Old Dominion University
ODU Digital Commons

Civil & Environmental Engineering Theses & Dissertations

Civil & Environmental Engineering

Spring 2016

Impacts of Operating Parameters on Extracellular Polymeric Substances Production in a High Rate Activated Sludge System with Low Solids Retention Times

Matthew S. Elliot
Old Dominion University, elliottvmi12@gmail.com

Follow this and additional works at: https://digitalcommons.odu.edu/cee_etds

Part of the Environmental Engineering Commons

Recommended Citation
Elliot, Matthew S.. "Impacts of Operating Parameters on Extracellular Polymeric Substances Production in a High Rate Activated Sludge System with Low Solids Retention Times" (2016). Master of Science (MS), thesis, Civil/Environmental Engineering, Old Dominion University, DOI: 10.25777/5mmx-c139
https://digitalcommons.odu.edu/cee_etds/8

This Thesis is brought to you for free and open access by the Civil & Environmental Engineering at ODU Digital Commons. It has been accepted for inclusion in Civil & Environmental Engineering Theses & Dissertations by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.

IMPACTS OF OPERATING PARAMETERS ON EXTRACELLULAR POLYMERIC SUBSTANCES PRODUCTION IN A HIGH RATE ACTIVATED SLUDGE SYSTEM WITH LOW SOLIDS RETENTION TIMES

by

Matthew S. Elliott
B.S. Biology May 2012, Virginia Military Institute

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

ENVIRONMENTAL ENGINEERING

OLD DOMINION UNIVERSITY
April 2016

Approved by:

Charles B. Bott

Gary Schafran

Peter Pommerenk

ABSTRACT

IMPACTS OF OPERATING PARAMETERS ON EXTRACELLULAR POLYMERIC SUBSTANCES PRODUCTION IN A HIGH RATE ACTIVATED SLUDGE SYSTEM WITH LOW SOLIDS RETENTION TIMES

Matthew S. Elliott
Old Dominion University, 2016
Director: Dr. Charles, Bott

The Adsorption/Bio-oxidation (A/B) process accomplishes carbon capture via bio-flocculation in the adsorption stage (A-stage) to maximize energy recovery while simultaneously providing an optimal carbon to nitrogen (C/N) ratio for denitrification in the Bio-oxidation stage (B-Stage). The present study evaluated the influence of the solids retention time (SRT), dissolved oxygen (DO) concentration, and production of extracellular polymeric substances (EPS) on bio-flocculation and subsequent carbon capture using a pilot-scale A-stage process. A mixed liquor suspended solids (MLSS)-based control strategy was implemented to manage carbon capture by maintaining a constant MLSS concentration of 3,000 mg/L in response to diurnal variations in organic loading. Bio-flocculation, in terms of colloidal organic matter removal and concentration of effluent suspended solids, was enhanced by operating at a 0.56 day SRT compared to a 0.26 day SRT regardless of the DO concentration. Increasing the DO concentration from 0.5 to 1.0 mg/L at a longer SRT resulted in maximum bio-flocculation and carbon capture without significantly increasing the amount of COD lost to mineralization. These operating conditions coincided with a large reduction in loosely bound EPS (LB-EPS) and slight reduction in tightly bound EPS (TB-EPS) leading to the lowest LB-EPS to TB-EPS (LB/TB) ratio. Further increasing the DO concentration to 1.5 mg/L did not enhance bio-flocculation or carbon capture. Although EPS may have contributed to enhancing bio-flocculation, correlations found between EPS production and bio-flocculation were not as strong compared to operating conditions such as the SRT, DO concentration, MLSS concentration and influent wastewater characteristics. On the other hand, EPS production showed strong correlations for suspended

solids removal and subsequent carbon capture in the A-stage pilot, especially when operated at a longer SRT.

Key words: A-stage, Bio-flocculation, Carbon capture, Extracellular polymeric substances.

©2016, by Matthew S. Elliott, All Rights Reserved.

ACKNOWLEDGMENTS

If someone asked me 5 years ago if I saw myself pursuing a master's degree focused on wastewater treatment, I probably would have responded with, "What is that"? Fortunately, while completing the requirements to earn my bachelor's degree in biology at the Virginia Military Institute, Dr. Tim Moore took the time to discuss such an opportunity with me. As a biologist with a strong passion for environmental conservation, I quickly jumped at the opportunity. Therefore, I must thank Tim as I would not be where I am today without his faith in me to make the switch to engineering.

Little did I realize that Tim would be introducing me to one of the smartest men that I know, Dr. Charles Bott, who undoubtedly became the best boss that I have ever had. Not only is Charles wickedly intelligent and passionate about his work, but more importantly, he effectively instills his passion to his young Padawans and makes a conscious effort to get down on each student's level to effectively expand their knowledge. I must thank Charles for an amazing experience and opening my eyes to a whole new world that was non-existent a few years ago.

After almost 4 years of working at the same pilot study, I have seen many fellow interns come and go but now it is my turn to walk out the door. Each and every one of the pilot crew had unique qualities that made working at the pilot one of the best experiences of my life. I need to thank all pilot crew members that helped me along the way including, Mark Miller (now Dr. Miller), Pusker Regmi (now Dr. Regmi), Ryder Bunce, Becky Holgate, Dana Fredericks, Claire Welling, Jon DeArmond, Johnnie Godwin, Michael Sadowski, Tyler Brickles, Warner Thomas, and Dr. Maureen Kinyua. I need to thank other interns that were not a part of the pilot but were instrumental in my 4 year stay at HRSD including Germano Salazar, Amanda Kennedy (Ford) and Arba Williamson (adopted intern). A special shout out is reserved for Mark who took the time away from his almost daily activities to answer all my questions and teach me the ways of the A-stage process. I think all interns would agree that without Mark, the pilot would not be where it is today and all of us are in a better place due to his vast knowledge, never ending generosity and selflessness in terms of lending a hand when needed.

Lastly, I would like to thank HRSD as a whole and Old Dominion University for providing me with this opportunity, as well as my committee members, Dr. Charles Bott, Dr. Gary Schafran and Dr. Peter Pommerenk, for taking the time to provide valuable insight on my research.

TABLE OF CONTENTS

Contents

ABSTRACT ii
ACKNOWLEDGMENTS v
TABLE OF CONTENTS vi
NOMENCLATURE viii
LIST OF TABLES x
LIST OF FIGURES xi
List of Equations xii
CHAPTER 1 1
CHAPTER 2 7
2.1 Activated Sludge Processes 7
2.1.1 High Rate Activated Sludge (HRAS) process 9
2.1.2 A/B process 11
2.2 Operating Conditions 16
2.2.1 Hydraulic Retention Time 18
2.2.2 Solids Retention Time 19
2.2.3 Dissolved Oxygen Concentration 24
2.2.4 Mixed Liquor Suspended Solids (MLSS) Concentration 28
2.2.5 Oxygen Requirement and Oxygen Uptake Rate 30
2.2.6 Food-to-Microorganism Ratio 32
2.3 Fate of COD 34
2.3.1 Assessment of COD Fractions 35
2.3.2 Bio-flocculation 36
2.3.3 Extracellular Polymeric Substances 40
2.3.4 Surface Properties and Divalent Cation Bridging 46
2.3.5 Adsorption 51
2.3.6 Storage 53
2.3.7 Oxidation/Mineralization 53
2.4 Present Study 54

CHAPTER 3 55
3.1 A-stage Pilot Configuration 55
3.2 System Controls 58
3.3 Analytical Methods 60
3.4 Data Analysis 63
CHAPTER 4 64
4.1 A-Stage Control 64
4.2 Overall System Performance 72
4.3 EPS Production 76
CHAPTER 5 80
5.1 Influence of SRT, DO and Influent Wastewater Characteristics on Overall System Performance 80
5.2 Influence of SRT, DO and Influent Wastewater Characteristics on EPS Production 82
5.3 Influence of EPS Production on Overall System Performance 85
CHAPTER 6 88
REFERENCES 91
APPENDIX: I 99
VITA 104

NOMENCLATURE

A/B	Adsorption/Bio-Oxidation process
ABAC	Aeration Based Ammonia Control
ANAMMOX	Anaerobic Ammonia Oxidizing Bacteria
ANOVA	Analysis of Variance
AOB	Ammonia Oxidizing Bacteria
APHA	American Public Health Association
ASE	Adsorption Stage Effluent
ASM	Activated Sludge Model
AvN	Ammonia vs NO_x-N
BMP	Biochemical Methane Potential
BNR	Biological Nutrient Removal
BOD	Biochemical Oxygen Demand
C/N	Carbon to Nitrogen Ratio
CAS	Conventional Activated Sludge
C_C	Pearson Product Moment Correlation Coefficient
cCOD	Colloidal Chemical Oxygen Demand
COD	Chemical Oxygen Demand
CST	Capillary Suction Time
DO	Dissolved Oxygen
DS	Dry Solids
EPS	Extracellular Polymeric Substances
G	Velocity Gradient (s^{-1})
F/M	Food to Microorganism Ratio
GC/MS	Gas Chromatography and Mass Spectrometry
ffCOD	Flocculated and Filtered Chemical Oxygen Demand
HWM	High Molecular Weight
HMW_{cCOD}	High Molecular Weight Colloidal Chemical Oxygen Demand
HRAS	High Rate Activated Sludge
HRT	Hydraulic Retention Time
k_h	Hydrolysis Rate Coefficient
K_{DO}	DO Half Saturation Coefficient
LB-EPS	Loosely Bound Extracellular Polymeric Substances
LB/TB	Loosely Bound to Tightly Bound Extracellular Polymeric Substances Ratio
MBR	Membrane Bioreactor
MCRT	Mean Cell Residence Time
MLE	Modified Ludzack-Ettinger Process
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
MOV	Mechanically Operated Valve
NOB	Nitrite Oxidizing Bacteria
OP	Orthophosphate
OTE	Oxygen Transfer Efficiency

OTR	Oxygen Transfer Rate
OUR	Oxygen Uptake Rate
PAO	Phosphate Accumulating Organism
PBS	Phosphate Buffering Solution
PCE	Primary Clarifier Effluent
pCOD	Particulate Chemical Oxygen Demand
PID	Proportional-Integral-Derivative Controls
PLC	Programmable Logic Controller
Pn	Proteins
Ps	Polysaccharides
Pn/Ps	Protein to Polysaccharide Ratio
Q	Flow Rate
Q_e	Effluent Flow Rate
Q_w	Wasting Flow Rate
RAS	Return Activated Sludge
rbCOD	Readily Biodegradable Chemical Oxygen Demand
sbCOD	Slowly Biodegradable Chemical Oxygen Demand
S-EPS	Soluble Extracellular Polymeric Substances
SLPM	Standard Liters Per Minute
SLR	Solids Loading Rate
SMP	Soluble Microbial Products
SOUR	Specific Oxygen Uptake Rate
SRT	Solids Retention Time
SS	Suspended Solids
SVI	Sludge Volume Index
TB-EPS	Tightly Bound EPS
tCOD	Total Chemical Oxygen Demand
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TSS	Total Suspended Solids
TSS_{sd}	Settleometer Decant Total Suspended Solids
V	Volume
VFD	Variable Frequency Drive
VLR	Volumetric Loading Rate
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge
WWT	Wastewater Treatment
WWTP	Wastewater Treatment Plant
X_a	Biomass Concentration in the Aeration Basin
X_e	Biomass Concentration in the Effluent
X_R	Biomass Concentration in the Return Activated Sludge

LIST OF TABLES

Table 2.1: Operating conditions and performance of CAS, HRAS and A-stage HRAS processes 17

Table 3.1: Average influent wastewater characteristics of the A-stage HRAS pilot..... 61

Table 4.1: Average A-stage HRAS operating conditions 71

LIST OF FIGURES

Figure 2.2: A-stage process schematic..... 20
Figure 3.1: A/B pilot process schematic located at the Chesapeake Elizabeth WWTP 57
Figure 3.2: MLSS-based control and Cascade DO control schematic 60
Figure 4.1: 24-hour average SRT values from 02/23/2016 to 04/21/2016 67
Figure 4.2: Daily measured MLSS concentrations from 02/23/2016 to 04/21/2016..... 68
Figure 4.3: 24-hour average DO concentrations from 02/23/2016 to 04/21/2016..... 69
Figure 4.4: 24-hour average airflow rates (SLPM) from 02/23/2016 to 04/21/2016 70
Figure 4.1: Average performance and standard deviations of the A-stage HRAS pilot.. 73
Figure 4.2: Fractions of COD captured in the WAS, lost in the effluent and lost to mineralization 75
Figure 4.3: Average concentrations of EPS components and fractions per operating condition 77
Figure 4.4: Average total EPS concentrations, Pn/Ps ratios and LB/TB ratios..... 79

LIST OF EQUATIONS

Equation 1 18
Equation 2 20
Equation 3 20
Equation 4 30
Equation 5 32
Equation 6 33
Equation 7 37
Equation 8 52
Equation 9 52

CHAPTER 1

INTRODUCTION

Activated sludge has been considered one of the core wastewater treatment processes (Jones and Shuler, 2010; Krzeminski et al., 2012) due to its technical simplicity, low cost and high removal efficiencies of pollutants (Zhang et al., 2014). On the other hand, in 2011, United States municipal wastewater treatment plants consumed approximately 0.8% of the nation's electricity (30.2 billion kWh) with over 50% attributed to aeration alone (WRF and EPRI, 2013). Therefore, reducing aeration requirements as well as increasing energy recovery potential from wastewater is a highly desirable practice.

The adsorption/bio-oxidation (A/B) process is a two-stage process that concentrates on the removal of organic matter, measured as chemical oxygen demand (COD), in the adsorption stage (A-stage) and nutrient removal in the bio-oxidation stage (B-stage) in a very small footprint (Böhnke et al., 1998). The A-stage was developed as a cost-effective biological buffer at wastewater treatment plants (WWTP) receiving high-strength (organic matter) industrial waste without completely removing COD which can be used as an internal carbon source for downstream denitrification (Böhnke and Diering, 1980). The A-stage is highly loaded with a food to microorganism ratio (F/M) of 2 to 10 gBOD/g VSS-day, short hydraulic retention time (HRT) of 30-60 minutes and short solids retention time (SRT) of 3-12 hours (based on aeration tank solids inventory only) (Böhnke, 1997b; Miller et al., 2014). As the name suggests, the primary mechanism of COD removal is by enmeshment and adsorption of particulate and colloidal matter into the activated sludge floc matrix, known as bio-flocculation. Since the

primary COD removal mechanism is associated with bio-flocculation instead of oxidation, the aeration demand of the A-stage is significantly reduced resulting in a low oxygen requirement (0.2 kg O_2/COD removed)(Jetten et al., 1997), limited carbon loss via mineralization (10-20% of total carbon removed)(Böhnke, 1997b; Haider et al., 2003) and 57-68% less volume required for aeration compared to a single-stage process (Muller-Rechberger et al., 2001). By utilizing bio-flocculation as the primary COD removal mechanism, a higher energy recovery potential can be achieved by concentrating the organic matter, known as carbon capture, into the waste activated sludge (WAS) which can be redirected to an energy recovery process, such as anaerobic digestion.

The A-stage has been observed as bio-flocculation limited by low colloidal COD (cCOD) removal efficiency, which may potentially be due to lack of extracellular polymeric substances (EPS) production (Jimenez et al., 2015). Without bio-flocculation, colloidal COD (cCOD) would not be incorporated into the activated sludge flocs resulting in a lower energy recovery potential. In general, EPS are primarily negatively charged substances which constitute anywhere from 50-80% of the organic fraction in activated sludge (Dignac et al., 1998; Wilén et al., 2003a) and serve as a microbial aggregate, structural backbone of the floc, and survival mechanism for bacteria against turbulent conditions, dehydration, nutrient deficiency and toxic substances (Lispidou and Rittmann, 2002). The fractions of EPS are categorized as soluble or bound EPS. Bound EPS is further characterized as loosely bound EPS (LB-EPS), or slime layer, and tightly bound EPS (TB-EPS), or capsular layer (Liao et al., 2001; Lispidou and Rittmann, 2002; Li and Yang, 2007; Sheng et al., 2010). Soluble EPS (S-EPS) can be considered as the equivalent of soluble microbial products (SMP) since both S-EPS and SMP are organic compounds produced

by microorganisms through substrate utilization (cell growth) or associated with biomass (cell lysis) (Laspidou and Rittman, 2002). Since the retention times of the A-stage process are very short, it is likely that a fraction of the S-EPS (SMP) found in the A-stage effluent is associated with the raw wastewater as bacteria in the collection system may produce EPS prior to reaching the treatment process. Noting that the diversity of microbial communities in activated sludge varies depending on the type of influent wastewater, geographical location, plant configuration, operating conditions and seasonality (Wilén et al., 2003b; Huang et al., 2010), the composition of EPS, surface properties of the floc and interactions with divalent cations are site specific. Furthermore, the microbial communities will likely vary at a given treatment plant, especially in temperate climate zones where seasons are drastically different throughout the year.

The specific influence of EPS production on bio-flocculation is contradictory as previous studies indicate that bio-flocculation and EPS concentration are positively (Ehlers and Turner, 2001; Urbain et al., 1993), negatively (Goodwin and Forster, 1985; Liao et al., 2001) or not correlated (Chao and Keinath, 1979). Jimenez et al (2007) operated a pilot-scale process with SRTs of 1, 2, 3, 5, and 10 days and found that increasing the SRT up to 3 days resulted in increased EPS production and effluent quality and remained relatively stable thereafter. Moreover, the same authors noted that operating at an SRT <2 days had a tremendous influence on bio-flocculation (TSS and cCOD removal) in which bio-flocculation became more limited as the SRT decreased. Li and Yang (2007) noted that increasing the SRT from 5 to 20 days resulted in increased settleability, bio-flocculation and dewaterability which were attributed to a decrease in LB-EPS. Operating a pilot-scale A-stage process, Jimenez et al. (2015) found that the removal

efficiencies of cCOD and particulate COD (pCOD), classified by particle size ranging from 0.001-0.45 microns and > 0.45 microns, respectively (Jimenez et al., 2005), showed similar trends to EPS production suggesting that bio-flocculation of pCOD and cCOD (carbon capture) was likely enhanced by EPS production. The same authors concluded that a DO concentration of 1 mg/L was required to maximize bio-flocculation and increasing the SRT from 0.3 to 1.0 days (DO = 1 mg/L) resulted in increased EPS production, pCOD removal and cCOD removal from approximately 50 to 105 mgCOD/gVSS, 30 to 65%, and 22 to 50%, respectively.

Liao et al. (2001) suggested that the concentrations of EPS are less important in understanding bio-flocculation compared to the components and surface properties of the EPS. The components of EPS include proteins, polysaccharides, humics, uronic acids and cellular material (DNA) with approximately 70-80% of extracellular organic carbon associated with proteins and polysaccharides (Dignac et al., 1998). Surface charge plays a role in bio-flocculation due to repulsive electrostatic forces (Liao et al., 2002) where highly negative charged flocs are weakly bound with high quantities of dispersed particles (Morgan et al., 1990; Daffonchio et al., 1995; Liu and Fang, 2002; Neyens et al., 2004; Xie et al., 2010). The key component of EPS contributing to the net negative charge varies throughout the literature as different authors report that negatively charged polysaccharides (Bruus et al., 1992), uronic acids (Forster and Dallas-Newton, 1980) and proteins (Sutherland, 1977; Frolund et al., 1995; Neyens et al., 2004) are responsible for binding with divalent cations. Increasing divalent cation concentrations increased the bound protein content, bio-flocculation, floc strength, resistance to shear and decreased bound water content but did not influence extracellular polysaccharides (Higgins and Novak, 1997). Bio-flocculation can be enhanced by divalent cations due to bridging between

the divalent cations and negatively charged particles (Higgins and Novak, 1997) or a decreased zeta potential and double layer compression resulting in decreased electrostatic repulsive forces (Liao et al., 2002).

More hydrophobic flocs, associated with hydrophobic amino acids on proteins (Jorand et al., 1998), contain less bound water and produce a higher degree of adhesion to the sludge flocs with lower effluent turbidity indicating better bio-flocculation, but showed no correlation with settleability (Zita and Hermansson, 1997a, 1997b; Liao et al., 2001). Since polysaccharides have been shown to have minimal influence on bio-flocculation, relatively high concentrations of polysaccharides and/or low concentrations of proteins may limit the bio-flocculation process. This may be attributed to non-beneficial polysaccharides occupying a large volume of the EPS matrix, thus, limiting the influence of proteins that would enhance bio-flocculation via interparticle forces. Therefore, it is reasonable to consider proteins and/or the protein to polysaccharide (Pn/Ps) ratio as key parameters for bio-flocculation and subsequent carbon capture. Although substantial work has been done to characterize EPS and its influence on system performance, EPS analysis has been done primarily on conventional activated sludge (CAS) systems with very limited literature on high-rate activated sludge (HRAS).

Since the A-stage process is operated at such a low SRT (<1 day), the performance of the A-stage is not typical of other activated sludge process. This is attributed to the fact that the SRT is shorter than typical diurnal variations in organic loading which results in variable MLSS and COD removal efficiency throughout a 24-hour period. Although the A-stage process was developed in the 1980's, there is relatively limited literature on how operating conditions of the

A-stage influence carbon capture. Therefore, the primary objective of the present study was to develop a better understanding of how operating conditions (SRT and DO) and process control strategies (MLSS-based control) influence the floc characteristics and carbon capture in a pilot-scale A-stage HRAS process. The MLSS-based control strategy was used to minimize daily variations in COD removal efficiencies in response to diurnal variations in organic loading by automatically adjusting the waste rate to maintain a MLSS concentration set-point of 3,000 mg/L. This control strategy differs from maintaining a constant waste rate, as to achieve a target SRT, which has been common practice at treatment plants that are operated at substantially longer SRTs than the A-stage process. The A-stage pilot was operated with constant HRTs of 30 and 60 minutes which corresponded to different SRTs of 0.26 $\pm$ 0.05 days and 0.56 $\pm$ 0.11 days, respectively. The influence of DO concentration on carbon capture in the A-stage was evaluated by applying constant DO concentrations of 0.5, 1.0 and 1.5 mg/L to both HRT/SRT conditions. Additional objectives of the A-stage pilot study were to determine 1) how operating conditions influence EPS production and 2) how EPS production influences carbon capture.

CHAPTER 2

LITERATURE REVIEW

2.1 Activated Sludge Processes

The primary objective of wastewater treatment (WWT) is the removal of pollutants that negatively affect ambient bodies of water receiving treated wastewater. In domestic wastewater, influent contaminants include organic matter measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorus (TP), ammonia, total suspended solids (TSS), volatile suspended solids (VSS), metals and synthetic organic compounds. The removal of these contaminants can be achieved by aggregating bacteria into larger colonies, referred to as activated sludge flocs, with a provided hydraulic retention time (HRT). Activated sludge has been considered one of the core treatment processes for purification of wastewater (Jones and Shuler, 2010; Krzeminski et al., 2012) due to its technical simplicity, low cost and high removal efficiencies of pollutants (Zhang et al., 2014). Activated sludge flocs that carry out the treatment process are formed in activated sludge processes through the collection of particles held together by different kinds of interparticle forces such as bridging by divalent cations, extracellular polymeric substances (EPS) and hydrophobic interactions (Urbain et al., 1993; Higgins and Novak, 1997; Sobeck and Higgins, 2002). These interparticle forces will be discussed in detail in following sections of this thesis. Once formed, these flocs enmesh particulate and colloidal compounds present in the wastewater resulting in the rapid removal of unbiodegradable and biodegradable particulate matter without significant mineralization depending on the mean cell residence time (MCRT) also known as the solids retention time (SRT).

