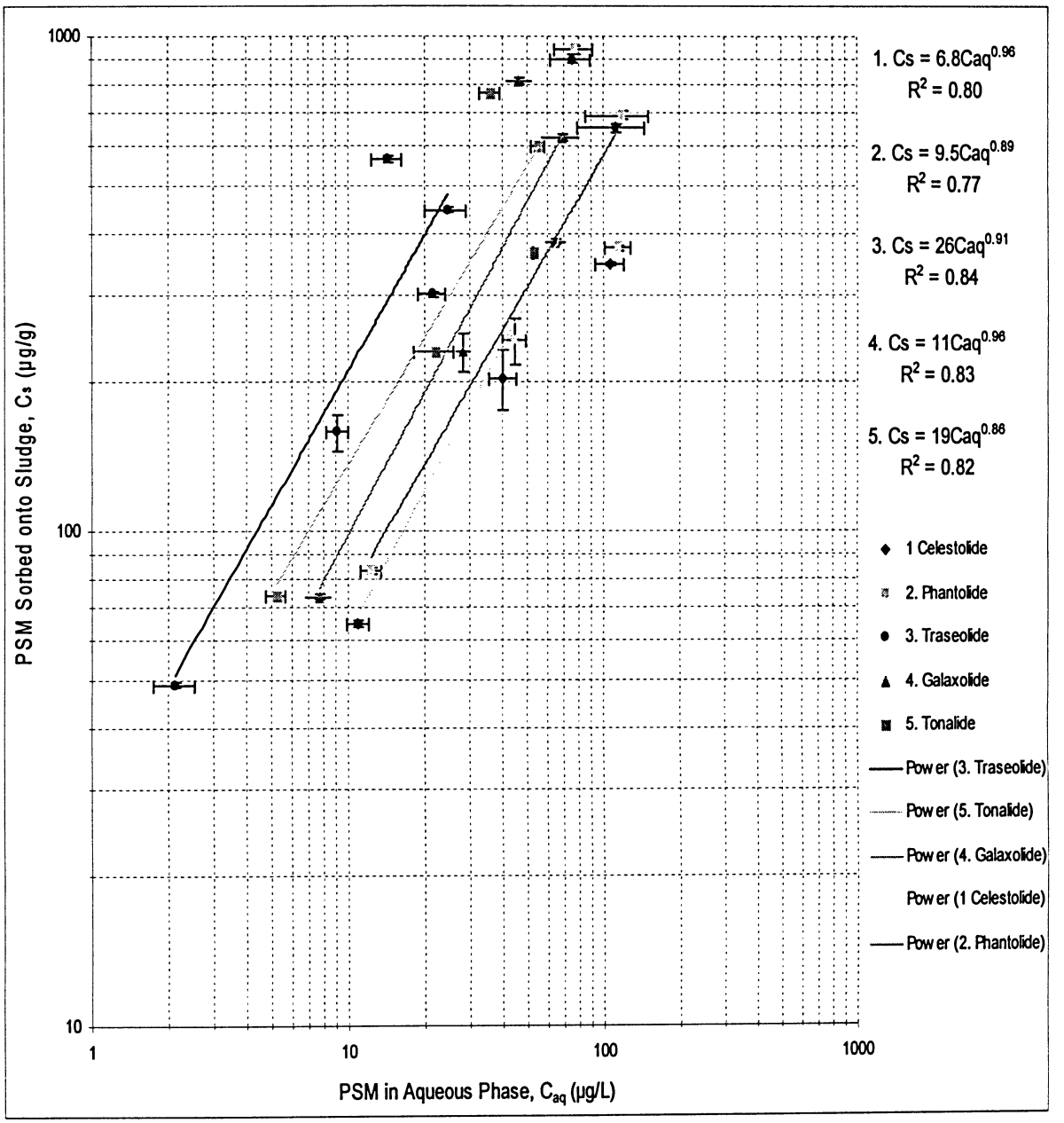


Figure 4-22. Freundlich equilibrium desorption isotherms (n=5), at T=25 °C at pH =7.4, of selected PSM using lyophilized sludge from the SBRs operated at the SRT of 3.5 days.

Figures 4-23 to 4-26 provide the logarithm plots from which the regression linear analyses were conducted to generate the regression coefficients and constants given in Tables 4-11 for SRT of 10.5 days. With the sludge at SRT of 10.5 days the aqueous concentration $C_{aq}^{sor}(eq)$, range of analysis was extended to about 2000 µg/L beyond the range of 330 µg/L used with SRT of 3.5 days.

Figure 4-23 shows that a linear range up to about the 100 µg/L is followed by a plateau at the 1000 µg/L level followed by a sharp decline at about 2000 µg/L. This curve extends beyond the solubility range of all the PSMs (< 0.6 mg/L) and micelle formation causing the PSMs to remain in solution may explain the increased aqueous concentration of the PSMs. A similar isotherm was observed in another study that showed that the aqueous concentration progressively increased with a change in pH (affecting the solubility) and the solids concentration decreased (Deng et al., 2006). This concentration range, beyond 1 mg/L of PSMs, is well beyond the typical cumulative environmental PSMs concentration found in aquatic environments (Herberer, 2002).

Figure 4-24 provides linear curves based on the linear portion of Figure 4-23. Only three points (n=3) could be extracted to generate Figure 4-24 and the linear range may actually extend beyond the 100 µg/L aqueous concentration $C_{aq}^{sor}(eq)$ level.

When Figure 4-24 is compared to Figure 4-22, for sludge with SRT of 3.5 days, the corresponding maximum PSM concentrations sorbed onto the sludge are equal at about 1000 µg/g d.m. however the corresponding aqueous concentration $C_{aq}^{sor}(eq)$ is 100 µg/L at the lower SRT versus about 300 µg/ for the higher SRT. This suggests that sludge at the higher SRT of 10.5 days has 3 times greater equilibrium sorption capacity than the lower SRT sludge.

No Cashmeran sorption or desorption isotherms could be generated for the sludge with the higher SRT. The raw data is however presented in Appendix J.

Figure 4-25 presents the general desorption trend of PSM from the sludge at SRT of 10.5 days. A linear range between the 10 to 100 µg/L aqueous concentration $C_{aq}^{sor}(eq)$ levels is

evident and this became clearer in Figure 4-26 from which average Freundlich isotherm parameters were determined (n=3) and tabulated in Table 4-11.

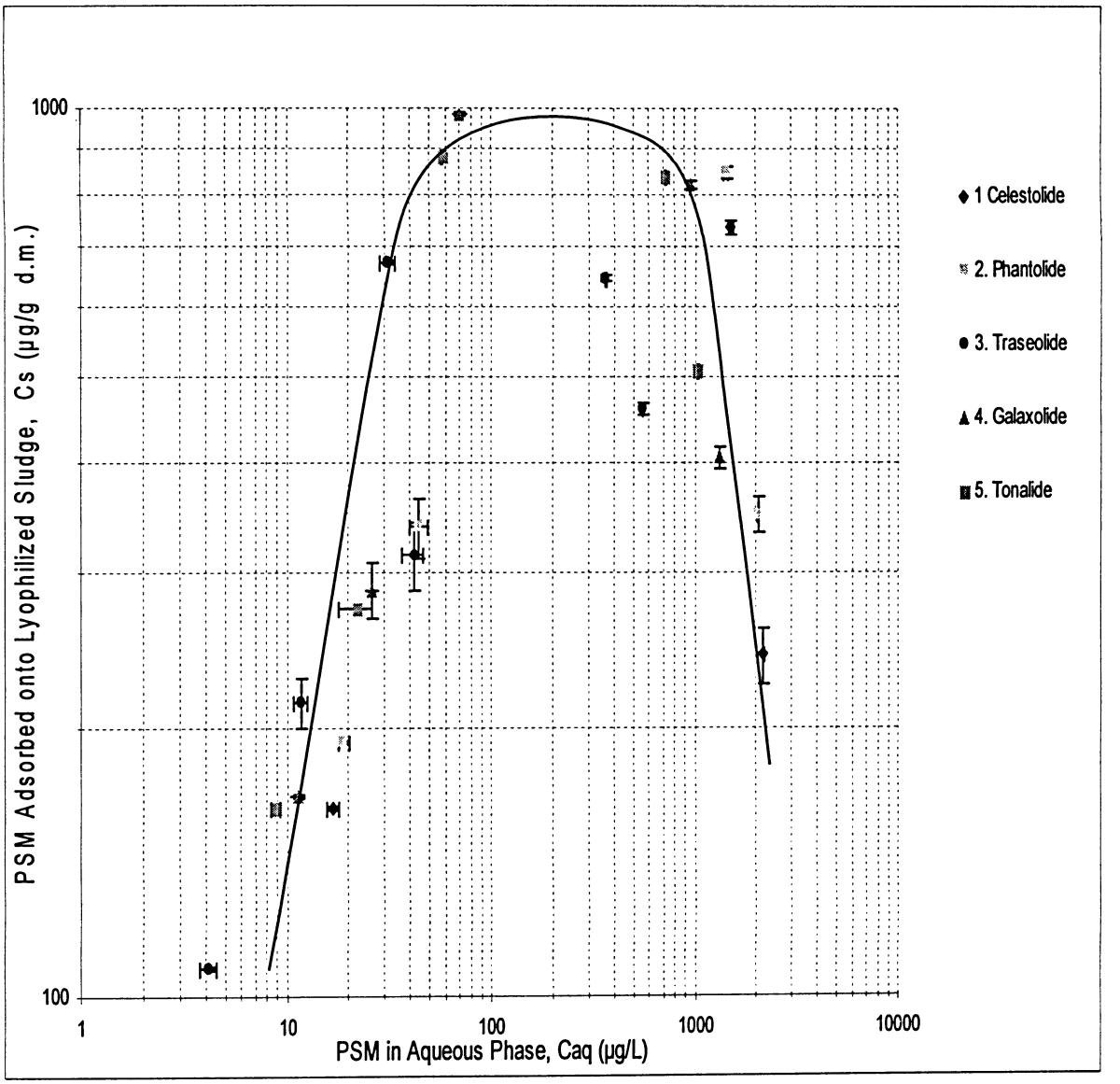


Figure 4-23. Freundlich equilibrium sorption isotherms (n=5), at T=25 °C at pH =7.4, of selected PSM using lyophilized sludge from the SBRs operated at the SRT of 10.5 days.

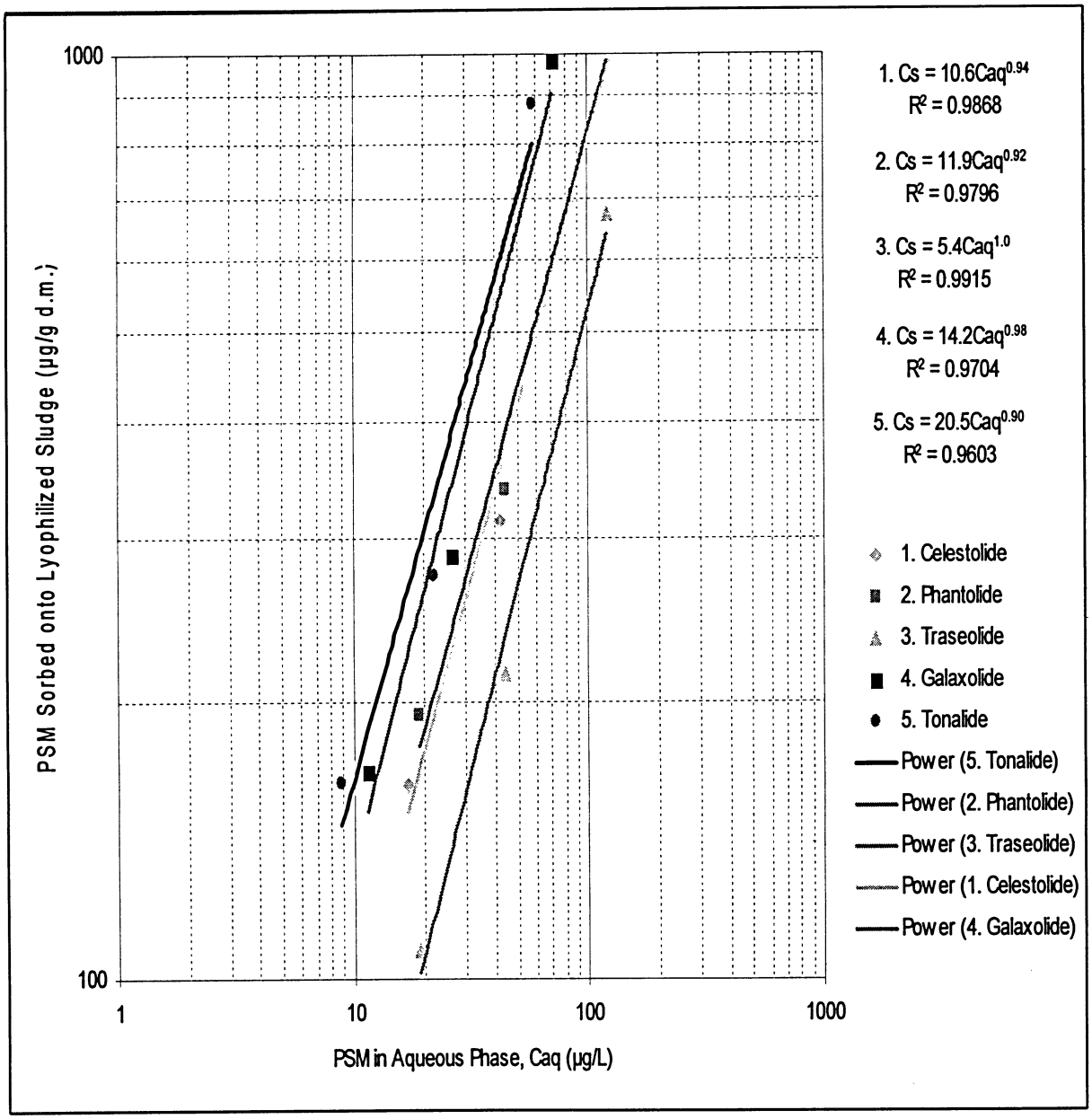


Figure 4-24. Freundlich equilibrium sorption isotherms (n=3), at T=25 °C at pH =7.4, of selected PSM using lyophilized sludge from the SBRs operated at the SRT of 10.5 days.

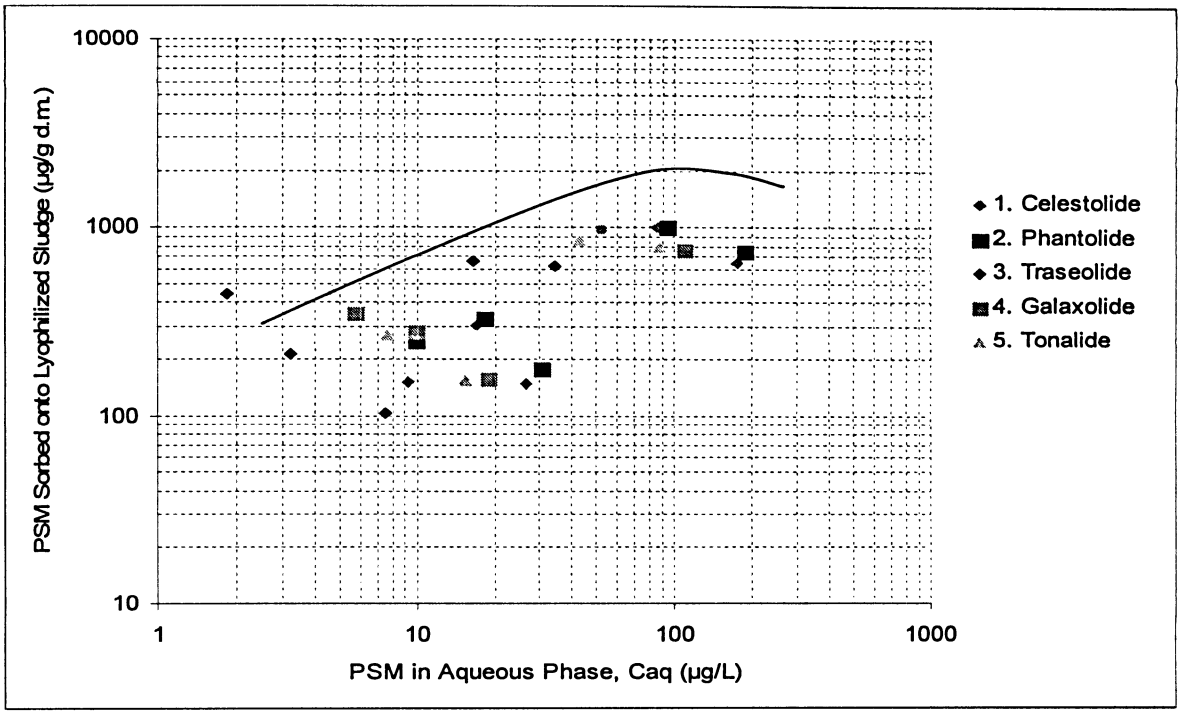


Figure 4-25. Equilibrium desorption trend (n=5), at T=25 °C at pH =7.4, of selected PSM using lyophilized sludge from the SBRs operated at the SRT of 10.5 days.

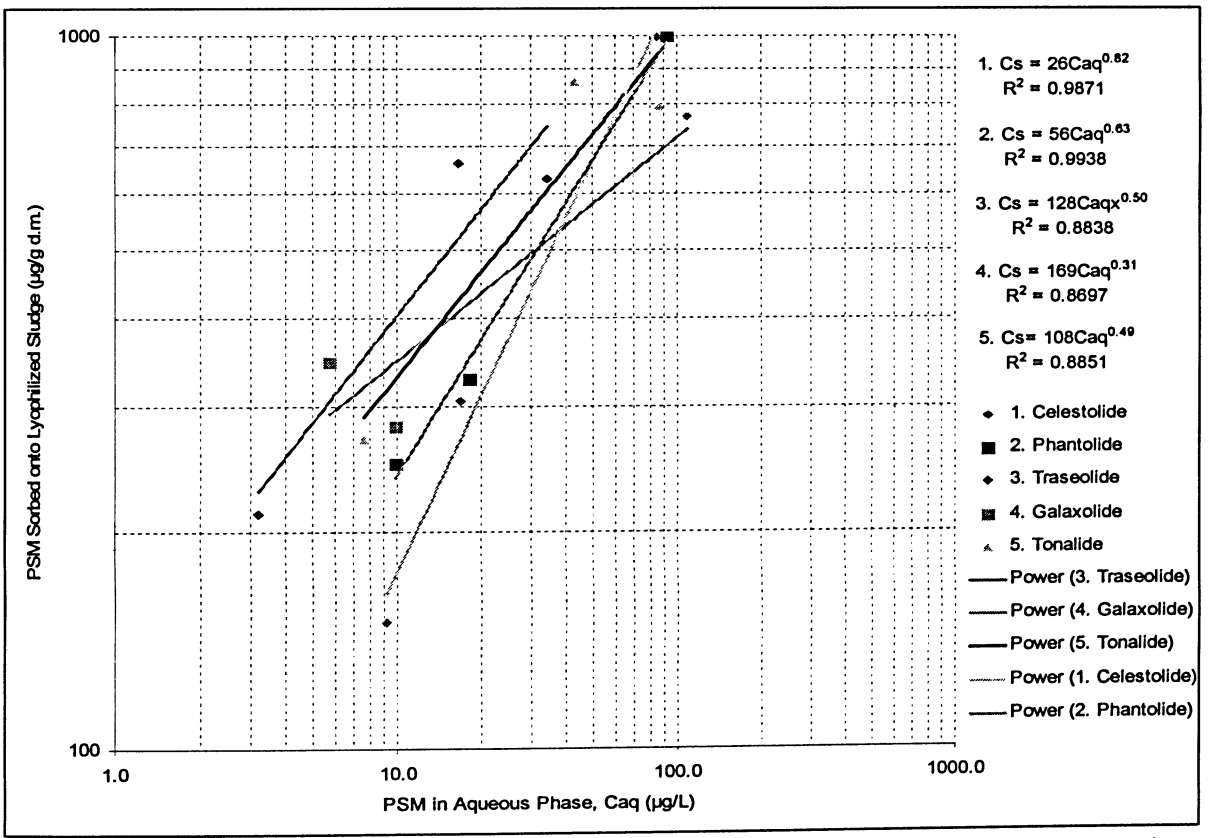


Figure 4-26. Freundlich equilibrium desorption isotherms (n=3), at T=25 °C at pH =7.4, of selected PSM using lyophilized sludge from the SBRs operated at the SRT of 10.5 days.

Table 4-11. Freundlich equilibrium sorption and desorption coefficients K_{sor}, K_{des}, regression constant n and linear correlation coefficient r^2 at SRT of 10.5 days, pH=7.4 and T=25 °C, (n=3)

Polycyclic Synthetic Musks (*Kow*)	K_{sor}	n	r^2	K_{dess}	n	r^2
Celestolide (6.6)	10.6	1.1	0.99	26	1.2	0.99
Phantolide (6.7)	11.9	1.1	0.98	56	1.6	0.99
Traseolide (8.1)	5.4	1.0	0.99	128	2.0	0.88
Galaxolide (7.2)	14.2	1.0	0.97	169	3.2	0.87
Tonalide (7.2)	20.5	1.1	0.96	108	2.0	0.89

4.7.3 Sorption and desorption predictions at 3.5 days SRT

Figure 4-27 and 4-28 present a comparative calculation of the predicted over the experimental PSMs sorbed onto sludge in SBR 1-1 and 2-1, operated at 3.5 days. The predicted PSMs sorbed onto sludge were calculated based on the mean effluent PSM, given in Table 4-3, representing the equilibrium aqueous PSM concentration $C_{aq}^{sor}(eq)$ (µg/L), and using Equation 4-1. The corresponding $C_s^{sor}(eq)$ (µg/g) and the ratio of the predicted over the actual sorbed PSM concentration was further computed and graphed in Figures 4-27 and 4-28.

Figures 4-27 and 4-28 show that the predicted PSM concentration sorbed onto sludge are generally equal to or greater than the measured values for all cases except Cashmeran. This suggests that there are other abiotic or biologically mediated processes which reduce the resulting PSM concentrations, found sorbed onto sludge, even further. This is not the case for Cashmeran. In the case of Cashmeran ®, the predicted sorbed value is overestimated by 70 to 80 % of the observed value. This difference may be considered to be within experimental error and thus is in good agreement with the experimental sorption concentration measured. Because of the excellent linearity observed with the isotherms it is assumed that the comparison of the K values, between the two different sludges, would also apply at the lower aqueous concentration levels of (20 to 300 ng/L).

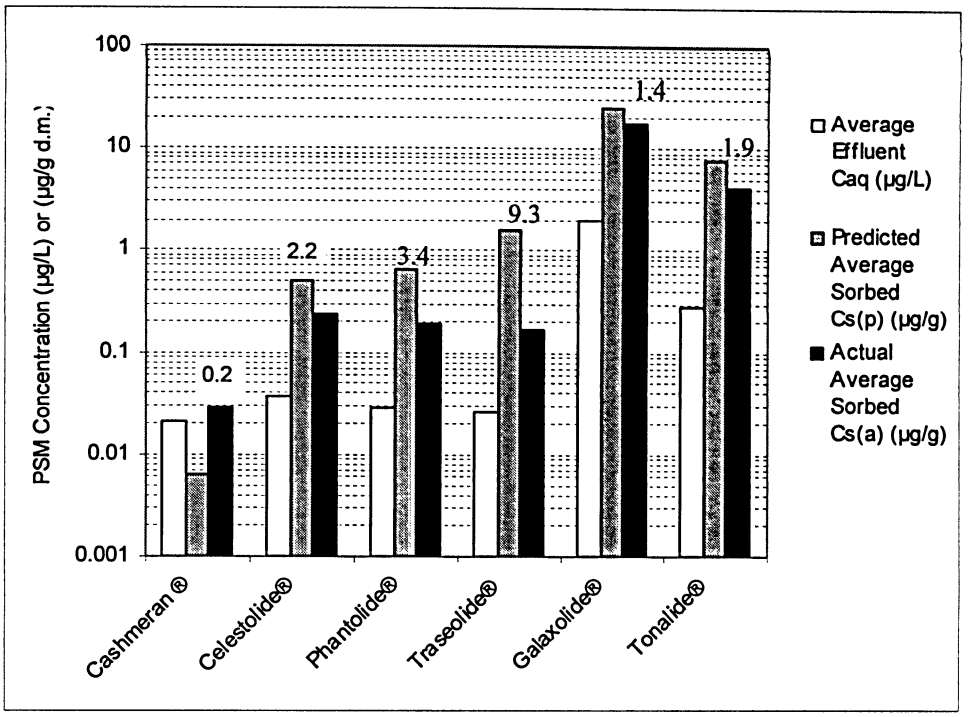


Figure 4-27. Comparison of the average predicted and experimental sorbed PSM on sludge from SBR 1-1 operated at the SRT of 3.5 days and 10 °C. The bar labels refer to the ratio between the predicted over the observed values.