Conventional activated sludge (CAS) is a process that was developed by Arden and Lockett in 1914 (Arden and Lockett, 1914) as a single-stage process primarily for nitrification. Typical CAS processes are operated at HRTs of 4-9 hours, SRTs of 3-15 days, food-to-microorganism ratio (F/M) of 0.2-0.4 $kgBOD_5$/kgMLSS-day (Tchobanoglous et al., 2003) and dissolved oxygen (DO) concentrations greater than 2 mg/L to ensure fast degradation of organics and complete nitrification (Wang et al., 2007).

By retaining the bacteria in the aeration basin, the CAS process could achieve near complete oxidation of COD via aeration, reducing the spatial area required for treatment, also known as the footprint. Typical operating parameters and the performance of CAS systems found in the literature is elaborated on in Section 2.2 of the literature review. With strict effluent limits of suspended solids and organic matter imposed by the United States Environmental Protection Agency (USEPA), operation of CAS systems would not meet these limits with additional aeration costs and land requirements. Therefore, a number of activated sludge processes and configurations have been developed to improve COD removal efficiency and reduce the required aeration volume for treatment, thus, reducing the overall footprint and operational costs of treatment facilities.

Modifications to the CAS process can be attributed to the difference in heterotrophic and nitrifying bacteria maximum specific growth rates of 6 d^{-1} and 0.2 to 1.0 d^{-1}, respectively. Due to the higher growth rates of heterotrophs, treatment processes focusing solely on COD and solids removal can operate with much shorter retention times (HRT and SRT) resulting in a reduced footprint with relatively high COD and TSS removal efficiencies. This process has been

termed as high-rate activated sludge (HRAS). However, operational parameters of the CAS process changed again when the need for enhanced nitrogen removal was brought forward in the 1980's and 1990's to combat the impact of eutrophication (Siegrest et al., 2008). To accommodate the lower specific growth rate of nitrifiers, the SRT needed to be increased (Salem et al., 2005). In addition to inducing denitrification for the removal on nitrogen, portions of the CAS system became anoxic and sometimes anaerobic zones resulting in biological phosphorus removal via polyphosphate accumulating organisms (PAO) (Olofsson et al., 1998; Henze et al., 2000).

Single-stage activated sludge processes were modified into a two-stage activated sludge process with the aim of reducing operational costs while maintaining efficient contaminant removal. This was achieved by selectively retaining heterotrophs in the first stage using HRAS, resulting in rapid carbon removal with less aeration demand and treatment volume. With the majority of COD removed in the first stage, the second stage can be operated to focus on biological nutrient removal (BNR). A two-stage treatment plant configuration is ideal for treating industrial wastewaters with high organic concentrations and chemical compounds inhibitory towards BNR, and processes where energy efficiency is desired. This thesis will only concentrate on the HRAS process.

2.1.1 High Rate Activated Sludge (HRAS) process

In order to reduce the footprint and meet effluent requirements, HRAS processes were developed by reducing the HRT to 1-3 hours and SRT to 1-4 days depending on temperature,

thus increasing the F/M to 1.5-2.0 kgBOD_5/kgMLSS-day (van Loosdrecht and Heijnen, 2002). The dissolved oxygen concentration is typically greater than 2 mg/L in order to ensure rapid removal of carbonaceous matter (Kher, 1960) via oxidation of soluble substrate and assimilation of particulate substrate which is removed by wasting. These operating parameters promote the retention of bacteria with increased growth and substrate utilization rates by selectively wasting microorganisms with slower growth rates, resulting in efficient solids and organic matter removal within a smaller footprint. The conventional HRAS process has a typical oxygen requirement of 0.6 kg O_2/kg COD removed (Jetten et al., 1997) making it a relatively affordable method of removing particulate (pCOD) and soluble COD (sCOD) from wastewater. Effluent limits of 30 mgBOD_5/L and 30 mgTSS/L are achieved when applying the HRAS process. It should be noted that the conventional HRAS process is designed for plants that do not have nitrogen limits allowing for a concentration on organic matter and suspended solids (SS) removal. A summary of typical operating conditions and system performance of conventional HRAS systems from the literature is elaborated on in Section 2.2 of this literature review.

If a HRAS process that maximizes carbon removal is upstream of certain BNR systems in a two-stage process, supplemental carbon addition can be necessary for denitrification leading to increased operational costs. Innovative nitrogen removal systems such as ammonia versus nitrite and nitrate (AvN) and side-stream anaerobic ammonia oxidation (ANAMMOX) for nitrogen polishing have been developed to address the need for organic carbon resulting in a 40% reduction in organic carbon demand (Regmi et al., 2014). However, it should be noted that BNR systems operated with aeration strategies such as ammonia-based aeration control (ABAC)

and DO control require a carbon to nitrogen (C/N) ratio >10 because heterotrophs in aerated zones consume COD limiting the availability of COD for denitrification (Sadowski, 2015).

Although two-stage processes require smaller footprint for treatment, the addition of intermediate clarifiers results in an increased overall footprint. To address the issue of increased footprint in a two-stage process, as well as carbon limitation in the BNR process operated under ABAC and DO control, the conventional HRAS two-stage operational parameters were modified to develop what has been termed the adsorption/biological oxidation (A/B) process. The A/B process will be further discussed in the following sections.

2.1.2 A/B process

The adsorption/bio-oxidation process, also known as the A/B process, was developed in Europe in the 1970's. It is a two-stage process that takes advantage of biological and physical removal mechanisms of the conventional HRAS process to optimize COD (A-stage) and nutrient (B-stage) removal in a very small footprint (Böhnke et al., 1998). Compared to older conventional two-stage processes, the A/B process requires much less or no supplemental carbon for nitrogen removal, less oxygen, and 34% less land space than a typical single sludge plant (Böhnke, 1983). By decreasing the required footprint, the A/B process can be retrofitted into existing infrastructure resulting in an increased throughput capacity of 20-50% significantly reducing construction costs without increasing the footprint of the plant (Schulze-Rettmer and Zuckut, 1998).

2.1.2.1 A-stage

The A-stage was developed as a cost-effective biological buffer at wastewater treatment plants (WWTP) receiving high strength (organic matter) industrial waste without completely removing COD which can be used as an internal carbon source for downstream denitrification. (Böhnke and Diering, 1980). As the name suggests, the primary mechanism of COD removal is by enmeshment and adsorption of particulate and colloidal matter into the activated sludge floc matrix, known as bio-flocculation. As bio-flocculation increases, the size of flocs increase and become denser, thus, promoting increased solid-liquid separation. As the flocs reach the sludge blanket in the clarifier, they compress into thicker sludge resulting in concentrated solids and organic matter per unit volume (mg/L) which can be removed from the system by wasting, known as waste activated sludge (WAS).

Since the A-stage was never designed to completely remove organic carbon, it has the potential to control removal performance by manipulating operating parameters in order to meet specific carbon removal criteria based on the downstream goals. The influence of A-stage effluent COD fractions on the downstream BNR process will be addressed in the following section. A summary of operating parameters and performance of A-stage HRAS in the literature is elaborated on in the following section (2.2). Unlike the conventional HRAS process, the A-stage HRAS is highly loaded with a F/M of 2 to 10 gBOD/g VSS-day, short HRT (30 minutes) and short SRT (3-12 hours), based on aeration tank solids inventory only) (Böhnke, 1997b; Miller et al., 2014). It was reported by Muller-Rechberger et al. (2001) that the required specific aeration volume could be reduced by 57-68% compared to a single-stage process. Therefore, the A-stage process can be described as treatment intensification in which required

aeration volume is decreased and operated with higher mixed liquor suspended solids (MLSS) concentrations (Boon and Thomas, 1998).

The A-stage functions to attenuate fluctuations of influent characteristics to provide a stable B-stage influent quality needed for the downstream BNR process. Böhnke et al. (1997a) showed that as the influent COD loading increased from 600 to 1,200 mg/L, the COD removal efficiencies in the A-stage increased from 45 to 65% resulting in more stable organic loading to the B-stage. This may be attributed to the fast reproduction (generation) rates of bacteria, often less than 30 minutes, likely resulting in rapid mutations in response to changes in loading characteristics (Böhnke et al.,1997a). Furthermore, Schulze-Rettmer and Zuckut (1998) found that the A-stage is capable of decomposing complex molecules resulting in the generation of short chain molecules that can be metabolized easier in the proceeding BNR process.

Another benefit of the A-stage is attributed to the primary COD removal mechanism being bio-flocculation instead of aeration (Jetten et al., 1997) resulting in less aeration necessary for treatment. The typical oxygen requirement of the A-stage is 0.2 kg O_2/COD removed which is significantly lower compared to 0.6 kg O_2/COD in conventional HRAS processes. Therefore, DO concentrations can be maintained at <1 mg/L resulting in limited carbon loss via mineralization which is typically responsible for 10-20% of the total carbon removed (Böhnke, 1997b; Haider et al., 2003; Miller et al., 2014). This coincides with data reported by Khiewwijit et al. (2015) showing that the A/B process would reduce CO_2 emissions from 0.43 to 0.28 kg-CO2/m^3 of wastewater treated when compared to a CAS system, thus, making the A/B process a more environmentally friendly process. Moreover, utilizing the kinetics of bio-flocculation results in

more COD being captured in the WAS that can be redirected to an energy recovery system such as anaerobic digesters for biogas production. Owen (1982) found that municipal wastewater with an influent concentration of 400-500 mgCOD/L contains a potential chemical energy of 1.5-1.9 kWh/m^3 of wastewater treated. This is significantly higher than typical energy consumption of an A/B and CAS process operated at 20°C that uses 0.23 and 0.37 kWh/m^3 of wastewater treated, respectively (Kheiwwijit et al., 2015). It should be noted that their study only incorporated energy consumed for aerating the biological treatment process and heating for anaerobic digestion. The decreased energy consumption of the A/B process was attributed to reduced aeration requirements. In addition to lower energy consumption, Kheiwwijit et al. (2015) reported a higher methane yield increasing from 24% to 34% and a net energy production for A-stage HRAS (0.24 kWh/m^3 of wastewater) compared to CAS (-0.08 kWh/m^3 of wastewater). Organic matter concentrated in the activated sludge is converted to biogas which is made up of approximately 60-70% methane (Mottet et al., 2010).

Operating sequencing batch reactors (SBR) with SRTs in the range of 7-11 days (CAS process), Martins et al. (2003) observed that limited DO concentrations (<1.1 mg/L) resulted in deteriorating settleability with the sludge volume index (SVI) reaching greater than 250 mL/g. Average SVI values of 85 $\pm$ 26 mL/g have been reported in an A-stage pilot study (Miller, 2015) which was similar to the range of 38 to 93 mL/g reported by Bohnke (1994) who evaluated the performance of ten full-scale A/B plants in Germany and the Strass A/B plant in Austria.

In summary, the A-stage is a promising wastewater treatment process as it efficiently removes organic matter in a small footprint with low aeration requirements, provides carbon for

downstream BNR, increases the energy recovery potential and produces sludge with good settling characteristics.

2.1.2.2 B-stage

The biological oxidation stage (B-stage) of the A/B process is operated as a BNR process focused on two-step nitrogen removal through nitrification and denitrification. To accommodate the slower growth rate of nitrifying organisms, the SRT in the B-stage is operated between 8-20 days with a F/M less than 0.1 gBOD/gVSS-day (Böhnke et al., 1997a; Böhnke et al., 1998).

The denitrification step requires organic carbon (COD) for the conversion of nitrate and/or nitrite to nitrogen gas that is released to the atmosphere. The B-stage can be operated as a shortcut nitrogen removal system to utilize the internal carbon provided from the A-stage effluent as well as decrease aeration requirements. Shortcut nitrogen removal can result in an effluent TN concentration < 5 mg/L and BOD_5 < 30mg/L which are the typical discharge limits established in the United States. If internal carbon is needed for denitrification in the B-stage, carbon capture in the A-stage can be managed and redirected to the B-stage to provide an optimal C/N ratio of 8-12 mgCOD/mgN depending on the downstream BNR operating conditions (Böhnke et al., 1997b; Miller, 2015; Sadowski, 2015).

If the BNR process is intermittently aerated with no designated anoxic zone, such as AvN, a higher pCOD fraction in the A-stage effluent is desired as the pCOD adsorbed to the flocs can persist during aeration and be hydrolyzed to readily biodegradable COD (rbCOD) when transitioned to an anoxic state resulting in denitrification occurring in all reactors during anoxic

cycles (Miller, 2015). Sadowski (2015) evaluated the influence of primary clarifier effluent (PCE) and A-stage effluent (ASE) on the nitrogen removal performance of B-stage configurations and aeration strategies. The two configurations consisted of AvN and Modified Ludzack-Ettinger (MLE), where the first tank in series (four total) in the MLE configuration was designated as an anoxic zone with the remaining tanks continuously aerated with DO control and ABAC. PCE was beneficial for both aeration strategies in a MLE configuration since rbCOD could be rapidly utilized for denitrification in the anoxic zone but resulted in excess carbon loaded to the system (Sadowski, 2015). Excess carbon resulted in heterotrophic competition for substrate and space leading to decreased nitrification activity and subsequent increase in SRT or HRT (Miller, 2015; Sadowski, 2015). Therefore, excess carbon should be removed prior to the BNR process.

2.2 Operating Conditions

There are numerous variations of activated sludge processes used to remove contaminants from wastewater in which a specific process can be selected based on the type of influent wastewater, available footprint and the effluent quality standards implemented in that specific region. The differences between these processes can be distinguished by the specific parameters in which the treatment plant is operated under; also known as the operating conditions. The operating conditions and their influence on wastewater treatment performance of interest in the present study include HRT, SRT, DO concentration, MLSS concentration, oxygen requirement, specific oxygen uptake rate (SOUR), and the F/M. Typical operating conditions of the CAS, conventional HRAS and A-stage HRAS processes are summarized in Table 2.1 and will be expanded on in the following sections.

Table 2.1: Operating conditions and performance of CAS, HRAS and A-stage HRAS processes

Parameter	Unit	CAS			Conventional HRAS			A-stage HRAS		
	Reference	Sawyer, 2015	Zielinska et al., 2012	Gonzalez -Martinez et al., 2016	Sawyer, 2015	Kehr, 1960	Emde et al., 1982	Böhnke, 1997; 1998	Shulze-Rettmer and Zuckut, 1998	Miller, 2015
HRT	Hours	> 5		15 - 35	2-4			0.5		0.5
SRT	Days		15 - 50	14 - 27			1-3	0.1 - 0.5	0.1 - 0.5	< 1.0
DO	mg/L		0.5 - 1.5	1.0 - 2.5		>2				< 1.5
MLSS	g/L	2.0	2.9 - 3.5	3.7 – 5.0		3 - 6	2 - 3			2 - 3
Aeration volume	$m^3/kgBOD_5$ removed				37-60			21-41	26-44	
Oxygen Requirement	$kgO_2/kgCOD$ removed						0.6 - 0.7			0.2-0.3
SOUR	mgO_2/ gVSS-h		3.6-4.7							25-200
BOD Loading	$kgBOD_5/d/m^3$	< 0.56			1.0 - 1.6	3.2 - 4.5	2.2 – 4.0			8.3 - 14.1
F/M	kgBOD/ kgMLSS-d	< 0.5				0.7-2.7			2-10	2-10
BOD removal	%	> 90		96-99	63-78	70 - 90	85	50 - 80	85	39 - 65
tCOD removal	%		80-90			50 - 70	77		48	35 - 61
TSS removal	%				70.5-72.2					41 - 69
SVI	mL/g					42 - 80	120	40 - 80	50	60 - 110

2.2.1 Hydraulic Retention Time

The average time that it takes for wastewater entering a system (influent) to reach the discharge (effluent) is referred to as the HRT. Assuming that the flow is constant throughout the system, HRT (hours) is approximated by the working volume of the reactors (m^3) divided by the influent flow rate (m^3/hour), as shown in Eq. (1).

$$\mathbf{HRT} = \frac{\mathbf{V}}{\mathbf{Q}} \qquad \text{Eq. 1}$$

Using a membrane bioreactor (MBR), Rodriguez et al. (2013) noted that increasing the HRT from 12 to 18 hours resulted in a slightly higher alpha factor which represents the relationship of oxygen transfer between clean and processed water. Higher alpha factor values indicate better oxygen transfer from the bulk liquid to microorganisms resulting in a lower aeration demand needed for cell growth (substrate utilization). However, Rodriguez et al. (2013) noted that the difference in alpha factor values at the two applied HRTs was not statistically significant (Multiple Linear Regression Analysis; $p = 0.124$). Therefore, increasing the HRT will result in increased volume of treatment without enhancing the oxygen transfer for substrate utilization leading to an overall increased aeration demand and subsequent operating cost to remove organic matter.

Jimenez et al. (2015) observed the impacts of increasing the HRT from 5 to 60 minutes while holding the SRT and DO concentrations constant at 1 day and 1.0 mg/L, respectively. Increasing the HRT from 5 to 20 minutes resulted in a rapid increase in EPS production from approximately

10 mgCOD/gVSS to 150 mgCOD/gVSS as the sCOD removal increased from approximately 50% to 88% but both variables remained relatively stable as the HRT further increased. pCOD removal was rapid at low HRT achieving removal efficiencies of approximately 25% and 75% at HRTs of 5 and 30 minutes, respectively, with effluent concentrations <30 mg/L in 10 minutes. Maximized colloidal COD (cCOD) removal required a longer HRT than sCOD and pCOD in which cCOD removals of approximately 18%, 55% and 70% were achieved at HRTs of 5, 30 and 45 minutes, respectively. This observation supported their theory that pCOD and cCOD removal is neither instantaneous nor complete at such a short HRT (< 60 minutes), which are common assumptions in typical activated sludge models (ASM) (Jimenez et al., 2015). Furthermore, findings from Jimenez et al. (2015) suggest that operating at a 30-minute HRT in the A-stage process is effective for targeting COD removal but is bio-flocculation limited in terms of cCOD removal efficiencies.

2.2.2 Solids Retention Time

The SRT represents the average duration that the activated sludge biomass is retained in the treatment process. Likewise, the aerobic SRT refers to how long the biomass is retained in the aeration basin and neglects biological reactions that occur in the anaerobic and/or anoxic zones of the clarifier. Reactions occurring in the clarifier could have a significant impact on processes such as dewatering and digestion, however, for the purpose of the present study; the following information will only cover aerobic SRT. If the WAS is wasted from the sludge return line, the aerobic SRT is calculated based on the aeration basin volume (V = m^3), MLSS concentration (X_a = g/m^3) in the aeration basin, wasting flow rate (Q_w= m^3/d), return activated sludge (RAS)

concentration (X_R = g/m^3), effluent flow rate (Q_e = m^3/d) and effluent concentration (X_e = g/m^3), as shown in Eq. (2).

$$\mathbf{SRT} = \frac{\mathbf{VX}}{\mathbf{Q_w X_R + Q_e X_e}} \qquad \text{Eq. (2)}$$

A schematic of an A-stage process showing the different parameters used for the calculation of SRT is shown in Figure (2.2).

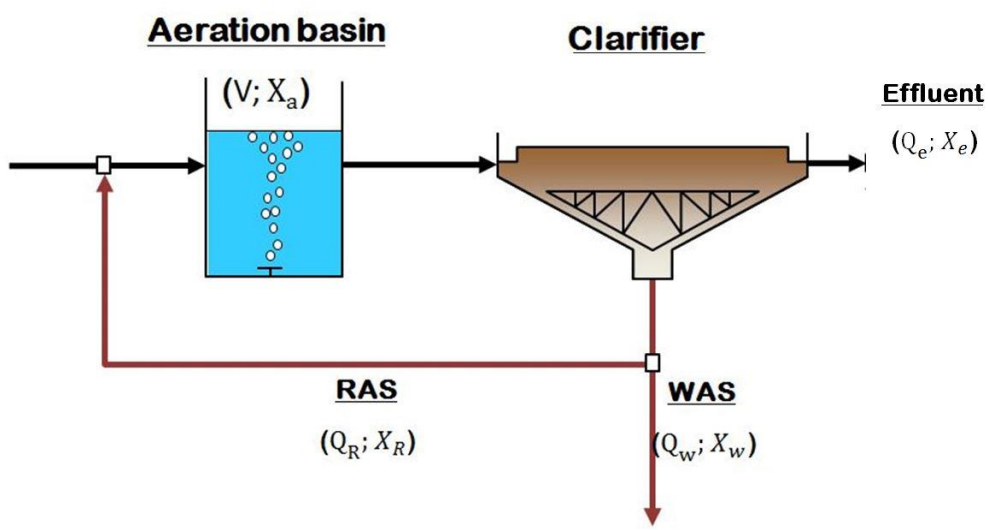

Figure 2.2: A schematic of an A-stage process outlining the different parameters used for the calculation of SRT.

Assuming that the effluent solids concentration is negligible in relation to the MLSS and RAS concentrations, the equation is simplified to Eq. (3).

$$\mathbf{SRT} = \frac{\mathbf{VX}}{\mathbf{Q_w X_R}} \qquad \mathbf{Eq.}\ (\mathbf{3})$$

The SRT is of great importance to an activated sludge system because it can influence the kinetics of bacteria, removal efficiencies and settling characteristics of the activated sludge. Operating at very low SRTs (<1 day) selectively retains the fastest growing microorganisms (bacteria) and removes more complex organisms from the system that exert an oxygen demand

without beneficial COD removal (Böhnke et al., 1997a). This was supported by Haider et al. (2003) in which the mean growth rates of heterotrophic bacteria were observed to be in the range of the maximum growth rates resulting in the removal of slower growing heterotrophic organisms depending on the applied SRT.

Heterotrophic organisms responsible for carbon removal require oxygen to convert COD into biomass and EPS while simultaneously oxidizing existing cellular material to produce energy for cell growth, known as endogenous respiration (Walker, 1971). Selectively retaining bacteria with high metabolic rates by decreasing the SRT resulted in a higher specific oxygen uptake rate (SOUR) by the retained microorganisms and subsequent maximum growth rates (substrate utilization) (Orthon et al., 2009; Frienrich et al., 2015). Therefore, lowering the aerobic SRT resulted in lower oxygen demand and energy requirements for aeration with increased organic matter converted into biomass that can be sent to an energy recovery system (McCarty et al., 2011). Moreover, the ratio of cell debris to active biomass increased from 0.41 to 2.0 when the SRT increased from 5 to 20 days, respectively, indicating a higher aeration demand with longer SRTs (Liu and Wang, 2015).

Using bench-scale SBR's operated at SRT's of 5, 10 and 20 days and MLSS concentration maintained at 2,000 mg/L, Li and Yang (2007) noted that decreasing the SRT resulted in decreased settleability and bio-flocculation indicated by higher SVI values and effluent suspended solids (ESS) concentrations, respectively. It should be noted that the SVI ranged from 32.4 $\pm$ 2.1 to 51 $\pm$ 3.6 which are indicative of good settling sludge regardless of the SRT

within the range of their study. A similar study conducted by Xie and Yang (2009) found that increasing the SRT from 5 to 10 days resulted in decreased loosely bound EPS (LB-EPS) concentrations from approximately 5 to 2 mgTOC/gMLSS (total organic carbon; TOC), tightly bound EPS (TB-EPS) from 41 to 30 mgTOC/gMLSS and SVI values from 100 to 75 mL/g. When the system was changed back to a 5-day SRT, a return in deteriorated settleability (SVI = 181 mL/g), increased LB-EPS to 6 mgTOC/gMLSS and TB-EPS to 40 mgTOC/gMLSS occurred (Xie and Yang, 2009). It should be noted that both studies (Li and Yang, 2007; Xie and Yang, 2009) related the deteriorated performance at a 5 day SRT to higher quantities of LB-EPS whereas increased TB-EPS concentrations had minimal influence. Both studies operated outside the typical SRT range of the A-stage HRAS process (<1 day), therefore, results may vary when operated at a very low SRT.