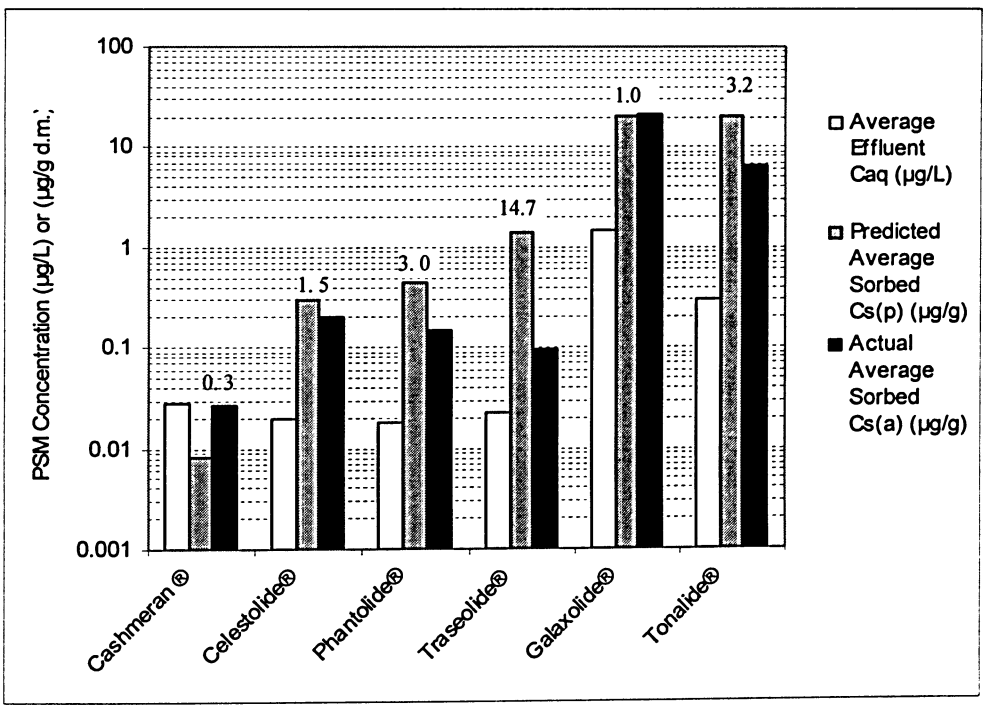


Figure 4-28. Comparison of the average predicted and actual sorbed PSM on sludge from SBR 2-1 operated at the SRT of 3.5 days and 20 °C. The bar labels refer to the ratio between the predicted over the observed values.

4.7.4 Sorption predictions at 10.5 days SRT

A comparative calculation of the predicted over the experimental PSMs sorbed onto sludge in SBR 1-2 and 2-2, operated at 11 and 10 days, respectively, is shown in Figures 4-29 and 4-30. The predicted PSMs sorbed onto sludge was calculated based on the mean effluent PSM, given in Table 4-3, representing the equilibrium aqueous PSM concentration $C_{aq}^{sor}(eq)$ (µg/L), and using Equation 4-1. The corresponding $C_s^{sor}(eq)$ (µg/g) and the ratio of the predicted over the actual sorbed PSMs concentration was determined and graphed in Figures 4-29 and 4-30.

Figures 4-29 and 4-30 show that the predicted PSM concentration sorbed onto sludge are generally greater than the actual measured values for all cases except Traseolide. This suggests that there are other abiotic or biologically mediated processes which reduce the observed PSM concentrations, found sorbed onto sludge, even further.

The ratio values are lower at 10 °C and are marginally lower than at the 20 °C operating conditions, well within the experimental error, suggesting that the temperature effect is minimal or not significant.

Further as in lower SRT conditions the high ratio values suggest that there are other significant abiotic or biologically mediated processes which reduce the resulting PSM concentrations, found sorbed onto sludge, even further. Traseolide appears to be the exception at the higher SRT as Cashmeran was at the lower SRT.

This is 2 to 3 orders of magnitude lower than typically observed in sewage effluent from this study and others (Heberer, 2002; Smyth et al., 2006 in print;). The total PSM desorbed is dominated by Celestolide, followed by Tonalide and then Galaxolide with a total of 2.3 ng/L.

These result suggests that the sludges at different SRTs tested behave significantly different in terms of desorption of selected PSMs.

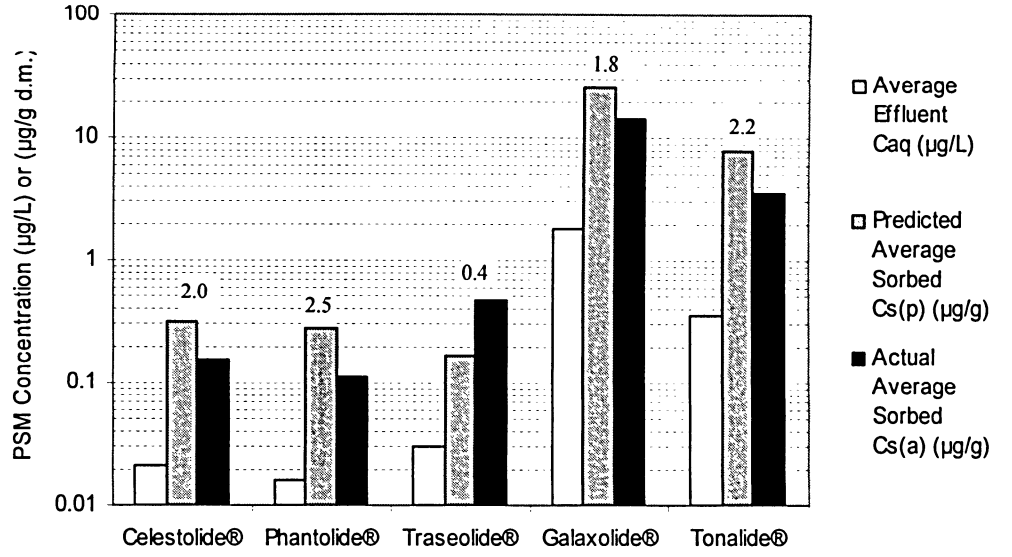


Figure 4-29. Comparison of the average predicted and actual sorbed PSM on sludge from SBR 1-2 operated at the SRT of 11 days and 10 °C. The bar labels refer to the ratio between the predicted over the observed values.

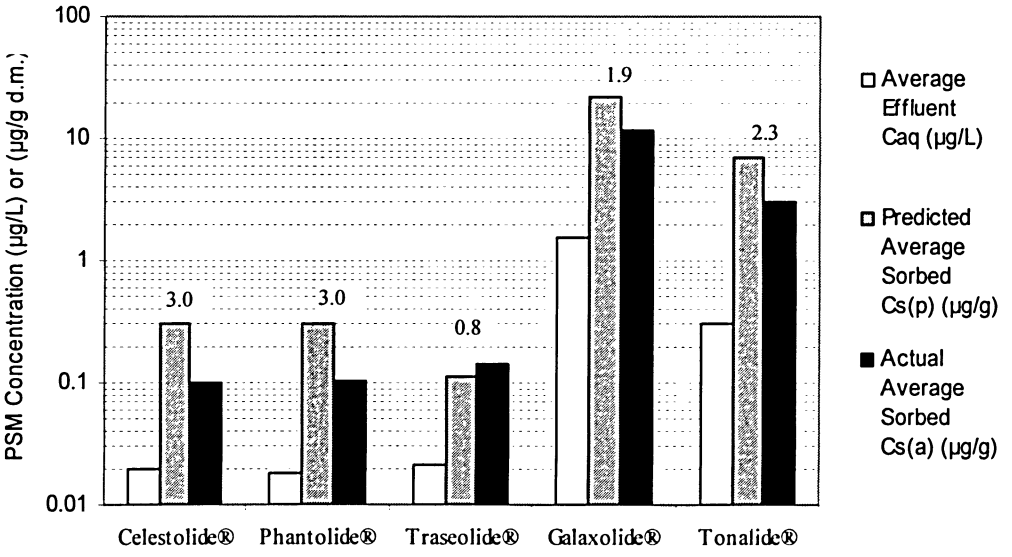


Figure 4-30. Comparison of the average predicted and actual sorbed PSM on sludge from SBR 2-2 operated at the SRT of 10 days and 20 °C. The bar labels refer to the ratio between the predicted over the observed values.

4.8 The Partition Coefficient *Kp*

A useful term, closely related to Freundlich partition coefficient K_F, is the partition coefficient *Kp* (L/g) which, for dilute systems typical of environmental aqueous environments, is defined as:

$$K_p = \frac{C_S}{C_L} \tag{4-3}$$

where C_S is the PSM concentration (μg/g) associated with solids and C_L is the PSM concentration (μg/L) in the aqueous phase.

In sludges and soils the degree to which an organic compound partitions between solids and the aqueous phase is often correlated to the organic content of the solid matrix. In sludges the organic content is commonly taken to be the VSS. The organic matter partition coefficient K_{OM} can be defined for sludges as:

$$K_{OM} = 100\frac{K_P}{\%VSS} \tag{4-4}$$

where K_P is the partition coefficient and %VSS is the percent volatile suspended solids of the sludge.

Sorption data are commonly correlated on the basis of the organic matter and thus for comparison purposes the partition coefficient is commonly redefined as:

$$K_P^{'} = 1000 \cdot K_{OM} \tag{4-5}$$

where $K_P^{'}$ (L/kg) is the modified partition coefficient in dilute aqueous solutions.

In the present study the average percent VSS ranged from 69 to 79 % and this is within the reported range of 40 to 85 % (Dobbs, Wang and Govind, 1989) typical of municipal sludges. It is reported that organic matter content has a significant impact on sorption and that there exists a significant difference in sorption capacity between soils and sludges commonly attributed to the organic component difference (Dobbs, Wang and Govind, 1989; Tsezos and Bell, 1989). In addition

in an effort to predict the fate and impact of organic contaminants the physical chemical properties and in particular the K_{OW} has proved useful in predicting the sorption, bioconcentration, lipophilic storage and biomagnifications of lipophilic compounds (Dobbs, Wang and Govind, 1989; Tsezos and Bell, 1989).

Table 4-12 compares the experimental K_{OM} and $\log K_P^{'}$ values and shows that at the 95% confidence level there is a significant difference in the partition coefficient for all the PSMs except Cashmeran (see also Appendix N).

Table 4-12. The comparison of equilibrium partitioning onto sludge K_{OM} and $\text{Log } K_P^{'}$ with reference to $\log Kow$[1]

Polycyclic Synthetic Musks	Log K_{OW}	K_{OM} (L/kg)[2]		Log $K_P^{'}$ (L/kg)		Two-tailed t-test p-value ($\alpha = 0.05$)
		SRT = 3.5 d (n=5)	SRT =10.5 d (n=3)	SRT = 3.5 d (n=5)	SRT =10.5 d (n=3)	
Cashmeran	4.5	308 ± 89	235 ± 317	2.5 ± 0.1	2.1 ± 0.7	0.791
Celestolide	5.4	5200 ± 1519	12529 ± 836	3.7 ± 0.1	4.1 ± 0.1	**0.002**
Phantolide	5.8	5695 ± 1807	12102 ± 2354	3.7 ± 0.1	4.1 ± 0.1	**0.008**
Traseolide	6.3	17061 ± 7410	32980 ± 4223	4.2 ± 0.2	4.5 ± 0.1	**0.026**
Galaxolide	5.9	8902 ± 2972	19661 ± 1947	3.9 ± 0.1	4.3 ± 0.1	**0.003**
Tonalide	5.7	10910 ± 4089	21087 ± 4287	4.0 ± 0.2	4.3 ± 0.1	**0.023**

1. The average percent VSS was 80 ± 25 %, 70 ± 6 % in the 3.5 and 10.5 days sludge, respectively (see Appendix A and B).
2. The experimental K_{OM} values were converted to L/kg for comparison with reported values.

Figure 4-31 and 4-32 show the experimental $\log K_P^{'}$ versus the $\log K_{OW}$ of the PSMs and provide a best linear fit curve. A good correlation (r^2 of 0.91 and 0.87) for sludge at SRT of 3.5 and 10.5 days, respectively, was observed.

The experimental $\log K_P^{'}$ values observed of Galaxolide and Tonalide of 3.9 to 4.3 are within one order of magnitude of the corresponding log organic carbon-water partition coefficient ($\log K_{OC}$ (L/kg)) of 4.9 and 4.8, respectively reported by Balk et al. (1999). Similarly the experimental organic matter partition coefficient K_{OM} (L/Kg) for Galaxolide and Tonalide of 9000 to

21000 L/kg is within one order of magnitude of the secondary sludge portioning in L/Kg reported by Ternes et al. (2004) of 2000 and 2400 L/kg, respectively.

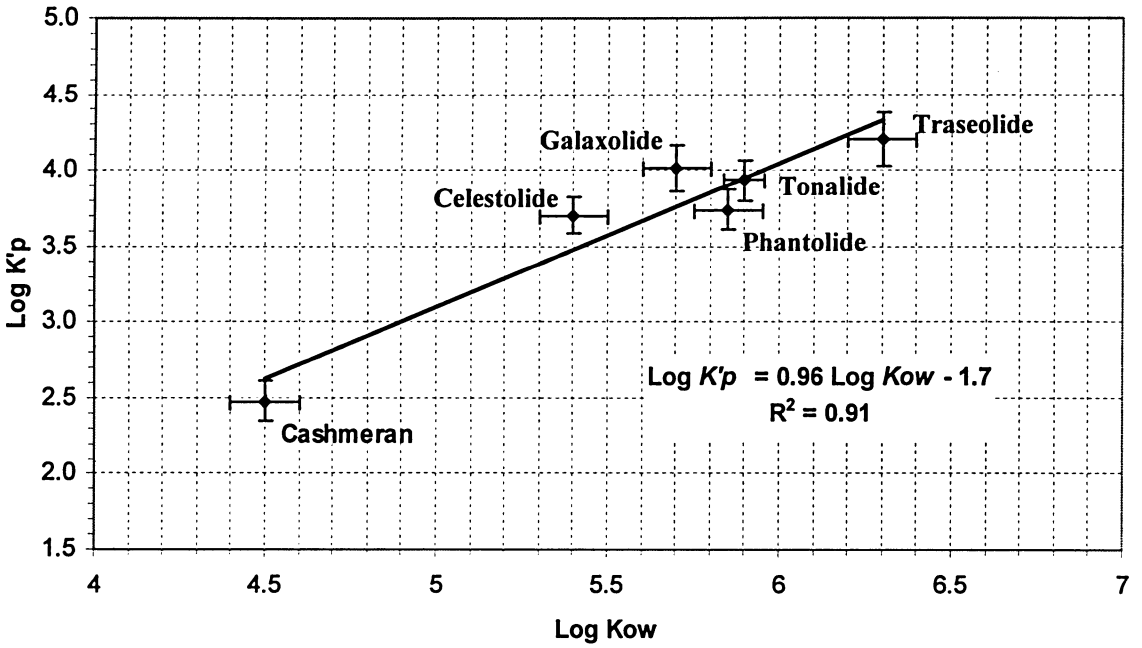


Figure 4-31. Correlation of the equilibrium sorption partition coefficient with the octanol-water partition coefficients with sludge from the SBR operated at an average SRT of 3.5 days.

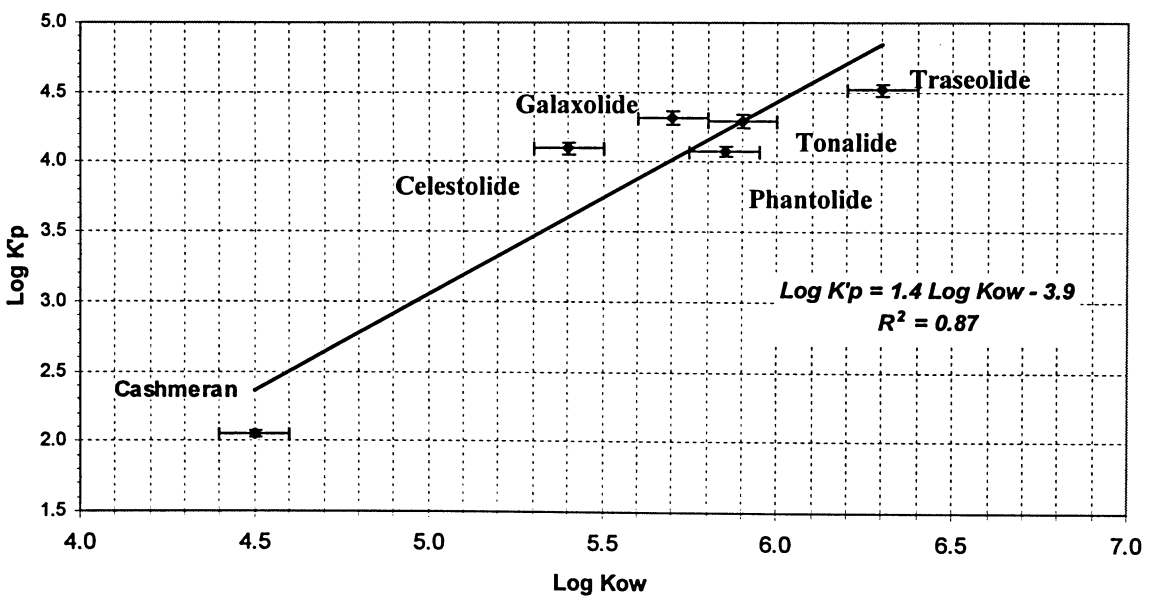


Figure 4-32. Correlation of the equilibrium sorption partition coefficient with the octanol-water partition coefficients with sludge from the SBR operated at an average SRT of 10.5 days.

4.9 Summary

In the operation of the SBRs stable operating conditions at the lower and higher SRTs of 3.5 and 10.5 days, took the expected time of about 3 SRTs to achieve stable operating conditions when upset occurrences (power outages and feed loss) were taken into account. The operating conditions provided sufficiently unique conditions that became evident in the activated sludge measured characteristics, conventional performance, the PSMs partitioning and sorption-desorption behaviour.

The AS morphological characteristics including the mean and 90 percentile floc size, the mean settling velocity (mm/s), floc porosity and excess density were found to be significantly different at the higher SRT as opposed to the lower SRT (see Table 4-3 and 4-4). No significant temperature effect, on these morphological properties, was observed. The mean floc size was smaller, less porous, more dense and had a higher settling velocity (all significant at the 95% level) than at the lower SRT. These morphological properties are considered to impact the advective transfer mechanisms, the mean diffusional distances of substrates and the available surface area to volume ratio readily available for sorption.

The EPS constituents showed a significant SRT effect, with increasing proteins and carbohydrates and a reduction of DNA and acidic polysaccharides at the higher SRT of 10.5 days. No significant T effect was observed. A significant difference during nitrification versus non-nitrification conditions, similar to the higher SRT, in the distribution of EPS constituents was observed. The one exception was EPS-proteins to carbohydrates ratio which did not show any significant difference under T, SRT or nitrification changes.

Two-factor ANOVA analyses were conducted on the EPS constituents and no significant T or interaction parameter, TxSRT, effects were observed. The single parameter Type I SS model (based on SRT) had a good to moderate (r^2 from 0.58 to 0.78) correlation coefficient (see Table 4-5) in predicting the EPS DNA, acidic polysaccharides and carbohydrates. On the basis of morphological and EPS constituents, it appears the SRT is the dominant influencing factor with nitrification and T

playing a secondary influence. Nitrification, as shown in Figure 4-1, is influenced by SRT and T at a ratio of approximately 2 days/ °C, which suggest that T has a more significant influence under nitrifying conditions. We observed only one nitrifying condition in the experimental set up at 20 °C and 10 days SRT and significance of T over SRT could not be tested.

The surface charge (SC) did not show any significant changes during SRT or T ranges of 3.5 to 10.5 days or 10 to 20 °C and the relative hydrophobicity (RH) showed a significant difference under the different SRT conditions. The ANOVA analysis did however indicate SRT, T and TxSRT, in descending priority, to be significant with respect to RH. Also the Type I SS three parameter Model (SRT, T and TxSRT) provided a very good prediction (r^2 =0.85, see Table 4-6) of the RH. RH is expected to influence the sorption and partitioning of PSMs due to hydrophobic interactions at the EPS level.

Galaxolide and Tonalide were found to represent more than 95% of the total PSMs in municipal sewage. The effluent PSMs were found to be reduced by 62% to 80% from the influent concentration with the nitrifying SBR providing the highest reduction. The PSMs solids concentrations were found to be the lowest in AS from the nitrifying reactor. T and SRT are both significant, however T was more significant than SRT in reducing PSMs concentration in the effluent and sludge.

Sorption equilibrium of PSMs on sludge at pH of 7.4 and T of 25 °C was observed to occur within 30 minutes. PSMs sorption and desorption isotherms were found to be slightly non-linear in the aqueous concentration range of 10 to 300 µg/L range and to fit the Freundlich model equation well. The sorption of the PSMs on sludge was essentially irreversibly under the experimental conditions and the PSMs were found to concentrate on sludge at a 5 to 10 L/g concentration ratio. This predominant concentration on sludge was suggested by the high *Kow* values. The sorption predictions provided by the isotherms at different SRTs suggest that other abiotic or biologically mediated processes must be reducing PSMs in the effluent and sludge beyond the sorption mechanisms alone. It is predicted by the Freundlich isotherms that the sludge at higher SRT will

desorb significantly less PSMs than the lower SRT sludge. The phenomena of PSMs partitioning is complicated but appears to be strongly correlated to the changes affected by SRT and T in the fundamental floc properties such as floc size, porosity, density, EPS proteins, carbohydrates, acidic polysaccharides, DNA, total EPS and relative hydrophobicity. Further consideration of the K_P to the K_{OW} suggests a strong correlation of the portioning behaviour of PSMs to K_{OW} values.

Relative hydrophobicity was significantly influence by the operating T conditions and is considered the most likely property to influence PSMs partitioning under our experimental conditions. The floc morphological properties and EPS constituents were most influenced by SRT and nitrifying conditions which also present opportunities to influence PSM partitioning. Surface charge appears to have a minimal direct impact on changing the behaviour of the partitioning of PSMs under the experimental conditions. The coupling of T and SRT during nitrification has complicated the issue of understanding which fundamental sludge properties primarily influence PSMs partitioning.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

This chapter provides the conclusions and recommendations on the findings of this study. The specific objectives consisted in investigating the:

(1) The effect of SRT and T on selected sludge floc properties;

(2) The correlation of the removal of selected polycyclic synthetic musks (PSMs) to sludge floc properties grown at different SRTs and Ts; and

(3) The competitive equilibrium adsorption-desorption behaviour of selected musks to the different sludge.

The purpose of the objectives was to test the central hypothesis which was that solids retention time (SRT) and temperature (T) change key activated sludge floc properties sufficiently to affect the sludge's capacity to influence removal of polycyclic synthetic musks (PSMs).

The key sludge floc properties investigated included sludge surface charge (SC), relative hydrophobicity (RH), EPS and EPS constituents, sludge volume index (SVI), mean particle size, size distribution, porosity and sorption-desorption characteristics of activated sludge from SBRs operated at 3.5 and 10.5 days SRT.

The conclusions and recommendations discussion of the results leads to various important conclusions and recommendations related to environmental strategies for dealing with the optimization of the activated sludge sewage treatment process and the management of waste municipal sludge as it relates to similar microcontaminants of environmental concern of which PSM are a subclass.

5.1 Conclusions

Operating the activated sludge SBRs at higher SRTs and Ts reduced the final PSMs concentration in the effluent and PSMs associated with sludge significantly and to the lowest levels. SRT followed by T played important roles in determining the partitioning and removal of PSMs. The coupling of T and SRT was evident under nitrifying conditions which provided the highest reduction of PSMs found in the aqueous and solids matrix but this also corresponded to the highest T and SRT operating conditions. The observed PSMs datasets were observed to follow a lognormal and in some cases non-parametric distribution and sufficient data points (≥ 5) are required to make meaningful comparisons when using less powerful statistical non-parametric methods.

Fundamental sludge floc properties changes were affected by SRT and T changes. The sludge floc properties considered to be the most important in priority sequence include: (1) sludge relative hydrophobicity; (2) floc size, porosity and, density; and (3) EPS constituents which include proteins, carbohydrates, acidic polysaccharide, DNA and total EPS. The sorptive and desorptive capacity of sludge is assumed to be a result of the fundamental properties (1) to (3) which influence the sorption and desorption coefficients of the sludge.

Freundlich isotherm predictions of the fate of PSMs in the AS process suggest that other abiotic or biologically mediated mechanisms are present which reduce PSMs further. The adsorption of PSMs is predicted to be effectively a nonreversible process. The experimental K_P values were determined and found to be well correlated to the PSMs K_{OW} and within one order of magnitude for Galaxolide and Tonalide. K_P values for other PSMs have not been previously reported on.

5.2 Recommendations

Due to typically non-parametric environmental trace sample datasets, the reduced power of non-parametric statistical methods and the difficulties associated with obtaining low MDL in trace analyses of complex matrices, sufficient number of replicate samples (>10 per sampling point) is recommended to conduct meaningful comparative controlled studies.

Galaxolide and Tonalide are typically over 95% of the PSMs and sampling and analyses for these two compounds will provide a good indicator of most PSMs in municipal wastewater in southern Ontario. Cashmeran although classified as a PSMs, for purposes of AS treatment process, should not be grouped with the rest of the PSMs. Cashmeran's higher water solubility and lower *Kow* make it behave significantly different than the other PSMs investigated in this study.

5.3 Engineering Implications

The present work suggest that upgrading activated sludge WWTPs to partially or fully nitrifying conditions can yield benefits in the reduction of PSMs in the final effluent and associated to sludges by about a 20%. Similar benefits are expected for other microcontaminants.

Engineering flocs (by appropriate selection of reactor conditions) to affect such properties as relative hydrophobicity and EPS constituents, by judicious selection of SRT, nutrients, reducing conditions and reactor configurations, may provide economic incentives over fully nitrifying conditions which provide similar benefits.

5.4 Future Investigations

Similar bench scale studies can be extended to monitor for PSM metabolites, conduct volatilization or air stripping studies to determine a mass balance around the reactors and if significant biotransformations occur. Considering sludge anoxic, anaerobic and aerobic treatment to determine if sludge treatment can further reduce PSMs is a natural extension especially if sludge land utilization is practiced. Considering anoxic conditions at different SRTs may also provide some further insights as to how differences in activated sludge properties and reducing conditions may effect the removal of PSMs suspended growth activated sludge systems.

Appendix A
Phase I SBRs Conventional Operating Data

Table A-1. Phase I MLSS, MLVSS and SRT during operating conditions

Date 2004	Day	MLSS SBR 1-1 (mg/l)	MLSS SBR 2-1 (mg/l))	MLVSS SBR 1-1 (mg/l)	MLVSS SBR 2-1 (mg/l))	SRT SBR 1-1 (days)	SRT SBR 2-1 (days)
23-Jul	0	-	-	-	-	-	-
27-Jul	4	-	-	-	-	-	-
29-Jul	6	-	-	-	-	-	-
3-Aug	11	-	-	-	-	-	-
4-Aug	12	-	-	-	-	-	-
5-Aug	13	-	-	-	-	-	-
9-Aug	17	164	44	117	32	0.10	0.03
10-Aug	18	634	552	471	412	2.78	3.62
11-Aug	19	897	559	682	440	3.35	2.06
12-Aug	20	960	739	722	556	3.60	3.67
13-Aug	21	993	361	740	283	3.66	0.28
16-Aug	24	624	625	469	625	2.09	2.43
17-Aug	25	1007	781	765	593	3.56	2.12
18-Aug	26	958	685	735	536	3.57	1.80
19-Aug	27	950	709	733	541	3.58	3.79
20-Aug	28	909	800	698	610	2.95	3.32
23-Aug	31	793	891	600	644	2.65	3.58
24-Aug	32	1007	981	771	719	3.07	3.90
25-Aug	33	963	1091	734	803	2.31	3.92
26-Aug	34	909	1035	683	747	1.11	2.44
27-Aug	35	823	1050	621	787	-	-
30-Aug	38	577	833	438	603	-	-
31-Aug	39	827	992	640	665	3.06	3.50
1-Sep	40	920	1075	713	817	2.71	3.34
2-Sep	41	841	1032	641	772	2.96	3.85
3-Sep	42	1132	1131	867	861	3.41	3.85
7-Sep	46	671	1027	521	776		3.62
8-Sep	47	963	824	736	635	-	-
9-Sep	48	1016	887	756	675	1.54	2.73
10-Sep	49	1170	1072	863	783	3.11	3.91
13-Sep	52	1650	1817	1196	1333	3.05	3.29
14-Sep	53	-	-	-	-	-	-
15-Sep	54	2490	2338	1782	1692	-	-
16-Sep	55	3333	2418	2413	1743	-	-
17-Sep	56	1556	1848	1017	1362	-	-

Date 2004	Day	MLSS SBR 1-1 (mg/l)	MLSS SBR 2-1 (mg/l))	MLVSS SBR 1-1 (mg/l)	MLVSS SBR 2-1 (mg/l))	SRT SBR 1-1 (days)	SRT SBR 2-1 (days)
20-Sep	59	1329	1512	994	1115	3.20	2.31
21-Sep	60	-	-	-	-	-	-
22-Sep	61	1657	2272	1217	1630	3.33	3.33
23-Sep	62	1942	2495	1467	1844	3.40	3.56
24-Sep	63	2114	2448	1587	1835	3.61	3.28
27-Sep	66	-	-	-	-	-	-
28-Sep	67	2161	2377	1646	1771	3.78	-
29-Sep	68	2092	2245	1565	1606	3.81	-
30-Sep	69	1952	2224	1455	1605	-	-
1-Oct	70	-	-	-	-	-	-
4-Oct	73	1553	1445	1224	1087	3.58	3.82
5-Oct	74	2109	1600	1528	1132	3.70	3.84
6-Oct	75	2055	1797	1540	1328	-	3.93
7-Oct	76	-	-	-	-	-	-
8-Oct	77	3320	2830	2430	2040	3.77	3.73
11-Oct	80	1794	1813	1310	1290	3.55	3.78
12-Oct	81	-	-	-	-	-	-
13-Oct	82	-	-	-	-	-	-
14-Oct	83	1442	1184	1104	932	3.29	3.58
15-Oct	84	1425	1255	1125	985	3.16	3.61
16-Oct	85	1510	1135	1195	910	3.36	3.74
17-Oct	86	1220	880	980	725	3.24	3.67
18-Oct	87	1150	735	895	615	3.15	3.71
19-Oct	88	1275	807	975	637	3.22	3.46
20-Oct	89	930	750	717	580	3.64	3.06
21-Oct	90	1260	870	945	670	3.58	3.53
22-Oct	91	1180	895	930	685	3.52	3.47
07-Jul	92	1942	1755	1486	1287		
08-Jul	93	1782	1657	1346	1223		
11-Jul	94	1883	1703	1436	1242		
12-Jul	95	2219	2098	1701	1551		
13-Jul	96	2047	1789	1533	1326		
14-Jul	97	1978	1745	1494	1297		
15-Jul	98	1998	2209	1536	1665		

Table A-2. Phase I MLSS, MLVSS and SRT descriptive statistics during operating conditions

Descriptive Statistics	MLSS SBR 1-1 (mg/l)	MLSS SBR 2-1 (mg/l))	MLVSS SBR 1-1 (mg/l)	MLVSS SBR 2-1 (mg/l))	SRT SBR 1-1 (days)	SRT SBR 2-1 (days)
Mean	1411.71	1334.32	1062.23	993.89	3.10	3.21
Standard Error	86.15	86.94	62.98	62.05	0.12	0.14
Median	1240	1083	960	810	3.31	3.57
Mode	1007	#N/A	#N/A	#N/A	3.58	3.62
Standard Deviation	644.69	650.62	471.30	464.31	0.76	0.91
Sample Variance	415619	423300	222123	215582	0.57	0.83
Kurtosis	1.02	-0.76	0.73	-0.70	6.37	4.97
Skewness	0.89	0.48	0.79	0.46	-2.36	-2.20
Range	3169	2786	2313	2008	3.71	3.9
Minimum	164	44	117	32	0.1	0.03
Maximum	3333	2830	2430	2040	3.81	3.93
Sum	79056	74722	59485	55658	124.11	128.46
Count	56	56	56	56	40	40

Table A-3. Phase I MLSS, MLVSS and SRT descriptive statistics during stable operating conditions

Descriptive Statistics	MLSS SBR 1-1 (mg/l)	MLSS SBR 2-1 (mg/l))	MLVSS SBR 1-1 (mg/l)	MLVSS SBR 2-1 (mg/l))	SRT SBR 1-1 (days)	SRT SBR 2-1 (days)
Mean	1717.71	1473.90	1306.19	1105.10	3.44	3.64
Standard Error	114.90	121.08	81.80	83.81	0.06	0.06
Median	1782	1600	1310	1132	3.52	3.69
Mode	#N/A	#N/A	#N/A	#N/A	3.58	#N/A
Standard Deviation	526.54	554.84	374.84	384.08	0.21	0.22
Sample Variance	277245	307846	140505	147519	0.05	0.05
Kurtosis	3.04	0.08	2.80	0.16	-1.55	2.96
Skewness	1.23	0.49	1.13	0.52	-0.05	-1.40
Range	2390	2095	1713	1460	0.62	0.87
Minimum	930	735	717	580	3.15	3.06
Maximum	3320	2830	2430	2040	3.77	3.93
Sum	36072	30952	27430	23207	44.76	50.93
Count	21	21	21	21	13	14

Table A-4. Phase I pH and T during operating conditions

SBR 1-1			SBR 2-1		
Date	pH	T	Date	pH	T
23-Jul	7.6	12.6	23-Jul	8.0	19.8
27-Jul	7.6	12.5	27-Jul	8.1	21.5
29-Jul	7.1	12.6	30-Jul	7.1	19.8
03-Aug	7.9	11.9	03-Aug	7.5	19.6
04-Aug	8.1	11.0	04-Aug	7.9	19.6
05-Aug	8.1	11.2	05-Aug	8.1	19.5
09-Aug	7.8	9.8	09-Aug	7.5	19.8
10-Aug	7.4	11.8	10-Aug	8.2	20.3
11-Aug	7.4	11.4	11-Aug	7.7	19.8
12-Aug	7.4	10.7	12-Aug	7.7	19.8
13-Aug	7.6	11.2	13-Aug	7.7	20.0
16-Aug	7.6	11.1	16-Aug	7.5	20.0
17-Aug	8.1	10.7	17-Aug	7.8	19.5
18-Aug	8.4	11.4	18-Aug	8.2	20.0
19-Aug	8.3	10.7	19-Aug	8.4	20.0
20-Aug	8.9	10.9	20-Aug	8.8	19.6
23-Aug	8.4	10.3	23-Aug	8.2	19.6
24-Aug	7.5	9.9	24-Aug	7.5	19.7
25-Aug	8.2	9.8	25-Aug	8.3	19.7
26-Aug	8.0	10.6	26-Aug	7.9	20.3
31-Aug	7.7	10.2	31-Aug	7.6	19.9
01-Sep	7.3	11.0	01-Sep	7.2	20.1
09-Sep	7.1	11.2	07-Sep	7.2	20.5
10-Sep	7.9	11.0	09-Sep	7.1	20.5
14-Sep	7.3	13.0	10-Sep	7.9	20.0
20-Sep	8.1	10.0	14-Sep	6.8	20.0
21-Sep	7.6	10.1	20-Sep	7.8	18.0
22-Sep	8.4	10.0	21-Sep	7.3	18.0
23-Sep	7.8	10.0	22-Sep	8.1	16.5
27-Sep	8.0	10.0	23-Sep	7.4	17.0
28-Sep	8.6	9.8	27-Sep	7.7	17.0
29-Sep	7.8	9.1	28-Sep	8.1	16.0
01-Oct	8.1	9.8	29-Sep	7.5	15.9
04-Oct	7.8	9.1	01-Oct	7.5	17.4
05-Oct	7.9	10.0	04-Oct	7.5	20.0
07-Oct	7.5	10.0	05-Oct	7.6	20.0
13-Oct	7.9	10.0	06-Oct	7.5	20.0
18-Oct	7.8	10.0	07-Oct	7.5	20.3
19-Oct	7.8	10.0	13-Oct	7.5	20.0
20-Oct	7.8	10.0	18-Oct	7.7	20.0
21-Oct	7.8	10.0	21-Oct	7.6	20.0
22-Oct	7.5	10.0	22-Oct	7.6	20.0
08-Jul-05	7.4	12.8	08-Jul-05	7.4	19.2
11-Jul-05	7.4	12.8	11-Jul-05	7.1	19.5
12-Jul-05	7.5	16.2	12-Jul-05	7.4	15.6
13-Jul-05	7.3	13.4	13-Jul-05	7.0	19.3
14-Jul-05	7.2	14.2	14-Jul-05	6.8	20.1
15-Jul-05	7.4	14.7	15-Jul-05	6.9	20.1

Table A-5. Phase I pH and T descriptive statistics during operating conditions

Descriptive Statistics	SBR 1-1		SBR 2-1	
	pH	T	pH	T
Mean	7.77	11.05	7.63	19.35
Standard Error	0.06	0.22	0.06	0.19
Median	7.77	10.7	7.56	19.8
Mode	8.1	10	7.45	20
Standard Deviation	0.39	1.49	0.44	1.33
Sample Variance	0.15	2.23	0.19	1.76
Kurtosis	0.31	2.21	0.11	1.81
Skewness	0.57	1.45	0.33	-1.63
Range	1.8	7.1	2.03	5.9
Minimum	7.08	9.1	6.81	15.6
Maximum	8.88	16.2	8.84	21.5
Sum	372.75	530.5	366.29	928.8
Count	48	48	48	48

Table A-6. Phase I pH and T descriptive statistics during stable operating conditions

Descriptive Statistics	SBR 1-1		SBR 2-1	
	pH	T	pH	T
Mean	7.6	12.0	7.3	19.5
Standard Error	0.1	0.7	0.1	0.4
Median	7.525	11.4	7.41	20
Mode	7.83	10	#N/A	20
Standard Deviation	0.23	2.28	0.28	1.28
Sample Variance	0.05	5.18	0.08	1.63
Kurtosis	-1.77	-1.18	-1.03	9.83
Skewness	0.02	0.54	-0.63	-3.05
Range	0.64	6.2	0.85	4.7
Minimum	7.23	10	6.82	15.6
Maximum	7.87	16.2	7.67	20.3
Sum	90.93	144.1	87.84	234.1
Count	12	12	12	12

Table A-7. Phase I SVI during stable operating conditions

Sampling Date	SBR 1-1 (SRT=3.4 d, T=10 °C) WAS SVI				SBR 2-1 (SRT=3.5 d, T=20 °C) WAS SVI			
	Initial Volume (mL)	Settled Volume (mL)	MLSS (mg/L)	SVI (mL/g)	Initial Volume (mL)	Settled Volume (mL)	MLSS (mg/L)	SVI (mL/g)
07-Jul-05	202	34	1942	87	200	27	1755	77
08-Jul-05	202	30	1782	83	202	25	1657	75
11-Jul-05	202	30	1883	79	200	24	1703	70
12-Jul-05	200	32	2219	72	200	27	2098	64
13-Jul-05	208	34	2047	80	206	28	1789	76
14-Jul-05	202	36	1978	90	200	28	1745	80
15-Jul-05	200	33	1998	83	202	30	2209	67

Table A-8. Phase I SVI descriptive statistics during stable operating conditions

Descriptive Statistics	*SBR 1-1 SVI (mL/g)*	*SBR 2-1 SVI (mL/g)*
Mean	81.93	72.84
Standard Error	2.19	2.15
Median	82.58	74.69
Mode	#N/A	#N/A
Standard Deviation	5.80	5.68
Sample Variance	33.64	32.29
Kurtosis	0.53	-1.11
Skewness	-0.40	-0.37
Range	18.00	15.88
Minimum	72.10	64.35
Maximum	90.10	80.23
Sum	573.53	509.86
Count	7	7

Appendix B
Phase II SBR 1-2 and SBR 2-2 Conventional Data

Table B-1. Phase II MLSS, MLVSS, ESS and SRT during poerating conditions

Date 2005	Opera-tional Days	MLSS SBR 1-2 (mg/L)	MLSS SBR 2-2 (mg/L)	MLVSS SBR 2-1 (mg/L)	MLVSS SBR 2-2 (mg/L)	ESS SBR 1-2 (mg/L)	ESS SBR 2-2 (mg/L)	SRT SBR 2-1 (days)	SRT SBR 2-2 (days)
01-Feb	1	3316	2693	2328	1813	10	17	10.8	9.8
02-Feb	2	3297	2796	2306	1892	12	5	10.6	11.3
03-Feb	3	3550	3044	2514	2117	7	5	11.2	11.3
04-Feb	4	3560	2928	2505	2000	6	5	11.3	11.3
07-Feb	7	4032	3610	2869	2488	8	13	11.2	10.6
08-Feb	8	3436	3117	2511	2192	9	4	11.0	11.5
09-Feb	9	3447	3094	2469	2148	8	5	11.1	11.3
10-Feb	10	3444	3212	2488	2229	7	3	11.2	11.6
11-Feb	11	3090	2825	2229	1967	6	13	11.2	10.3
14-Feb	14	3278	3074	2416	2178	5	3	11.4	11.6
15-Feb	15	3093	3035	2308	2165	6	4	11.2	11.5
16-Feb	16	2841	2786	2096	1989	7	5	11.0	11.3
17-Feb	17	3215	2557	2300	1765	8	4	11.0	11.4
18-Feb	18	2708	2334	1933	1611	9	3	10.7	11.5
21-Feb	21	2692	2323	1926	1597	10	4	10.6	11.3
22-Feb	22	2702	2566	1955	1811	7	2	11.0	11.7
23-Feb	23	2980	2429	2112	1679	7	3	11.1	11.5
24-Feb	24	2982	2615	2123	1811	8	4	10.9	11.4
28-Feb	28	2723	3060			8	4	10.8	11.5
01-Mar	29	2811	2825	2017	1969	8	12	10.9	10.4
02-Mar	30	2624	2437	1891	1691	8	10	10.8	10.4
03-Mar	31	2766	2574	1969	1762	8	10	10.9	10.5
04-Mar	32	3048	2637	2190	1846	6	12	11.2	10.3
07-Mar	35	3068	2872			10	28	10.7	8.9
08-Mar	36	3153	2785	2260	2049	7	26	11.1	9.0
09-Mar	37	2738	2401	1943	1672	4	28	11.4	8.4
10-Mar	38	2969	2471	2100	1726	6	27	11.2	8.6
11-Mar	39	3196	2507	2311	1803	15	25	10.3	8.8
14-Mar	42	3274	2881	2379	2084	13	16	10.5	10.0
15-Mar	43	3221	3034	2334	2163	6	15	11.2	10.2
16-Mar	44	3294	4216	2413	3008	6	14	11.3	10.7
17-Mar	45	3431	2771	2490	1987	4	37	11.5	8.1
18-Mar	46	3519	2676	2520	1920	3	12	11.6	10.3
21-Mar	49	3231	2315	2313	1648	4	39	11.5	7.4
22-Mar	50	3314	2060	2361	1457	4	19	11.5	9.0
23-Mar	51	3054	1878	2163	1337	3	3	11.6	11.3
29-Mar	57	3383	2130	2779	1187	5	7	11.4	10.7
30-Mar	58	3434	2210	2486	1571	5	5	11.4	11.1
31-Mar	59	3454	2782	2509	2030	5	7	11.5	10.7
01-Apr	60	3008	2439	2157	1718	5	5	11.3	10.9
04-Apr	63	2695	1859	1927	1286	5	7	11.2	10.9
06-Apr	65	2756	2332	1921	1540	7	8	11.3	10.8
07-Apr	66	3212	2998	2260	2018	9	10	11.1	10.9
08-Apr	67	2944	2681	2080	1823	4	10	11.4	10.6
11-Apr	70	3071	3118	2203	2118	5	12	11.3	10.5
12-Apr	71	2949	3082	2140	2107	4	10	11.3	10.5
13-Apr	72	2828	2947	2045	2009		7		10.7

Date 2005	Opera-tional Days	MLSS SBR 1-2 (mg/L)	MLSS SBR 2-2 (mg/L)	MLVSS SBR 2-1 (mg/L)	MLVSS SBR 2-2 (mg/L)	ESS SBR 1-2 (mg/L)	ESS SBR 2-2 (mg/L)	SRT SBR 2-1 (days)	SRT SBR 2-2 (days)
14-Apr	73	2911	3029	2125	2056	4	14	11.4	11.1
15-Apr	74	2699	2783	2020	1933	4	28	11.4	10.1
19-Apr	78	2676	2940	1990	2062	5	21	11.2	9.5
20-Apr	79	2818	2934	2095	2026	5	19	11.3	9.7
21-Apr	80	2728	2877	2058	1995	6	17	11.1	9.9
22-Apr	81	2546	2899	1895	2045	6	18	11.1	9.8
28-Apr	87	2676		1939		5	9	11.2	

Table B-2. Phase II MLSS, MLVSS, ESS and SRT descriptive statistics during operating conditions

Descriptive Statistics	MLSS SBR 1-2 (mg/L)	MLSS SBR 2-2 (mg/L)	MLVSS SBR 1-2 (mg/L)	MLVSS SBR 2-2 (mg/L)	ESS SBR 1-2 (mg/L)	ESS SBR 2-2 (mg/L)	SRT SBR 1-2 (days)	SRT SBR 2-2 (days)
Mean	3072	2745	2224	1904	6.6	12.1	11.1	10.5
Standard Error	43	56	32	42	0.3	1.2	0.0	0.1
Median	3061	2785	2196.5	1967	6	10	11.2	10.7
Mode	2676	2825	2260	1811	5	5	11.2	#N/A
Standard Deviation	316	405	233	303	2.5	9.1	0.3	1.0
Sample Variance	99623	164349	54333	91509	6.1	82.8	0.1	1.0
Kurtosis	-0.03	2.59	-0.11	2.91	1.8	0.9	0.6	0.7
Skewness	0.46	0.54	0.54	0.48	1.2	1.2	-0.8	-1.1
Range	1486	2357	978	1821	12	37	1.4	4.2
Minimum	2546	1859	1891	1187	3	2	10.3	7.4
Maximum	4032	4216	2869	3008	15	39	11.6	11.7
Sum	165885	145478	115671	97098	352	653	590.3	554.2
Count	54	53	52	51	53	54	53	53

Table B-3. Phase II MLSS, MLVSS, ESS and SRT descriptive statistics during stable operating conditions

Descriptive Statistics	MLSS SBR 1-2 (mg/L)	MLSS SBR 2-2 (mg/L)	MLVSS SBR 1-2 (mg/L)	MLVSS SBR 2-2 (mg/L)	ESS SBR 1-2 (mg/L)	ESS SBR 2-2 (mg/L)	SRT SBR 1-2 (days)	SRT SBR 2-2 (days)
Mean	2834	2780	2057	1910	5.3	13.0	11.3	10.4
Standard Error	46	93	28	65	0.4	1.7	0.0	0.1
Median	2818	2917	2058	2014	5.0	10.0	11.3	10.5
Mode	2676	#N/A	#N/A	#N/A	5.0	10.0	11.2	#N/A
Standard Deviation	179	350	109	241	1.4	6.4	0.1	0.5
Sample Variance	32044	122368	11970	58315	1.9	40.9	0.0	0.3
Kurtosis	-0.16	2.72	-0.81	2.52	3.2	0.5	-0.4	-1.2
Skewness	0.53	-1.69	0.16	-1.72	1.6	1.0	-0.3	-0.5
Range	666	1259	365	832	5.0	23.0	0.4	1.5
Minimum	2546	1859	1895	1286	4.0	5.0	11.1	9.5
Maximum	3212	3118	2260	2118	9.0	28.0	11.4	11.1
Sum	42517	38918	30855	26736	74.0	195.0	157.7	146.0
Count	15	14	15	14	14	15	14	14