Chao and Keinath (1979) noted that non-filamentous bulking occurred between a 2-day and 5-day SRT as well as lower than 1.9 days but good settling occurred past 5 days and around 2 days. Operating an A-stage pilot (SRT < 1 day), Miller (2015) reported an average SVI of 85 $\pm$ 26 mL/g (n=414) with less than 10 sample days (1 data point per day) reaching SVI values greater than 150 mL/g. Furthermore, COD removal was positively correlated with SRT until reaching a maximum COD removal between 70-80% (Miller, 2015) which was achieved between a 0.3-day SRT. Based on similar studies conducted on HRAS processes (Ge et al., 2013; Jimenez et al., 2015), it is likely that the COD removal efficiency would increase to 85-95% if the SRT increased past a one-day SRT. Miller (2015) further suggested that COD removal becomes a function of hydrolysis at SRTs between 0.5-1 days since adsorption of pCOD and cCOD was maximized but

limited by the number of available adsorption sites that would be created via hydrolysis in a system operating under a longer SRT.

Jimenez et al. (2015) noted that operating at a lower SRT (0.1 days) resulted in a low oxygen requirement compared to operating at a higher SRT (2 days) with values of 0.23 +/- 0.08 and 0.52 +/- 0.13 kg O2/kgCOD removed, respectively. Furthermore, SRT was positively correlated with mineralization (calculated as the difference between the influent COD concentration and the COD concentration found in the WAS and effluent, where 67%, 37% and 14% of the total COD being mineralized at SRTs of 2, 0.5 and 0.1 days, respectively. Using lab-scale MBRs operated with a 0.7 hour HRT and SRT varying from 0.125 to 5 days, Faust et al. (2014b) found that the extent of bio-flocculation, measured as the suspended COD (pCOD and cCOD) in the concentrate, increased from 59% to 98% at SRTs of 0.125 and 5 days, respectively. The same authors further noted increased mineralization from 1% to 32% as the SRT increased from 0.125 to 5 days, respectively, leading to an optimal range for bio-flocculation and energy recovery in the range of 0.5 to 1 day SRT (Faust et al., 2014b).

Operating SBRs with synthetic wastewater at SRTs in the range of 5 to 20 days and DO concentrations ranging from 0.4 to 4 mg/L, Liu and Wang (2015) showed that mixed liquor volatile suspended solids (MLVSS) concentration increased with SRT for all DO concentrations. Conversely, their study showed that the endogenous OUR remained stable under oxygen limitation (<0.5 mg/L) and increased with DO = 4 mg/L but at a slower rate than the increase of MLVSS. This indicates that there are increased quantities of cell debris at higher SRTs and more

active biomass at lower SRTs regardless of DO concentration (Liu and Wang, 2015). Therefore, lowering the SRT of a HRAS process resulted in an increased sludge yield and higher COD content on the WAS which suggests that less hydrolysis of pCOD and cCOD as well as the storage of sCOD occurs at lower SRT operations (Jimenez et al., 2015). The rate of hydrolysis is a slow process with a hydrolysis rate coefficient (k_h) of 0.013 d^{-1} (Liu and Wang, 2015), indicating that operating at a low SRT will not provide enough time for significant hydrolysis to occur. Limiting the amount of hydrolysis resulted in increased COD content captured in the WAS and energy recovery potential when sent to an anaerobic digester. Using the A/B process, Meerburg et al. (2015) showed that operating at a SRT of 0.41 day resulted in a specific methane yield of 484 $mLCH_4$/gTSS which was significantly higher than the 389 $mLCH_4$/gTSS they observed when operated at 1.31 day SRT.

Therefore, the A-stage HRAS process can be considered as a cost effective means for carbon removal by lowering the aeration demand and increasing the energy recovery potential by diverting COD captured in the WAS to an anaerobic digester.

2.2.3 Dissolved Oxygen Concentration

The dissolved oxygen (DO) concentration represents the mass of oxygen (mg) present in a volume of liquid (L). Oxygen is an electron acceptor utilized by heterotrophic bacteria, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) to consume organic matter (COD) for cell growth and maintenance.

In 2011, United States municipal wastewater treatment facilities consumed approximately 0.8% of the nation's electricity (30.2 billion kWh) with over 50% attributed to aeration alone (WRF and EPRI, 2013). Supplying DO at a rate greater than the demand by the microorganisms provides no advantage to biochemical oxidation (Boon and Thomas, 1998) leading to increased operational expenditures with no further increase in contaminant removal. The rate limiting DO concentration for carbonaceous oxidation and nitrification are approximately 0.5 and 1.5 mg/L, respectively (Boon and Thomas, 1998). Therefore, efficient COD removal might be achieved by the A-stage HRAS process operating with DO concentrations < 1 mg/L lowering the overall aeration requirements compared to conventional HRAS and CAS systems.

Martins et al. (2003b) evaluated the influence of DO concentration on settling characteristics by operating SBRs with a total cycle time of 4 hours, SRT of 10 days, aerobic feed times between 3 and 15 minutes and different DO concentrations of >2.5 mg/L and < 1.1 mg/L. Their results showed that DO concentrations < 1.1 mg/L resulted in deteriorating settleability with SVI values greater than 250 mL/g compared to less than 100 mL/g with DO concentrations >2.5 mg/L. This was attributed to oxygen limitation producing porous and irregularly shaped flocs with finger-like filamentous structures whereas operating without oxygen limitation produced firm, round, and compact flocs with very few filaments (Martins et al., 2003b). Similar results were reported by Wilén and Balmér (1998) operating a pilot study at a 5-day SRT and varying the DO concentration from 0.5 to 2 mg/L. Therefore, operating with low DO and high SRT (CAS) could be detrimental to clarifier performance but the settling characteristics could vary for very low SRT (<1 day) systems. Over a period of 600 days of operating an A-stage pilot study, no

correlation was found between DO concentration and SVI values although sludge bulking was observed at a DO < 0.1 mg/L (Miller, 2015). The bulk DO concentration likely did not negatively impact the system since heterotrophs have a very low DO half saturation coefficient (k_{DO} < 0.05) (Miller, 2015). Good settling sludge was promoted by a plug-flow configuration (Miller, 2015) compared to typical complete-mix HRAS processes which are known to have poor settling characteristics when operated with a low SRT or high F/M (Stewart, 1964; Bisogni and Lawrence, 1971; Chao and Keinath, 1979).

Operating a pilot-scale process with a constant SRT (1 day) and varying the DO concentration, Jimenez et al. (2015) reported that a DO concentration of 1 mg/L was needed to maximize bio-flocculation and subsequent pCOD and cCOD removal whereas a sCOD removal was controlled at a much lower DO concentration of approximately 0.1 mg/L. Therefore, the DO set-point in the A-stage process can be adjusted to remove specific COD fractions based on the downstream BNR operating conditions as previously discussed (section 2.1.2.2).

The A-stage HRAS process is typically operated with a DO concentration near zero promoting the growth of bacteria that are capable of breaking down complex chemical pollutants (Shulze-Rettmer et al., 1998). Low DO concentrations not only reduce oxygen input but also enhance the driving force for oxygen mass transfer by maintaining a large oxygen deficit which results in high oxygen transfer efficiency (OTE) (Lee et al., 2015; Liu and Wang, 2015). Moreover, only 10-20% of the COD removed in the A-stage is attributed to oxidation (Böhnke, 1997b; Haider et al., 2003; Miller et al., 2014) resulting in minimal aeration requirements. Increasing the DO

concentration from 0.5 to 1.5 mg/L resulted in increased COD oxidation (Zielinska et al., 2012) with less COD being captured in the WAS. Liu and Wang (2015) investigated the influence of DO concentration on the degradation of cell debris by operating SBRs with a 12-hour HRT, SRTs ranging from 5 to 40 days, and DO concentrations from 0.4 to 4 mg/L using synthetic wastewater without additional TSS. Their study found that long-term low DO concentrations (< 2 mg/L) significantly inhibited the heterotrophs hydrolysis rate of cell debris (k_h = 2.1) but did not impact the heterotrophs endogenous decay rate ($k_{d,H}$ approximately 0). This resulted in increased carbon capture and active biomass production which has been shown to reduce aeration demand and increased energy recovery potential (McCarty et al., 2011; Ge et al., 2013; Liu and Wang, 2015). Faust et al. (2014a) operated 2 lab-scale MBRs and found that EPS production increased from 122 to 175 mgEPS/gVSS when operated at DO concentrations of 1.0 and 4.0 mg/L, respectively. This translated into bio-flocculation efficiencies (quantified as cCOD removed) of 65% and 91%, respectively, (Faust et al., 2014a) which contradicts findings from Li and Yang (2007) and Xie and Yang (2009) noting that increased EPS concentrations resulted in deteriorated bioflocculation. These conflicting reports may be a result of extracting EPS from different processes in which the EPS concentrations and components were likely different. Increased EPS production and subsequent bio-flocculation can be attributed to increased sCOD uptake (Lispidou and Rittmann, 2002; Jimenez et al., 2015) or higher DO concentrations producing more turbulence in which bacteria excrete more EPS to resist floc shearing (Sheng et al., 2010).

2.2.4 Mixed Liquor Suspended Solids (MLSS) Concentration

The total mass of solids (mg) present in a completely mixed liquid (L) is referred to as the MLSS and is typically expressed as total suspended solids (TSS) per liter (mgTSS/L). The concentration of MLSS in a wastewater treatment process is critical because it represents the quantity of active bacteria, cellular debris, influent TSS and influent inert VSS present in the system for subsequent removal of contaminants.

Increasing the MLSS concentration resulted in decreasing the alpha factor and subsequent oxygen transfer (Rodriquez et al, 2013) but was not found to be significantly different (Multiple Linear Regression Analysis, $p = 0.09$). Zhang et al. (2014) investigated the influence of MLSS concentration on the specific adsorption capacity of activated sludge by operating SBRs with HRTs ranging from 5 to 30 minutes, MLSS concentrations ranging from 2,250 - 4,500 mg/L and a constant substrate concentration of 600 mgCOD/L. Their study showed that the specific adsorption capacity of organic matter was steady while MLSS increased from 2,250 to 2,570 mg/L but decreased from approximately 0.17 to 0.105 mgCOD/mgMLSS as the MLSS concentration increased from 2,570 to 4,500 mg/L, respectively. It is likely that the adsorption sites at lower MLSS concentrations were fully saturated but the specific adsorption capacity declined when MLSS was increased because there was no additional substrate to be adsorbed.

Similar results were found by Miller (2015) who noted that COD removal increased from 20 to 60% as the MLSS increased from 500 to 3,000 mg/L but did not increase when the MLSS concentration exceeded 3,000 mg/L. It is likely that the A-stage was not limited by biomass

concentration but more so associated with a lack of EPS production and available adsorption sites at low SRT (<0.5 day) based on previous findings from Jimenez et al. (2007). EPS possess high concentrations of extracellular enzymes that hydrolyze particulate and colloidal organic matter within the floc matrix but at a slower rate than adsorption (Frolund et al., 1995). Therefore, the increase in COD removal from 20-60% observed by Miller (2015) was associated with readily biodegradable substrate consumption whereas COD removal in the absence of rbCOD was dependent on EPS production and subsequent hydrolysis of pCOD and cCOD, resulting in slower COD removal.

Mikkelsen and Keiding (2002b) found that the ratio of dispersed particles to total mass of activated sludge increased as the MLSS concentration increased from 0-4 g/L which negatively impacted dewaterability measured as increased capillary suction time (CST). Increased sludge dispersion was thought to be associated with surface shear as the floc network structures develop (Mikkelsen and Keiding, 2002b). This conflicts with Parker et al. (1970) suggesting that higher MLSS concentrations increase the collision frequency between particles, thus promoting flocculation. It is possible that there was an optimal MLSS concentration that promoted bio-flocculation, and increasing past that optimal concentration resulted in increased dispersion.

Based on the fore mentioned studies, it can be suggested that increasing the MLSS concentration up to approximately 3,000 mg/L would benefit bio-flocculation without significantly inhibiting the oxygen transfer rates, resulting in optimal MLSS concentrations for carbon capture.

2.2.5 Oxygen Requirement and Oxygen Uptake Rate

The amount of oxygen that is required to remove organic matter is referred to as the oxygen requirement (kgO_2/kgCOD removed) whereas the oxygen uptake rate (OUR) represents the rate at which the oxygen is being utilized by bacteria per unit time and unit volume. In the majority of activated sludge models (ASM), parameter sets used for growth kinetics are obtained from systems with a SRT in the range of 3 to 20 days (Henze et al., 1987; Gujer et al., 1999). In these models, the maximum specific OUR ($1.17 \frac{mgO_2}{mg\ X_{OHO} * d}$) of ordinary heterotrophic organisms (OHO) refers to the maximum OUR (OUR_m) performed by a specific concentration of OHOs (X_{OHO}) and is dependent on the OHO yield (Y_{OHO}), maximum growth rate ($\mu_{m,OHO}$), decay rate (b_{OHO}) and endogenous respiration residue fraction (f_U) (McKinney, 1960) as shown in Eq. (4).

$$\mathbf{\frac{OUR_m}{X_{OHO}} = \frac{1 - Y_{OHO}}{Y_{OHO}} * \mu_{m,OHO} + (1 - f_u) * b_{OHO}} \qquad \mathbf{Eq.\,(4)}$$

This calculation was based on using default values (WRC, 1984) of OHOs ($Y_{OHO} = 0.67$ gCOD/gCOD, $\mu_{m,OHO} = 2d^{-1}$, $b_{OHO} = 0.24\ d^{-1}$, and $f_U = 0.2$).

There are several concerns associated with using this method in ASMs including the use of constant growth rates and neglecting physiological adaption of microorganisms over a period of time (Friedrich et al., 2015). In systems with a very low SRT (1 day), ASMs predict 80% active biomass fraction compared to < 20% with an SRT of 50 days with each system differing in composition of constituents but having the same physiological properties (μ_m and OUR_m) which is unlikely in activated sludge (Friedrich et al., 2015). The variability of growth rates was confirmed by altering constants in a calibration exercise by Orhon et al. (2009) who reported

that low SRT systems had a higher maximum growth rate than high SRT systems when calibrating ASM1 and ASM3. To elucidate physiological adaption of X_{OHO}, Friedrich et al. (2015) obtained the endogenous decay rate (b_e) from the endogenous respiration rate (OUR_e) and compared the results to OUR_m and subsequent μ_m. Their results showed that endogenous respiration better represented the OHO biomass than maximum respiration due to their association with degradable organic matter. The decrease in OUR_e with decreasing b_e over a period of time represented the decrease in biomass whereas the decrease in OUR_m with decreasing b_m reflected a decrease in growth potential. Friedrich et al. (2015) further noted that the b_e was smaller than b_m for highly loaded systems whereas low loaded systems had similar decay rates which can be attributed to bacteria in highly loaded systems utilizing internal substrate in the absence of external substrate, thus reducing their growth potential faster.

Witzig et al. (2002) proposed that the value for OUR is equivalent to the overall metabolic activity of the activated sludge community and the oxygen requirement depends on the rate at which sewage is treated (Boon and Thomas, 1998). Therefore, a smaller tank volume containing higher MLSS concentrations will have a higher OUR per unit volume of the aeration tank (Boon and Thomas, 1998) resulting in efficient substrate utilization with decreased aeration and footprint. This was supported by Haider et al (2003) operating SBRs with a SRT ranging from 0.4 to 1.0 days and found that as sludge age increased, the ratio of initial to maximum OUR decreased and that the absolute values for OUR increased with sludge age as the MLSS concentration increased from 1.5 to 3.0 g/L. Operating a pilot-scale A-stage, Miller

(2015) noted that increasing the SRT resulted in increased OUR until a maximum OUR of 150-200 mgO_2/L-hr was reached. The OUR in full scale plants typically peak around 150 mgO_2/L-hr which could explain why A-stage processes can only achieve 70-80% COD removal (Miller, 2015). According to Zielinska et al. (2012), the OUR is dependent on the composition of influent wastewater and showed that addition of acetate (organic carbon) increased the OUR at a DO concentration of 0.5mg/L compared to an influent containing only inorganic carbon which predictably remained at zero.

2.2.6 Food-to-Microorganism Ratio

The amount of organic matter (BOD or COD) that is loaded to a WWTP operating with a certain sludge concentration (MLSS) per day is known as the food-to-microorganism ratio (F/M) (kgBOD/kgMLSS-day). The F/M is calculated by dividing the product of influent flow rate (Q; m^3/d) and substrate concentration (S_o; kg/m^3) by the concentration of biomass (X; kg/m^3) occupying a specific volume of treatment (V; m^3) which is shown in Eq. (5).

$$\mathbf{F/M} = \frac{\mathbf{Q*S_o}}{\mathbf{X*V}} \qquad \text{Eq. (5)}$$

Typical F/M's for CAS, conventional HRAS and A-stage HRAS are 0.2-0.4 kgBOD/kgMLSS-day, 1.5-2.0 $kgBOD_5$/kgMLSS-day and 2.0-10 kgBOD/kgMLVSS-day, respectively (Böhnke, 1997b; van Loosdrecht and Heijnen, 2002; Miller et al., 2014). Bacteria in highly loaded systems, such as the A-stage HRAS process, base their survival on maximizing their growth rates (substrate utilization rate) at the expense of increasing their decay rate, whereas bacteria in low loaded

systems reduce their decay rate at the expense of lower maximum growth rates (Friedrich et al., 2015). The specific adsorption capacity (q_c; mgCOD/mgMLSS), refers to how much organic matter is adsorbed to the surface of the flocs in a specific concentration of biomass. The difference between the total substrate found in the influent (S_o; mg/L) and effluent (S; mg/L) divided by the biomass concentration (X; g/L) reflects the specific adsorption capacity of activated sludge as shown in Eq. (6).

$$\mathbf{q_c} = \frac{\mathbf{S_o - S}}{\mathbf{X}} \qquad \text{Eq. (6)}$$

As seen in Eq. (7), q_c is inversely correlated with the MLSS concentration but directly related to substrate concentration and has been confirmed through experimental studies (Zhang et al., 2014). Zhang et al. (2014) further noted that the specific adsorption capacity is linearly correlated with the F/M. Assuming that the influent substrate and biomass concentration in a given volume are relatively stable, increasing the influent flow rate (i.e. increased F/M) would result in a higher specific adsorption capacity with more carbon being captured in the WAS and diverted to an energy recovery system.

Van Dierdonck et al. (2012) varied the F/M using SBRs operated at a 20 day SRT and MLSS concentration of 4.5 g/L and found that operating at a low F/M (0.024 kgBOD/kgMLSS-day), activated sludge flocs were fragmented (broken down into smaller flocs) resulting in a significant increase in effluent suspended solids (ESS) when compared to operating at a high F/M (0.24 kgBOD/kgMLSS-day). However, both conditions obtained similar SVI values

(approximately 52 mL/g). Based on their results, increasing the F/M promotes better bio-flocculation with less suspended solids reaching the effluent. Martins et al. (2003a) evaluated the influence of an aerobic selector by operating 2L SBRs at a total cycle time of 4 hours, DO concentration > 2mg/L and SRT of 10 days with aerobic feed times ranging from 3 to 90 minutes. Their study found that decreasing the substrate gradient (increased aerobic feed time) had a strong negative impact on settleability. Again, these operating conditions (Martins et al., 2003a; Van Dierdonk et al., 2012) were not representative of a HRAS process and the influence of F/M on floc structure and solids removal with lower retention times (higher F/M) could vary from these results.

2.3 Fate of COD

Microorganisms can remove a wide range of contaminants from wastewater resulting in biological treatment being the most widely used and cost-effective methods for wastewater treatment. To effectively remove contaminants, the retention of microorganisms in the treatment process is required. This can be accomplished by keeping the biomass in suspension (i.e., activated sludge) or providing additional surfaces for the biomass to adhere to known as attached growth. For the purpose of the present study, the focus of the following sections is on suspended growth systems. Organic matter (carbon) present in the wastewater is commonly measured as BOD and COD in which COD is a more useful measure when attempting to make correlations among substrate, biomass and DO in terms of electron equivalence (Orhon et al., 1997). COD represents the amount of oxygen required to oxidize organic matter into carbon dioxide and water (McCarty et al., 2011) and can be removed from wastewater by several

different mechanisms including bio-flocculation, adsorption, intracellular storage and oxidation. However, COD can overestimate the biodegradable fraction of organic matter as is also measures oxidizable constituents such as ammonia and ammonium.

2.3.1 Assessment of COD Fractions

In order to determine the fate of COD removal, it is necessary to analyze the fractions of COD present in the wastewater. The total COD (tCOD) present in wastewater consist of slowly biodegradable COD (sbCOD) and readily biodegradable COD (rbCOD) as well as other oxidizable constituents as previously mentioned. sbCOD is characterized as particulate COD (pCOD) which in turn is defined as the sum of suspended solids (SS) and colloidal organic matter (cCOD). For the purpose of this paper, pCOD refers to the SS fraction and cCOD is the truly colloidal fraction which is too small to settle by gravitational forces alone. The rbCOD fraction consists of soluble COD (sCOD) and is considered as the truly soluble organic matter (Jimenez et al., 2005). The colloidal fraction can further be divided into two fractions, as defined by Jimenez et al (2005), consisting of high molecular weight cCOD (HMW_{cCOD}) and low molecular weight cCOD (LMW_{cCOD}) categorized by the size of particles ranging from 0.45-0.01 and 0.01-0.001 microns, respectively.

For the purpose of this paper, the mechanisms responsible for the removal of COD are characterized as carbon capture and carbon oxidation. Carbon capture refers to mechanisms in which COD is enmeshed, adsorbed or absorbed to the activated sludge (bio-flocculation, adsorption and storage) whereas carbon oxidation (mineralization) refers to COD that is

converted to CO_2 and lost to the system. By optimizing the mechanisms responsible for carbon capture in a HRAS process, increased quantities of COD can be redirected to an energy recovery system with the potential for WWTP's to become energy neutral or even energy positive.

2.3.2 Bio-flocculation

The aggregation of bacteria into biological flocs, known as bio-flocculation, has been associated with several interparticle forces such as extracellular polymeric substances (EPS), divalent cation bridging and hydrophobic interactions (Urbain et al., 1993; Higgins and Novak, 1997; Sobeck and Higgins, 2002). Not only do these interparticle forces act to aggregate bacteria into flocs, but also enmesh pCOD, cCOD and exoenzymes that are associated with hydrolysis (Laspidou and Rittman, 2002). In other words, bio-flocculation is responsible for the removal and conversion of biodegradable substrate into biomass and EPS which acts as the structural backbone of the activated sludge flocs (Jimenez et. al., 2005; La Motta et al., 2007; Miller, 2015).