Table B-4. Phase II COD, and NH_3-N during operating conditions

Date	Day	Primary Sewage COD (mg/L)	SBR 1-2 Effluent Total COD (mg/L)	SBR 2-2 Effluent Total COD (mg/L)	Primary Sewage NH_3-N (mg/L)	SBR 1-2 Effluent NH_3-N (mg/L)	SBR 2-2 Effluent NH_3-N (mg/L)
03-Feb-05	3	230	29	43			
18-Feb-05	49				15.7	13.3	0.1
23-Feb-05	54					14.8	0.1
01-Mar-05	60				23.6	18.5	0.7
04-Mar-05	63				23.5	11.6	0.0
11-Mar-05	70				21.1	10.4	1.1
17-Mar-05	76						0.1
18-Mar-05	77	179	3	59			0.0
21-Mar-05	80						0.1
22-Mar-05	81						0.0
24-Mar-05	83				26.3	7.2	0.1
13-Apr-05	103	307	27	25			

Table B-5. Phase II MLSS, MLVSS and SRT descriptive statistics during poerating conditions

Descriptive Statistics	Primary Sewage COD (mg/L)	SBR 1-2 Effluent Total COD (mg/L)	SBR 2-2 Effluent Total COD (mg/L)	Primary Sewage N-NH3 (mg/L)	SBR 1-2 Effluent N-NH_3 (mg/L)	SBR 2-2 Effluent N-NH_3 (mg/L)
Mean	238.7	19.7	42.3	22.0	12.6	0.2
Standard Error	37.2	8.4	9.8	1.8	1.6	0.1
Median	230	27	43	23.5	12.465	0.1
Mode	#N/A	#N/A	#N/A	#N/A	#N/A	0.1
Standard Deviation	64.44	14.47	17.01	3.99	3.87	0.37
Sample Variance	4152.33	209.33	289.33	15.95	14.99	0.14
Kurtosis	#DIV/0!	#DIV/0!	#DIV/0!	1.61	0.30	3.14
Skewness	0.59	-1.69	-0.18	-1.12	0.21	1.99
Range	128	26	34	10.6	11.3	1.1
Minimum	179	3	25	15.7	7.2	0
Maximum	307	29	59	26.3	18.5	1.1
Sum	716	59	127	110.2	75.78	2.25
Count	3	3	3	5	6	10

Table B-6. Phase II primary sewage TSS and NH_3-N during operating conditions

Date	Day	Primary Sewage TSS (mg/L)	Primary Sewage NH_3-N (mg/L)	Date	Day	Primary Sewage TSS (mg/L)	Primary Sewage NH_3-N (mg/L)
07-Feb-05	7	135		24-Mar-05	52	80	26.3
18-Feb-05	18	98	15.7	29-Mar-05	57	114	
21-Feb-05	21	100		01-Apr-05	60	128	
23-Feb-05	23	93		04-Apr-05	63	99	
25-Feb-05	25	102		08-Apr-05	67	116	
28-Feb-05	28	74		11-Apr-05	70	127	
01-Mar-05	29		23.6	13-Apr-05	72	145	
02-Mar-05	30	96		15-Apr-05	74	150	
04-Mar-05	32	112	23.5	18-Apr-05	77	181	
07-Mar-05	35	72		19-Apr-05	78	216	
09-Mar-05	37	88		20-Apr-05	79	227	
11-Mar-05	39	114	21.1	21-Apr-05	80	171	
14-Mar-05	42	109		22-Apr-05	81	147	
16-Mar-05	44	135		25-Apr-05	84	164	
18-Mar-05	46	135		26-Apr-05	85	145	
21-Mar-05	49	118		27-Apr-05	86	150	
23-Mar-05	51	103		28-Apr-05	87	190	

Table B-7. Phase II primary sewage TSS and NH_3-N descriptive statistics during operating onditions

Descriptive Statistics	Primary Sewage TSS (mg/L)	Primary Sewage N-NH3 (mg/L)
Mean	128.3	22.0
Standard Error	6.6	1.8
Median	118	23.5
Mode	135	#N/A
Standard Deviation	38.19	3.99
Sample Variance	1458.72	15.95
Kurtosis	0.50	1.61
Skewness	0.87	-1.12
Range	155	10.6
Minimum	72	15.7
Maximum	227	26.3
Sum	4234	110.2
Count	33	5

Table B-8. Phase II DO profiles during daily SBR cycle times

SBR 1-2						SBR 2-2					
20-Jan-05		*08-Feb-05*		*22-Feb-05*		*18-Jan-05*		*08-Feb-05*		*22-Feb-05*	
Time	DO (mg/L)	Time	DO (mg/L)	Time	DO (mg/L)	Time	DO (mg/L)	Time	DO (mg/L)	Time	DO (mg/L)
9:04	0.68	9:34	0	9:25	1.07	9:14	0.37	9:35	0.17	9:21	0.27
9:12	0.38	9:41	0	9:33	1.48	9:22	0.59	9:42	0.26	9:29	0.3
9:21	0.05	9:48	0	9:41	2.1	9:31	0.82	9:49	0.19	9:37	0.44
9:29	0.3	9:55	0	9:49	2.62	9:39	0.77	9:57	0.25	9:46	0.53
9:38	0.61	10:03	0	9:58	3.17	9:47	1.01	10:04	0.28	9:54	0.61
9:46	0.86	10:10	0.18	10:06	3.71	9:56	1.14	10:11	0.35	10:03	0.75
9:54	1.1	10:17	0.68	10:14	4.18	10:04	1.35	10:19	0.46	10:11	0.88
10:03	1.25	10:24	1.12	10:23	4.83	10:13	1.54	10:26	0.58	10:20	1.06
10:11	1.61	10:32	1.55	10:31	5.08	10:21	1.86	10:33	0.65	10:28	1.25
10:20	1.91	10:39	1.98	10:39	5.25	10:30	2.05	10:41	0.72	10:36	1.39
20:28	2.2	10:46	2.41	10:47	5.34	10:38	2.32	10:48	0.85	10:45	1.5
10:36	2.56	10:53	2.75	10:56	5.55	10:46	2.55	10:56	1.07	10:53	1.61
10:45	2.74	11:01	3.19	11:04	5.73	10:55	2.74	11:03	1.19	11:02	1.83
10:53	2.93	11:08	3.31	11:12	5.84	11:03	3.01	11:10	1.34	11:10	2.04
11:02	3.03	11:16	3.46	11:29	5.92	11:12	3.22	11:18	1.45	11:19	2.2
11:10	2.94	11:23	3.57	11:37	6.26	11:20	3.37	11:25	1.69	11:27	2.37
11:19	2.98	11:30	3.76	11:46	7.37	11:29	3.39	11:32	1.9	11:35	2.59
11:27	2.95	11:37	3.89	11:54	8.18	11:37	3.54	11:40	2.13	11:44	3.54
11:35	3.02	11:44	4.68	12:02	8.55	11:45	3.98	11:47	3.25	11:52	5.53
11:52	4.61	11:52	5.26	12:10	8.98	11:54	4.5	11:55	4.8	12:00	6.4
12:01	5.29	11:59	5.75	12:19	9.17	12:02	5.18	12:02	5.74	12:09	6.78
12:09	5.69	12:06	6.13	12:27	9.27	12:11	6.25	12:09	6.19	12:17	7.01
12:17	6.02	12:13	6.4	12:35	9.4	12:19	6.85	12:17	6.42	12:26	7.08
12:26	6.12	12:21	6.59	12:44	9.48	12:27	7.14	12:24	6.69	12:34	7.22
12:34	6.36	12:28	6.64	12:52	9.43	12:36	7.34	12:31	6.74	12:42	7.31
12:43	6.41	12:35	6.87	13:00	9.48	12:44	7.46	12:39	6.86	12:51	7.53
12:51	6.51	12:42	6.95	13:08	9.48	12:53	7.49	12:46	6.84	12:59	7.51
12:59	6.62	12:50	7	13:17	9.54	13:01	7.4	12:54	6.93	13:08	7.54
13:08	6.64	12:57	7.06	13:25	9.56	13:10	7.46	13:01	6.93	13:17	7.57
13:16	6.49	13:04	7.06	13:33	9.59	13:18	7.52	13:08	6.89	13:25	7.55
13:25	6.69	13:11	7.1	13:42	8.82	13:26	7.53	13:16	6.99	13:33	7.61
13:33	6.77	13:19	7.1	13:50	5.3	13:35	7.56	13:23	6.93	13:41	7.47
13:41	6.82	13:26	7.12	13:58	0.64	13:43	7.45	13:30	6.99	13:50	3.9
13:50	4.89	13:33	7.11			13:52	2.94	13:38	6.93	13:58	1.29
13:58	1.43	13:41	7.09					13:45	7.01		
		13:48	5.06					13:52	3.52		
		13:55	2.38					14:00	0.54		

Table B-9. Phase II DO profiles during daily SBR cycle times descriptive statistics

Descriptive Statistics	SBR 1-2 DO Profile (mg/L)	SBR 2-2 DO Profile (mg/L)
Mean	4.63	3.79
Standard Error	0.39	0.45
Median	4.70	3.07
Mode	#N/A	7.32
Standard Deviation	2.39	2.75
Sample Variance	5.72	7.56
Kurtosis	-1.23	-1.72
Skewness	-0.21	0.17
Range	7.17	7.11
Minimum	0.58	0.27
Maximum	7.75	7.38
Sum	171.39	140.34
Count	37	37

Appendix C
Phase I and II EPS Protein Data

Table C-1. Phase I and II EPS protein in sludge samples during stable operating conditions

SBR	SRT (d)	T (°C)	Sampling Date	Measurement Date	Calculated Denaturing Factor	EPS Protein (mg/g MLSS)	
						Average	SD
SBR 1-1	3.5	10	15-Jul-05	29-Dec-05	6.7	36	7
SBR 1-1	3.5	10	19-Oct-04	09-Nov-04	1.0	94	2
SBR 1-1	3.5	10	20-Oct-04	09-Nov-04	1.0	82	2
SBR 1-1	3.5	10	22-Oct-04	09-Nov-04	1.0	102	2
SBR 2-1	3.5	20	15-Jul-05	29-Dec-05	6.7	39	3
SBR 2-1	3.5	20	19-Oct-04	09-Nov-04	1.0	94	0
SBR 2-1	3.5	20	20-Oct-04	09-Nov-04	1.0	135	0
SBR 2-1	3.5	20	22-Oct-04	09-Nov-04	1.0	83	8
SBR 1-2	10.5	10	14-Mar-05	29-Dec-05	11.6	200	14
SBR 1-2	10.5	10	15-Mar-05	29-Dec-05	11.6	179	6
SBR 1-2	10.5	10	16-Mar-05	29-Dec-05	11.6	102	13
SBR 1-2	10.5	10	17-Mar-05	29-Dec-05	11.5	67	15
SBR 2-2	10.5	20	14-Mar-05	29-Dec-05	11.6	231	8
SBR 2-2	10.5	20	15-Mar-05	29-Dec-05	11.6	126	7
SBR 2-2	10.5	20	16-Mar-05	29-Dec-05	11.6	234	29
SBR 2-2	10.5	20	17-Mar-05	29-Dec-05	11.5	76	21

Table C-2. Summary EPS protein averages and standard deviation

SBR	SRT (d)	T (°C)	Number of 24-hour Composite Samples	Proteins (mg/g MLSS)	
				Ave	SD
SBR 1-1	3.5	10	4	79	29
SBR 2-1	3.5	20	4	88	39
SBR 1-2	10.5	10	5	103	58
SBR 2-2	10.5	20	5	145	84

Table C-3. Phase I EPS protein comparison for denatured samples factor

Analysis Date	Sample Reactor	Sampling Date	Protein in EPS Nov 10-04 (mg/g MLSS)
Nov 10-04	SBR 1-1	Oct 19-04	94
	SBR 1-1	Oct 20-04	91
	SBR 2-1	Oct 19-04	94
	SBR 2-1	Oct 20-04	135
Analysis Date	Sample Reactor	Sampling Date	Protein in EPS Dec 29-04 (mg/g MLSS)
Dec 29-05	SBR 1-1	Oct 17-04	34
	SBR 1-1	Oct 18-04	39
	SBR 2-1	Oct 17-04	70
	SBR 2-1	Oct 18-04	68

Table C-4. Phase I EPS protein denaturing factor calculation

	Protein in EPS Nov 10-04	*Protein in EPS Dec 29-04*	Denaturing Factor	Denaturing Factor Calculation
Mean	103	53	2.0	mg/ MLSS g/L/ (49d)
Standard Deviation	440	350		
Observations	4	4	2004/11/10	Start Date
			2004/12/29	End Date
			49.00	days
			0.04	mg/ MLSS(g/L)/d

Table C-5. Phase I and II EPS protein replicate samples and analysis results

SBR	SRT (d)	T (°C)	Sampling Date	Measurement Date	Calculated Denaturing Factor	MLSS (g/L)	Protein per MLSS (mg/g)	Adjusted Protein per MLSS (mg/g)	Protein per MLSS (mg/g)	
									Ave	SD
SBR 1-1	3.5	10	15-Jul-05	29-Dec-05	6.7	4.98	5	32	36	7
SBR 1-1	3.5	10	15-Jul-05	29-Dec-05	6.7	4.98	6	41		
SBR 1-2	10.5	20	14-Mar-05	29-Dec-05	11.6	3.37	19	225	231	8
SBR 1-2	10.5	20	14-Mar-05	29-Dec-05	11.6	3.37	20	237		
SBR 1-2	10.5	10	15-Mar-05	29-Dec-05	11.6	3.70	10	122	126	6
SBR 1-2	10.5	10	15-Mar-05	29-Dec-05	11.6	3.70	11	130		
SBR 2-1	3.5	20	15-Jul-05	29-Dec-05	6.7	5.48	6	37	39	3
SBR 2-1	3.5	20	15-Jul-05	29-Dec-05	6.7	5.48	6	41		
SBR 2-2	10.5	10	14-Mar-05	29-Dec-05	11.6	2.77	18	209	200	14
SBR 2-2	10.5	10	14-Mar-05	29-Dec-05	11.6	2.77	18	207		
SBR 2-2	10.5	20	15-Mar-05	29-Dec-05	11.6	3.77	16	184	179	7
SBR 2-2	10.5	20	15-Mar-05	29-Dec-05	11.6	3.77	15	175		
SBR 1-2	10.5	10	16-Mar-05	29-Dec-05	11.6	3.37	10	111	102	13
SBR 1-2	10.5	10	16-Mar-05	29-Dec-05	11.6	3.37	8	93		
SBR 1-2	10.5	10	16-Mar-05	29-Dec-05	11.6	3.37	7	87		
SBR 2-2	10.5	20	16-Mar-05	29-Dec-05	11.6	2.77	19	221	234	29
SBR 2-2	10.5	20	16-Mar-05	29-Dec-05	11.6	2.77	23	268		
SBR 2-2	10.5	20	16-Mar-05	29-Dec-05	11.6	2.77	18	213		
SBR 1-2	10.5	10	17-Mar-05	29-Dec-05	11.5	3.70	5	57	67	15
SBR 1-2	10.5	10	17-Mar-05	29-Dec-05	11.5	3.70	5	59		
SBR 1-2	10.5	10	17-Mar-05	29-Dec-05	11.5	3.70	7	84		
SBR 2-2	10.5	20	17-Mar-05	29-Dec-05	11.5	3.77	8	98	76	21
SBR 2-2	10.5	20	17-Mar-05	29-Dec-05	11.5	3.77	6	73		
SBR 2-2	10.5	20	17-Mar-05	29-Dec-05	11.5	3.77	5	56		
SBR 1-1	3.5	10	19-Oct-04	09-Nov-04	1.0	3.33	95	95	94	2
SBR 1-1	3.5	10	19-Oct-04	09-Nov-04	1.0	3.33	92	92		
SBR 2-1	3.5	20	19-Oct-04	09-Nov-04	1.0	1.67	94	94	94	0
SBR 2-1	3.5	20	19-Oct-04	09-Nov-04	1.0	1.67	94	94		
SBR 1-1	3.5	10	20-Oct-04	09-Nov-04	1.0	5.00	84	84	82	2
SBR 1-1	3.5	10	20-Oct-04	09-Nov-04	1.0	5.00	80	80		
SBR 2-1	3.5	20	20-Oct-04	09-Nov-04	1.0	1.97	135	135	135	0
SBR 2-1	3.5	20	20-Oct-04	09-Nov-04	1.0	1.97	135	135		
SBR 1-1	3.5	10	22-Oct-04	09-Nov-04	1.0	3.48	104	104	102	2
SBR 1-1	3.5	10	22-Oct-04	09-Nov-04	1.0	3.48	100	100		
SBR 2-1	3.5	20	22-Oct-04	09-Nov-04	1.0	2.26	89	89	83	8
SBR 2-1	3.5	20	22-Oct-04	09-Nov-04	1.0	2.26	78	78		

Appendix D

Phase I and II EPS, Surface Charge and Relative Hydrophobicity Data

Table D- 1. Phase I and II EPS and EPS constituents

SBR	Operational Condition	SRT (days)	T	EPS Protein (mg/g MLSS)	EPS Acidic Polysaccharides (mg/g MLSS)	EPS Carbohydrates (mg/g MLSS)	EPS DNA (mg/g MLSS)	Total EPS (mg/g MLSS)	Ratio of EPS-PR to EPS-OCH
SBR 1-2	par-NI	10.50	10	200	3.67	45.8	0.5	250	4.4
SBR 1-2	par-NI	10.50	10	179	2.74	34.7	0.3	217	5.2
SBR 1-2	par-NI	10.50	10	102	1.20		0.1	103	
SBR 1-2	par-NI	10.50	10	67	1.17	30.9	0.0	99	2.2
SBR 2-2	NI	10.50	20	231	4.65	80.6	1.5	318	2.9
SBR 2-2	NI	10.50	20	126	3.10		0.9	130	
SBR 2-2	NI	10.50	20	234	1.23	15.0	0.6	251	15.6
SBR 2-2	NI	10.50	20	76	1.40	103.6	0.9	182	0.7
SBR 1-1	non-Ni	3.45	10	36	2.76	9.2	7.6	56	3.9
SBR 1-1	non-Ni	3.45	10	94	3.94	12.1	7.8	118	7.8
SBR 1-1	non-Ni	3.45	10	82	8.02	13.8	4.4	108	5.9
SBR 1-1	non-Ni	3.45	10	102	7.39	18.2	3.4	131	5.6
SBR 2-1	non-Ni	3.45	20	39	4.97	9.9	8.5	62	3.9
SBR 2-1	non-Ni	3.45	20	94	6.72	21.6	4.2	127	4.3
SBR 2-1	non-Ni	3.45	20	135	6.69	8.0	4.4	154	16.9
SBR 2-1	non-Ni	3.45	20	83	8.72	15.5	4.2	111	5.4

Table D- 2. Phase I sludge relative hydrophobicity

Relative Hydrophobicity					
Sampling and Analysis Dates	Number of Replicates (n)	SBR 1-1 10 °C, SRT=3.4 days		SBR 2-1 20 °C, SRT= 3.5 days	
		Average	Standard Deviation	Average	Standard Deviation
11-Jul-05	3	0.65	0.00	0.75	0.02
12-Jul-05	3	0.66	0.09	0.77	0.02
13-Jul-05	3	0.68	0.04	0.78	0.03
14-Jul-05	3	0.69	0.04	0.73	0.01
15-Jul-05	3	0.70	0.02	0.78	0.01

Table D- 3. Phase II sludge relative hydrophobicity

Relative Hydrophobicity					
Sampling Dates	Number of Replicates (n)	SBR 1-2 10 °C, SRT=11 days		SBR 2-2 20 °C, SRT=10 days	
		Average	Standard Deviation	Average	Standard Deviation
14-Mar-05	3	0.87	0.03	0.83	0.06
15-Mar-05	3	0.86	0.06	0.84	0.03
16-Mar-05	4	0.81	0.02	0.84	0.06
21-Mar-05	4	0.76	0.05	0.82	0.01
22-Mar-05	4	0.81	0.01	0.81	0.06
23-Mar-05	4	0.81	0.04	0.86	0.01

Table D- 4. Phase I sludge surface charge

Surface Charge (meq/g MLSS)					
Sampling and Analysis Dates	Number of Replicates (n)	SBR 1-1 10 °C, SRT=3.4 days		SBR 2-1 20 °C, SRT= 3.5 days	
		Average	Standard Deviation	Average	Standard Deviation
11-Jul-05	3	-0.85	0.06	-0.93	0.12
12-Jul-05	3	-0.71	0.05	-0.80	0.27
13-Jul-05	3	-0.38	0.07	-0.46	0.05
14-Jul-05	3	-0.57	0.10	-0.68	0.24
15-Jul-05	3	-0.42	0.02	-0.51	0.01

Table D- 5. Phase II sludge surface charge

Surface Charge (meq/g MLSS)					
Sampling and Analysis Dates	Number of Replicates (n)	SBR 1-2 10 °C, SRT=11 days		SBR 2-2 20 °C, SRT=10 days	
		Average	Standard Deviation	Average	Standard Deviation
14-Mar-05	3	-0.40	0.07	-0.46	0.05
15-Mar-05	3	-0.63	0.09	-0.32	0.06
16-Mar-05	3	-0.53	0.09	-0.49	0.06
22-Mar-05	2	-0.58	0.05	-0.50	0.02
23-Mar-05	2	-0.88	0.06	-0.54	0.05

Table D- 6. Phase I and II EPS results and analysis

Operational Conditions and p-value	Total EPS and EPS Components (mg/g MLSS)					
	EPS DNA	EPS Acidic Polysac-charides	EPS Carbohy-drates	EPS Protein	EPS Proteins to Carbohy-drates Ratio	Total EPS
Low SRT (n=8)	6 ± 2	6 ± 2	14 ± 5	83 ± 33	7 ± 4	106 ± 34
High SRT (n=8)	0.6 ± 0.5	2 ± 1	52 ± 34	152 ± 68	5 ± 5	194 ± 79
p-value	**0.001**	**0.001**	**0.008**	**0.03**	0.6	**0.02**
Low T (n=8)	3 ± 3	4 ± 3	24 ± 14	108 ± 55	5 ± 2	135 ± 65
High T (n=8)	3 ± 3	5 ± 3	36 ± 39	127 ± 71	7 ± 6	167 ± 82
p-value	0.86	0.38	0.24	0.50	0.50	0.33
Nitrification (n=4)	1.0 ± 0.4	3 ± 2	66 ± 46	167 ± 79	6 ± 8	220 ± 82
Non-Nitrification (n=8)	5 ± 2	6 ± 2	14 ± 5	83 ± 33	7 ± 4	108 ± 34
p-value	**0.001**	**0.01**	**0.006**	**0.02**	0.93	**0.006**

ANOVA	Results for EPS DNA		EPS-DNA Model Analysis (Type I, SS)		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	**40.9**	**0.0001**	**0.78**	**13.8**	**0.0003**
T	0.03	0.86			
TxSRT	0.61	0.45			

ANOVA	Results for EPS Acidic Polysaccharides		EPS-AP Model Analysis (Type I, SS)		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	**17.3**	**0.001**	**0.60**	**61**	**0.009**
T	0.82	0.38			
TxSRT	0.22	0.65			

ANOVA	Results for EPS Carbohydrates		EPS-CH Model Analysis (Type I, SS)		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	**11.1**	**0.008**	**0.58**	**4.7**	**0.03**
T	1.3	0.29			
TxSRT	1.6	0.24			

ANOVA	Results for EPS Protein		EPS-P Model Analysis (Type I, SS)		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	**6.0**	**0.03**	0.28	2.2	0.139
T	0.48	0.50			
TxSRT	0.13	0.72			

ANOVA	Results for EPS Protein to CH Ratio		EPS-P to CH Model Analysis (Type I, SS)		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	0.33	0.58	0.087	0.32	0.81
T	0.61	0.45			
TxSRT	002	0.91			

ANOVA	Results for Total EPS		Total EPS Model Analysis (Type I, SS)		
Parameters	F-value	p-value	r^2	Fisher Value	p-value
SRT	**7.6**	**0.02**	0.433	3.1	0.07
T	1.1	0.33			
TxSRT	0.47	0.51			

Appendix E

Figures of Conventional Parameters of the SBRs

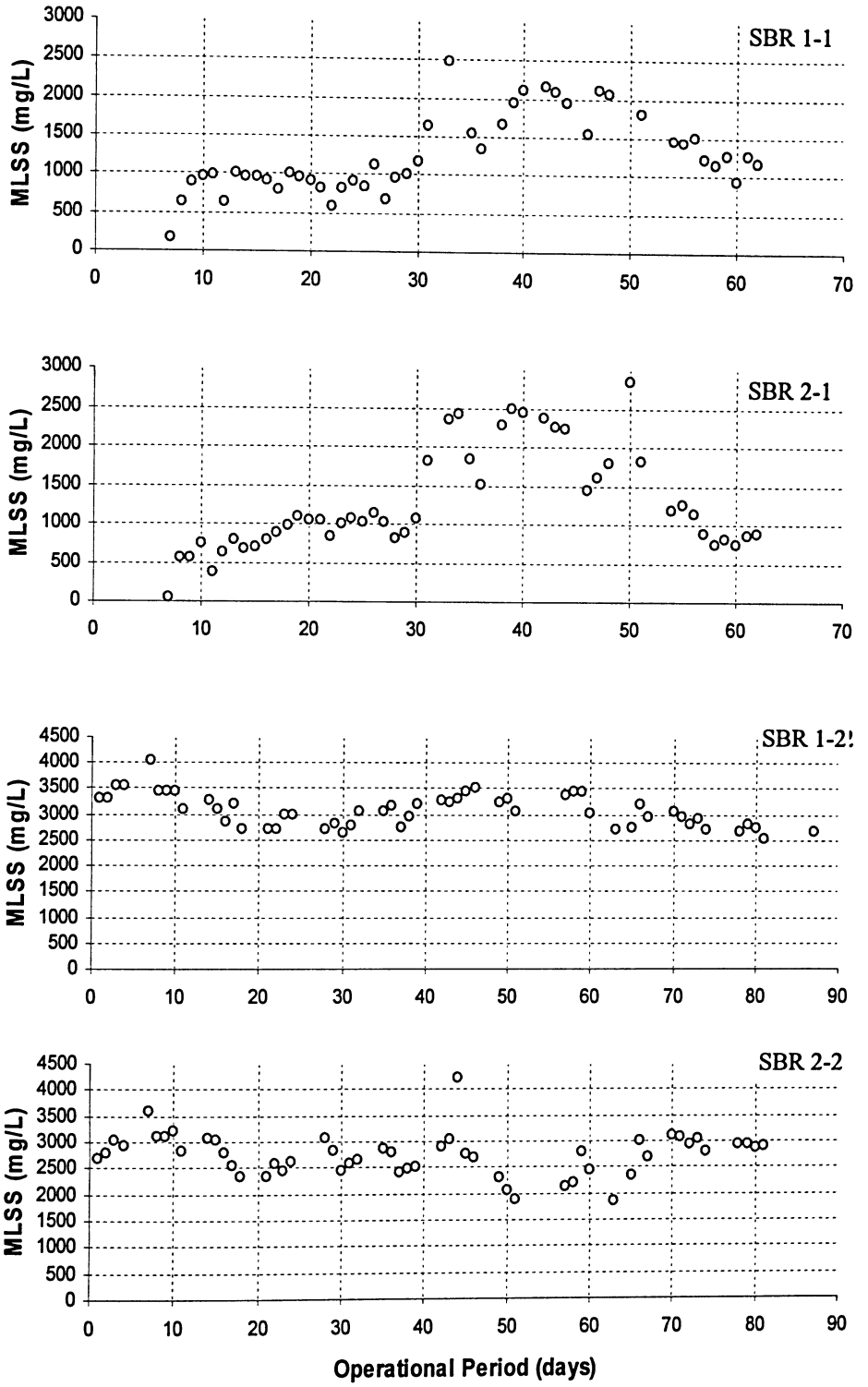


Figure E- 1. Mixed liquor suspended solids (MLSS) during the operation of the four sequencing batch reactors (SBRs).

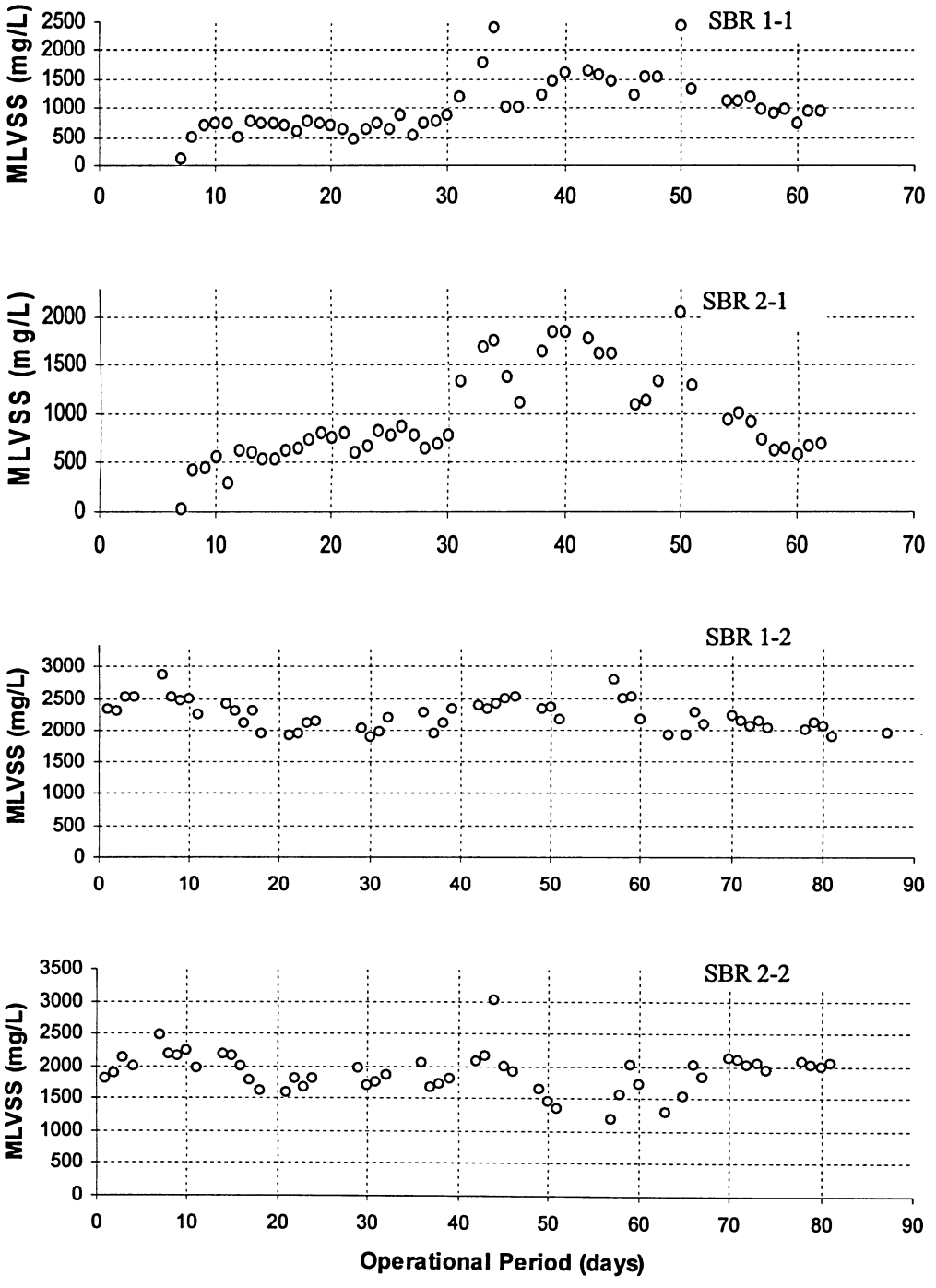


Figure E-2. Mixed liquor volatile suspended solids (MLVSS) during the operation of the four sequencing batch reactors (SBRs).

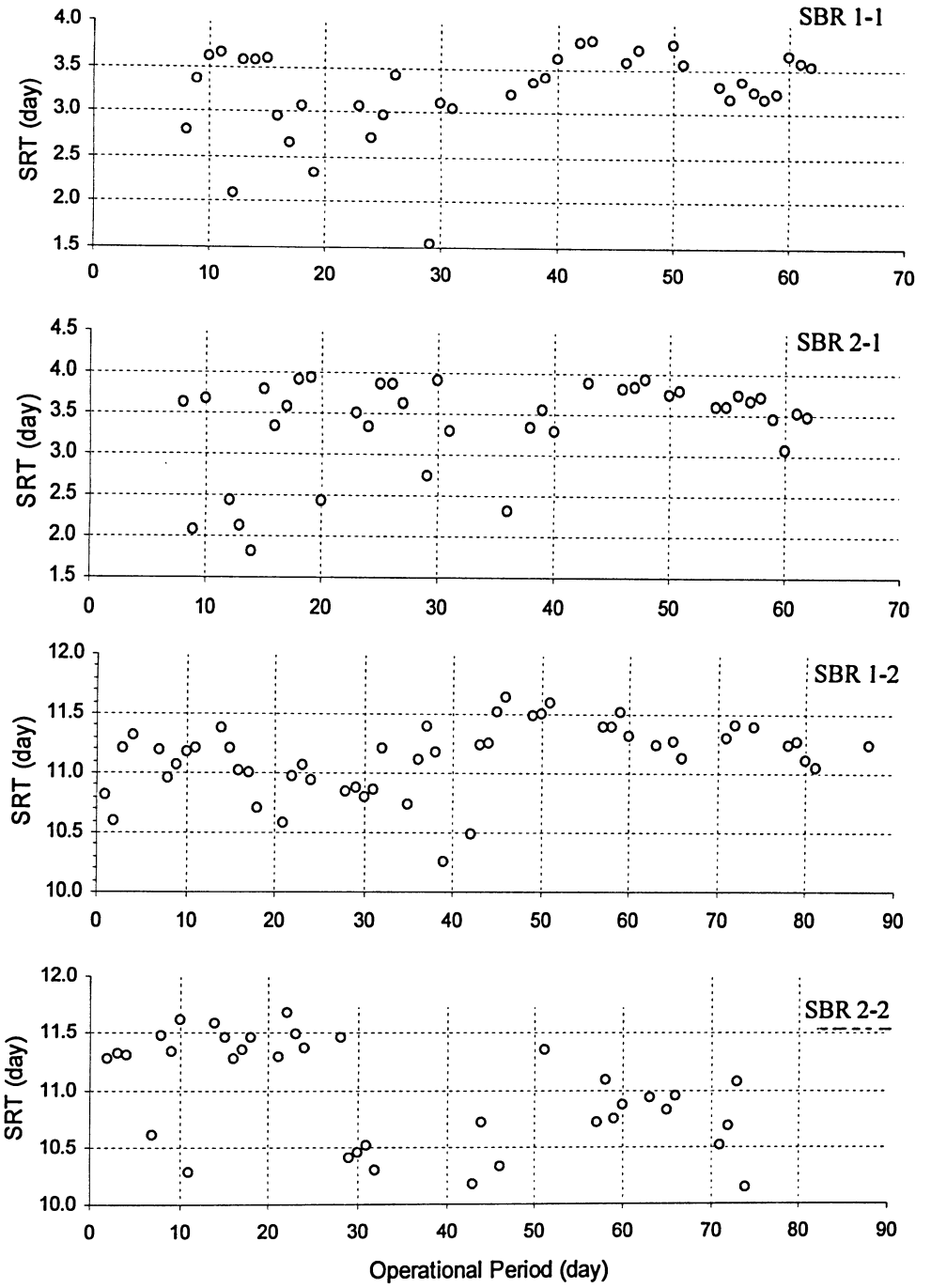


Figure E-3. Solids retention time (SRT) during the operation of the four sequencing batch reactors (SBRs).

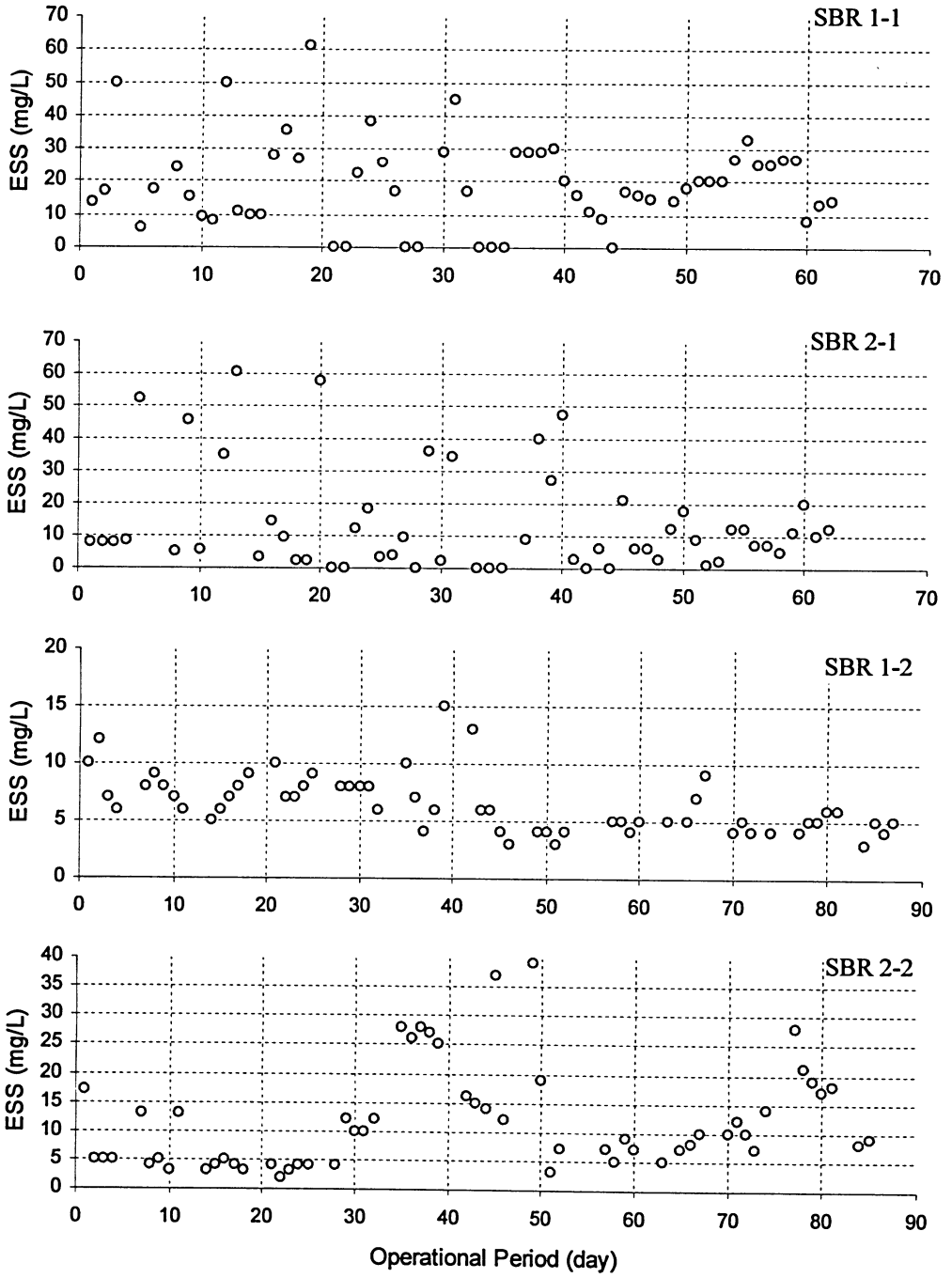


Figure E-4. Effluent suspended solids (ESS) from the sequencing batch reactors during their operation.

Appendix F

Phase I and II Polycyclic Synthetic Musks in Influent, Effluent and Solids

Table F- 1. Phase I and II influent PSMs

SRT=11 d T= 10 °C	Sampling Date	Concentration in Influent (ng/L.)					
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
SBR 2-1	18-Apr-05	34.8	77.8	43.9	22.5	7490	1710
SBR 2-1	19-Apr-05	40	67.7	36.6	149	5630	1290
SBR 2-1	21-Apr-05	28.4	61.8	30.5	165	5880	1270
SBR 2-1	20-Apr-05	31.2	59	31.6	120	5850	1200
SBR 2-1	22-Apr-05	21.7	58.9	53	195	5470	1160
Average		31	65	39	130	6064	1326
Standard Deviation		7	8	9	66	815	221
SRT=10 d T= 20 °C	Sampling Date	Concentration in Influent (ng/L.)					
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
SBR 2-2	19-Apr-05	34.8	77.8	43.9	22.5	7490	1710
SBR 2-2	18-Apr-05	40	67.7	36.6	149	5630	1290
SBR 2-2	20-Apr-05	28.4	61.8	30.5	165	5880	1270
SBR 2-2	19-Apr-05	31.2	59	31.6	120	5850	1200
SBR 2-2	22-Apr-05	21.7	58.9	53	195	5470	1160
Average		31	65	39	130	6064	1326
Standard Deviation		7	8	9	66	815	221
SRT=3.4 d T= 10 °C	Sampling Date	Concentration in Influent (ng/L.)					
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
SBR 1-1	18-Jul-05	25.8	42.55	27.2	68.15	5690	559
SBR 1-1	19-Jul-05	24.7	40.5	20.5	70	4690	706
SBR 1-1	20-Jul-05	35.8	50.3	26	85.5	5300	909
SBR 1-1	21-Jul-05	27.3	47.3	25.8	0	4640	970
SBR 1-1	22-Jul-05	26	48.65	27.05	96.7	6340	1071
Average		28	46	25	64	5332	843
Standard Deviation		5	4	3	38	713	207
SRT=3.5 d T= 20 °C	Sampling Date	Concentration in Influent (ng/L.)					
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
SBR 1-2	18-Jul-05	25.8	42.55	27.2	68.15	5690	559
SBR 1-2	19-Jul-05	24.7	40.5	20.5	70	4690	706
SBR 1-2	20-Jul-05	35.8	50.3	26	85.5	5300	909
SBR 1-2	21-Jul-05	27.3	47.3	25.8	0	4640	970
SBR 1-2	22-Jul-05	26	48.65	27.05	96.7	6340	1071
Average		28	46	25	64	5332	843
Standard Deviation		5	4	3	38	713	207

Table F- 2. Phase I and II effluent PSMs

SRT=11 d T= 10 °C SBR 1-2	Sampling Date	Concentration in Effluent (ng/L.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 1-2	18-Apr-05	33.6	19.7	17.5	23.5	1570	302	1933
SBR 1-2	19-Apr-05	32.6	22.1	17.5	24.1	1700	332	2096
SBR 1-2	21-Apr-05	27.5	19.3	17.5	19.8	1490	317	1864
SBR 1-2	20-Apr-05	21.1	16.3	17.5	14.3	1230	243	1521
SBR 1-2	22-Apr-05	24.6	20.2	17.5	25.2	1780	322	2165
Average		28	20	18	21	1554	303	1916
Standard Deviation		5	2	0	4	213	35	252
SRT=10 d T= 20 °C SBR 2-2	Sampling Date	Concentration in Effluent (ng/L.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 2-2	19-Apr-05	21.1	16.3	17.5	22	1320	255	1631
SBR 2-2	18-Apr-05	27.8	16.3	17.5	30.9	1370	285	1720
SBR 2-2	20-Apr-05	21.1	16.3	17.5	18.4	972	225	1249
SBR 2-2	19-Apr-05	21.1	16.3	17.5	21.4	1270	269	1594
SBR 2-2	22-Apr-05	21.1	16.3	17.5	17.7	1040	215	1307
Average		22	16	18	22	1194	250	1500
Standard Deviation		3	0	0	5	177	29	209
SRT=3.4 d T= 10 °C SBR 1-1	Sampling Date	Concentration in Effluent (ng/L.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 1-1	18-Jul-05	18.8	48.6	37.4	36.8	2720	401	3244
SBR 1-1	19-Jul-05	17.3	40.3	31.4	26	1710	287	2095
SBR 1-1	20-Jul-05	31.8	38.2	29.4	24.6	2030	304	2426
SBR 1-1	21-Jul-05	14.1	30.7	24.3	21.4	1730	249	2055
SBR 1-1	22-Jul-05	17.5	28.7	21.4	21.2	1720	193	1984
Average		20	37	29	26	1982	287	2361
Standard Deviation		7	8	6	6	434	77	522
SRT=3.5 d T= 20 °C SBR 2-1	Sampling Date	Concentration in Effluent (ng/L.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 2-1	18-Jul-05	14.7	16.3	12.7	13.4	1090	150	1282
SBR 2-1	19-Jul-05	17.1	17.3	12.9	33.2	1130	192	1385
SBR 2-1	20-Jul-05	20.8	15.4	11.1	9.7	1040	155	1231
SBR 2-1	21-Jul-05	18.5	14.6	9.52	9.38	1080	149	1263
SBR 2-1	22-Jul-05	16.2	16.1	13	21.2	1220	230	1500
Average		17	16	12	17	1112	175	1332
Standard Deviation		2	1	2	10	68	35	110

Table F- 3. Phase I and II PSMs in mixed liquor suspended solids

SRT=11 d T= 10 °C	Sampling Date	Concentration in Sludge (ng/g d.m.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 1-2	18-Apr-05	14	237	179	79	26433	6821	33748
SBR 1-2	19-Apr-05	16	198	148	110	18603	5153	24212
SBR 1-2	20-Apr-05	13	246	157	83	22375	5860	28720
SBR 1-2	21-Apr-05	12	211	171	71	21481	5765	27699
SBR 1-2	22-Apr-05	14	144	102	143	17183	4503	22074
Average		14	207	151	97	21215	5620	27291
Standard Deviation		2	40	30	30	3596	864	4489
SRT=10 d T= 20 °C	Sampling Date	Concentration in Sludge (ng/g d.m.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 2-2	18-Apr-05	15	109	122	125	13436	3439	17232
SBR 2-2	19-Apr-05	16	96	102	157	11600	3040	14995
SBR 2-2	20-Apr-05	13	115	102	130	11310	2962	14620
SBR 2-2	21-Apr-05	13	94	107	126	12164	3164	15655
SBR 2-2	22-Apr-05	13	87	84	160	9486	2442	12259
Average		14	100	103	140	11599	3009	14952
Standard Deviation		1	12	13	17	1435	365	1807
SRT=3.4 d T= 10 °C	Sampling Date	Concentration in Sludge (ng/g d.m.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 1-1	18-Jul-05	28	189	141	201	14776	3183	18489
SBR 1-1	19-Jul-05	29	355	306	96	26228	6258	33244
SBR 1-1	20-Jul-05	30	180	141	215	12509	2947	15993
SBR 1-1	21-Jul-05	28	172	132	209	14042	2975	17530
SBR 1-1	22-Jul-05	29	222	175	168	16894	3795	21255
Average		29	224	179	178	16890	3832	21302
Standard Deviation		1	76	73	49	5454	1399	6945
SRT=3.5 d T= 20 °C	Sampling Date	Concentration in Sludge (ng/g d.m.)						
		Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide	Total PSMs
SBR 2-1	18-Jul-05	33	144	115	287	12199	3102	15847
SBR 2-1	19-Jul-05	31	234	214	145	20276	5298	26167
SBR 2-1	20-Jul-05	27	108	85	314	9136	2280	11923
SBR 2-1	21-Jul-05	29	119	21	1371	13744	3202	18457
SBR 2-1	22-Jul-05	26	171	128	207	16126	3875	20507
Average		29	155	112	465	14296	3551	18580
Standard Deviation		3	50	70	511	4196	1129	5317