One of the most commonly used methods to remove suspended matter from wastewater is by gravity settling due to its simplicity and low operational costs. Efficient primary sedimentation tanks can remove influent TSS, BOD and COD in the ranges of 50 to 70%, 25 to 40%, and 20 to 35%, respectively. On the other hand, primary sedimentation fails to remove cCOD and sCOD which could be used as a source for biogas production. If bio-flocculation does not occur in the activated sludge process, colloidal particles are not incorporated into the floc matrix via enmeshment (La Motta et al., 2007). Moreover, cCOD not captured in the WAS would result in

lower biogas production with more COD reaching the effluent. Therefore, bio-flocculation is a crucial mechanism for optimizing carbon capture and energy recovery from wastewater and should be included when modeling activated sludge systems for the most accurate results. The rate of bio-flocculation as defined by Jimenez et al. (2005) is shown in Eq. (7).

$$\boldsymbol{r = k(C - a) * X} \qquad \text{Eq. (7)}$$

As seen in Eq. (7), the flocculation rate (r) follows first-order rate kinetics with a flocculation constant (k), and depends on the concentration of particles in the supernatant after 30 minutes of settling (C; mg/L), residual concentration of particles (a; mg/L) which includes shearing effects, and the MLSS concentration (X; mg/L) of the reactor.

The removal of pCOD can be influenced by operating conditions such as HRT, SRT, F/M, DO concentration and mixing intensity (G^{-1}). Jimenez et al. (2005) evaluated the influence of DO on COD removal by operating two SBR's with DO concentrations of 0.02-0.05 mg/L and >1.5 mg/L with additional mixing applied (G = 40 s^{-1}) in both reactors to keep the sludge in suspension. Their results showed the cCOD concentration decreased from 50 mg/L at the start of the experiment to approximately 22 mg/L and 25 mg/L for the aerated and non-aerated reactors, respectively. Since DO concentration did not significantly influence cCOD removal, the only remaining parameter that would influence cCOD was attributed to continuous mixing. It should be noted that their study did not evaluate the influence of EPS which likely played a significant role on bio-flocculation. This may be attributed to mixing in the absence of DO kept

the sludge in suspension allowing the EPS of dispersed particles and flocs to interact. However, it was not well established whether the presence of DO (>1.5 mg/L) or potential increased mixing intensity provided by aeration was responsible for the slightly better COD removal efficiency in the aerated reactor as only the mixing intensity of the mechanical mixing device was calculated by Jimenez et al. (2005). Although there was only a slight increase in bio-flocculation under aerated conditions, Jimenez et al. (2015) suggested that a DO concentration of 1.0 mg/L is necessary in order to maximize bio-flocculation. The same authors noted that EPS production increased almost linearly with DO concentration from 0.1 to 2.0 mg/L suggesting the DO enhances substrate uptake (sCOD) and EPS production. However, pCOD and cCOD removal was maximized around 90% and 80%, respectively, with a DO concentration of 1.0 mg/L and EPS production of approximately 80 mgCOD/gVSS. Since the COD removal efficiencies did not increase with DO concentration or EPS production, it is likely that providing a DO concentration of 1.0 mg/L resulted in an optimal mixing intensity combined with increased EPS production for bio-flocculation. Jimenez et al. (2005) further noted that the residual concentration of HMW_{cCOD} (0.01-0.45 microns) was minimal with lower velocity gradient (G = 30 s^{-1}) and comparable to SS residual concentrations, whereas LMW_{cCOD} (0.001-0.01 microns) residual concentrations remained relatively stable from G = 10-75 s^{-1} but significantly increased at high G values (G = 100 s^{-1}). Therefore, highly turbulent environments can induce floc shearing resulting in a release of organic matter into solution and subsequent decreased effluent quality.

In the majority of systems operated with a SRT > 3 days, bio-flocculation of pCOD and cCOD is rapid and complete resulting in most activated sludge models (ASM) neglecting the influence of bio-flocculation kinetics on carbon removal (Miller, 2015). Modelling flocculation kinetics initially focused on floc break up and reflocculation to improve suspended solids removal in secondary clarifiers (Parker et al., 1971; Whalberg et al., 1994). La Motta et al. (2003a) expanded on this work and developed a model that incorporated EPS with a first-order rate expression for describing the removal of pCOD and cCOD in the aeration basin. Jimenez et al. (2005) evaluated the validity of this model in terms of pCOD and cCOD removal in a HRAS pilot and found that pCOD removal was rapid but not instantaneous and the removal of cCOD was slower than pCOD with removal rates similar to rbCOD. The model developed by La Motta et al. (2003a) is limited by the assumption that the ratio of EPS to MLSS is constant and adsorption sites are readily available. As previously mentioned, decreasing the SRT resulted in less adsorption capacity and availability of adsorption sites which has been postulated as a lack of EPS production at low SRT (Miller, 2015). Therefore this model may not accurately estimate carbon removal for the A-stage HRAS process. A non-steady state model developed by Laspidou and Rittman (2002) was based on the assumption that the EPS production rate is proportional to soluble substrate consumption but neglected the removal of pCOD and cCOD associated with EPS production and bio-flocculation. Most recently, Nogaj et al. (2013) developed a model in attempt to couple the production of EPS with pCOD and cCOD removal.

2.3.3 Extracellular Polymeric Substances

The influence of EPS is a relatively new aspect for evaluating the performance of biological wastewater treatment and has been gaining more attention in recent years. Although substantial work has been done in attempt to characterize EPS and its influence on system performance, the kinetics and mechanisms behind EPS production are not well understood with conflicting reports throughout the literature. EPS analysis has been done primarily on CAS systems with very limited literature on HRAS processes resulting in more informational gaps that need to be filled in order to fully understand the kinetics of EPS production and how it influences contaminant removal from wastewater. Moreover, there are many different methods to extract EPS from activated sludge that vary in extraction efficiency making comparisons between studies very difficult.

In general, EPS are primarily negatively charged, high molecular weight (HMW) substances that are incorporated in the floc matrix through active secretion from microorganisms, products of cell lysis, hydrolysis of macromolecules and adsorbed from the environment (Wingender et al., 1999). These substances serve as a microbial aggregate, structural backbone of the floc, and survival mechanism for bacteria against turbulent conditions, dehydration, nutrient deficiency and toxic substances (Lispidou and Rittmann, 2002).

EPS have been considered the third largest component in activated sludge, behind water and cells (Li and Ganczarczyk, 1990), which constitute anywhere from 50-80% of the organic fraction in activated sludge (Dignac et al., 1998; Wilén et al., 2003a). The fractions of EPS are

categorized as soluble or bound EPS. Bound EPS is further characterized as loosely bound EPS (LB-EPS), or slime layer, and tightly bound EPS (TB-EPS), or capsular layer (Li and Yang, 2007; Liao et al., 2001; Sheng et al., 2010; Lispidou and Rittmann, 2002). Soluble EPS (S-EPS) can be considered as the equivalent of soluble microbial products (SMP) since both S-EPS and SMP are organic compounds produced by microorganisms through either substrate utilization (cell growth) or associated with biomass (cell lysis) (Laspidou and Rittman, 2002). SMP are associated with the majority of COD that are not incorporated into the flocs, therefore, decreasing the effluent quality of the system (de Silva and Rittman, 2000). Concentrations of LB-EPS make up 10 to 20% of the total EPS and are found in significantly lower concentrations than TB-EPS but are more influential on the bio-flocculation and settling characteristics of the sludge (Li and Yang, 2007; Xie and Yang, 2009). It should be noted that both studies (Li and Yang, 2007; Xie and Yang, 2009) did not quantify the S-EPS fraction leading to TB-EPS making up approximately 80 to 90% of the total EPS.

The formation of EPS requires oxygen (electron acceptor), therefore if a significant fraction of the oxygen demand is directed to EPS production, growth rates will decline, and neglecting the kinetics of EPS production would lead to an overestimation of cell yield (Laspidou and Rittmann, 2002). However, the exact mechanisms responsible for EPS production are not well understood with conflicting reports throughout previous literature.

It has been proposed by Logan and Hunt (1988) that the only common condition inducing EPS production is microbial starvation and EPS production increased with increasing the SRT (Evans

et al., 1994; Sheng et al., 2010), suggesting increased EPS production during the endogenous growth phase. This conflicts with the model developed by Laspidou and Rittman (2003a) showing that EPS is produced in direct proportion to soluble substrate utilization. Furthermore, Liao et al. (2001) showed that large amounts of EPS were extracted at low SRTs (4 days) and the total EPS concentration was independent of SRT (ANOVA, $p > 0.05$) indicating that EPS production is not limited to the stationary and endogenous growth phases associated with longer SRTs (4-20 days). Ehlers and Turner (2011) noted that the F/M was negatively correlated with EPS production which was attributed to the fact that readily available substrate is rarely limited at higher F/M ratios, thus not fully inducing EPS production (Miller, 2015). Therefore, the high F/M (low SRT) of the A-stage HRAS process is potentially bio-flocculation limited due to low EPS production.

The influence of EPS production on bio-flocculation is contradictory as previous studies have reported that bio-flocculation and EPS concentration are positively (Urbain et al., 1993; Ehlers and Turner, 2001), negatively (Goodwin and Forster, 1985; Liao et al., 2001) or not correlated at all (Chao and Keinath, 1979). Differences in these studies may be attributed to differences in influent wastewater characteristics and/or to the operating conditions of the specific treatment plant ((Wilén et al., 2003b; Huang et al., 2010). Huang et al. (2010) analyzed the organic composition found in domestic wastewater (Shanghai, China) and reported 90 different organic compounds measured using Gas Chromatography and Mass Spectrometry (GC/MS) and noted that the composition of wastewater varies by different living habits throughout the world. Moreover, there are many various types of bacteria, each of which possess different metabolic

characteristics resulting in the growth and production of flocs with different densities and structures, as well as different quantities and compositions of EPS (Wilén et al., 2003b). To get a better understanding on how EPS influences bio-flocculation, settleability and dewaterability, operating conditions, such as the SRT and DO concentration, should be taken into consideration.

Bisogni and Lawrence (1971) showed that 13-35% dispersed growth occurred when operating completely mixed activated sludge reactors with SRTs in the range of 0.25-0.5 day, suggesting a lack of EPS and aggregation, whereas operating at an SRT >1 day resulted in < 5 % dispersed growth.. Using a pilot-scale HRAS process (HRT = 30 minutes; DO = 1 mg/L) Jimenez et al. (2015) showed a linear increase in EPS production from approximately 50 mgCOD/gVSS to 105 mgCOD/gVSS when the SRT increased from 0.3 to 1.0 days but remained constant after reaching a 2 day SRT at about 125 mg EPS/g VSS. The same author reported that cCOD and pCOD removal efficiencies had similar trends compared to EPS production and in which increasing the SRT from 0.3 to 1.0 days resulted in increased removal efficiencies of pCOD from 30 to 65% and cCOD from 22 to 50%, respectively. Li and Yang (2007) noted that increasing the SRT from 5 to 20 days resulted in decreased LB-EPS whereas the TB-EPS fraction remained relatively unchanged and coincided with increased settleability, bio-flocculation and dewaterability of activated sludge (Li and Yang, 2007). Liao et al. (2001) found no significant change in EPS concentration with SRT ranging from 4 to 20 days but showed that increasing SRT significantly lowered ESS and SVI values (all flocs settled well implying that the influence of SRT on SVI is of no practical interest). Morgan et al. (1990) did not differentiate between bound EPS

fractions but noted that increased total EPS resulted in decreased settling characteristics. Based on findings from Li and Yang (2007), deteriorating settleability with increasing total EPS can be associated with an increased concentration of LB-EPS. This has been attributed to structural differences between the bound EPS fractions in which LB-EPS are more filamentous and porous containing higher bound water content compared to TB-EPS (Yang and Li, 2009) resulting in decreased bio-flocculation and ability to compact in the clarifier. Based on these findings (Bisogni and Lawrence, 1971; Morgan et al., 1990; Liao et al., 2001; Li and Yang, 2007), operating at very low SRTs (<1 day) would likely contain a higher ratio of LB-EPS to TB-EPS resulting in poor bio-flocculation, settleability and dewaterability.

Operating a pilot-scale HRAS process at a constant SRT of 1 day, Jimenez et al. (2015) found that operating at DO concentrations of 0.5, 1.0 and 2.0 mg/L, the average EPS productions were 50 ± 18, 90 ± 10 and 120 ± 22 mgCOD/gVSS, respectively. Increasing the DO concentration from 0.5 to 1.0 mg/L resulted in increased pCOD removal from approximately 70 to 95% and cCOD removal from approximately 45 to 79%. The higher substrate removal efficiencies found by Jimenez et al. (2015) when operated at a higher DO concentration (1.0 mg/L) were likely attributed to increased mixing intensities with additional air supply as Jimenez et al. (2005) found similar cCOD removal efficiencies between SBRs operated under aerated and non-aerated conditions with supplemental mechanical mixing.

Liao et al. (2001) suggested that the concentrations of EPS fractions are less important in understanding bio-flocculation compared to the components and surface properties of the EPS. The surface properties of activated sludge will be covered in the following section. The

components of EPS include proteins, polysaccharides, humics, uronic acids and DNA. Approximately 70-80% of extracellular organic carbon associated with proteins and polysaccharides with more than 65% of the total organic carbon (TOC) associated with proteins (Dignac et al., 1998). The large presence of proteins in EPS can be associated with large quantities of exoenzymes produced by cells during substrate utilization (Frolund et al., 1995) which in turn are enmeshed within the EPS matrix. Noting that the composition of raw wastewater varies with geographical location and type of waste received (Huang et al., 2010) and the fact that WWTP's differ in treatment processes, it can be assumed that the components of EPS will differ depending on the influent wastewater characteristics and treatment process applied. Using SBRs with SRTs ranging from 4 to 20 days, Liao et al. (2001) showed that total EPS concentrations were not correlated with SRT but the concentration of proteins were significantly lower (ANOVA, $p<0.05$) and carbohydrate concentration significantly higher (ANOVA, $p<0.05$) when operating at SRTs of 4 and 9 days compared to SRTs >9 days. This could be related to the change in growth rates (substrate utilization) where carbon sources are not completely consumed at shorter SRTs (Liao et al., 2001). It should be noted that the 4-day and 9-day SRTs applied by Liao et al. (2001) were significantly higher than what would be seen when operating the A-stage process (SRT < 1 day), thus, conclusions from their study may not be applicable to such a high-rate process.

In summary, EPS production is very site specific as there have been many contradictory studies even on processes operating under similar conditions. Therefore, it is difficult to determine the

exact cause for the change in EPS production and its influence on system performance based on previous reports.

2.3.4 Surface Properties and Divalent Cation Bridging

Bio-flocculation can be influenced by the surface properties of the activated sludge flocs including surface charge and hydrophobicity, but the specific mechanisms behind these interparticle forces are not well understood with conflicting reports throughout the literature. In general, activated sludge flocs possess a net negative surface charge (attributed to EPS) that can bind with cations to enhance bio-flocculation. Since divalent cations carry two positive charged ions, they can bind to two separate negatively charged bacteria improving flocculation and floc stability compared to monovalent cations (+1) which can only occupy one negatively charged site. The influence of the monovalent to divalent cation (M/D) ratio was studied by Higgins and Novak (1997) and noted that as the M/D ratio increased >2, settling and dewatering characteristics deteriorated. The same authors further noted that when the addition of sodium (Na+) increased past 10 milliequivalent per liter (meq/L) bound polysaccharide and protein concentrations decreased by 30 and 60%, respectively. When sodium increased to >20 meq/L, the SVI could not be measured due to deflocculation and floatation problems in which the membrane functions of the bacteria were likely compromised leading to cell death. Therefore, it has been suggested that the ratio of monovalent to divalent cations (M/D) should be < 2 for good bio-flocculation, settleability and dewaterability of activated sludge (Böhnke, 1997; Higgins and Novak, 1997).

Both extracellular polysaccharides and proteins have a variety of functional groups that carry a positive, negative or even no charge, as well as vary in hydrophobic and hydrophilic nature. Furthermore, the diversity of microbial communities in activated sludge varies between treatment plants depending on the type of influent wastewater, geographical location, and seasonality (Wilén et al., 2003b; Huang et al., 2010). Therefore, the composition of activated sludge, EPS, surface properties of the floc and interactions with divalent cations are site specific making comparisons to other studies difficult with the possibility of conflicting results.

Surface charge plays a role in bio-flocculation due to repulsive electrostatic forces can prevent the aggregation of particles (Liao et al., 2002). Therefore, flocs with a highly negative surface charge are weakly bound with high quantities of dispersed particles (Morgan et al., 1990; Daffonchio et al., 1995; Liu and Fang, 2002; Neyens et al., 2004; Xie et al., 2010). It has been shown that increased quantities of dispersed particles not only decrease effluent quality but impair settleability and dewaterability of activated sludge (Mikkelsen and Keiding, 2002a; Neyens et al., 2004). As the surface charge becomes more neutral, repulsive electrostatic forces can be overcome by other interparticle forces such as divalent cation bridging, thus, enhancing bio-flocculation. As bio-flocculation improves, the floc size, density and structural integrity increase resulting in better settling and dewatering characteristics. This has been attributed to less bound water filling the void spaces in tightly bound flocs allowing increased interactions between particles and divalent cations, better compression in the clarifier, less water needed to be removed in the dewatering process and a reduction of fine particles that could clog the filter medium.

The key component of EPS contributing to the net negative charge is controversial as different authors report that negatively charged polysaccharides (Bruus et al., 1992), uronic acids (Forster and Dallas-Newton, 1980) and proteins (Sutherland, 1977; Frolund et al., 1995; Neyens et al., 2004) are responsible for binding with divalent cations. Increasing divalent cation concentrations has been shown to increase the bound protein content, bio-flocculation, floc strength, resistance to shear and decreased bound water content (Higgins and Novak, 1997). With high quantities of exoenzymes in the floc matrix, proteins are predominant in activated sludge and contain a relatively high content of negatively charged amino acids that could preferentially bind with divalent cations over polysaccharides (Sutherland, 1977; Frolund et al., 1995; Dignac et al., 1998; Neyens et al., 2004). Once bound with divalent cations, the negatively charged amino acids become neutralized and subsequently lower the repulsive electrostatic forces of the floc (Higgins and Novak, 1997; Huang et al., 1999). Therefore, it is reasonable to consider protein as the most influential component of EPS for bio-flocculation, floc structure and stability. This was further supported by Higgins and Novak (1997) who showed that removing proteins from the floc via protein degrading enzymes (pronase) resulted in deflocculation and subsequent deterioration of settleability and dewaterability whereas polysaccharide degrading enzymes had minimal impact. Mikkelsen and Keiding (2002a) found that increasing EPS concentrations resulted in larger flocs with low shear sensitivity but carried a higher zeta potential (surface charge). Since electrostatic repulsion increased with increased negative surface charge, findings from Mikkelsen and Keiding (2002a) suggests that forces other than surface charge alone govern bio-flocculation and floc structure. Other studies indicated

that increasing protein, polysaccharide and total EPS concentrations overwhelm electrostatic forces due to their polymeric nature (van Loodstredtch et al., 1990; Wilén et al., 2003a/b).

Shin et al. (2001) operated three SBRs with a 7 day SRT with each reactor receiving different airflow rates of 0.8, 2.0 and 4.0 L/min and found that increasing the airflow rate resulted in increased total EPS concentration and deteriorated settleability. However, Shin et al. (2001) did not monitor the subsequent DO concentration associated with the different airflow rates making it difficult to determine whether the increased airflow rate or higher DO concentrations were responsible for increased EPS production and deteriorated settleability. Increased airflow produces more turbulence in the system which has been found to be the main cause of erosion in flocculated suspensions (Mikkelsen and Keiding, 2002b). Shin et al. (2001) found that the SBR operated at 0.8 L/min had stable SVI values around 90 mL/g over a seven day period indicating good settling sludge whereas reactors operated at 2.0 and 4.0 L/min increased from 90 mL/g to 160 and 500 mL/g after four days, respectively, and 2.0 L/min further increased to >400 mL/g after 6 days of operation (Shin et al., 2001). The increase in total EPS was associated with increased polysaccharide concentrations whereas protein concentrations were stable under all three conditions (Shin et al., 2001). The same authors reported that the change in EPS component concentrations was attributed to the growth phase under elevated DO concentrations and that the ratio of polysaccharides to proteins during the settling phases (starvation) was smaller than during the growth phase.

The bound water content, whether chemical bound or entrapped in the floc matrix, influences bio-flocculation by increasing void space and subsequently limiting the available binding sites

for bio-flocculation. More hydrophobic activated sludge flocs contain less bound water and produce a higher degree of adhesion between sludge flocs with lower effluent turbidity indicating better bio-flocculation, but shows no correlation with settleability (Zita and Hermansson, 1997a, 1997b; Liao et al., 2001). It has been thought that hydrophobicity includes a mechanism mediated by extracellular proteins and is not associated with extracellular polysaccharides (Jorand et al., 1998). Xie et al. (2010) found that increasing batch culture time resulted in increased hydrophobicity of the sludge and suggested that more proteins are accumulated in the floc matrix as the production of EPS increases and/or polysaccharides are degraded once the available substrate is depleted (Shin et al., 2001). Since polysaccharides do not significantly influence the hydrophobicity of activated sludge (Jorand et al., 1998), a relatively higher polysaccharide content, and subsequent lower protein to polysaccharide ratio (Pn/Ps), may decrease the hydrophobic nature of the floc resulting in deteriorated bio-flocculation. This is in agreement with findings from Liao et al. (2001) who found that higher Pn/Ps ratios coincided with a higher hydrophobicity and less suspended solids found in the effluent. However, increased polysaccharide concentrations may still promote bio-flocculation as they are polymeric in nature (van Loodstredtch et al., 1990; Wilén et al., 2003a/b). Pn/Ps ratios have been found in the range of 1.6-1.9 for S-EPS and approximately 2.4 for bound EPS (Comte et al., 2006) but a review by Liu and Fang (2003) showed the ratio of Pn/Ps varied between 0.5-21.2 depending on the influent wastewater, applied treatment and method used to extract EPS. In general, higher Pn/Ps ratio values are an indication of contamination by intracellular materials lysed during extraction (Comte et al., 2006) which may result in misinterpreting the influence of EPS production on system performance.

As previously mentioned, most of these studies were conducted on CAS systems and the reported results may differ when applying HRAS operating conditions.

2.3.5 Adsorption

Adsorption is the process in which organic molecules are physically adsorbed to binding sites located in the floc matrix. Haider (2002) examined the uptake of sCOD (as flocculated and filtered COD according to (Mamais et al., 1993) after spiking cyanide to stop biological activity and found that soluble substrate did not physically adsorb to flocs. Therefore, the adsorption processes is associated with slowly-biodegradable COD (sbCOD), measured as pCOD and cCOD, but not sCOD. Since sbCOD is too large to be actively transported across the cell membrane, this fraction must first adsorb to the floc matrix and then be hydrolyzed to rbCOD before it can be utilized for cell growth (Jimenez et al., 2005; Ni and Yu, 2008). Not only does EPS act to enmesh (adsorb) sbCOD into the floc matrix but it also captures exoenzymes which are necessary for hydrolysis (Frølund et al., 1995; Morgenroth et al., 2002). Once adsorption sites of the floc become occupied, the bio-flocculation rate becomes limited by the rate of hydrolysis to make adsorption sites available again (Jimenez et al., 2005). The rate of hydrolysis refers to how quickly sbCOD is converted into rbCOD which is transported across the cell membrane for cell growth (substrate utilization) making hydrolysis the rate-limiting step in the bio-flocculation process. The change in concentration of rbCOD over time is calculated as the product of the hydrolysis rate (k_h; d^{-1}) and either the fraction of inert particulate matter in the influent (X_I; mgCOD/L) or inert particulate matter that is produced from endogenous decay (X_P; mgCOD/L) as shown in Eq. (8) and Eq. (9) (Lubello et al., 2009).

$$\frac{dX_I}{dt} = -k_h X_I \qquad \text{Eq. (8)}$$

$$\frac{dX_P}{dt} = -k_h X_P \qquad \text{Eq. (9)}$$

In the A/B process, the A-stage is termed the adsorption stage, yet Böhnke et al. (1998) reported that for an overall COD removal of 55%, only 10% of COD removed in the A-stage is attributed to adsorption. As previously mentioned, the low contribution of adsorption for COD removal may be attributed to a lack of EPS production under short SRTs (Miller, 2015). Furthermore, since rbCOD is rarely limited in the A-stage providing sufficient substrate for cell growth, it is reasonable to assume that hydrolysis of pCOD and cCOD would be limited resulting in occupied adsorption sites and subsequent decrease in removal associated with adsorption. pCOD particles that are large enough to be removed by sedimentation alone would not require additional EPS production whereas small pCOD and cCOD particles must be adsorbed to the floc matrix for subsequent removal. Therefore, the A-stage HRAS process reveals itself as being bio-flocculation limited based on lack of available adsorption sites for cCOD removal. When the SRT is increased (> 1.5 days), there are adequate quantities of EPS with available adsorption sites allowing adsorption to be rapid and complete within 15 to 20 minutes (Whalberg et al., 1994; Zhao et al., 2000; La Motta et al., 2004; Jimenez et al., 2007). It has been shown that the specific adsorption capacity decreases with increased MLSS concentrations but increases with F/M (Zhang et al., 2014). This suggests that the substrate was rapidly adsorbed until the flocs were saturated and increasing the MLSS concentration would require additional substrate (higher F/M) to maintain the same specific adsorption capacity due to low hydrolysis rates preventing adsorption of new substrate at such a low SRT.

2.3.6 Storage

While conducting aerobic digestion batch tests on the return activated sludge (RAS) from 7 full scale WWTPs, Friedrich and Takács (2013) observed a rapid decrease in the initial stage of aerobic digestion which varied from typical exponential decreases in respiration. These authors determined that the rapid respiratory decrease was attributed to the degradation of internally stored organic matter as the sludge that contained the highest quantity of stored and aerobically degraded substrate was taken from the only plant which successfully ran with excess biological phosphorus removal. Although stored organic matter only represents 2-5% of the degradable organic matter in activated sludge, it is responsible for up to 50% of the initial total OUR (Friedrich et al., 2015). Storage of substrate occurs when either uptake rates are higher than consumption rates or when there is substrate limitation such as nutrients or electron acceptors (Majone et al., 1999). Because the A-stage process is operated at such a low SRT (< 1 day), substrate is rarely a limiting factor, thus, storage does not likely play a key role on the removal of COD in the A-stage.

2.3.7 Oxidation/Mineralization

Mineralization of organic matter refers to the conversion of soluble substrate (sCOD) into biomass, EPS, water, carbon dioxide and other byproducts. Since the organic matter (carbon) is converted into a gas, COD removed via oxidation results in a loss of COD to the system that cannot be captured and diverted to an energy recovery process. Therefore, it is important to decrease the quantity of COD being oxidized in order to increase energy recovery potential. Mineralization in the A-stage has been estimated and found to attribute 12 to 20% of COD

removed (Bohnke, 1994; Muller-Rechberger et al., 2001; Haider, 2002) whereas increasing the SRT from 2 to 4 days resulting in increased mineralization from 25 to 50%, respectively (Ge et al., 2013). Low DO concentrations have been shown to increase the oxygen deficit and subsequent oxygen transfer efficiency as well as inhibit the hydrolysis of cell debris, thus increasing biomass production and carbon captured in the WAS that can be redirected to an energy recovery system (Liu and Wang, 2015). Therefore, the A-stage process operating under low SRT (<1 day) and DO (<1 mg/L) is suitable for minimizing the aeration demand and mineralization, as well as promoting carbon capture that can be redirected to an energy recovery system.

2.4 Present Study

Since there is limited research available on the A-stage process and EPS production in such a high-rate process (SRT < 1 day), the present study was developed to analyze the influence of various operating conditions (SRT and DO concentration) of the A-stage HRAS process on overall system performance (in terms of COD removal, bio-flocculation, carbon capture and settleability) as well as the influence on EPS production. EPS production, including the components and concentrations of EPS, was analyzed under each operating condition to determine any relationships between EPS production and overall system performance of the A-stage HRAS process. Based on previous findings discussed in the literature review, it can be expected that operating at a longer SRT would result higher EPS production leading to better bio-flocculation and carbon capture that can be redirected to an energy recovery process.

CHAPTER 3

MATERIALS AND METHODS

3.1 A-stage Pilot Configuration

The A-stage pilot was located at the Chesapeake Elizabeth wastewater treatment plant (Hampton Roads Sanitation District, Virginia Beach, Virginia) and consisted two identical configurations (trains) receiving the same influent wastewater. A schematic of the pilot study showing the A-stage train that was used upstream of the B-stage process is shown in Figure 3.1. The second A-stage train was identical to the schematic presented in Figure 3.1 with the only difference attributed to the effluent from the storage tank was discharged to the floor drain. The influent wastewater used in the present A-stage pilot study was pumped from the headwork building of the treatment plant after preliminary screening without primary clarification. Each train contained three completely mixed, vertical aeration tanks in series (total working volume = 0.51 m^3), an intermediate clarifier (working volume = 1.7 m^3) and effluent storage tank. The aerobic HRTs were constant at 30 and 60 minutes per train (in terms of aerobic contact time in the aeration basins only). With a total working volume of 2.21 m^3, the total HRTs are calculated as 130 minutes and 260 minutes for the 30 and 60 minute aerobic HRTs, respectively. Additional preliminary treatment was done prior to feeding the pilot-scale aeration tanks which included grit removal, screening (2-3 mm openings) and temperature adjustment to 20°C using submersible heaters (OEM OTS, Minneapolis, MN) or a water chiller (Aqualogic MT-9, San Diego, CA). The flow rates for the influent and return activated sludge (RAS) were flow-paced using progressive cavity pumps (Seepex BW5, Bottrop, Germany) with variable frequency drives (VFDs) and magnetic flow meters (Rosemount 8705, Houston, TX). A

digital, speed-controlled peristaltic pump (Masterflex L/S, Vernon Hills, IL) was used to remove waste activated sludge (WAS) from the underflow of the clarifier. An optical DO sensor (InsiteIG Model 10, Slidell, LA) was installed in the middle reactor in attempt to prevent low DO conditions in the first reactor. An infrared MLSS sensor (s::can soli::lyser, Vienna, Austria) was mounted in the last reactor. Compressed air supplied aeration to the reactors through a mechanically operated valve (MOV; v-notch ball valve) to fine-pore membrane disc diffusers (17.8 cm diameter) mounted on the bottom of each aeration tank. Airflow was monitored using a gas mass flow meter (Alicat M-Series, Tucson, AZ; standard temperature pressure = 25°C, 1 atm) placed on the compressed air line upstream of the MOV. Airflow was balanced in each reactor using separate needle valves downstream of the MOV in attempt to maintain the same DO concentration throughout all three reactors.

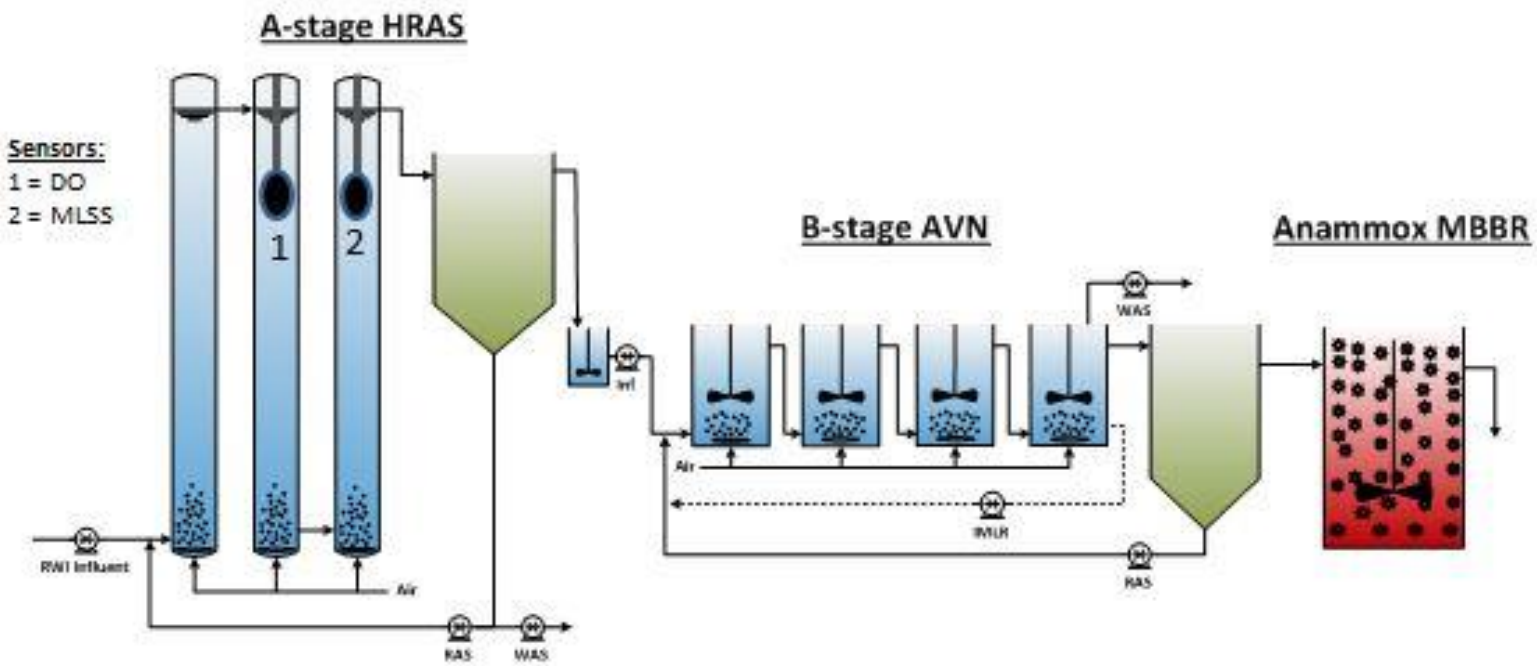

Figure 3.1: Schematic of the A/B pilot process located at the Chesapeake Elizabeth WWTP in Virginia Beach, Virginia.

3.2 System Controls

COD removal efficiency achieved by the A-stage process in response to organic loading is not typical of other activated sludge processes as bacteria are retained in the process for a shorter time frame than typical diurnal variations of the influent wastewater. Operating the A-stage with constant wasting rates, so as to achieve a target SRT, would result in variable COD removal efficiencies over the course of a diurnal loading cycle and subsequent variable effluent C/N ratios that can deteriorate the denitrification efficiency of the downstream BNR process. This can be attributed to the fact that COD removal efficiency is inversely related to the specific loading rate (SLR; kgCOD/kgMLSS-d)(Böhnke, 1977) and that operating at an SRT of < 1 day would result in a change in organic loading before the MLSS had time to stabilize (Miller, 2015). Therefore, a MLSS-based control strategy was implemented to maximize carbon capture for increased energy recovery as well as minimize daily variations in COD removal efficiency by maintaining a MLSS concentration of 3,000 mg/L. The MLSS concentration was maintained by using a programmable logic controller (PLC) and proportional-integral-derivative (PID) controls (Allen-Bradley SLC 500, Milwaukee, WI) to automatically adjust the wasting rates in response to variations in organic loading. A schematic of the MLSS-based control strategy is depicted in Figure 3.2. To verify the accuracy of the MLSS probe, the probe reading was recorded at the same time that a grab sample of mixed liquor was taken from the same aeration basin in which the MLSS probe was located and measured for TSS as described in the following section (3.3). Maintenance was performed on the MLSS sensor by manually cleaning the sensor with a clean rag when the measured lab value (TSS) was $\pm$ 20% different than the sensor reading.

A Cascade DO control strategy was also implemented, using the PLC and PID controls, to prevent low DO filament growth during high organic loading conditions and reduce energy consumption via aeration during low organic loading conditions. A schematic of the Cascade DO control strategy is depicted in Figure 3.2. Airflow was bound between 20-90 standard liters per minute (SLPM) to maintain well mixed reactors and mimic full-scale aeration oxygen transfer rates (OTR) which is typically limited to 150 mg/L-h. The accuracy of the DO sensor was monitored by using a handheld DO probe (HACH LDO101, Loveland, CO) by simultaneously taking measurements from the DO sensor and DO probe once per day. Maintenance was performed on the DO sensor by manually cleaning the sensor with a clean rag when the measurements between the DO sensor and handheld DO probe differed by > 0.2 mg/L.

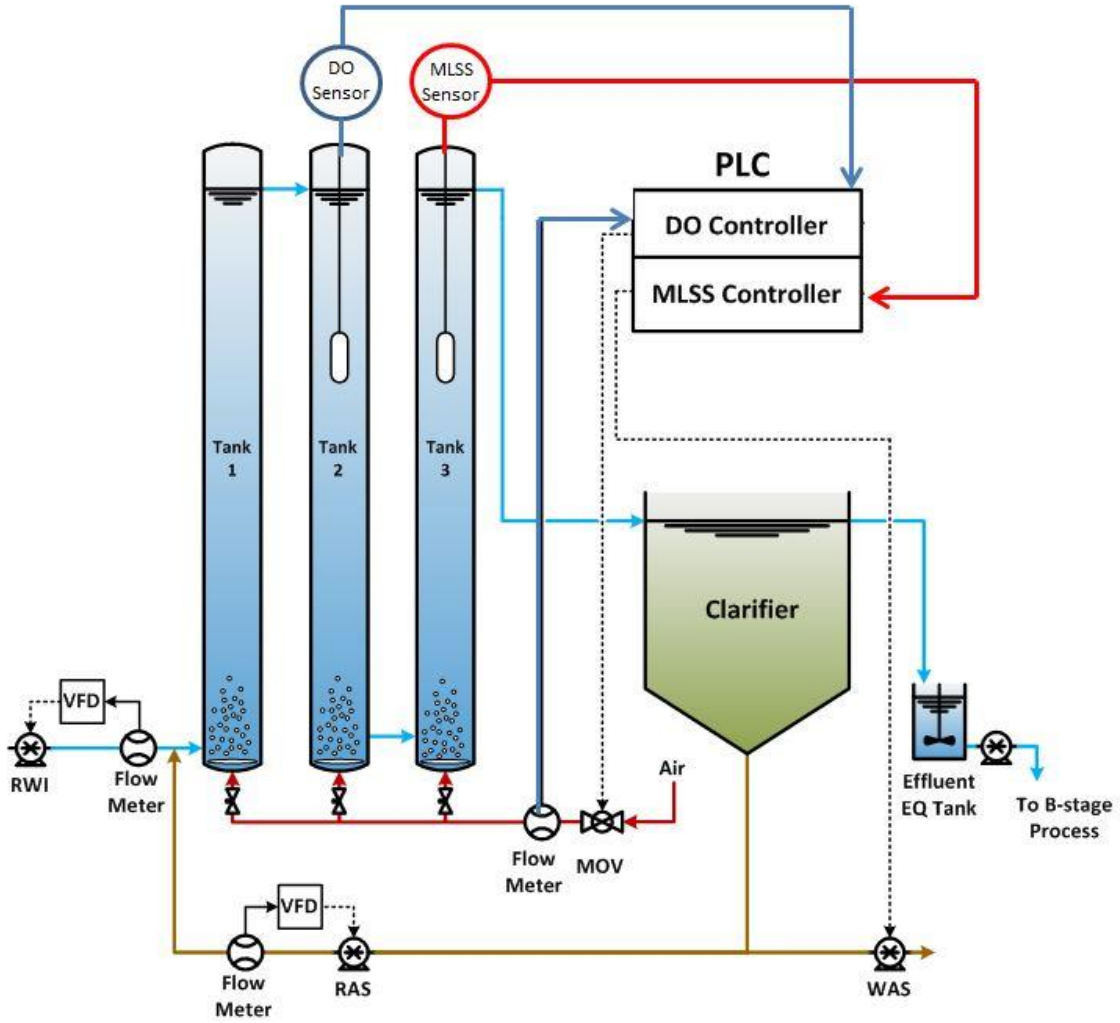

Figure 3.2: Schematic of the logic behind the MLSS-based control and Cascade DO control strategies.

3.3 Analytical Methods

To assess overall performance of the A-stage pilot, 24-hour flow-weighted composite samples of the influent and effluent were stored at approximately 4°C and analyzed for total COD (tCOD), soluble COD (1.5 µm glass microfiber filtered), TSS, volatile suspended solids (VSS), total ammonia nitrogen (TAN), and orthophosphate (OP), according to Standard Methods (APHA,

2012). Particulate COD (pCOD) was calculated as the difference between tCOD and soluble COD (1.5 µm glass microfiber filtered). The sludge volume index (SVI) was calculated according to Standard Methods (APHA, 2012). Immediately after 30 minutes of settling (SVI_{30}), decant was analyzed for turbidity and TSS, referred to as the settleometer decant TSS (TSS_{sd}). Flocculated and filtered COD (ffCOD) was analyzed according to Mamais et al. (1993) using a 0.45 μm cellulose membrane filter. The ffCOD was considered as truly soluble COD where the difference between ffCOD and sCOD (1.5 μm glass microfiber filtered) represented the colloidal COD (cCOD) fraction. The average influent wastewater characteristics are shown on Table 3.1. This study started on February 25th (2016) and ended on April 21st (2016) with each condition lasting approximately 3-4 weeks.

Table 3.1: Average influent wastewater characteristics of the A-stage HRAS pilot during the 3 different applied DO set-points.

Parameter	**Units**	**RWI**	**RWI**	**RWI**
DO set-point	mg/L	0.5	1.0	1.5
# of Samples		16	14	15
tCOD	mg/L	575 $\pm$ 67	635 $\pm$ 63	595 $\pm$ 54
ffCOD	mg/L	149 $\pm$ 18	138 $\pm$ 5	155 $\pm$ 16
pCOD	mg/L	345 $\pm$ 43	417 $\pm$ 68	370 $\pm$ 45
cCOD	mg/L	85 $\pm$ 20	75 $\pm$ 12	74 $\pm$ 18
TSS	mg/L	211 $\pm$ 26	250 $\pm$ 55	206 $\pm$ 30
VSS	mg/L	197 $\pm$ 29	226 $\pm$ 48	188 $\pm$ 26
pH		7.0 $\pm$ 0.1	6.7 $\pm$ 0.1	6.6 $\pm$ 0.1

MLSS composite samples were taken in the same manner previously mentioned and analyzed for TSS, VSS and extracellular polymeric substances (EPS). EPS fractions were extracted based

on the heat extraction method described in Li and Yang (2007) with various modifications. 50 mL of the MLSS composite sample was centrifuged for 10 minutes at G = 6,000 s^{-1} in which supernatant was filtered through a 1.5 μ_m glass microfiber filter which was used as the soluble EPS (S-EPS) sample. The sludge pellet was then re-suspended to 50 mL with a preheated phosphate buffering solution (PBS) to ensure the suspension was at 50°C. The PBS consisted of 0.164 g Na_3PO_4, 0.272 g KH_2PO_4, 0.2625 g NaCl and 0.037 g KCl in 500 mL of deionized water, and was adjust to a pH of approximately 7.2 using NaOH. After ensuring the PBS stock was well mixed, the PBS was adjusted to match the wastewater specific conductivity (SPC) of approximately 980 SPC, using tap water. After re-suspending the sludge pellet with the preheated and diluted PBS solution, loosely bound EPS (LB-EPS) was extracted by centrifuging the sample for 10 minutes at G = 6,000 s^{-1} and filtered as previously mentioned. To measure the concentration of tightly bound EPS (TB-EPS), the sludge pellet was re-suspended again (50 mL) with the PBS, heated in a water bath at 60°C for 30 minutes, followed by centrifugation and filtration in the same manner listed above. All EPS fractions were analyzed for COD (APHA, 2012), proteins (FrØlund et al., 1995) and polysaccharides (Dubois et al., 1956) reflecting the components of extracted EPS. Each of the EPS components was measured in triplicates in which the average of the three samples was used for data analysis. As a method for quality assessment and control (QA/QC), if one of the three samples was noticeably different from the other samples, it was discarded and the average of the two remaining samples was used for data analysis. MLSS composite samples were also analyzed for filamentous bacteria abundance once per week.

Carbon capture efficiency was determined by conducting a mass balance on the concentrations of COD found in the influent, effluent, WAS and amount of COD lost to mineralization. In order to close the carbon mass balance, mineralization was calculated by subtracting the sum of total COD found in the WAS and effluent of the process from the concentration of total COD found in the influent raw wastewater.

3.4 Data Analysis

Statistical analysis was performed using Microsoft Excel and spot-checked using SigmaPlot 12.5 (Systat Software, San Jose, CA). Analysis of variance (ANOVA) was used to determine the normality. Statistical differences between parameters were assessed by using the one-tailed t-test. Correlations between independent and dependent variables were assessed using Pearson Product Moment Correlation in which the correlation coefficient (C_C) ranges from -1 to 1. Values closer to -1 represent a strong negative relationship and values closer to 1 represent a strong positive relationship. Any data points collected during non-steady state operations were removed from the data set as they would not accurately represent the operating conditions of interest in this study. The process was considered to be at steady state when the applied operating conditions were maintained for a minimum of 24 hours.

CHAPTER 4
RESULTS

Results of the A-stage HRAS pilot, in terms of system performance, EPS production and COD fractionation, are reported per operating condition. There were 6 different operating conditions in the present study which were denoted as Low_0.5, Low_1.0, Low_1.5, High_0.5, High_1.0 and High_1.5. "Low" and "High" represented the applied HRTs of 30 and 60 minutes, respectively, whereas the numerical value represented the targeted DO concentrations of 0.5, 1.0 and 1.5 mg/L. Average operating conditions, including the volumetric loading rates (VLR), are shown in Table 4.1. Each operating condition was run for approximately 3-4 weeks in which data collection for the new operating condition was not used for analysis until the system reached steady state (minimum of 24 hours after change in conditions).

4.1 A-Stage Control

The effectiveness of the MLSS-based control strategy in terms of minimizing variations in COD removal efficiencies over the course of a diurnal loading cycle was not directly measured in the present study. Miller (2015) measured variations in COD removal efficiency over 24-hour period (n = 12) in a pilot-scale A-stage process (SRT = 0.15 days; DO = 0.5 mg/L) with a constant waste rate and MLSS set-point control and reported that MLSS control reduced COD removal variations by 90% compared to constant wasting rates. Therefore, the standard deviations of daily COD removal efficiencies, based on influent and effluent 24-hour composite samples, were used as surrogates to evaluate the effectiveness of the MLSS-based control strategy. Each

operating condition had a minimum of 11 days in which steady state data was collected and analyzed. Across all DO concentrations, operating at a shorter SRT resulted in standard deviations ($\pm$) of approximately 7%, 15%, 10% and 4% for the removal of tCOD, cCOD, pCOD and ffCOD (sCOD), respectively, whereas operating at a longer SRT resulted in $\pm$ 5%, 20%, 7%, and 4.5%, respectively. These results suggest that MLSS-based control was an effective strategy for minimizing variations in COD removal efficiencies regardless of the DO concentration but slightly less effective for minimizing variations in bio-flocculation in terms of cCOD removal.

The average SRT of the 30 minute (Low) and 60 minute (High) HRT across all DO concentrations was 0.26 $\pm$ 0.05 days and 0.54 $\pm$ 0.11 days, respectively. A time series data plot of the average SRT for each system is shown in Figure 4.1. As shown in Table 4.1, the MLSS concentrations were relatively stable for each operating condition as all conditions produced a standard deviation of < 400 mg/L. A time series data plot of the measured MLSS concentrations for each applied HRT is shown in Figure 4.2. The lower MLSS concentrations found at Low_0.5 and High_0.5 were attributed to poorly calibrated sensors that were re-calibrated when the DO set-point was adjusted from 0.5 to 1.0 mg/L.