Appendix G
Phase I and II Floc Distribution Data

Table G-1. Phase I floc size distribution in SBR 1-1 (SRT=3.4 days, T= 10 °C)

Floc Size	SBR 1-1 Oct 20/04	SBR 1-1 Oct 21/04	SBR 1-1 Oct 22/04	SBR 1-1: SRT =3.4 d, T=10 °C		
	#	#	#	Sum #	% #	Cum % #
7.7	1001	463	480	1944	12.71%	12.71%
8.9	703	376	351	1430	9.35%	22.07%
10.3	969	582	580	2131	13.94%	36.00%
12.0	667	489	556	1712	11.20%	47.20%
13.9	530	479	543	1552	10.15%	57.35%
16.2	366	495	474	1335	8.73%	66.08%
18.7	221	359	319	899	5.88%	71.96%
21.6	122	296	231	649	4.24%	76.21%
25.1	105	246	230	581	3.80%	80.01%
29.1	71	227	186	484	3.17%	83.17%
33.7	66	200	174	440	2.88%	86.05%
39.1	42	199	126	367	2.40%	88.45%
45.3	37	149	141	327	2.14%	90.59%
52.6	18	136	110	264	1.73%	92.32%
60.9	22	100	105	227	1.48%	93.80%
70.6	21	63	124	208	1.36%	95.16%
81.8	18	47	81	146	0.95%	96.12%
94.8	17	34	71	122	0.80%	96.91%
109.9	14	35	57	106	0.69%	97.61%
127.6	12	32	56	100	0.65%	98.26%
147.8	13	24	31	68	0.44%	98.71%
171.2	10	14	24	48	0.31%	99.02%
198.9	11	18	19	48	0.31%	99.33%
231.2	5	12	9	26	0.17%	99.50%
268.6	6	3	12	21	0.14%	99.64%
312.1	2	4	11	17	0.11%	99.75%
362.7	6	4	5	15	0.10%	99.85%
421.5	4	1	1	6	0.04%	99.89%
489.8	4	0	3	7	0.05%	99.93%
569.4	6	1	0	7	0.05%	99.98%
662.0	2	0	0	2	0.01%	99.99%
769.5	1	0	0	1	0.01%	100.00%
894.0	0	0	0	0	0.00%	100.00%
1039.5	0	0	0	0	0.00%	100.00%
	5092	5088	5088	15290	100.00%	

Table G-2. Phase I floc size distribution in SBR 2-1 (SRT=3.5 days, T= 20 °C)

Size	SBR 2-1 Oct 20/04	SBR 2-1 Oct 21/04	SBR 2-1 Oct 22/04	SBR 2-1: SRT =3.5 days , T=20 °C		
	#	#	#	Sum #	% #	Cum % #
7.7	676	657	692	2025	13.30%	13.30%
8.9	490	470	404	1364	8.96%	22.25%
10.3	647	636	599	1882	12.36%	34.61%
12.0	478	489	455	1422	9.34%	43.94%
13.9	364	371	441	1176	7.72%	51.66%
16.2	337	322	379	1038	6.82%	58.48%
18.7	222	287	296	805	5.29%	63.76%
21.6	215	237	243	695	4.56%	68.33%
25.1	171	192	207	570	3.74%	72.07%
29.1	146	196	193	535	3.51%	75.58%
33.7	158	184	184	526	3.45%	79.04%
39.1	159	166	174	499	3.28%	82.31%
45.3	150	153	151	454	2.98%	85.29%
52.6	141	146	140	427	2.80%	88.10%
60.9	137	117	125	379	2.49%	90.58%
70.6	128	116	95	339	2.23%	92.81%
81.8	103	91	95	289	1.90%	94.71%
94.8	77	61	65	203	1.33%	96.04%
109.9	76	65	52	193	1.27%	97.31%
127.6	49	47	37	133	0.87%	98.18%
147.8	38	29	18	85	0.56%	98.74%
171.2	15	22	20	57	0.37%	99.11%
198.9	11	18	8	37	0.24%	99.36%
231.2	12	14	12	38	0.25%	99.61%
268.6	6	4	3	13	0.09%	99.69%
312.1	7	8	4	19	0.12%	99.82%
362.7	1	6	4	11	0.07%	99.89%
421.5	3	4	1	8	0.05%	99.94%
489.8	0	1	3	4	0.03%	99.97%
569.4	1	2	1	4	0.03%	99.99%
662.0	1	0	0	1	0.01%	100.00%
769.5	0	0	0	0	0.00%	100.00%
894.0	0	0	0	0	0.00%	100.00%
1039.5	0	0	0	0	0.00%	100.00%
	5019	5111	5101	15231	100.00%	

Table G-3. Phase II floc size distribution in SBR 1-2 and SBR 2-2 (SRT =10 and 11 d, T=10 and 20°C)

Size	SBR 1-2 and SBR 2-2 Mar 7/05	SBR 1-2 and SBR 2-2 Mar 8/05	SBR 1-2 and SBR 2-2 Mar 9/05	SBR 1-2 and 2-2 SRT =10.5 days, T=10 & 20 °C		
	#	#	#	Sum #	% #	Cum % #
7.7	980	1105	870	2955	19.29%	19.29%
8.9	646	678	660	1984	12.95%	32.25%
10.3	804	890	860	2554	16.67%	48.92%
12.0	530	676	649	1855	12.11%	61.03%
13.9	456	434	450	1340	8.75%	69.78%
16.2	314	332	371	1017	6.64%	76.42%
18.7	234	220	219	673	4.39%	80.81%
21.6	176	158	167	501	3.27%	84.08%
25.1	148	126	125	399	2.60%	86.69%
29.1	131	120	94	345	2.25%	88.94%
33.7	146	93	117	356	2.32%	91.26%
39.1	90	66	78	234	1.53%	92.79%
45.3	81	44	58	183	1.19%	93.99%
52.6	57	25	66	148	0.97%	94.95%
60.9	54	14	44	112	0.73%	95.68%
70.6	45	19	39	103	0.67%	96.36%
81.8	50	10	38	98	0.64%	97.00%
94.8	33	8	31	72	0.47%	97.47%
109.9	26	10	23	59	0.39%	97.85%
127.6	23	9	19	51	0.33%	98.19%
147.8	17	2	21	40	0.26%	98.45%
171.2	16	13	24	53	0.35%	98.79%
198.9	16	6	13	35	0.23%	99.02%
231.2	21	4	14	39	0.25%	99.28%
268.6	8	7	7	22	0.14%	99.42%
312.1	13	6	8	27	0.18%	99.60%
362.7	8	6	5	19	0.12%	99.72%
421.5	4	7	4	15	0.10%	99.82%
489.8	4	2	4	10	0.07%	99.88%
569.4	2	0	2	4	0.03%	99.91%
662.0	2	1	1	4	0.03%	99.93%
769.5	1	0	3	4	0.03%	99.96%
894.0	3	1	0	4	0.03%	99.99%
1039.5	1	1	0	2	0.01%	100.00%
	5140	5093	5084	15317	100.00%	

Appendix H
Phase I and II Floc Distribution Data

Table H-1. Phase I floc size distribution in SBR 1-1 (SRT=3.4 days, T= 10 °C)

Floc Size	SBR 1-1 Oct 20/04	SBR 1-1 Oct 21/04	SBR 1-1 Oct 22/04	SBR 1-1: SRT =3.4 d, T=10 °C		
	#	#	#	Sum #	% #	Cum % #
7.7	1001	463	480	1944	12.71%	12.71%
8.9	703	376	351	1430	9.35%	22.07%
10.3	969	582	580	2131	13.94%	36.00%
12.0	667	489	556	1712	11.20%	47.20%
13.9	530	479	543	1552	10.15%	57.35%
16.2	366	495	474	1335	8.73%	66.08%
18.7	221	359	319	899	5.88%	71.96%
21.6	122	296	231	649	4.24%	76.21%
25.1	105	246	230	581	3.80%	80.01%
29.1	71	227	186	484	3.17%	83.17%
33.7	66	200	174	440	2.88%	86.05%
39.1	42	199	126	367	2.40%	88.45%
45.3	37	149	141	327	2.14%	90.59%
52.6	18	136	110	264	1.73%	92.32%
60.9	22	100	105	227	1.48%	93.80%
70.6	21	63	124	208	1.36%	95.16%
81.8	18	47	81	146	0.95%	96.12%
94.8	17	34	71	122	0.80%	96.91%
109.9	14	35	57	106	0.69%	97.61%
127.6	12	32	56	100	0.65%	98.26%
147.8	13	24	31	68	0.44%	98.71%
171.2	10	14	24	48	0.31%	99.02%
198.9	11	18	19	48	0.31%	99.33%
231.2	5	12	9	26	0.17%	99.50%
268.6	6	3	12	21	0.14%	99.64%
312.1	2	4	11	17	0.11%	99.75%
362.7	6	4	5	15	0.10%	99.85%
421.5	4	1	1	6	0.04%	99.89%
489.8	4	0	3	7	0.05%	99.93%
569.4	6	1	0	7	0.05%	99.98%
662.0	2	0	0	2	0.01%	99.99%
769.5	1	0	0	1	0.01%	100.00%
894.0	0	0	0	0	0.00%	100.00%
1039.5	0	0	0	0	0.00%	100.00%
	5092	5088	5088	15290	100.00%	

Table H-2. Phase I floc size distribution in SBR 2-1 (SRT=3.5 days, T= 20 °C)

Size	SBR 2-1 Oct 20/04	SBR 2-1 Oct 21/04	SBR 2-1 Oct 22/04	SBR 2-1: SRT =3.5 days , T=20 °C		
	#	#	#	Sum #	% #	Cum % #
7.7	676	657	692	2025	13.30%	13.30%
8.9	490	470	404	1364	8.96%	22.25%
10.3	647	636	599	1882	12.36%	34.61%
12.0	478	489	455	1422	9.34%	43.94%
13.9	364	371	441	1176	7.72%	51.66%
16.2	337	322	379	1038	6.82%	58.48%
18.7	222	287	296	805	5.29%	63.76%
21.6	215	237	243	695	4.56%	68.33%
25.1	171	192	207	570	3.74%	72.07%
29.1	146	196	193	535	3.51%	75.58%
33.7	158	184	184	526	3.45%	79.04%
39.1	159	166	174	499	3.28%	82.31%
45.3	150	153	151	454	2.98%	85.29%
52.6	141	146	140	427	2.80%	88.10%
60.9	137	117	125	379	2.49%	90.58%
70.6	128	116	95	339	2.23%	92.81%
81.8	103	91	95	289	1.90%	94.71%
94.8	77	61	65	203	1.33%	96.04%
109.9	76	65	52	193	1.27%	97.31%
127.6	49	47	37	133	0.87%	98.18%
147.8	38	29	18	85	0.56%	98.74%
171.2	15	22	20	57	0.37%	99.11%
198.9	11	18	8	37	0.24%	99.36%
231.2	12	14	12	38	0.25%	99.61%
268.6	6	4	3	13	0.09%	99.69%
312.1	7	8	4	19	0.12%	99.82%
362.7	1	6	4	11	0.07%	99.89%
421.5	3	4	1	8	0.05%	99.94%
489.8	0	1	3	4	0.03%	99.97%
569.4	1	2	1	4	0.03%	99.99%
662.0	1	0	0	1	0.01%	100.00%
769.5	0	0	0	0	0.00%	100.00%
894.0	0	0	0	0	0.00%	100.00%
1039.5	0	0	0	0	0.00%	100.00%
	5019	5111	5101	15231	100.00%	

Table H-3. Phase II floc size distribution in SBR 1-2 and SBR 2-2 (SRT =10 and 11 d, T=10 and 20°C)

Size	SBR 1-2 and SBR 2-2 Mar 7/05	SBR 1-2 and SBR 2-2 Mar 8/05	SBR 1-2 and SBR 2-2 Mar 9/05	SBR 1-2 and 2-2 SRT =10.5 days, T=10 & 20 °C		
	#	#	#	Sum #	% #	Cum % #
7.7	980	1105	870	2955	19.29%	19.29%
8.9	646	678	660	1984	12.95%	32.25%
10.3	804	890	860	2554	16.67%	48.92%
12.0	530	676	649	1855	12.11%	61.03%
13.9	456	434	450	1340	8.75%	69.78%
16.2	314	332	371	1017	6.64%	76.42%
18.7	234	220	219	673	4.39%	80.81%
21.6	176	158	167	501	3.27%	84.08%
25.1	148	126	125	399	2.60%	86.69%
29.1	131	120	94	345	2.25%	88.94%
33.7	146	93	117	356	2.32%	91.26%
39.1	90	66	78	234	1.53%	92.79%
45.3	81	44	58	183	1.19%	93.99%
52.6	57	25	66	148	0.97%	94.95%
60.9	54	14	44	112	0.73%	95.68%
70.6	45	19	39	103	0.67%	96.36%
81.8	50	10	38	98	0.64%	97.00%
94.8	33	8	31	72	0.47%	97.47%
109.9	26	10	23	59	0.39%	97.85%
127.6	23	9	19	51	0.33%	98.19%
147.8	17	2	21	40	0.26%	98.45%
171.2	16	13	24	53	0.35%	98.79%
198.9	16	6	13	35	0.23%	99.02%
231.2	21	4	14	39	0.25%	99.28%
268.6	8	7	7	22	0.14%	99.42%
312.1	13	6	8	27	0.18%	99.60%
362.7	8	6	5	19	0.12%	99.72%
421.5	4	7	4	15	0.10%	99.82%
489.8	4	2	4	10	0.07%	99.88%
569.4	2	0	2	4	0.03%	99.91%
662.0	2	1	1	4	0.03%	99.93%
769.5	1	0	3	4	0.03%	99.96%
894.0	3	1	0	4	0.03%	99.99%
1039.5	1	1	0	2	0.01%	100.00%
	5140	5093	5084	15317	100.00%	

Appendix I
Phase I and II Sludge Floc Physical Properties

Table I-1. Phase I mean floc diamter t-test comparison results

d_{50}	d_{50} (μm) SBR 1-1	d_{50} (μm) SBR 2-1
Oct 20	10	13
Oct 21	14	13.6
Oct 22	14.6	13.7
t-Test: Paired Two Sample for Means		
	d50 (μm) SBR 1-1	d50 (μm) SBR 2-1
Mean	13	13.4
Variance	6	0.1
SD	3	0.4
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.4625101	
P(T<=t) one-tail	0.34457863	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.68915727	
t Critical two-tail	4.30265273	

Table I-2. Phase I and II mean floc diamter z-test comparison results

SBR	SBR 1-1 & SBR 2-1	SBR 1-2 & SBR 2-2
Oct 20	10	10
Oct 21	14	10.7
Oct 22	14.6	10.7
Oct 20	13	
Oct 21	13.6	
Oct 22	13.7	
Average	13.2	10.5
Standard Deviation	1.6	0.4
Variance	2.655	0.1633333
z-Test: Two Sample for Means		
	SBR 1-1 & SBR 2-1	*SBR 1-2 & SBR 2-2*
Mean	13.15	10.46667
Known Variance	2.655	0.1633
Observations	6	3
Hypothesized Mean Difference	0	
z	3.806498	
P(Z<=z) one-tail	7.05E-05	
z Critical one-tail	1.644854	
P(Z<=z) two-tail	0.000141	
z Critical two-tail	1.959964	

Table I-3. Phase I 90 percentile floc diameer t-test comparison results

Sampling Date	d_{90} (μm) SBR 1-1	d_{90} (μm) SBR 2-1
Oct 20	22	54
Oct 21	46	59
Oct 22	60	63
t-Test: Paired Two Sample for Means		
	d90 (μm) SBR 1-1	*d90 (μm) SBR 2-1*
Mean	43	59
Variance	369	20
Standard Deviation	19	5
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	-1.88	
P(T<=t) one-tail	0.10	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.20	
t Critical two-tail	4.30	

Table I-4. Phase I and II 90 percentile floc diamter z-test comparison results

Sampling Date	d_{90} SBR 1-1 & SBR 2-1	d_{90} SBR 1-2 & SBR 2-2
Oct 20	22	24
Oct 21	46	34
Oct 22	60	37
Oct 20	54	
Oct 21	59	
Oct 22	63	
Average	51	32
Standard Deviation	15	7
Variance	233	46
z-Test: Two Sample for Means		
	d_{90} SBR 1-1 & SBR 2-1	*d_{90} SBR 1-2 & SBR 2-2*
Mean	50.6666667	31.666667
Known Variance	233	46
Observations	6	3
Hypothesized Mean Difference	0	
z	2.58159164	
P(Z<=z) one-tail	0.00491729	
z Critical one-tail	1.64485363	
P(Z<=z) two-tail	0.00983459	
z Critical two-tail	1.95996398	

Table I-5. Phase I sludge mean floc size analysis

Number of Flocs	SBR 1-1, SRT =3.4 d, T = 10 °C					SBR 2-1, SRT =3.5 d, T = 20 °C				
	Sampling Date: Oct 20-22, 2004					Sampling Date: Oct 20-22, 2004				
	ρ_s (g/L)	D (μm)	V_s (mm/s)	% ε	ρ_e (g/L)	ρ_s (g/L)	D (μm)	V_s (mm/s)	% ε	ρ_e (g/L)
1	1.41	40	0.37	37	0.410	1.37	40	0.33	44	0.367
2	1.29	59	0.56	55	0.292	1.10	99	0.56	84	0.104
3	1.20	73	0.59	68	0.205	1.07	104	0.41	89	0.069
4	1.15	99	0.83	76	0.155	1.09	113	0.64	86	0.092
5	1.12	99	0.66	81	0.123	1.05	117	0.37	92	0.050
6	1.05	104	0.31	92	0.053	1.08	122	0.66	88	0.080
7	1.09	105	0.53	87	0.088	1.06	133	0.57	91	0.059
8	1.09	113	0.62	86	0.088	1.02	135	0.25	96	0.025
9	1.10	115	0.75	84	0.105	1.08	139	0.88	87	0.084
10	1.08	117	0.60	88	0.081	1.07	143	0.74	90	0.066
11	1.22	117	1.63	66	0.220	1.05	146	0.52	93	0.045
12	1.06	120	0.49	90	0.062	1.07	151	0.81	90	0.066
13	1.08	122	0.69	87	0.085	1.07	151	0.81	90	0.066
14	1.06	122	0.52	90	0.063	1.06	154	0.83	90	0.064
15	1.08	124	0.71	87	0.085	1.05	155	0.66	92	0.050
16	1.05	125	0.44	92	0.051	1.07	160	0.98	89	0.070
17	1.05	125	0.41	93	0.048	1.05	160	0.74	92	0.052
18	1.07	127	0.62	89	0.070	1.04	160	0.58	94	0.041
19	1.09	128	0.81	86	0.091	1.02	160	0.30	97	0.022
20	1.09	130	0.82	86	0.090	1.03	161	0.46	95	0.033
21	1.06	133	0.62	90	0.065	1.03	162	0.41	96	0.029
22	1.04	135	0.41	94	0.041	1.03	162	0.45	95	0.031
23	1.04	135	0.35	95	0.035	1.05	162	0.67	93	0.047
24	1.04	139	0.41	94	0.039	1.05	164	0.72	92	0.049
25	1.06	139	0.68	90	0.064	1.04	165	0.59	94	0.040
26	1.05	142	0.59	92	0.054	1.05	166	0.73	92	0.049
27	1.06	143	0.67	91	0.060	1.05	167	0.70	93	0.046
28	1.04	143	0.49	93	0.044	1.02	168	0.34	97	0.022
29	1.04	146	0.50	93	0.043	1.05	168	0.74	93	0.048
30	1.18	147	2.12	72	0.180	1.01	170	0.23	98	0.015
31	1.19	147	2.23	71	0.190	1.03	170	0.41	96	0.026
32	1.06	148	0.76	90	0.064	1.03	170	0.45	96	0.028
33	1.15	148	1.77	77	0.149	1.02	171	0.37	96	0.023
34	1.05	149	0.63	92	0.052	1.04	172	0.67	94	0.042
35	1.17	150	2.09	74	0.170	1.05	172	0.84	92	0.052
36	1.05	150	0.66	92	0.053	1.05	172	0.77	93	0.048
37	1.04	151	0.51	94	0.042	1.04	174	0.59	94	0.036
38	1.10	153	1.23	85	0.096	1.02	174	0.34	97	0.020
39	1.03	154	0.34	96	0.026	1.03	174	0.56	95	0.034
40	1.06	155	0.82	90	0.063	1.05	176	0.82	93	0.048
41	1.05	155	0.59	93	0.045	1.01	176	0.18	98	0.011

Table I-1 Sludge mean floc size analysis (continued)

Number of Flocs	SBR 1-1, SRT =3.4 d, T = 10 °C					SBR 2-1, SRT =3.5 d, T = 20 °C				
	Sampling Date: Oct 20-22, 2004					Sampling Date: Oct 20-22, 2004				
	ρ_s (g/L)	D (μm)	V_s (mm/s)	% ε	ρ_e (g/L)	ρ_s (g/L)	D (μm)	V_s (mm/s)	% ε	ρ_e (g/L)
42	1.05	156	0.68	92	0.051	1.03	178	0.51	95	0.030
43	1.07	158	1.00	89	0.074	1.04	178	0.77	93	0.045
44	1.08	159	1.14	87	0.083	1.10	178	1.74	85	0.100
45	1.04	159	0.55	94	0.040	1.03	178	0.47	96	0.027
46	1.02	160	0.35	96	0.025	1.05	182	0.83	93	0.046
47	1.20	160	2.77	69	0.199	1.06	182	1.11	91	0.061
48	1.05	160	0.65	93	0.046	1.02	184	0.45	96	0.025
49	1.06	160	0.78	91	0.056	1.03	186	0.47	96	0.025
50	1.02	160	0.23	97	0.016	1.02	187	0.33	97	0.017
51	1.03	161	0.41	95	0.029	1.03	187	0.62	95	0.033
52	1.05	161	0.69	93	0.048	1.04	189	0.83	93	0.043
53	1.14	162	2.05	78	0.143	1.03	189	0.49	96	0.026
54	1.12	162	1.72	82	0.120	1.07	189	1.39	89	0.071
55	1.04	162	0.51	94	0.036	1.04	189	0.75	94	0.038
56	1.02	162	0.34	96	0.023	1.03	190	0.53	96	0.027
57	1.03	162	0.47	95	0.033	1.03	190	0.57	96	0.029
58	1.06	163	0.85	91	0.059	1.03	193	0.52	96	0.025
59	1.17	163	2.45	74	0.168	1.09	194	1.91	86	0.094
60	1.06	164	0.86	91	0.059	1.04	194	0.82	94	0.040
61	1.03	166	0.45	95	0.030	1.02	195	0.42	97	0.020
62	1.03	167	0.51	95	0.034	1.02	197	0.45	97	0.022
63	1.05	168	0.78	92	0.050	1.04	198	0.84	94	0.039
64	1.03	168	0.53	95	0.034	1.05	199	0.99	93	0.046
65	1.04	170	0.58	94	0.036	1.06	199	1.24	91	0.057
66	1.12	170	1.87	82	0.118	1.02	199	0.54	96	0.025
67	1.04	171	0.59	94	0.037	1.03	201	0.56	96	0.025
68	1.04	172	0.66	94	0.041	1.04	204	0.81	94	0.036
69	1.03	172	0.51	95	0.032	1.03	204	0.75	95	0.033
70	1.04	174	0.63	94	0.038	1.02	205	0.49	97	0.021
71	1.04	176	0.73	93	0.043	1.02	205	0.52	97	0.023
72	1.03	176	0.49	96	0.029	1.03	205	0.69	95	0.030
73	1.05	178	0.80	93	0.046	1.03	206	0.64	96	0.028
74	1.12	178	2.03	82	0.118	1.03	206	0.66	96	0.028
75	1.14	178	2.51	78	0.144	1.07	208	1.55	90	0.066
76	1.05	180	0.83	93	0.047	1.02	208	0.58	96	0.025
77	1.05	181	0.96	92	0.054	1.02	209	0.44	97	0.018
78	1.02	182	0.39	97	0.022	1.03	209	0.77	95	0.032
79	1.04	182	0.66	94	0.036	1.02	210	0.44	97	0.018
80	1.11	185	2.11	83	0.113	1.04	210	0.87	94	0.036
81	1.05	185	0.84	93	0.045	1.03	210	0.64	96	0.027
82	1.13	186	2.42	80	0.129	1.05	211	1.14	93	0.047
83	1.05	187	0.96	92	0.050	1.03	211	0.66	96	0.027
84	1.04	187	0.82	93	0.043	1.02	211	0.48	97	0.020
85	1.03	187	0.58	95	0.030	1.02	211	0.45	97	0.018