A time series data plot showing the 24-hour average DO concentrations for each applied HRT is shown in Figure 4.3. Each DO set-point was maintained within the bounded airflow range with the exception of Low_1.5 in which the airflow rate was maxed out at 90 SLPM and the DO concentration dropped from 1.39 mg/L to 0.29 mg/L from the 5th to 8th day of operation. A time series data plot showing the 24-hour average airflow rates for each operating condition is shown in Figure 4.4. On day 9, the maximum airflow rate was increased to 120 SLPM and by

days 10 and 11, the average DO concentration increased up to 1.37 mg/L and 1.52 mg/L, respectively. Therefore, data from day 6 through 10 (including 2 days without data collection over the weekend) were removed from the data set as those days were not considered to be at steady state. Since the SRT of Low_1.5 was < 0.3 days, there was a minimum of 3 sludge cycles per day allowing for sufficient data analysis on the remaining data set (n = 12). A time series data plot showing the daily variations in airflow rates over a 24-hour time span for each of the DO set-points is shown in Figure 4.3.

It should be noted that the average airflow rates for Low_0.5 and Low_1.0 (Table 4.1) were practically identical ($p = 0.498$) even though the DO concentration was doubled for Low_1.0. This was likely attributed to frequent DO sensor fouling issues during operations at a DO concentration of 0.5 mg/L. A fouled probe would likely read a lower DO concentration than what is present in the system, thus, requiring higher airflow rates than would be necessary to maintain a 0.5 mg/L DO concentration. Throughout the first two weeks of this study, manual cleaning of the sensors was only performed when the handheld DO probe and DO sensor values differed by > 0.2 mg/L, but due to frequent fouling, maintenance was adjusted to manually clean the sensors daily.

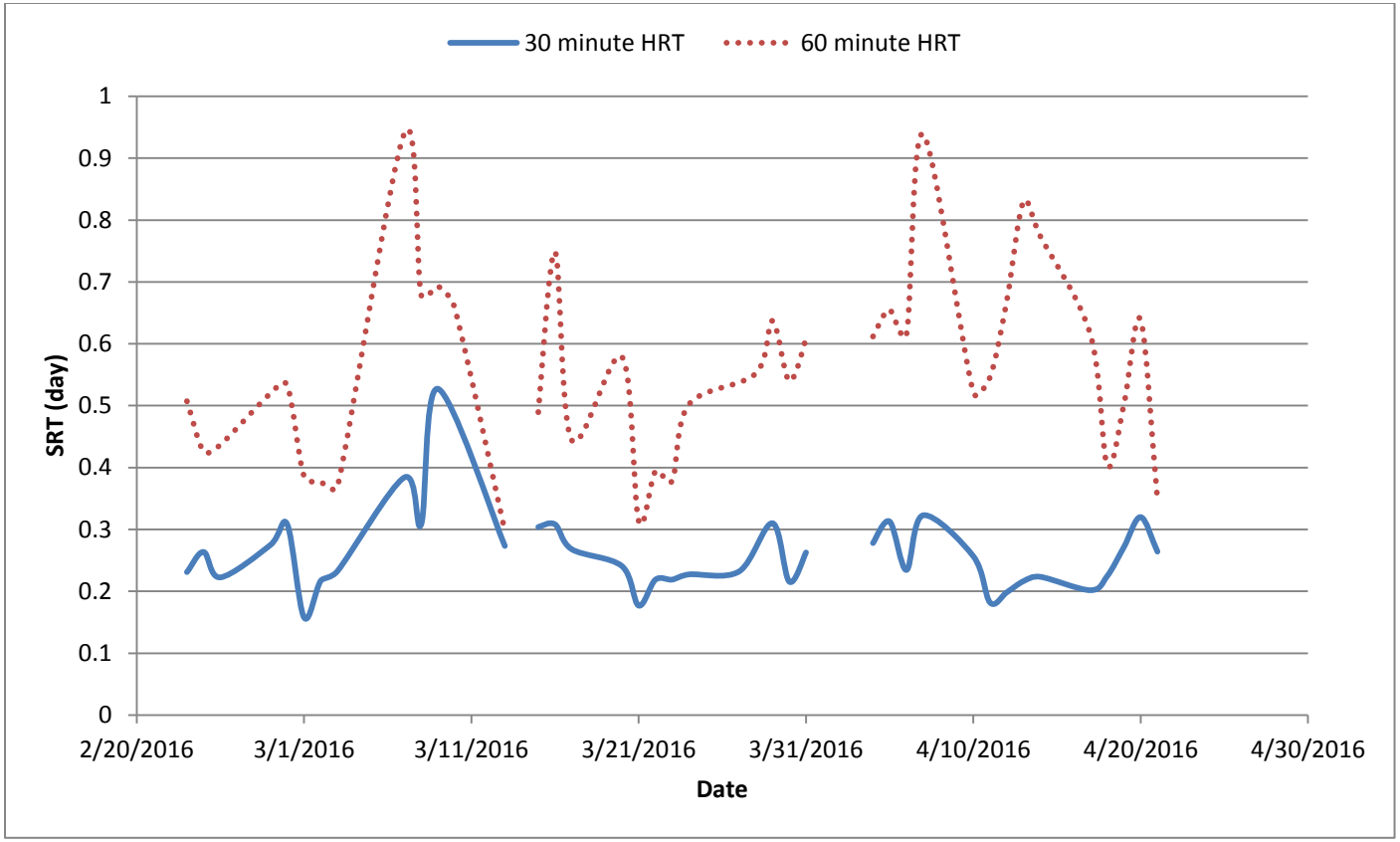

Figure 4.1: 24-hour average SRT values from 02/23/2016 to 04/21/2016. Data shown was prior to removing outliers and data collected during non-steady state conditions. Gaps in the lines reflect the change in the applied DO concentration.

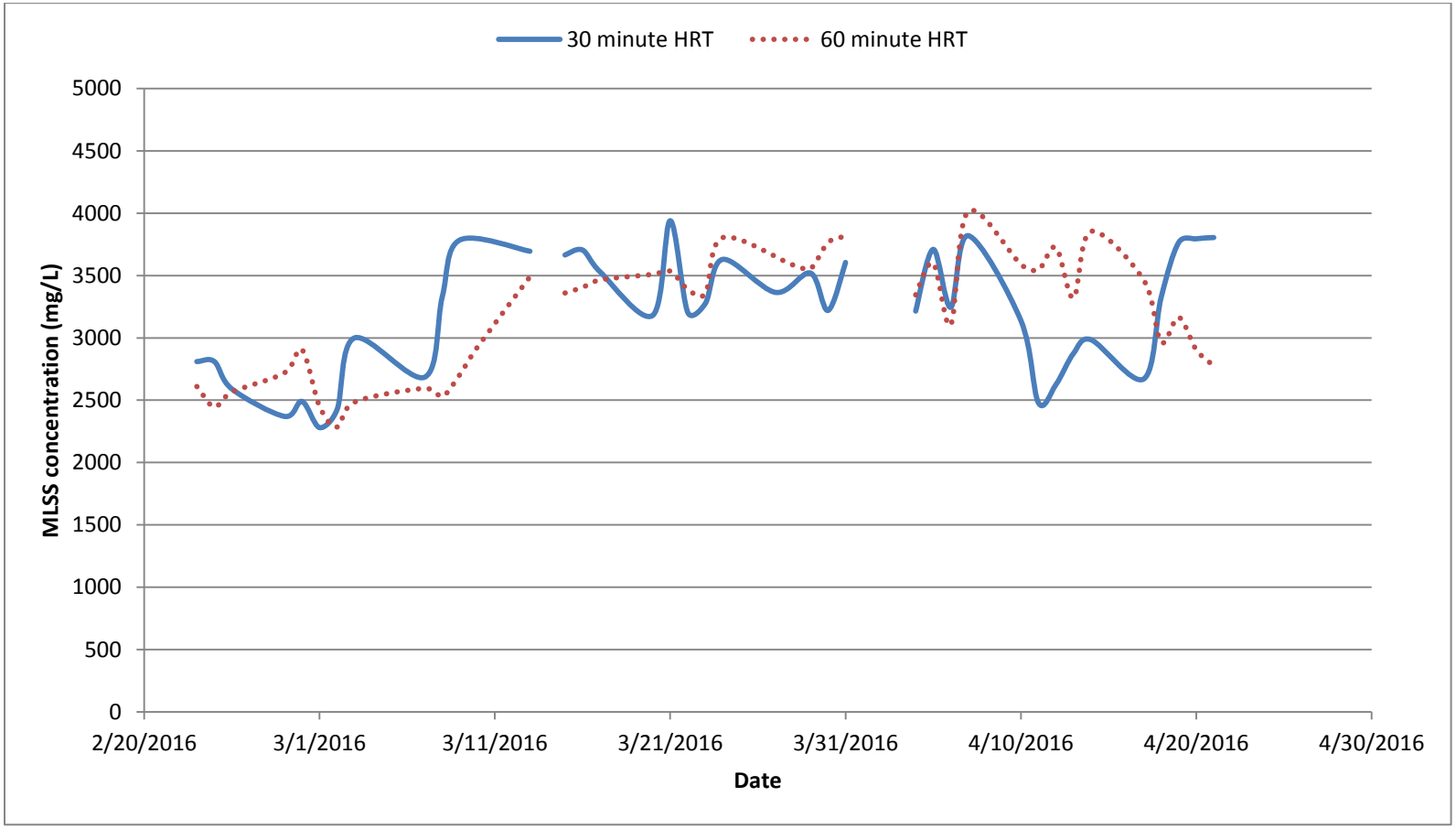

Figure 4.2: Daily measured MLSS concentrations from 02/23/2016 to 04/21/2016. Data shown was prior to removing outliers and data collected during non-steady state conditions. Gaps in the lines reflect the change in the applied DO concentration.

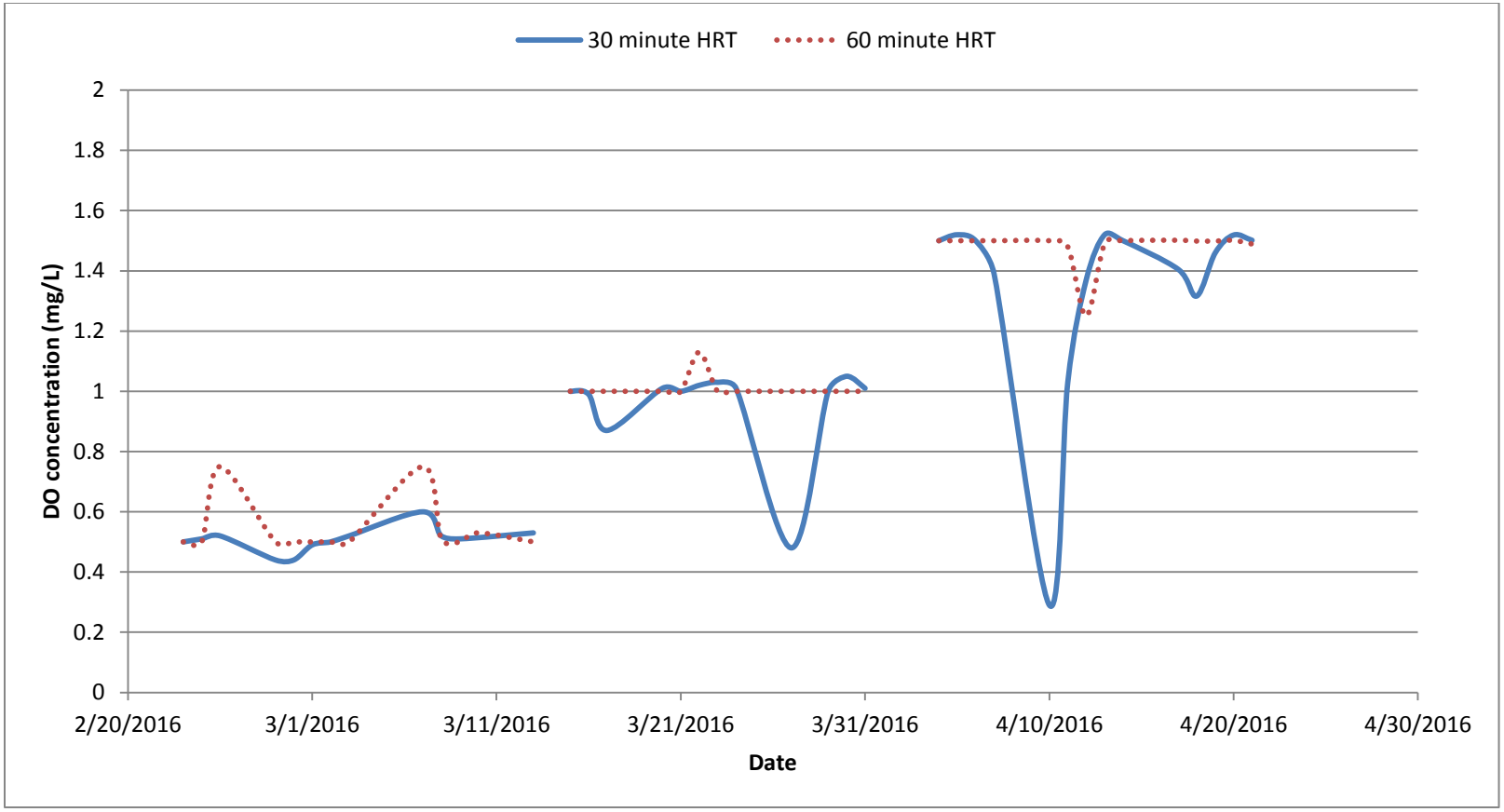

Figure 4.3: 24-hour average DO concentrations from 02/23/2016 to 04/21/2016. Data shown was prior to removing outliers and data collected during non-steady state conditions. Gaps in the lines reflect the change in the applied DO concentration.

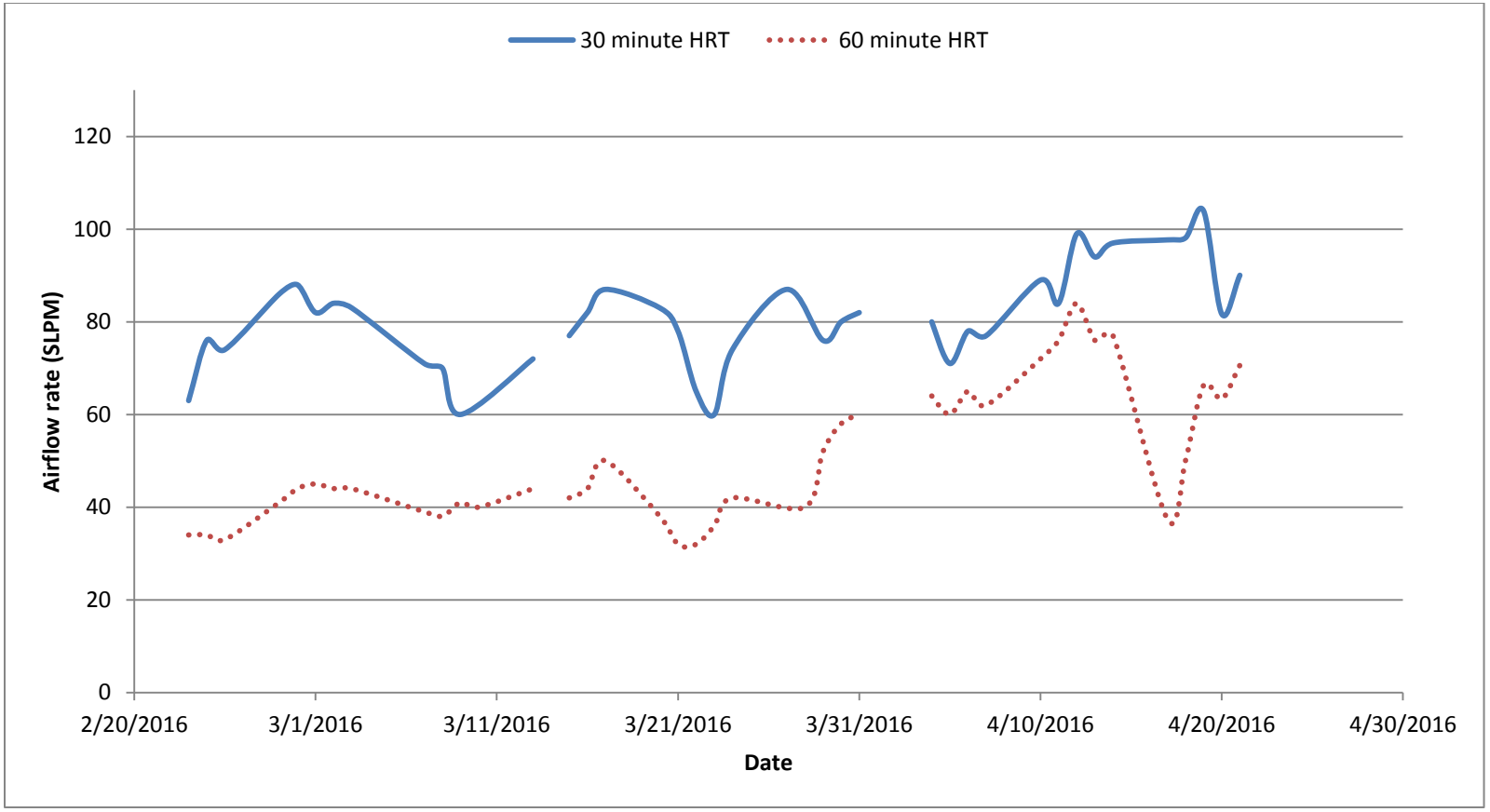

Figure 4.4: 24-hour average airflow rates (SLPM) from 02/23/2016 to 04/21/2016. Data shown was prior to removing outliers and data collected during non-steady state conditions. Gaps in the lines reflect the change in the applied DO concentration.

Table 4.1: Average A-stage HRAS operating conditions after outliers and non-steady state data removed.

Parameter	**Units**	**Low_0.5**	**High_0.5**	**Low_1.0**	**High_1.0**	**Low_1.5**	**High_1.5**
HRT	min	30	60	30	60	30	60
# of Samples		11	14	13	13	12	13
SRT	days	0.26 $\pm$ 0.06	0.51 $\pm$ 0.11	0.25$\pm$ 0.04	0.52 $\pm$ 0.11	0.27 $\pm$ 0.04	0.59 $\pm$ 0.09
DO	mg/L	0.50 $\pm$ 0.04	0.52 $\pm$ 0.06	0.99 $\pm$ 0.05	1.01 $\pm$ 0.03	1.49 $\pm$ 0.07	1.49 $\pm$ 0.06
MLSS	mg/L	2679 $\pm$ 304	2571 $\pm$ 194	3514 $\pm$ 219	3540 $\pm$ 156	3409 $\pm$ 393	3383 $\pm$ 288
Airflow	SLPM	77.9 $\pm$ 7.4	37.9 $\pm$ 5.4	76.4 $\pm$ 7.8	43.7 $\pm$ 8	87.9 $\pm$ 10.7	62.8 $\pm$ 12.5
VLR	kgCOD/ m^3-d	27.7 $\pm$ 3.7	13.7 $\pm$ 1.6	30.8 $\pm$ 2.5	15.2 $\pm$ 1.5	28.3 $\pm$ 2.6	14.3 $\pm$ 1.2

4.2 Overall System Performance

The overall performance of each operating condition for the A-stage HRAS pilot was evaluated in terms of TSS and COD removal, fate of COD removed, bio-flocculation and settleability. Bio-flocculation is characterized as cCOD removal efficiency and TSS_{sd} concentration. Settleability is characterized by the SVI_{30}. The average performance of each operating condition in the A-stage HRAS pilot study is shown in Figure 4.1.

In general, operating at a longer SRT resulted in slightly better COD removal efficiencies but substantially worse settleability which was attributed to a higher abundance of filaments. An average filament abundance of 3.2 ± 0.4 was found when operating at a longer SRT which was significantly higher ($p < 0.001$) than 1.9 ± 0.5 when operated at a shorter SRT. Thiothrix sp. II was always the most dominant filamentous bacteria found when operating at the longer SRT whereas operating at the shorter SRT varied primarily between Thiothrix sp. II and Type 1863 with only 1 of the 10 MLSS samples showing dominance of Thiothrix sp. I.

For each applied SRT, it was found that operating at a DO concentration of 1.0 mg/L resulted in the highest COD removal efficiencies with the exception of cCOD removal which was not influenced by further increasing the DO concentration to 1.5 mg/L. Similar to cCOD removal, TSS_{sd} concentrations decreased for both SRTs as the DO concentration increased from 0.5 to 1.0 mg/L but remained stable as the DO further increased to 1.5 mg/L.

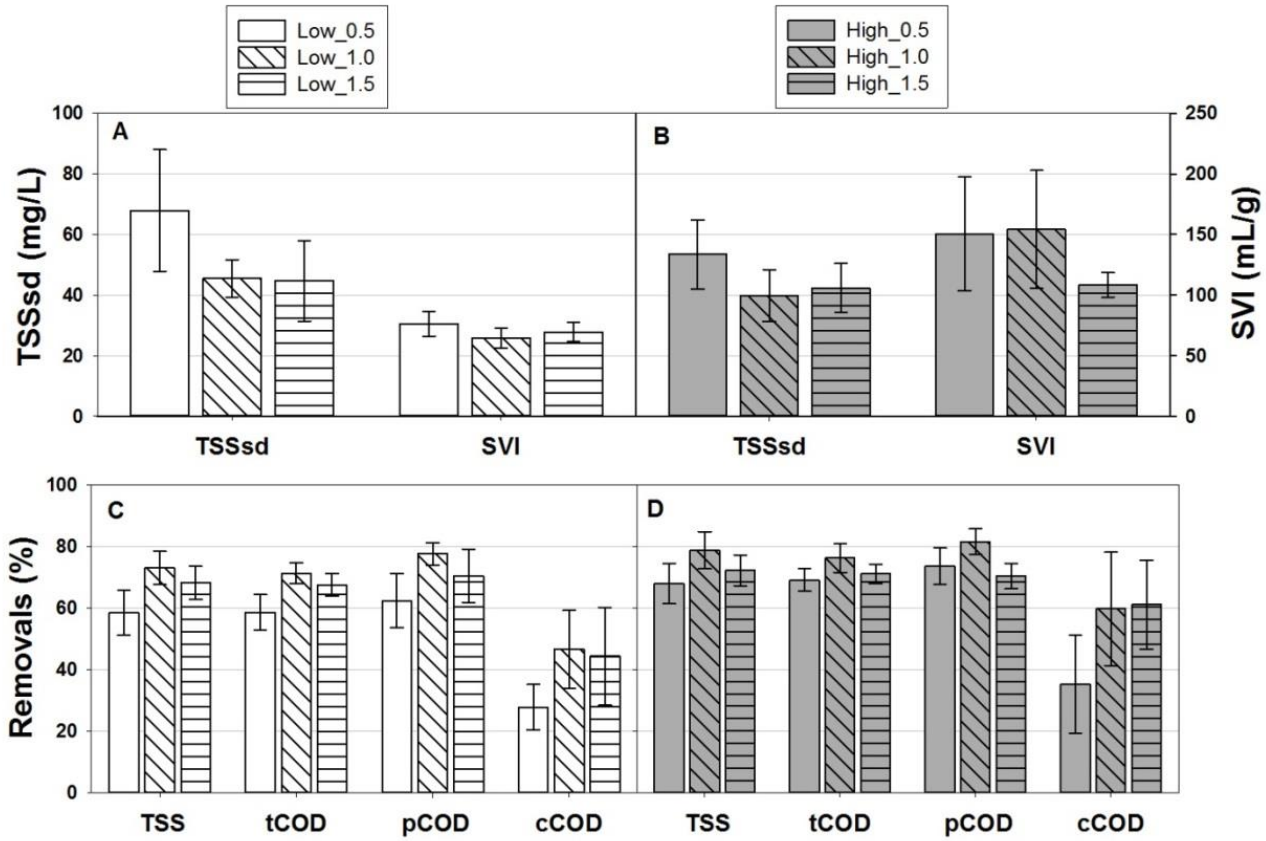

Figure 4.1: Average performance and standard deviations of the A-stage HRAS pilot. Figures A/B are based on values obtained from a settleometer using MLSS grab samples from the aeration basin. Figures C/D are based on the wastewater characteristics found in the influent and overflow from the clarifier (n = 11-14; per operating condition).