Table I-1 Phase I sludge mean floc size analysis (continued)

Number of Flocs	SBR 1-1, SRT =3.4 d, T = 10 °C					SBR 2-1, SRT =3.5 d, T = 20 °C				
	Sampling Date: Oct 20-22, 2004					Sampling Date: Oct 20-22, 2004				
	ρ_s (g/L)	D (μm)	V_s (mm/s)	% ε	ρ_e (g/L)	ρ_s (g/L)	D (μm)	V_s (mm/s)	% ε	ρ_e (g/L)
86	1.02	189	0.35	97	0.018	1.06	212	1.37	91	0.056
87	1.02	189	0.41	97	0.021	1.02	212	0.48	97	0.020
88	1.04	189	0.81	94	0.042	1.02	213	0.53	97	0.021
89	1.02	189	0.30	98	0.016	1.07	213	1.73	89	0.070
90	1.09	189	1.67	87	0.085	1.06	215	1.41	91	0.056
91	1.04	190	0.74	94	0.038	1.04	215	0.89	95	0.036
92	1.04	190	0.70	95	0.035	1.01	217	0.17	99	0.007
93	1.07	192	1.40	89	0.069	1.01	217	0.24	99	0.009
94	1.08	192	1.59	88	0.079	1.04	218	1.06	94	0.041
95	1.02	194	0.41	97	0.020	1.05	219	1.41	92	0.054
96	1.02	194	0.49	96	0.024	1.02	219	0.55	97	0.021
97	1.05	195	1.00	93	0.048	1.02	221	0.59	97	0.022
98	1.03	197	0.74	95	0.035	1.02	221	0.57	97	0.022
99	1.03	197	0.66	95	0.031	1.01	221	0.30	98	0.011
100	1.10	197	2.22	84	0.105	1.02	223	0.43	98	0.016
101	1.02	198	0.46	97	0.022	1.04	225	0.99	94	0.036
102	1.03	198	0.57	96	0.027	1.03	227	0.83	95	0.030
103	1.14	199	2.96	79	0.137	1.02	227	0.60	97	0.021
104	1.05	200	1.20	92	0.055	1.02	227	0.56	97	0.020
105	1.06	201	1.32	91	0.060	1.03	227	0.89	95	0.032
106	1.05	202	1.02	93	0.046	1.03	229	0.81	96	0.029
107	1.05	202	1.07	93	0.048	1.02	231	0.67	96	0.023
108	1.03	204	0.66	96	0.029	1.01	232	0.41	98	0.014
109	1.04	205	1.01	93	0.044	1.01	232	0.44	98	0.015
110	1.02	205	0.57	96	0.025	1.05	233	1.37	93	0.046
111	1.10	206	2.30	85	0.099	1.02	233	0.55	97	0.019
112	1.02	206	0.45	97	0.020	1.02	235	0.75	96	0.025
113	1.10	207	2.35	85	0.101	1.02	236	0.58	97	0.019
114	1.07	208	1.71	89	0.073	1.02	238	0.48	98	0.016
115	1.08	208	1.83	88	0.078	1.03	239	0.99	95	0.032
116	1.06	209	1.47	90	0.062	1.02	240	0.52	97	0.016
117	1.04	209	0.86	94	0.036	1.03	241	1.03	95	0.032
118	1.02	211	0.39	97	0.016	1.02	241	0.66	97	0.021
119	1.04	211	0.99	94	0.041	1.02	242	0.66	97	0.021
120	1.03	211	0.77	95	0.032	1.02	244	0.52	98	0.016
121	1.04	212	0.97	94	0.039	1.03	245	0.98	95	0.030
122	1.03	212	0.66	96	0.027	1.02	245	0.75	96	0.023
123	1.11	213	2.83	82	0.115	1.02	247	0.52	98	0.016

Table I-1. Phase I sludge mean floc size analysis (continued)

Number of Flocs	SBR 1-1, SRT =3.4 d, T = 10 °C					SBR 2-1, SRT =3.5 d, T = 20 °C				
	Sampling Date: Oct 20-22, 2004					Sampling Date: Oct 20-22, 2004				
	ρ_s	D	V_s	% ε	ρ_e	ρ_s	D	V_s	% ε	ρ_e
	(g/L)	(μm)	(mm/s)		(g/L)	(g/L)	(μm)	(mm/s)		(g/L)
123	1.11	213	2.83	82	0.115	1.02	248	0.74	97	0.022
124	1.02	215	0.39	98	0.015	1.02	248	0.58	97	0.017
125	1.06	215	1.55	91	0.062	1.01	248	0.43	98	0.013
126	1.02	217	0.49	97	0.019	1.02	249	0.77	96	0.023
127	1.10	218	2.61	85	0.101	1.05	249	1.66	92	0.049
128	1.01	219	0.39	98	0.015	1.02	250	0.53	98	0.016
129	1.02	219	0.60	96	0.023	1.02	250	0.52	98	0.015
130	1.02	221	0.45	97	0.017	1.02	251	0.61	97	0.018
131	1.03	221	0.83	95	0.031	1.01	253	0.41	98	0.012
132	1.05	222	1.47	92	0.055	1.01	253	0.29	99	0.008
133	1.03	223	0.79	96	0.029	1.03	256	1.06	95	0.030
134	1.03	223	0.69	96	0.026	1.03	256	1.02	96	0.029
135	1.03	223	0.75	96	0.027	1.03	256	0.99	96	0.028
136	1.03	224	0.76	96	0.028	1.02	257	0.67	97	0.019
137	1.02	225	0.57	97	0.021	1.01	260	0.47	98	0.013
138	1.05	225	1.44	92	0.052	1.02	260	0.90	96	0.024
139	1.03	226	0.83	95	0.030	1.01	261	0.54	98	0.015
140	1.01	227	0.35	98	0.012	1.02	263	0.74	97	0.020
141	1.05	227	1.41	92	0.050	1.02	263	0.61	98	0.016
142	1.02	227	0.58	97	0.020	1.02	264	0.67	97	0.018
143	1.03	228	0.88	95	0.031	1.01	265	0.49	98	0.013
144	1.03	231	0.99	95	0.034	1.01	265	0.39	98	0.010
145	1.02	231	0.49	97	0.017	1.01	267	0.54	98	0.014
146	1.02	232	0.64	97	0.022	1.01	268	0.51	98	0.013
147	1.03	233	0.82	96	0.028	1.02	269	0.66	97	0.017
148	1.04	233	1.06	94	0.036	1.04	271	1.47	94	0.037
149	1.02	235	0.62	97	0.021	1.05	273	1.92	93	0.047
150	1.02	235	0.49	97	0.016	1.02	274	0.75	97	0.018
151	1.03	237	1.05	95	0.034	1.01	275	0.47	98	0.012
152	1.05	238	1.58	92	0.051	1.01	275	0.52	98	0.013
153	1.07	238	2.04	90	0.066	1.01	276	0.26	99	0.006
154	1.03	239	0.99	95	0.032	1.01	280	0.51	98	0.012
155	1.02	240	0.65	97	0.021	1.01	281	0.52	98	0.012
156	1.03	240	0.94	95	0.030	1.01	283	0.52	98	0.012
157	1.01	241	0.43	98	0.014	1.01	284	0.49	98	0.011
158	1.03	241	0.86	96	0.027	1.01	284	0.54	98	0.012
159	1.03	242	0.81	96	0.026	1.01	285	0.54	98	0.012
160	1.03	242	0.84	96	0.026	1.01	287	0.60	98	0.013
161	1.05	242	1.55	93	0.048	1.01	287	0.43	99	0.010
162	1.02	245	0.74	97	0.023	1.03	290	1.47	95	0.032
163	1.03	246	0.90	96	0.027	1.01	290	0.44	99	0.010
164	1.02	248	0.59	97	0.018	1.04	291	1.66	94	0.036
165	1.02	248	0.62	97	0.019	1.02	291	0.73	98	0.016
166	1.04	248	1.29	94	0.039	1.02	293	1.16	96	0.025
167	1.03	249	0.86	96	0.026	1.01	295	0.57	98	0.012

Table I-6. Phase II sludge floc distribution

SBR 2-2 , SRT = 11 d, 20 °C & SBR 1-2, SRT=10d, 10 °C						
Size	Mar 7-05	Mar 8-05	Mar 9-05			
	Sample 1	Sample 2	Sample 3	Total	n=3	n=3
	No. of Flocs	No. of Flocs	No. of Flocs	No. of Flocs	Average No. of Flocs	SD No. of Flocs
7.7	980	1105	870	2955	985	118
8.9	646	678	660	1984	661	16
10.3	804	890	860	2554	851	44
12.0	530	676	649	1855	618	78
13.9	456	434	450	1340	447	11
16.2	314	332	371	1017	339	29
18.7	234	220	219	673	224	8
21.6	176	158	167	501	167	9
25.1	148	126	125	399	133	13
29.1	131	120	94	345	115	19
33.7	146	93	117	356	119	27
39.1	90	66	78	234	78	12
45.3	81	44	58	183	61	19
52.6	57	25	66	148	49	22
60.9	54	14	44	112	37	21
70.6	45	19	39	103	34	14
81.8	50	10	38	98	33	21
94.8	33	8	31	72	24	14
109.9	26	10	23	59	20	9
127.6	23	9	19	51	17	7
147.8	17	2	21	40	13	10
171.2	16	13	24	53	18	6
198.9	16	6	13	35	12	5
231.2	21	4	14	39	13	9
268.6	8	7	7	22	7	1
312.1	13	6	8	27	9	4
362.7	8	6	5	19	6	2
421.5	4	7	4	15	5	2
489.8	4	2	4	10	3	1
569.4	2	0	2	4	1	1
662.0	2	1	1	4	1	1
769.5	1	0	3	4	1	2
894.0	3	1	0	4	1	2
1039.5	1	1	0	2	1	1
Sum:	*5140*	*5093*	*5084*	*15317*		

Appendix J
Adsorption and Desorption Isotherm Data

Table J-1. Equlibrium adsorption isotherm data at SRT of 3.5 days

Cashmeran									
Co		M		Ce		X		X/M	
Musks Initial Concentration In Solution		Mass of WAS		Musk Equilibrium Concentration in Solution		Musks Sorbed Onto WAS (Co - Ce)xVol		Mass of Musks Sorbed per Gram of WAS	
Average	Standard Error	Average	Standard Error	Average	Standard Error	Average	Standard Error	Average	Standard Error
(µg/L)	(µg/L)	(g)	(g)	(µg/L)	(µg/L)	(µg)	(µg)	(µg/g)	(µg/g)
378	40	0.10	0.01	366	49	0.6	3.2	6	165
877	308	0.10	0.01	654	198	11	18	112	302
2549	423	0.10	0.01	1685	357	43	28	432	182
3376	515	0.10	0.01	2075	207	65	28	651	125
Celestolide									
156	6	0.10	0.01	15	1	7.0	0.3	70	1
486	153	0.10	0.01	37	5	22	8	224	28
811	13	0.10	0.01	117	14	35	1	347	4
1609	124	0.10	0.01	190	33	71	6	710	13
2148	174	0.10	0.01	272	14	94	9	938	18
Phantolide									
195	10	0.10	0.01	17	1	9	1	89	1
569	157	0.10	0.01	39	5	27	8	265	26
866	11	0.10	0.01	117	14	37	1	374	4
1693	136	0.10	0.01	191	33	75	7	751	14
2216	155	0.10	0.01	263	13	98	8	976	16
Traseolide									
103	2	0.10	0.01	3.3	0.3	5.0	0.1	50	1
333	90	0.10	0.01	7	1	16	4	163	14
628	6	0.10	0.01	25	4	30	0	301	3
962	41	0.10	0.01	47	8	46	2	458	6
1212	74	0.10	0.01	69	2	57	4	572	8
Galaxolide									
165	8	0.10	0.01	10	1	7.7	0.4	77	1
510	132	0.10	0.01	22	2	24	7	244	20
839	6	0.10	0.01	70	8	38	1	384	4
1426	85	0.10	0.01	109	21	66	4	658	9
1828	109	0.10	0.01	154	7	84	5	837	12
Tonalide									
161	7	0.10	0.01	7	1	7.7	0.4	77	1
501	110	0.10	0.01	18	1	24	6	242	15
789	4	0.10	0.01	56	7	37	0	366	4
1350	90	0.10	0.01	93	17	63	5	628	10
1709	91	0.10	0.01	130	4	79	5	790	11

Table J-2. Equlibrium desorption isotherm data at SRT of 3.5 days

Cashmeran						
M	Ce		X		X/M	
Mass of WAS	Musks in Solution After 90 min Desorption (µg/L)		Mass of Musks On WAS After 90 min Desorption (µg/L)		Mass of Musks Sorbed per Gram of WAS (µg/g)	
(g)	Mean	SD	Mean	SD	Mean	SD
0.1	134	28	-6.1	56.6	-61	56.6
0.1	371	59	-7	208	-74	207.5
0.1	719	154	-9	144	-88	143.9
0.1	594	116	13	376	135	376.1
0.1	363	58	47	217	469	217.0
Celestolide						
0.1	11	2	6.5	2.1	65	2.1
0.1	40	10	20	13	204	13.4
0.1	106	25	35	14	347	13.6
0.1	112	21	65	39	654	39.3
0.1	75	12	90	20	901	19.9
Phantolide						
0.1	12	2	8.3	2.3	83	2.3
0.1	45	11	24	14	243	14.0
0.1	115	29	37	14	374	13.9
0.1	118	23	69	40	692	40.4
0.1	77	11	94	19	938	18.6
Traseolide						
0.1	2	0	4.9	0.4	49	0.4
0.1	9	4	16	6	158	6.1
0.1	21	5	30	4	301	3.9
0.1	24	4	45	9	445	9.4
0.1	14	2	56	5	565	4.9
Galaxolide						
0.1	8	1	7.4	1.4	74	1.4
0.1	28	7	23	10	230	9.8
0.1	65	16	38	8	384	8.2
0.1	69	14	62	25	624	25.3
0.1	47	7	81	11	814	11.3
Tonalide						
0.1	5	1	7.4	1.0	74	1.0
0.1	22	7	23	9	231	8.9
0.1	53	15	37	7	366	7.2
0.1	56	10	60	20	601	20.0
0.1	36	5	77	8	772	7.7

Table J-3. Equlibrium adsorption isotherm data at SRT of 10.5 days

Cashmeran									
Co		M		Ce		X		X/M	
Musks Initial Concentration In Solution		Mass of WAS		Musk Equilibrium Concentration in Solution		Musks Sorbed Onto WAS (Co - Ce)xVol		Mass of Musks Sorbed per Gram of WAS	
Average	Standard Error	Average	Standard Error	Average	Standard Error	Average	Standard Error	Average	Standard Error
(µg/L)	(µg/L)	(g)	(g)	(µg/L)	(µg/L)	(µg)	(µg)	(µg/g)	(µg/g)
792	147	0.10	0.01	406	8	19	12	193	9
1368	232	0.10	0.01	75	16	65	15	647	11
3334	2214	0.10	0.01	1083	7	113	47	1125	33
5233	609	0.10	0.01	9237	1029	-200	25	-2002	17
4871	289	0.10	0.01	11898	3327	-351	17	-3514	12
Celestolide									
342	66	0.10	0.01	17	1	16	8	162	6
671	171	0.10	0.01	37	7	32	13	317	9
2204	1317	0.10	0.01	123	3	104	36	1040	26
2968	380	0.10	0.01	1497	106	74	20	735	14
2659	80	0.10	0.01	2175	562	24	9	242	6
Phantolide									
405	78	0.10	0.01	19	1	19	9	193	6
720	168	0.10	0.01	49	10	34	13	335	9
2215	1199	0.10	0.01	121	1	105	35	1047	24
3128	354	0.10	0.01	1436	139	85	19	846	13
2741	91	0.10	0.01	2043	426	35	10	349	7
Traseolide									
219	46	0.10	0.01	4	0	11	7	107	5
439	139	0.10	0.01	10	5	21	12	215	8
1373	600	0.10	0.01	31	1	67	24	671	17
1656	211	0.10	0.01	367	86	64	15	645	10
1475	118	0.10	0.01	556	130	46	11	459	8
Galaxolide									
347	67	0.10	0.01	11	0	17	8	168	6
600	169	0.10	0.01	23	8	29	13	288	9
2040	1046	0.10	0.01	71	3	98	32	985	23
2592	331	0.10	0.01	951	130	82	18	821	13
2139	102	0.10	0.01	1329	315	40	10	405	7
Tonalide									
335	69	0.10	0.01	9	0	16	2	163	24
570	152	0.10	0.01	23	8	27	12	274	123
1824	824	0.10	0.01	58	4	88	29	883	291
2397	254	0.10	0.01	722	122	84	10	838	100
2044	106	0.10	0.01	1031	175	51	7	506	72

Table J-4. Equlibrium desorption isotherm data at SRT of 10.5 days

Cashmeran							
M		Ce		X		X/M	
Mass of WAS		Musks in Solution		Mass of Musks On		Mass of Musks	
Average	Standard Error	Average	Standard Error	Average	Standard Error	Average	Standard Error
(µg/L)	(µg/L)	(g)	(g)	(µg/L)	(µg/L)	(µg)	(µg)
0.1	0.01	248	48	7	48	69	482
0.1	0.01	195	116	29	170	292	1696
0.1	0.01	535	231	86	172	858	1722
0.1	0.01	9237	1029	-248	32	-2477	323
0.1	0.01	937	66	-398	13	-3982	130
Celestolide							
0.1	0.01	27	18	15	18	149	184
0.1	0.01	17	6	31	13	306	133
0.1	0.01	86	39	100	43	998	429
0.1	0.01	1497	106	65	11	649	108
0.1	0.01	184	50	15	6	150	56
Phantolide							
0.1	0.01	30	21	18	21	178	209
0.1	0.01	18	7	33	14	329	144
0.1	0.01	92	42	100	42	1001	423
0.1	0.01	1436	139	75	13	753	126
0.1	0.01	198	49	25	5	251	49
Traseolide							
0.1	0.01	8	7	10	7	104	72
0.1	0.01	3	1	21	9	212	93
0.1	0.01	17	7	66	16	662	159
0.1	0.01	367	86	63	17	628	172
0.1	0.01	37	10	44	3	441	30
Galaxolide							
0.1	0.01	19	14	16	14	159	141
0.1	0.01	10	3	28	12	282	123
0.1	0.01	52	25	96	32	959	317
0.1	0.01	951	130	77	15	766	153
0.1	0.01	115	32	35	4	347	43
Tonalide							
0.1	0.01	15	12	16	13	155	126
0.1	0.01	8	3	27	11	270	114
0.1	0.01	43	21	86	25	861	254
0.1	0.01	722	122	79	17	795	168
0.1	0.01	94	24	46	3	460	34

Table J-5. Calibration curves in aqueous phase

PSMs (μg/L)	Ratio of Chromatograph Peak Intensities (As/Ais)					
	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
0	0	0	0	0	0	0
17	0.5	1.5	1.9	1.1	1.4	1.7
33	0.8	3.0	3.9	2.5	2.9	3.4
67	1.2	6.6	8.2	5.9	6.6	7.5
133	2.4	14.8	19.1	14.1	15.0	17.8
233	3.9	26.0	32.7	24.8	26.2	30.5
333	4.6	34.0	42.9	33.0	34.9	40.8
Slope	0.0152	0.10560	0.13330	0.10140	0.10730	0.12560
R^2	0.97140	0.99570	0.99570	0.99590	0.99670	0.99630

Table J-6. Equilbration plateau determination in aqueous phase

PSM Concentration in the Aqueous Phase (μg/L)												
Time (min)	Cashmeran		Celestolide		Phantolide		Traseolide		Galaxolide		Tonalide	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	326	62	287	34	298	33	247	18	289	19	262	18
30	365	2	126	19	123	18	60	18	77	17	75	16
60	336	1	121	9	119	4	60	3	76	4	73	4
120	61	0	147	0	138	1	77	3	93	1	90	3
180	305	6	135	9	132	6	91	2	99	3	100	2
240	384	15	124	5	117	1	51	0	70	1	68	3

Table J-7. Calibration curves in solid phase[1, 2]

PSMs (ng/g)	Ratio of Chromatograph Peak Intensities Times Concentration of Internal Standard Concentration added (AsCis/Ais)					
	Cashmeran	Celestolide	Phantolide	Traseolide	Galaxolide	Tonalide
0	0	0	0	0	0	0
2400	52	116	143	44	72	73
4800	122	284	346	109	167	173
9600	471	706	830	248	364	384
19200	392	1150	1450	530	690	783
24000	792	1470	1860	659	832	968
38400	1322	2649	3327	1176	1465	1714
Slope	0.0325	0.0659	0.0827	0.0292	0.037	0.0428
R^2	0.9358	0.9909	0.9923	0.9923	0.9963	0.9945

1. Internal standard used was Phenanthrene-d10 at a concentration of 0.1 µg/mL (100 ppb) volume of 50 µL to 3 mL of PBS with 0.1 g of dried solids from SBR 1-1. The mixture was equilibrated overnight in 22 mL headspace vials with different musk standards additions and mixed with a micro flea mixers and frozen at -22 °C until ready for analyzes using HS SPME in the RUAC.
2. Adjusted values for the sorbent control are tabulated.

Table J-8. Concentration of PSMs in lyophilized and unspiked sludge using SAM[1]

PSMs	Initial PSMs in Lyophilized Sludge Co (µg/g)[2]	SAM Linear Equation ($Cs = m \cdot Co + B$)[3] (duplicates of n=7 points)	r^2
Cashmeran	0.04	Cs = 0.033Co – 11.95	0.936
Celestolide	0.47	Cs = 0.067Co – 31.41	0.992
Phantolide	0.63	Cs = 0.085Co – 53.69	0.994
Traseolide	0.87	Cs = 0.0305Co – 30.32	0.996
Galaxolide	1.8	Cs = 0.0376Co – 67.01	0.997
Tonalide	0.35	Cs = 0.0443Co – 15.59	0.996

1. SAM means standard addition method and is used in the absence of a blank matrix. We do not have a blank matrix to work with in our case.
2. Co (µg/g) represents the PSM concentration associated with the unspiked and lyophilized sludge and calculated from the SAM curve by –B/m.
3. Cs (µg/g) is the added PSM musk spike to the solids (0.1g) and PBS mixture (3 mL). The Cs values are given in Table J-6. The SAM linear curves are based on the linear plot of data in Table J-6.