The settleability in response to DO concentration differed between the two SRTs as Low_1.0 and High_1.5 produced the lowest SVI values of 64 $\pm$ 8 mL/g and 108 $\pm$ 10 mL/g, respectively. It is noteworthy that only 6 of the 38 measured SVI values for the longer SRT exceeded 200 mL/g, each of which occurred at High_0.5 or High_1.0 mg/L. All measured SVI values for High_1.5 were below 130 mL/g and all values at the shorter SRT were below 90 mL/g, which is indicative of good settling sludge.

The fraction of mineralized COD (%) was calculated by performing a COD mass balance in which the influent tCOD concentration was considered equal to the sum of effluent COD, WAS COD and mineralized COD, as described by Akanyeti et al. (2010). The WAS COD fraction represented the amount of influent carbon captured by the activated sludge that could be redirected to an energy recovery process. WAS COD was calculated by multiplying the WAS VSS concentration by 1.5 based on an average pCOD/VSS ratio of 1.5 $\pm$ 0.34 measured in the A-stage pilot over a wide range of operating conditions from August 2013 through April 2016. Results of the COD mass balance for each operating condition are shown in Figure 4.2. The lowest fraction of COD lost to mineralization was achieved by Low_0.5 (15.5 $\pm$ 5.6%). The largest fraction of COD directed to the WAS (55.4 $\pm$ 5.5 %) occurred when operating at High_1.0 and coincided with the lowest quantity of COD reaching the effluent (23.1 $\pm$ 5.1 %).

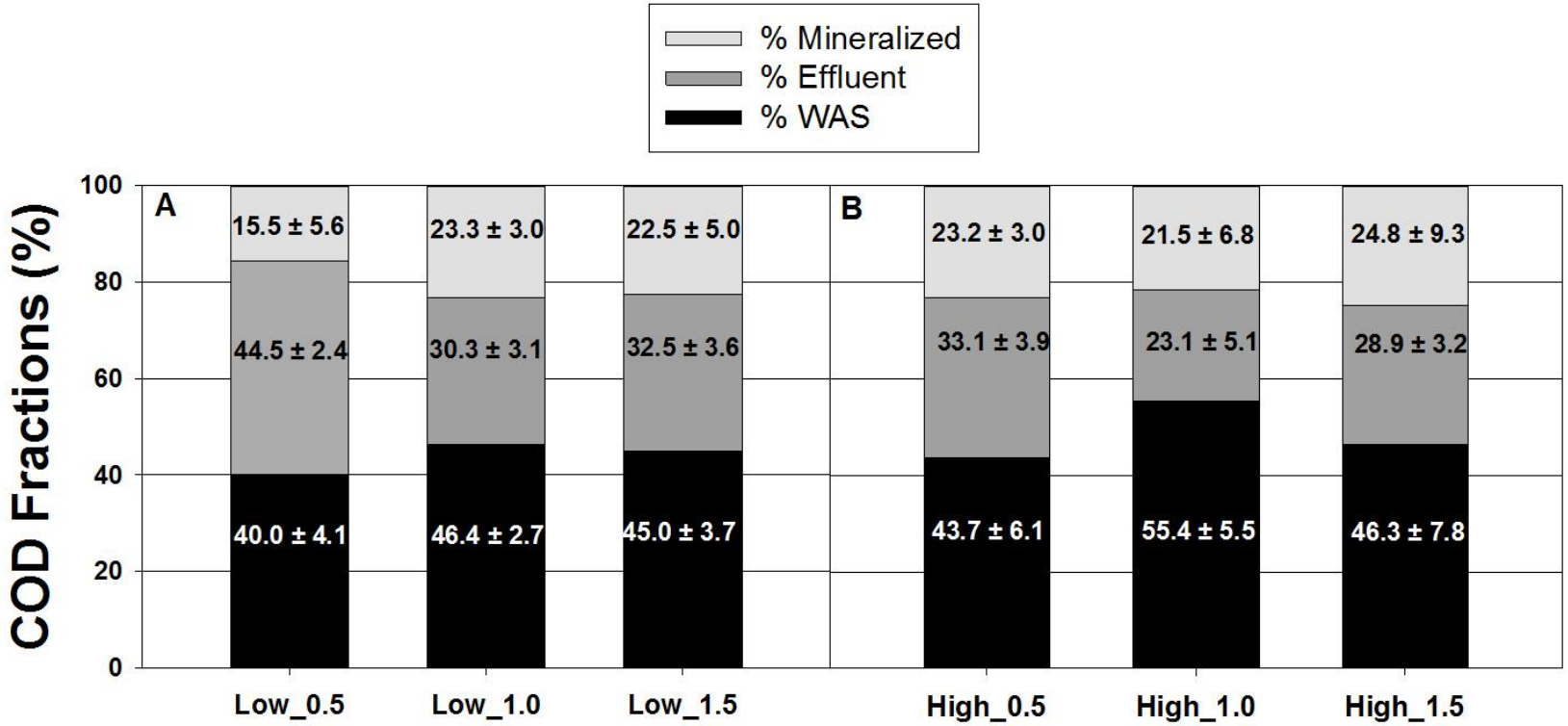

Figure 4.2: Fractions of COD captured in the WAS, lost in the effluent and lost to mineralization (n = 6-12 days per condition; 1 data point per day).

4.3 EPS Production

The average EPS component concentrations of COD, proteins (Pn), polysaccharides (Ps) and the ratio of proteins to polysaccharides (Pn/Ps) are shown in Figure 4.3. It should be noted that EPS extraction was conducted 1-2 times per week for all operating conditions leading to a total of 3-4 data points for EPS analysis which was not enough to accurately capture the random error of the experiment. Moreover, the system was not always at steady state when the EPS extraction was performed in which those data points were removed from the data set leading to the analysis of extracted EPS using only 2-3 total data points. All extracted EPS concentrations were normalized per gram of VSS. Pn and Ps were converted to COD using conversions factors of 1.5 and 1.07, respectively. Since Pn and Ps typically make up 70-80% of extracellular organic carbon (Dignac et al., 1998), each component was specifically measured to evaluate how operating conditions influence their production and in turn, how these components influenced system performance. Conversely, COD was measured to quantify all components of the EPS as a whole and evaluate any correlations with EPS in a more general manner.

Increasing the DO concentration at a longer SRT resulted in decreased Pn, Ps, and COD concentrations found in each fraction of the EPS (S-EPS, LB-EPS and TB-EPS) but showed no trend in the Pn/Ps ratios. The same trends were noticed at the shorter SRT as the DO concentration increased from 0.5 to 1.0 mg/L, however, further increasing the DO concentration to 1.5 mg/L resulted in increased Pn concentrations as well as increased concentrations of Ps and COD found in the LB-EPS.

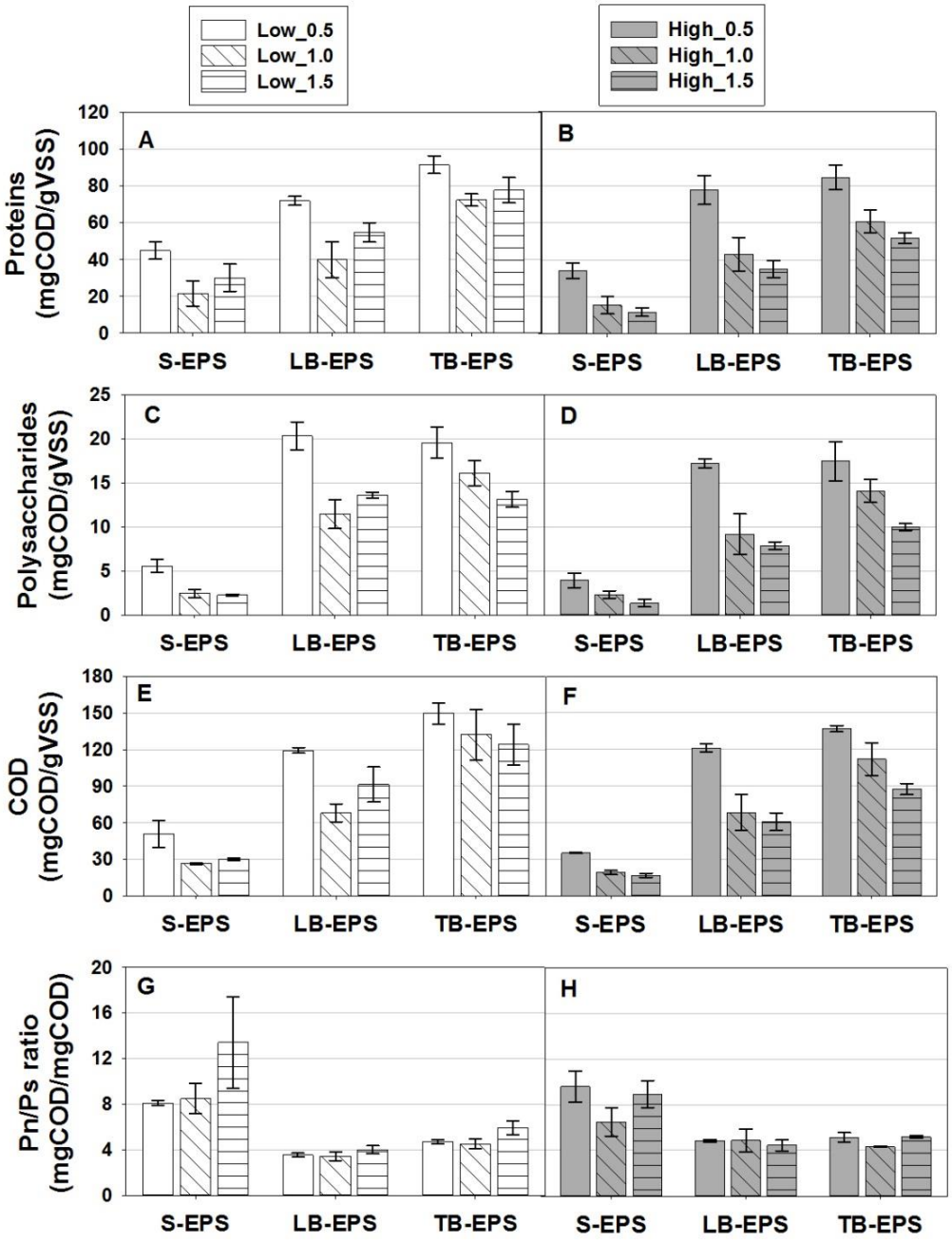

Figure 4.3: Average EPS concentrations denoted by SRT (fill color) and DO set point (fill design) (n = 2-3 sample days per operating condition; 1 data point based on the average value of triplicate samples per day).

Total EPS was calculated by adding component concentrations from the S-EPS, LB-EPS and TB-EPS in order to compare results of previous literature as many reports did not attempt to selectively extract individual EPS fractions. The average total EPS concentrations as well as the LB/TB ratios are shown in Figure 4.4. The S-EPS, LB-EPS and TB-EPS made up 8-19%, 24-42% and 44-65% of the total EPS, respectively. Total EPS followed the same trends as the individual EPS fractions with the exception of Ps between Low_1.0 and Low_1.5 due to an increase in LB-Ps and decrease in TB-Ps. Total COD concentrations of the extracted EPS decreased from 319.6 $\pm$ 0.1 to 227 $\pm$ 13 mgCOD/gVSS when operating conditions switched from Low_0.5 to Low_1.0, respectively, and from 293.3 $\pm$ 5.6 to 199.8 $\pm$ 1.6 mgCOD/gVSS when switched from High_0.5 to High_1.0, respectively. Interestingly, total EPS further decreased to 165.2 $\pm$ 10.9 mgCOD/gVSS at High_1.5 whereas Low_1.5 showed an increase in total EPS (245.6 $\pm$ 30.9 mgCOD/gVSS). Throughout all operating conditions, Pn made up approximately 63% of the total COD content whereas Ps only made up approximately 13% of the total COD content. In general, the highest and lowest LB/TB ratios occurred when operating at a DO concentration of 0.5 and 1.0 mg/L, respectively.

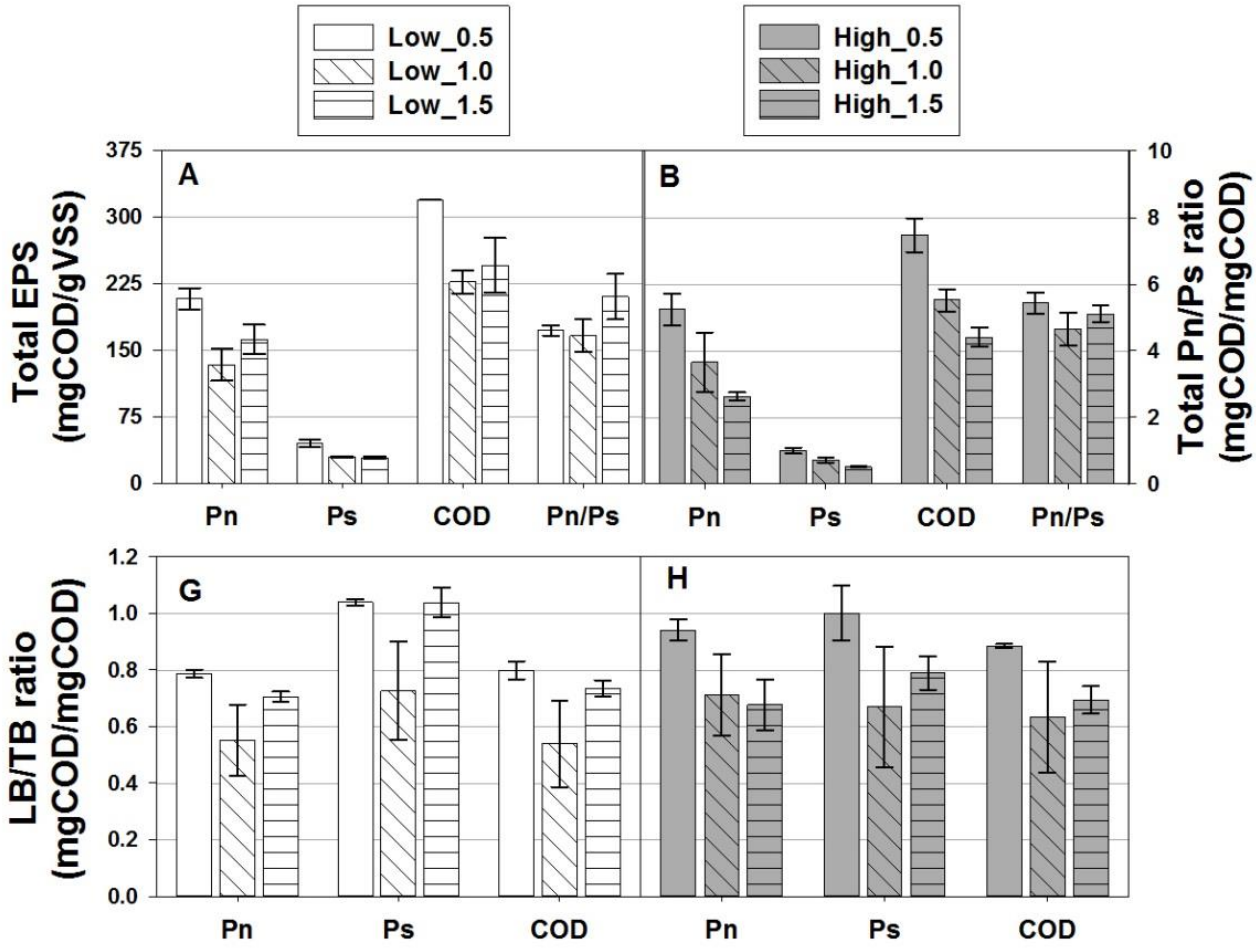

Figure 4.4: Average total EPS concentrations, Pn/Ps ratios and LB/TB ratios (n = 2-3 days per operating condition; 1 data point per day).

CHAPTER 5

DISCUSSION

5.1 Influence of SRT, DO and Influent Wastewater Characteristics on Overall System Performance

This study showed that increasing the DO concentration resulted in similar responses for each SRT with slightly better bio-flocculation and suspended solids removal efficiencies for the longer SRT but substantially worse settleability. This was consistent with previous reports which showed that bio-flocculation, measured as cCOD removal increased as the SRT increased from 0.125 day (Faust et al., 2014b) and 0.3 day (Jimenez et al., 2015) up to a 1.0 day SRT. It was postulated that a longer retention time provided additional time for particles and flocs to interact but allowed for filamentous bacterial growth. Filamentous bacteria can act as a structural backbone for flocs (Nielsen et al., 2004) resulting in increased floc diameter which in turn acts as a sweep floc capturing dispersed particulate and colloidal matter as they settle in the clarifier, thus, increasing bio-flocculation. On the other hand, due to the elongated and rigid structure of filamentous organisms, the ability to compact in the sludge blanket becomes deteriorated, resulting in increased SVI values and potential sludge bulking.

Interestingly, increasing the DO concentration from 1.0 to 1.5 mg/L did not improve pCOD or cCOD removal efficiencies for either applied SRT. In fact, the pCOD and subsequent tCOD removals decreased as the DO concentration increased to 1.5 mg/L whereas cCOD removal remained relatively stable. This is in contrast to previous reports that showed enhanced bio-flocculation when the DO concentration increased under constant SRTs (Faust et al., 2014a; Jimenez et al., 2015). The decreased pCOD removal efficiencies could have been a result of floc

shearing, but since the cCOD removals and TSS_{sd} concentrations were unaffected, it was postulated that higher organic loads during operations at a DO concentration of 1 mg/L (Table 3.1) had an additional benefit on bio-flocculation and carbon capture. This theory coincides with Böhnke (1997b) who noted that higher COD loading to the A-stage results in higher removal efficiencies. Furthermore, Zhang et al. (2014) found that the maximum specific adsorption capacity was obtained with influent substrate concentrations > 700 mg/L. The influence of influent characteristics on system performance was further emphasized by comparing the correlation coefficients for each SRT across all DO concentrations and for each individual DO concentration applied (Appendix A.1.4.). Operating at a DO concentration of 1.0 mg/L showed the highest correlations with influent TSS and tCOD concentrations compared to a DO concentration of 0.5 and 1.5 mg/L as well as all DO concentrations combined. Based on these findings and the fore mentioned literature, it was suggested that the influent wastewater characteristics played a supplemental role to DO concentration in terms of COD removal, bio-flocculation and subsequent carbon capture. However, the majority of correlations associated with DO concentrations were mild or weak, suggesting that other parameters, such as EPS, played a more significant role on bio-flocculation.

Increased bio-flocculation at the longer SRT, indicated by greater cCOD removal and lower TSSsd concentrations, resulted in less COD in the effluent and more COD captured in the WAS. Therefore, the highly concentrated organic matter in the WAS at a longer SRT would result in a higher energy recovery potential when diverted to an energy recovery process, such as anaerobic digestion. In contrast to Jimenez et al. (2015) who reported that increasing the SRT from 0.3 days to 0.5 days (DO = 1 mg/L) resulted in mineralization fractions of approximately

23% and 37%, respectively, results from the present study showed no significant difference in mineralization between Low_1.0 and High_1.0. Since both Low_1.0 and High_1.0 received the same influent wastewater, the potential for COD loading enhancing COD removal was neglected which further indicated that carbon capture in the A-stage is enhanced by better bio-flocculation at longer SRTs. Therefore, for this specific wastewater, operating at a longer SRT and a DO concentration of 1 mg/L would benefit carbon capture via bio-flocculation without significantly increasing the quantity of COD lost to mineralization.

5.2 Influence of SRT, DO and Influent Wastewater Characteristics on EPS Production

The total COD content of extracted EPS was significantly lower when operated at a longer SRT for each DO condition ($p < 0.03$). This is in contrast to findings from Jimenez et al. (2015) who showed that increasing the SRT from 0.3 to 1.0 day resulted in increased EPS production from 50 ± 25 mgCOD/gVSS to 105 ± 16 mgCOD/gVSS, respectively. The conflicting results could be a product of operating with different raw wastewater and/or operating at different SRTs as their study was maintained around a 1 day SRT and this study had average SRTs of 0.28 and 0.56 days. Furthermore, EPS extraction methods differed as this study used a modified heat extraction and Jimenez et al. (2015) used cation exchange resin to extract EPS from the activated sludge.

This study showed that operating at very low SRTs resulted in a noticeably higher content of LB-EPS compared to CAS processes with LB-EPS making up anywhere from 24-42% and 10-20% of the total EPS, respectively (Li and Yang, 2007; Xie and Yang, 2009). It was postulated that bacteria associated with low SRTs would produce more EPS as a defense mechanism against variations of the influent wastewater (Faust et al., 2014b) whereas bacteria associated with longer SRTs would either produce less EPS or degrade EPS for cell maintenance. Since the substrate concentration is rarely limited in the A-stage, lower EPS concentrations at the longer SRT can be associated with less EPS being produced by the bacteria compared to the decomposition of EPS as an energy source.

The LB/TB ratios for both SRTs decreased as the DO concentration increased from 0.5 to 1.0 mg/L, but increased again as the DO concentration further increased to 1.5 mg/L (Figure 4.4). Since LB-EPS concentrations increased for Low_1.5 and decreased for High_1.5, the only parameter that showed similar trends to the LB/TB ratios was the influent wastewater characteristics in which operating with the highest organic loading resulted in lowest LB/TB ratios.

On days in which EPS was extracted, combining data from all 6 operating conditions showed that the EPS fractions and components were significantly reduced as the SRT increased ($p < 0.05$), with the exception of LB Pn ($p = 0.152$). The SRT showed the strongest correlation with the Pn concentration in the TB-EPS ($C_C = -0.797$). Increasing the DO concentration also

negatively correlated with each EPS component across all EPS fractions. On the other hand, the DO concentration showed good correlations with the Ps concentrations found in the S-EPS (C_C = -0.794), TB-EPS (C_C = -0.823) and total EPS (C_C = -0.797). Interestingly, the opposite occurred in relation to the Pn/Ps ratios in which all ratios were positively correlated with DO concentrations, but showed primarily weak correlations with only one mild correlation for the TB-EPS Pn/Ps ratio (C_C = 0.446). Based on these results, it was suggested that operating at longer SRTs would result in a more substantial decrease in Pn concentrations whereas increasing the DO concentration would result in a more substantial decrease in Ps concentrations.