Appendix K

PSMs Effluent Comparison Using the Kruskal-Wallis and Mann-Whitney Non-parametric Test and the Student's t-test

Table K-1. The PSMs effluent sampling data from the SBR during stable operating conditions

SBR	Sampling Date	SRT (days)	T (°C)	Cashmeran (ng/L)	Celestolide (ng/L)	Phantolide (ng/L)	Traseolide (ng/L)	Galaxolide (ng/L)	Tonalide (ng/L)
SBR 2-1	18-Apr-05	10.5	10	33.6	19.7	18	23.5	1570	302
SBR 2-1	19-Apr-05	10.5	10	32.6	22.1	17	24.1	1700	332
SBR 2-1	21-Apr-05	10.5	10	27.5	19.3	18	19.8	1490	317
SBR 2-1	20-Apr-05	10.5	10	21.1	16.3	17	14.3	1230	243
SBR 2-1	22-Apr-05	10.5	10	24.6	20.2	18	25.2	1780	322
SBR 2-2	19-Apr-05	10.5	20	21.1	16.3	17	22	1320	255
SBR 2-2	18-Apr-05	10.5	20	27.8	16.3	18	30.9	1370	285
SBR 2-2	20-Apr-05	10.5	20	21.1	16.3	17	18.4	972	225
SBR 2-2	19-Apr-05	10.5	20	21.1	16.3	18	21.4	1270	269
SBR 2-2	21-Apr-05	10.5	20	21.1	16.3	17	17.7	1040	215
SBR 1-1	18-Jul-05	3.45	10	18.8	48.6	37.4	36.8	2720	401
SBR 1-1	18-Jul-05	3.45	10	17.3	40.3	31.4	26	1710	287
SBR 1-1	20-Jul-05	3.45	10	31.8	38.2	29.4	24.6	2030	304
SBR 1-1	19-Jul-05	3.45	10	14.1	30.7	24.3	21.4	1730	249
SBR 1-1	19-Jul-05	3.45	10	17.5	28.7	21.4	21.2	1720	193
SBR 1-2	19-Jul-05	3.45	20	14.7	16.3	12.7	13.4	1090	150
SBR 1-2	21-Jul-05	3.45	20	17.1	17.3	12.9	33.2	1130	192
SBR 1-2	20-Jul-05	3.45	20	20.8	15.4	11.1	9.7	1040	155
SBR 1-2	22-Jul-05	3.45	20	18.5	14.6	9.52	9.38	1080	149
SBR 1-2	18-Jul-05	3.45	20	16.2	16.1	13	21.2	1220	230

Table K-2. The mean PSMs effluent comparison results by the Student's t-test for Traseolide, Galaxolide, Tonalide and total PSMs

XLSTAT 2006.5 - Two-sample t-test and z-test						
Hypothesized difference (D): 0						
Significance level (%): 5	Degrees of Freedom: 8					
t-test for two independent samples / Two-tailed test: Log transformed datasets						
	SBR 1-1 vs SBR 1-2	SBR 1-1vs SBR	SBR 1-1 vs SBR 2-2	SBR 1-2 vs SBR 2-1	SBR 1-2 vs SBR 2-2	SBR 2-1 vs SBR 2-2
Traseolide						
Difference	-0.084	0.219	0.071	0.135	-0.014	-0.148
t (Observed value)	-1.346	1.925	1.156	1.177	-0.222	-1.308
t (Critical value)	2.306	2.306	2.306	2.306	2.306	2.306
p-value (Two-tailed)	0.215	0.090	0.281	0.273	0.830	0.227
Galaxolide						
Difference	-0.102	0.244	0.115	0.143	0.217	0.028
t (Observed value)	-2.128	6.025	2.814	4.705	4.430	0.866
t (Critical value)	2.306	2.306	2.306	2.306	2.306	2.306
p-value (Two-tailed)	0.066	0.0003	0.023	0.002	0.002	0.411
Tonalide						
Difference	0.034	-0.208	0.050	0.242	0.084	0.158
t (Observed value)	0.591	-3.250	0.876	5.452	2.505	3.614
t (Critical value)	2.306	2.306	2.306	2.306	2.306	2.306
p-value (Two-tailed)	0.571	0.012	0.406	0.001	0.037	0.007
Total PSMs						
Difference	-0.086	0.242	0.193	0.156	0.106	-0.049
t (Observed value)	-1.814	5.717	4.009	4.994	2.755	-1.542
t (Critical value)	2.306	2.306	2.306	2.306	2.306	2.306
p-value (Two-tailed)	0.107	0.0004	0.004	0.001	0.025	0.162

Table K- 3. The mean PSMs effluent comparison results by the Student's t-test for Traseolide, Galaxolide, Tonalide and total PSMs

XLSTAT 2006.5 - Two-sample t-test and z-test			
Hypothesized difference (D): 0			
Significance level (%): 5		Degrees of Freedom (DF): Variable	
t-test for two independent samples / Two-tailed test: Log transformed datasets			
	Low T vs High T (DF =18)	Low SRT vs High SRT (DF =18)	Nitrification vs Non-nitrification (DF=13)
Traseolide			
Difference	0.103	-0.032	0.039
t (Observed value)	1.522	-0.449	0.397
t (Critical value)	2.101	2.101	2.160
p-value (Two-tailed)	0.145	0.659	0.697
Galaxolide			
Difference	0.180	0.037	-0.094
t (Observed value)	5.556	0.705	-1.391
t (Critical value)	2.101	2.101	2.160
p-value (Two-tailed)	< 0.0001	0.490	0.188
Tonalide			
Difference	0.146	-0.096	0.054
t (Observed value)	3.361	-1.899	0.794
t (Critical value)	2.101	2.101	2.160
p-value (Two-tailed)	0.003	0.074	0.441
Total PSMs			
Difference	0.174	0.019	-0.072
t (Observed value)	5.497	0.360	-1.064
t (Critical value)	2.101	2.101	2.160
p-value (Two-tailed)	< 0.0001	0.723	0.307

Table K-4. The mean PSMs effluent comparison using Kruskal-Wallis test results for Cashmeran, Celestolide and Phantolide

XLSTAT 2006.5 - Comparison of k=4 samples (Kruskal-Wallis, Test)				
Hypothesized difference (D): 0 and Significance level (%): 5				
Kruskal-Wallis test: Cashmeran				
K (Observed value)	K (Critical value)	DF	p-value (Two-	alpha
11.562	7.815	3	0.009	0.05
Multiple pair wise comparisons using the Dunn's procedure / Two-tailed test:				
Sample	SBR 2-1	SBR 2-2	SBR 1-2	SBR 1-1
Frequency	5	5	5	5
Sum of ranks	25.000	38.000	65.000	82.000
Mean of ranks	5.000	7.600	13.000	16.400
Table of pair wise differences:	SBR 2-1	SBR 2-2	SBR 1-1	SBR 1-2
SBR 2-1	0	-8.000	-2.600	-11.400
SBR 2-2	-8.000	0	5.400	-3.400
SBR 1-1	-2.600	5.400	0	-8.800
SBR 1-2	-11.400	-3.400	-8.800	0
Kruskal-Wallis test: Celestolide				
K (Observed value)	K (Critical	DF	p-value (Two-	alpha
11.562	7.815	3	0.009	0.05
Multiple pair wise comparisons using the Dunn's procedure / Two-tailed test:				
Sample	SBR 2-1	SBR 2-2	SBR 1-2	SBR 1-1
Frequency	5	5	5	5
Sum of ranks	25.000	38.000	65.000	82.000
Mean of ranks	5.000	7.600	13.000	16.400
Table of pair wise differences:	SBR 2-1	SBR 2-2	SBR 1-1	SBR 1-2
SBR 2-1	0	-2.200	-13.200	-7.400
SBR 2-2	-2.200	0	-11.000	-5.200
SBR 1-1	-13.200	-11.000	0	5.800
SBR 1-2	-7.400	-5.200	5.800	0
Kruskal-Wallis test: Phantolide				
K (Observed value)	K (Critical value)	DF	p-value (Two-	alpha
15.492	7.815	3	0.001	0.05
Multiple pair wise comparisons using the Dunn's procedure / Two-tailed test:				
Sample	SBR 2-1	SBR 2-2	SBR 1-2	SBR 1-1
Frequency	5	5	5	5
Sum of ranks	24.000	35.000	61.000	90.000
Mean of ranks	4.800	7.000	12.200	18.000
Table of pair wise differences:	SBR 2-1	SBR 2-2	SBR 1-1	SBR 1-2
SBR 2-1	0	0.000	-7.500	7.500
SBR 2-2	0.000	0	-7.500	7.500
SBR 1-1	-7.500	-7.500	0	15.000
SBR 1-2	7.500	7.500	15.000	0
Critical difference: 9.8715				
Bonferroni corrected significance level: 0.0083				

Table K-5. The mean PSMs effluent comparison using the Mann-Whitney test results for Cashmeran, Celestolide and Phantolide

XLSTAT 2006.5 - Mann-Whitney test / Two-tailed test:			
Significance level (%): 5	Continuity correction: Yes		Hypothesized difference (D): 0
	Low T vs High T (DF =18)	Low SRT vs High SRT (DF =18)	Nitrification vs Non-nitrification (DF=13)
Cashmeran			
U	65.000	8.000	5.000
Expected value	50.000	50.000	25.000
Variance (U)	172.368	172.368	65.476
p-value (Two-tailed)	0.276	0.001	0.012
Celestolide			
U	70.000	59.000	32.500
Expected value	50.000	50.000	25.000
Variance (U)	167.105	167.632	62.500
p-value (Two-tailed)	0.159	0.520	0.384
Phantolide			
U	87.500	50.000	25.000
Expected value	50.000	50.000	25.000
Variance (U)	153.289	153.289	64.286
p-value (Two-tailed)	0.003	0.961	0.948

Table K-6. Normal distribution test results for the difference of PSMs from each SBR

Normality Tests	tPSMs (n=20)	Cashmeran (n=20)	Celestolide (n=20)	Phantolide (n=20)	Traseolide (n=20)	Galaxolide (n=20)	Tonalide (n=20)
Chi-square test:							
Chi-square (Observed value)	18.567	22.888	15.256	35.437	26.158	20.414	25.407
Chi-square (Critical value)	27.587	27.587	27.587	27.587	27.587	27.587	27.587
DF	17	17	17	17	17	17	17
p-value	0.354	0.153	0.577	0.005	0.072	0.254	0.086
alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ho: The sample set follows a Normal distribution	Y	Y	Y	N	Y	Y	Y
Kolmogorov-Smirnov test:							
D	0.123	0.196	0.148	0.190	0.098	0.136	0.146
p-value	0.902	0.386	0.738	0.423	0.987	0.823	0.757
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ho: The sample set follows a Normal distribution	Y	Y	Y	Y	Y	Y	Y

REFERENCES

Andreadakis, A. D. (1993). "Physical and chemical properties of activated sludge floc." *Water Res.*, 27(12), 1707-1714.

Artola-Garicano, E., Hermens, J. L., and Vaes, W. H. (2003). "Evaluation of Simple Treat 3.0 for two hydrophobic and slowly biodegradable chemicals: polycyclic musks HHCB and AHTN." *Water Res.*, 37(18), 4377-4384.

Azeredo, J., Oliveira, R., and Lazarova, V. (1998). "A new method for extraction of exopolymers from activated sludges." *Water Science and Technology,* 37(4-5), 367-370.

Balacko, G., Horenstein, B., Marshall, L., Muirhead, W., O'Neil, J. (1994) "Basic Activated Sludge Process Control", WEF Probe

Barbusiński, B. and Kościelniak, H. (1995) "Influence of substrate loading intensity on floc size in activated sludge process" *Wat Res.* 29 (7) pp. 1703-1710.

Bell, J. P., Tsezos, M. (1987), "Removal of hazardous organic pollutants by biomass adsorption", *Journa of WPCF,* 59 (4) 191-198.

Berg, U. T., and Nyholm, N. (1996). "Biodegradability simulation studies in semicontinuous activated sludge reactors with low (microgram/L range) and standard (ppm range) chemical concentrations." *Chemosphere,* 33(4), 711-735.

Berset, J. D., Kupper, T., Etter, R., and Tarradellas, J. (2004). "Considerations about the enantioselective transformation of polycyclic musks in wastewater, treated wastewater and sewage sludge and analysis of their fate in a sequencing batch reactor plant.", *Chemosphere,* 57(8), 987-996.

Bester, K. (2005). "Polycyclic musks in the Ruhr catchment area--transport, discharges of waste water, and transformations of HHCB, AHTN and HHCB-lactone." *J.Environ.Monit.,* 7(1), 43-51.

Bossier, P., Verstraete, W. (1996) "Triggers for microbial aggregation in activated sludge?" *Appl Microbiol Biotechnol* 45: 1-6.

Brown, M. J. and Lester, J. N. (1979) "Metal removal in activated sludge: The role of bacterial extracellular polymers *Water Research* 13, 817-837.

Buerge, I. J., Buser, H. R., Muller, M. D., and Poiger, T. (2003). "Behavior of the polycyclic musks HHCB and AHTN in lakes, two potential anthropogenic markers for domestic wastewater in surface waters." *Environ.Sci.Technol.,* 37(24), 5636-5644.

Bura, R., Cheung, M., Liao, B., Finlayson, J., Lee, B. C., Droppo, I. G., Leppard, G. G., and Liss, S. N. (1998). "Composition of extracellular polymeric substances in the activated sludge floc matrix" *Water Science and Technology,* 37(4-5), 325-333.

Chrysi, S. Laspidou, Rittman, Bruce E. (2002) "A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass" *Water Research,* 36, 2711-2720.

Daughton, G. C. and Ternes, T. A. (1999) "Pharmaceuticals and personal care products in the environment: agents of subtle change?" *Enviornmental health perspectives,* Special Report. Vol 107, Sup 6, 907-938.

Decho, A. W. (1990) "Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes." *Oceanogr. Mar. Biol. Annu. Rev.* 28, 73–154.

Deng, S., Ting, Y.P. and Yu, G.(2006) "Chromate sorption and reduction kinetics onto an aminated biosorbent" 54 (10) 1-8, *Waer Science and Technology,* IWA

Dignac, M. -., Urbain, V., Rybacki, D., Bruchet, A., Snidaro, D., and Scribe, P. (1998). "Chemical description of extracellular polymers: implication on activated sludge floc structure." *Water Science and Technology,* 38(8-9), 45-53.

Droppo, I. G., (2002) "A new definition of suspended sediment: Implications for the measurement and prediction of sediment transport", NWRI

Droppo, I. G., Leppard, G. G., Flannigan, D. T., and Liss, S. N. (1997). "The Freshwater Floc: A Functional Relationship of Water and Organic and Inorganic Floc Constituents Affecting Suspended Sediment Properties." *Water Air Soil Pollut.,* 99(1-4), 43-54.

Esparza-Soto, M., and Westerhoff, P. (2003). "Biosorption of humic and fulvic acids to live activated sludge biomass." *Water Res.,* 37(10), 2301-2310.

Finlayson, J. C., Liao, B., Droppo, I. G., Leppard, G. G., and Liss, S. N. (1998). "The relationship between the structure of activated sludge flocs and the sorption of hydrophobic pollutants." *Water Science and Technology,* 37(4-5), 353-357.

Frølund, B., Palmgren, R., Keiding, K., and Nielsen, P. H. (1996). "Extraction of extracellular polymers from activated sludge using a cation exchange resin." *Water Res.,* 30(8), 1749-1758.

Goodwin, J. A. S., and Forster, C. F. (1985). "A further examination into the composition of activated sludge surfaces in relation to their settlement characteristics." *Water Res.*, 19(4), 527-533.

Grady, C.P.L. Jr., Daigger, G. T., Lim, H. C. (1999) "Biological Wastewater Treatment"', Marcel Decker, Inc., Second Edition, Revised and Expanded.

Guellil, A., Block, J. –C., Urbain, V. (1998) "Adaptation of the microbial adhesion to hydrocarbon test (MATH) for measuring activated sludge hydrophobicity", *Wat. Sci. Tech.* (37) 4-5, 359-362.

Halalsheh, M., Koppes, J., den Elzen, J., Zeeman, G., Fayyad, M., and Lettinga, G. (2005). "Effect of SRT and temperature on biological conversions and the related scum-forming potential." *Water Res.*, 39(12), 2475-2482.

Heissenberger, A., Leppard, G. G., Hemdl, G. J. (1997) "Ultrastructure of marine snow. II. Microbiological considerations" (44) Issue:1 pp 30

Jin, B., Wilén, B., and Lant, P. (2003). "A comprehensive insight into floc characteristics and their impact on compressibility and settleability of activated sludge." *Chem.Eng.J.*, 95(1-3), 221-234.

Jorand, F., Zartarian, F., Thomas, F., Block, J. C., Bottero, J. Y., Villemin, G., Urbain, V. and Manem, J. (1998). "Chemical and structural (2D) linkage between bacteria within activated sludge flocs." *Water Research,* 329(7), 1639-1647.

Jorand, F., Boué-Bigne, F., Block, J. C., and Urbain, V. (1998). "Hydrophobic/hydrophilic properties of activated sludge exopolymeric substances." *Water Science and Technology,* 37(4-5), 307-315.

Kanda, R., Griffin, P., James, H. A., and Fothergill, J. (2003). "Pharmaceutical and personal care products in sewage treatment works." *J.Environ.Monit.*, 5(5), 823-830.

Keiding, K., and Nielsen, P. H. (1997). "Desorption of organic macromolecules from activated sludge: Effect of ionic composition." *Water Res.*, 31(7), 1665-1672.

Kerr, M. K, Larson, R. J., McAvoy, D. C. (2000) "Evaluation of an inactivation procedure for determinating the sorption of organic compounds to activated sludge", *Ecotox. Environ. Safety*, 47, 314-322.

Kördel, W. Hennecke, D. , Hermann, M. (1997) "Application of the HPLC-screening method for the determination of the adsorption coefficient on sewage sludges", *Chemosphere* 35 (1/2) 121-127.

Kupper, T., Berset, J. D., Etter-Holzer, R., Furrer, R., and Tarradellas, J. (2004). "Concentrations and specific loads of polycyclic musks in sewage sludge originating from a monitoring network in Switzerland." *Chemosphere,* 54(8), 1111-1120.

Laspidou, C. S., and Rittmann, B. E. (2002). "A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass." *Water Res.,* 36(11), 2711-2720.

Lee, Hing-Biu, Peart, T. E., Sarafin, Kurtis (2003). "Occurrence of Polycyclic and Nitro Musk Compounds in Canadian Sludge and Wastewater Samples." *Water Qual. Res. J. Canada, 38(4), 683-702.*

Li D.–H and Ganczarczyk J. (1987) Stroboscopic determination of settling velocity, size and porosity of activated sludge flocs. *Wat. Res.(21), 257-262.*

Liao, B. Q. (2000) Physicochemical studies of microbial flocs, Ph.D. thesis, University of Toronto, Canada.

Liao, B. Q., Allen, D. G., Droppo, I. G., Leppard, G. G., and Liss, S. N. (2001). "Surface properties of sludge and their role in bioflocculation and settleability." *Water Res.,* 35(2), 339-350.

Liao, B. Q., Allen, D. G., Leppard, G. G., Droppo, I. G., and Liss, S. N. (2002). "Interparticle Interactions Affecting the Stability of Sludge Flocs." *J.Colloid Interface Sci.,* 249(2), 372-380.

Lishman, L., Smyth, S. A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, H. B., Servos, M., Beland, M., Seto, P. (in print). "Occurrence and reduction of PPCPs and Estrogens by Municipal WWTPs in Ontario, Canada.

Liss, S. N. , Droppo, I. G., Flannigan, D. T. and Leppard, G. G. (1996). "Floc architecture in wastewater and natural riverine systems. *Environ. Sci. Tech.* 30 (2), 680-686.

Llompart M, Garcia-Jares C, Salgado C, Polo M, Cela R. 2003. Determination of musk compounds in sewage treatment plant sludge samples by solid-phase microextraction. J. Chromatogr. A 999:185-193.

Luckenbach, T. and Epel, D. (2005) "Nitromusk and Polycyclic Musk Compounds as Lorg-Term Inhibitors of Cellullar Xenobiotic Defense Systems Mediated by Multidrug Transporters" *Envrionmental Health Perscpectives*, 113(1), 17-24

Magara, Y., Nambu, S., and Utosawa, K. (1976). "Biochemical and physical properties of an activated sludge on settling characteristics." *Water Res.,* 10(1), 71-77.

Melcer, H., EnviroSim Associates Limited, Stensel, D. H., Wilson, W. A., Sun, P., Bury, S. (2003) Methosds of Wastewater Characterization in Activated Sludge Modeling', WERF, 99-WWF-3.

Metcalf and Eddy Inc., (2003), "Wastewater Engineering, Treatment and Reuse", McGraw-Hill, Fourth edition

Mikkelsen, L. H. (2003). "Applications and limitations of the colloid titration method for measuring activated sludge surface charges." *Water Res.*, 37(10), 2458-2466.

Mikkelsen, L. H., and Keiding, K. (2002). "Physico-chemical characteristics of full scale sewage sludges with implications to dewatering." *Water Res.*, 36(10), 2451-2462.

Moretti, C. J., Neufeld, D. R., (1989) "PAH patitioning mechanisms with activated sludge", *Wat. Res.* , 23 (1) 93-102.

OECD/OCDE 106 (2000). "OECD Guideline for the testing of Chemicals, Adsorption-Desorption Using Batch Equilibrium method"

Osemwengie, L. I., and Gerstenberger, S. L. (2004). "Levels of synthetic musk compounds in municipal wastewater for potential estimation of biota exposure in receiving waters." *J.Environ.Monit.*, 6(6), 533-539.

Paasivirta, J., Sinkkonen, S., Rantalainen, A-L., Broman, D. and Zebühr, Y. (2002) "Temperature dependent properties of enviornmentally important synthetic musks". *ESPR-Enviorn Sci & Pollut Res* 9 (5) 345-355.

Pommepuy, M., Dumas, F., Caprais, M. P., Camus, P., Le Mennec, C., Parnaudeau, S., Haugarreau, L., Sarrette, B., Vilagines, P., Pothier, P., Kholi, E., and Le Guyader, F. (2004). "Sewage impact on shellfish microbial contamination." *Water Sci.Technol.*, 50(1), 117-124.

Raszka, A., Chrvatova, M., Wanner J. (2006) "The role and significance of extracellular polymers in activated sludge. Part I: Literature review" *Acta hydrochim. Hydrobiol.* 34, 411-424.

Rittmann, B. E. and McCarty, P. L. , (2001) "Enviornmental Biotechnology: Principles and Applications", McGraw-Hill

Sawyer, C. L., McCarty, P. L. and Parkin, G. F., (2003), "Chemistry for Enviornmental Engineering and Science", McGraw-Hill Higher Education, Fifth edition

Sezgin, M. (1982). "Variation of sludge volume index with activated sludge characteristics." *Water Res.*, 16(1), 83-88.

Smyth, S. A., Lishman, L., McBean, E., Kleywegt, S., Yang, J.J., Svoboda, V. Pileggi, L., Lee, H. B., Seto, P. (in print, 2007). "Polycyclic and nitro musks in Canadian municipal wastewaters: Part I: Occurrence and removal through the stages of wastewater treatment.

Sponza, D. T. (2002). "Extracellular polymer substances and physicochemical properties of flocs in steady and unsteady-state activated sludge systems." *Process Biochemistry,* 37(9), 983.

Sponza, D. T. (2003). "Investigation of extracellular polymer substances (EPS) and physicochemical properties of different activated sludge flocs under steady-state conditions." *Enzyme Microb.Technol.,* 32(3-4), 375-385.

Stratton, H., Seviour, B., and Brooks, P. (1998). "Activated sludge foaming: what causes hydrophobicity and can it be manipulated to control foaming?" *Water Science and Technology,* 37(4-5), 503-509.

Svobota, M. L., Yang, J-J., Faletta, P., Lee Hing-Biu (EC internal manuscript, 2006), "A Microwave-assisted Extraction Method for the Determination of Synthetic Musks", Environment Canda.

Ternes, T. A., Janex-Habibi, M-L., Knacker, T., Kreuzinger, N., Siergrist, H. (2004) "Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse", POSEIDON

Urbain, V., Block, J. C., and Manem, J. (1993). "Bioflocculation in activated sludge: an analytic approach." *Water Res.,* 27(5), 829-838.

Voice, T. C., Weber, W. J. Jr., (1983) "Sorption of hydrophobic compounds by sediments, soils and suspended solids – I", *Water Research Vol.* 17(10) 1433-1441.

Weng., L., Govind, R., and Dobbs, R. A. (1993) "Sorption of toxic organic compounds on wastewater solids: mechanism and modeling", *Environ. Sci. Technol.* (27) 152-158.

Wilén, B., Jin, B., and Lant, P. (2003a) "Impacts of structural characteristics on activated sludge floc stability" *Water Res.,* 37, 3632-3645.

Wilén, B., Jin, B., and Lant, P. (2003b) "The influence of key chemical constituents in activated sludge on surface and flocculating properties." *Water Res.,* 37(9), 2127-2139.

Wu, R. M., Lee, D. J., Waite, T. D., Guan J. (2002). "Multilevel structure of sludge flocs." *Journal of Colloidal and Interface Science,* 252, 383-392.

Zar, H. J., (1996) "Biostatiscal Analysis", Third edition, Prentice Hall