Comparing the influence of each HRT applied in this study, across all DO concentrations, showed that operating at a longer average SRT showed strong negative correlations between the DO concentration and all EPS components but had mild to weak correlations with the Pn/Ps ratios. Similar correlations were found between EPS components and the SRT with the exception of LB-EPS Ps and COD concentrations which had mild negative correlations of C_C = -0.75 and C_C = -0.74, respectively. It should be noted that the correlation coefficients between EPS components and airflow rates at the longer SRT were in the range of C_C = -0.694 and C_C = -0.804, for all EPS components. Therefore, EPS production at a longer SRT can be influenced by the airflow rates but to a slightly lesser extent compared to the DO concentration and daily SRT.

Conversely, operating at a shorter SRT showed that the DO concentration only had strong correlations with Ps concentrations in the S-EPS (C_C = -0.822), TB-EPS (C_C = -0.883) and total EPS

(C_C = -0.825) whereas the SRT did not strongly correlate with any EPS component. Furthermore, airflow showed weak correlations with all EPS components at a shorter SRT, with the majority of correlations deemed as not significant ($p > 0.05$).

Therefore, it is suggested that the SRT and DO concentrations play a more significant role on total EPS production when operated at a longer average SRT whereas only Ps production is influenced by the DO concentration at a shorter SRT. This may indicate that other parameters, such as the influent characteristics, play a more significant role on EPS production when operated at a shorter SRT.

5.3 Influence of EPS Production on Overall System Performance

Based on previous literature (Bisogni and Lawrence, 1971; Liao et al., 2001; Li and Yang, 2007), it was postulated that operating at a very low SRT (< 1 day) would result in deteriorated bio-flocculation and suspended solids due to a higher LB-EPS content compared to CAS processes. This can be attributed to the fact that LB-EPS contains a high water content which can block binding sites on the floc (Zita and Hermansson, 1997a, 1997b; Liao et al., 2001). However, since the LB-EPS components did not follow the same trend between the two applied SRTs in the present study, results indicated that the LB/TB ratio was more influential on system performance than LB-EPS alone. The best overall performance in terms of suspended solids removal and bio-flocculation (Figure 4.1) as well as carbon capture (Figure 4.2) all occurred at a DO concentration of 1.0 mg/L for each SRT and coincided with the lowest LB/TB ratios (Figure 4.4). As the DO further increased to 1.5 mg/L, the LB/TB ratio also increased and coincided with

decreased TSS, tCOD and pCOD removal efficiencies. Since the only common factor associated with EPS between Low_1.5 and High_1.5 was a decrease in Ps and COD in the TB-EPS, it was postulated that suspended solids (TSS and pCOD) removal was associated with the TB-EPS. Conversely, bio-flocculation remained stable as the DO concentrations increased from 1.0 to 1.5 mg/L indicating that neither operating conditions nor EPS production influenced bio-flocculation as the conditions changed. Therefore, it is likely that operating at a DO concentration of 1.0 mg/L maximized bio-flocculation in which adsorption sites were fully saturated and further bio-flocculation was limited by the hydrolysis rate of the adsorbed organic matter (Jimenez et al., 2015). Similar results were found in terms of carbon capture which was maximized at Low_1.0 and High_1.0. Therefore, carbon capture was likely enhanced by a low LB/TB ratio based on the fore mentioned discussions.

System performance in terms of suspended solids removal, bio-flocculation and carbon capture was enhanced by operating at a longer SRT and coincided with lower total EPS concentrations. As previously discussed, decreasing the EPS concentrations would result in decreased bound water content, thus enhancing bio-flocculation and removal of suspended solids by allowing easier access to adsorption sites. Therefore, it was suggested that the A-stage process could be operated at longer SRTs, up to a limiting maximum value, to increase carbon capture as well as decrease the energy required for aeration.

Previous literature (Higgins and Novak, 1997; Jorand et al, 1998; Liao et al., 2001) has suggested that increasing the Pn content of EPS would result in better bio-flocculation which is also attributed to a decrease in the bound water content. Based on the results from this study, it was suggested that EPS production and/or the EPS components were not the primary factors responsible for bio-flocculation. However, EPS production seemed to play a more significant role in the removal of TSS, tCOD and pCOD, especially when operating at the shorter SRT. Although correlations were found between EPS production and system performance, it is still likely that these factors play a supplemental role compared to the influence of operating conditions (SRT, DO concentration and potentially influent wastewater characteristics).

CHAPTER 6

CONCLUSION

This study evaluated the influence of A-stage operating conditions on the overall system performance and EPS production as well as determined how EPS production correlated with the overall system performance. An A-stage HRAS pilot process was operated with constant HRTs of 30 minutes and 60 minutes, DO set-points of 0.5, 1.0 and 1.5 mg/L and a MLSS set-point of 3,000 mg/L. The average SRTs in the 30 and 60 minute HRT processes were 0.26 $\pm$ 0.05 days and 0.54 $\pm$ 0.11 days, respectively. In general, EPS production influenced the overall system performance but played a supplemental role to other parameters, such as operating conditions (SRT and DO) and influent wastewater characteristics. Specific influences and correlations found in this study are as follows:

- Bio-flocculation was enhanced by operating at a longer SRT resulting in increased carbon capture and decreased effluent COD. There was no significant increase in COD lost to mineralization.
- Operating at a longer SRT resulted in significantly worse settleability.
- Operating at a DO concentration of 1.0 mg/L resulted in the best performance for each SRT applied. This was potentially attributed to receiving the highest organic loading during this operational period. Although organic loading was not likely the primary parameter influencing system performance, it did contribute to additional removals resulting in the best overall performance.

- EPS production showed weak correlations to bio-flocculation at both SRTs, but showed mild and strong correlations with TSS, tCOD and pCOD removal efficiencies. This finding was more pronounced when operated at a lower SRT.
- Operating at a longer average SRT showed strong negative correlations between SRT and DO concentration on overall system performance suggesting that a decrease in total EPS would benefit suspended solids removal, bio-flocculation and subsequent carbon capture at a longer SRT.
- Operating at a short SRT showed that the DO concentration had strong negative correlations with Ps production suggesting that decreased Ps concentrations at a shorter SRT would enhanced suspended solids removal, bio-flocculation and subsequent carbon capture, but to a lesser extent than operating at a longer SRT.

Based on these conclusions, it can be suggested that the A-stage should be operated at an SRT around 0.5 days and a DO concentration of at least 1.0 mg/L to maximize bio-flocculation and subsequent carbon capture. Increasing the SRT up to 1 day may result in better carbon removal but at the expense of increasing mineralization instead of enhancing bio-flocculation, which may result in lower carbon capture efficiency. This study was limited by the fact that higher SRTs were achieved by operating with a longer HRT, which is not realistic for full scale treatment plants. Therefore, additional research should be performed on evaluating the influence of SRTs up to 1 day and EPS production on carbon capture by operating at different MLSS set-points to achieve different SRT ranges.

REFERENCES

1. Akanyeti, I., Temmink, H., Remy, M., and Zwijnenburg, A. (2010) Feasibility of bio-flocculation in a high-loaded membrane bioreactor for improved energy recovery from sewage. *Water Science & Technology* **61**(6), 1433-1439.
2. American Public Health Assoc., American Water Works Assoc., and the Water Environment Federation. (2012) Standard Methods for the Examination of Water and Wastewater, 22nd Ed. Published by American Public Health Assoc. Washington, DC (2-66, 2-67, 2-90 : 2-93).
3. Ardern, E. and Lockett, W.T. (1914) Experiments on the oxidation of sewage without the aid of filters. *Journal of the Society of Chemical Industry* **33**(10), 523-539.
4. Bisgoni Jr., J., and Lawrence, A. (1971) Relationships Between Biological Solids Retention Time and Settling Characteristics of Activated Sludge. *Water Research* **5**(9), 753-763.
5. Böhnke, B. (1983) Development of a competitive two-stage biological treatment system - results of pilot plants and full scale plants, pp. 419-431.
6. Böhnke, B. (1994) Stickstoffelimination in Adsorption-Belebungsanlagen (AB-Anlagen). *Korrespondenz Abwasser* **41**(6), 900-907.
7. Böhnke, B., and Diering, B.C. (1980) Two-stage activated sludge process. *Conference Proceedings*, Canada.
8. Böhnke, B., Schulze-Rettmer, R. and Zuckut, S.W. (1998) Cost-effective reduction of high-strength wastewater by adsorption-based activated sludge technology. *Water Engineering & Management* **145**(12), 31-34.
9. Böhnke, B., Diering, B. and Zuckut, S.W. (1997a) Ab process removes organics and nutrients. *Water Environment & Technology* **9**(3), 23-27.
10. Böhnke, B., Diering, B. and Zuckut, S.W. (1997b) Cost-effective wastewater treatment process for removal of organics and nutrients. *Water Engineering & Management* **144**(5), 30-35.
11. Boon, A.G. (1995) Septicity in Sewers: Causes, Consequences and Containment. *Water Science Technology* **31**(7), 237-253.
12. Bruss, J.H., Nielsen, P.H., and Keiding, K. (1992) On the stability of activated sludge flocs with implications to detwatering. *Water Research* **26**(1), 1597-1604.
13. Chao, A.C., and Keinath, T.M. (1979) Influence of process loading intensity on sludge clarification and thickening characteristics. *Water Research* **13**(12), 1213-1223.
14. Comte, S., Guibaud, G., and Baudu, M. (2006) Biosorption properties of extracellular polymeric substances (EPS) resulting from activated sludge according to their type: Soluble or bound. *Process Biochemistry* **41**, 815-823.
15. Daffonchio, D., Thaveesri, J., and Verstraete, W. (1995) Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. *Applied Environmental Microbiology* **61**(10), 3676-3680.
16. de Silva, D.G.V., and Rittman, B.E. (2000) Interpreting the response to loading changes in a mixed-culture compltely stirred tank reactor. *Water Research* **36**, 2711-2720.

17. Dignac, M.-F., Urbain, V., Rybacki, D., Bruchet, A., Snidaro, D., and Scribe, P. (1998) Chemical description of extracellular polymers: Implications on activated sludge floc structure. *Water Science & Technology* **38**(8-9), 45-53.
18. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, P. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**(3), 350-356.
19. Ehlers, G.A.C., and Turner, S.J. (2011) Evaluation of extracellular biopolymer and its impact on bio-flocculation in activated sludge bioreactors. *Water Science & Technology* **63**(4), 689-694.
20. Evans, E., Brown, M.R.W., and Gilbert, P. (1994) Iron chelator, exopolysaccharide and protease production in *Staphylococcus epidermis*: a comparative study of the effects of specific growth rate in biofilm and planktonic culture. *Microbiology* **140**, 153-157.
21. Faust, L., Temmink, H., Zwijnenburg, A., Kemperman, A.J.B., and Rijnaarts, H.H.M. (2014a) Effect of dissolved oxygen concentration on the bio-flocculation process in high loaded MBRs. *Water Research* **66**(0), 199-207.
22. Faust, L.; Temmink, H., Zwijnenburg, A., Kemperman, A.J.B., and Rijnaarts, H.H.M. (2014b) High loaded MBRs for organic matter recovery from sewage: Effect of solids retention time on bio-flocculation and on the role of extracellular polymers. *Water Research* **56**(0), 258-266.
23. Forster, C.F., and Dallas-Newton, J. (1980) Activated sludge sttlement – some suppositionsa nd suggestions. *Water Pollution & Control* **79**, 338-351.
24. Friedrich, M., and Takács, I. (2013) A new interpretation of endogenous respiration profiles for the evaluation of the endogenous decay rate of heterotrophic biomass in activated sludge. *Water Research* **47**(15), 5639-5646.
25. Friedrich, M., Takács, I., and Tranckner, J. (2015) Physiological adaption of growth kinetics in activated sludge. *Water Research* **85**, 22-30.
26. Frølund, B., Griebe, T., and Nielsen, P.H. (1995) Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology* **43**(4), 755-761.
27. Ge, H., Batstone, D.J. and Keller, J. (2013) Operating aerobic wastewater treatment at very short sludge ages enables treatment and energy recovery through anaerobic sludge digestion. *Water Research* **47**(17), 6546-6557.
28. Gonzalez-Martinez, A., Rodriguez-Sanchez, A., Lotti, T., Carcia-Ruiz, M. –J., Osorio, F., Gonzalez-Lopez, J., and van Loostredtch, M.C.M. (2016) Comparison of bacterial communities of conventional and A-stage activated sludge systems. *Scientific Reports.* **6**, 18786; doi: 10.1038/srep18786
29. Goodwin, J.A.S., and Forster, C.F. (1985) A further examination into the composition of activated sludge surfaces and relation to their settlement characterisitcs. *Water Research* **19**, 527-533.
30. Gori, R., Jiang, L., Sobhani, R., and Rosso, D. (2011) Effects of soluble and particulate substrate on the carbon and energy footprint of wastewater treatment processes. *Water Research* **45**, 5858-5872.
31. Gujer, W., Henze, M., Mino, T., and van Loosdrecht, M. (1999) Activated Sludge Model No. 3. *Water Science & Technology* **39**(1), 183-193.

32. Hach Company. (2014) Dissolved Oxygen: Hach LDO® Probe, Model 2. Webpage accessed Sept. 9th, 2014. http://www.hach.com/hach-ldo-model-2-optical-process-dissolved-oxygen-probe/product-downloads?id=10182183350&callback=pf
33. Haider, S., Svardal, K., Vanrolleghem, P.A., and Kroiss, H. (2003) The effect of low sludge age on wastewater fractionation (SS, SI). *Water Science & Technology* **47**(11), 203-209.
34. Henze, M., Grady, C.P.L., Gujer, W., Marais, G.v.R., and Matsuo, T. (1987) *Activated Sludge Model No. 1.* OAWPRC scientific and technical report no. 1; International association on water pollution research and control. London.
35. Henze, M., Gujer, W., Mino, T., and Loosdrecht, M.V. (2000) Activated Sludge Models ASM1, ASM2, ASM2d and ASM3, IWA Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment, Great Britain.
36. Higgins, M.J., and Novak, J.T. (1997) The Effect of Cations on the Settling and Dewatering of Activated Sludges: Laboratory Results. *Water Environment Research* **69**(2), 215-224.
37. Huang, M.-H., Li, Y.-M., and Gu, G.-W. (2010) Chemical composition of organic matters in domestic wastewater. *Desalination* **262**, 36-42.
38. Insite Instrumentation Group, Inc. InsiteIG FAQs. Webpage accessed Sept. 5th, 2014. http://www.insiteig.com/faqs-dissolved-oxygen.htm
39. Jetten, M., Horn, S., and van Loosdrecht, M. (1997) Towards a More Sustainable Municipal Wastewater Treatment System. *Water Science Technology* **35**(9), 171-180.
40. Jimenez, Jose A., Lamotta, Enrique J., Parker, and Denny S. (2007) Effect of Operational Parameters on the Removal of Particulate Chemical Oxygen Demand in the Activated Sludge Process. *Water Environment Research* **79**(9), 984-990.
41. Jimenez, J.A., La Motta, E.J., and Parker, D.S. (2005) Kinetics of Removal of Particulate Chemical Oxygen Demand in the Activated-Sludge Process. *Water Environment Research* **77**(5), 437-446.
42. Jimenez, J., Miller, M., Bott, C., Murthy, S., De Clippeleir, H., and Wett, B. (2015) High-rate activated sludge system for carbon management – Evaluation of crucial process mechanisms and design parameters. *Water Research,* http://dx.doi.org/10.1016/j.watres.2015.07.032.
43. Jones, P.A., and Shuler, A.J. (2010) Seasonal variability of biomass density and activated sludge settleability in full-scale wastewater treatment systems. *Chemical Engineering* **164**(1), 16-22.
44. Jorand, F., Boué-Bigne, F., Block, J.C., and Urbain, V. (1998) Hydrophobic/hydrophilic properties of activated sludge exopolymeric substances. *Water Science & Technology* **37**(4-5), 307-315.
45. Kher, D., and von der Emde, W. (1960) Experiments on the high-rate activated sludge process. *Water Environment Federation* **32**(10), 1066-1080.
46. Khiewwijit, R., Temmink, H., Rijnaarts, H., and Keesman, K.J. (2015) Energy and nutrient recovery from municipal wastewater treatment: How to design a feasible plant layout?. *Environmental Modelling & Software* **68**, 156-165.
47. Krezeminski, P., Iglesias-Obelleiro, A., Madebo, G., Garrido, J.M., van der Graaf, J.H.J.M., and van Lier, J.B. (2012) Impact of temperature on raw wastewater composition and activated sludge filterability in full-scale MBR systems for municipal sewage treatment. *Membrane Science* **423-424**, 348-361.

48. La Motta, E., Jiménez, J., Parker, D., and McManis, K. (2003a) Removal of particulate COD by bio-flocculation in the activated sludge process. *Water Pollution VII: Modelling, Measuring and Prediction* **65**, 349-358.
49. La Motta, E.J., McCorquodale, J.A., and Rojas, J.A. (2007) Using the kinetics of bio-flocculation and the limiting flux theory for the preliminary design of activated sludge systems. I: Model development. *Environmental Engineering* **133**(1), 104-110.
50. Laspidou, C.S., and Rittmann, B.E. (2002) Non-steady state modeling of extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research* **36**(8), 1983-1992.
51. Lee, S.-H., Kang, H.-J., and Park, H.-D. (2015) Influence of influent wastewater communities on temporal variation of activated sludge communities. *Water Research* **73**, 132-144.
52. Li, X.Y., and Yang, S.F. (2007) Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research* **41**(5), 1022-1030.
53. 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 bio-flocculation and settleability. *Water* Research **35**(2), 339-350.
54. 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. *Colloid and Interface Science* **249**, 372-380.
55. Liu, Y., and Fang, H.P. (2003) Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge. *Environmental Science & Technology* **33**(3), 237-273.
56. Logan, B., and Hunt, J. (1988) Bio-flocculation as a microbial response to substrate limitations. *Biotechnology Bioengineering* **31**(2), 91-101.
57. Lubello, C., Caffaz, S., Gori, R., and Munz, G. (2009) A modified activated sludge model to estimate solids production at low and high solids retention time. *Water Research* **43**, 4539-4548.
58. Majone, M., Dircks, K., and Beun, J.J. (1999) Aerobic storage under dynamic conditions in activated sludge processes. The state of the art. *Water Science & Technology* **39**(1), 61-73.
59. Mamais, D., Jenkins, D., and Pitt, P. (1993) A rapid physical-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater. *Water Research* **27**(1), 195-197.
60. Martins, A.M., Heijnen, J.J., and van Loostredtch, M.C. (2003a) Effect of feeding pattern and storage on sludge settleability under aerobic conditions. *Water Research* **37**, 2555-2570.
61. Martins, A.M., Heijnen, J.J., and van Loostredtch, M.C. (2003b) Effect of dissolved oxygen concentration on sludge settleability. *Applied Microbiology and Biotechnology* **62**, 586-593.
62. McCarty, P.L., Bae, J., and Kim, J. (2011) Domestic Wastewater Treatment as a Net Energy Producer–Can This be Achieved? *Environmental Science & Technology* **45**(17), 71007106.

63. McKinney, R.E. (1960) Complete mixing activated sludge. Water Sewage Works 107(2), 69.
64. Meerburg, F.A., Boon, N., Van Winckel, T., Vercamer, J.A.R., Nopens, I., and Vlaeminck, S.E. (2015) Toward energy-neutral wastewater treatment: A high-rate contact stabilization process to maximally recover sewage organics. *Bioresource Technology* **179**, 373-381.
65. Mikkelsen, L.H., and Keiding, K. (2002a) Physio-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Research* **36**, 2451-2462.
66. Mikkelsen, L.H., and Keiding, K. (2002b) The shear sensitivity of activated sludge: an evaluation of the possibility for a standard floc strength test. *Water Research* **36**, 2931-2940.
67. Miller, M.W., Regmi, P., Jimenez, J., Murthy, S., Wett, B., and Bott, C.B. (2014) Optimizing adsorption-style high rate activated sludge for BNR and energy recovery. *Proceedings of the IWA Specialist Conference Global Challenges: Sustainable Wastewater Treatment and Resource Recovery*, Kathmandu, Nepal, October 26-30, 2014.
68. Miller, M.W. (2015) Optimizing high-rate activated sludge: Organic substrate for biological nutrient removal and energy recovery. Dissertation submitted to Virginia Polytechnical Institute and State University.
69. Morgan, J.W., Forster, C.F., and Evison, L. (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Research* **24**(6), 743-750.
70. Morgenroth, E., Kommedal, R., and Harremoës, P. (2002) Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment -a review. *Water Science & Technology* **45**(6), 25-40.
71. Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., and Carrère, H. (2010) Estimating anaerobic biodegradability indicators for waste activated sludge. *Chemical Engineering Journal* **160**(2), 488-496.
72. Müller-Rechberger, H., Wandl, G., Winkler, S., Svardal, K., and Matsché, N. (2001) Comparison of different operational modes of a two-stage activated sludge pilot plant for the extension of the Vienna STP. *Water Science & Technology* **44**(1), 137-144.
73. Neyens, E., Baeyens, J., Dewil, R., and De heyder, B. (2004) Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *Journal of Hazardous Materials* **106**(2–3), 83-92.
74. Nogaj, T.M., Randall, A.A., Jimenez, J.A., Takács, I., Bott, C.B., Miller, M.W., Murthy, S., and Wett, B. (2013) Mathmatical modeling of carbon removal in the high-rate activated sludge system: model presentation and application. *Proceedings of the 86th Annual Water Environment Federation Technical Exposition and Conference*, Chicago, Illinois.
75. Ni, B.-J., and Yu, H.-Q. (2008) Simulation of heterotrophic storage and growth processes in activated sludge under aerobic conditions. *Chemical Engineering Journal* **140**(1–3), 101-109.

76.Nielsen, P.H., Thomsen, T.R., and Nielsen, J.L. (2004) Bacterial composition of activated sludge – importance for floc and sludge properties. *Water Science & Technology* **49**(10), 51-58.
77.Olofsson, A.C., Zita, A., and Hermansson, M. (1998) Floc stability and adhesion of green-fluorescent-protein-marked bacteria to flocs in activated sludge. *Microbiology* **144**(2), 519-528.
78.Orhon, D., and Cokgör, E.U. (1997) Fractionation in wastewater characterization – the state of the art. *Biotechnology* **68**, 283-293.
79.Owen, W.F. (1982) Energy in wastewater treatment. Prentice-Hall Inc. Englewood Cliffs, NJ.
80.Parker, D.S, Kauffman, W.J., and Jenkins, D. (1970) Characteristics of biological flocs in turbulent regimes. SERL Report No. 70-5, University of California, Berkley.
81.Regmi, P. (2014) Feasibility of Mainstream Nitrite Oxidizing Bacteria Out-Selection and Anammox Polishing for Enhanced Nitrogen Removal. Dissertation submitted to Old Dominion University.
82.Rodriguez, F.A., Reboleiro-Rivas, P., Osorio, F., Martinez-Toledo, M.V., Hontoria, E., and Poyatos, J.M. (2013) Influence of mized liqupr suspended solids and hydraulic retention time on oxygen transfer efficiency and viscosity in a submerged membrane bioreactor using pure oxygen to supply aerobic conditions. *Biochemical Engineering* **60**, 135-141.
83.Sadowski, M.S. (2015) Comparison of aeration strategies for optimization of nitrogen removal in an adsorption/bio-oxidation process with an emphasis on ammonia vs NOx control. Thesis submitted to Virginia Polytechnical Institute and State University.
84.Salem, S., Moussa, M.S., and van Loosdretch, M.C.M. (2005) Determination of the decay rate of nitrifying bacteria. *Biotechnology & Bioengineering* **94**(2), 252-262.
85.Schulze-Rettmer, R., and Zuckut, S.W. (1998) Treatment of textile dyeing wastewater by adsorption/bio-oxidation process. Textile Chemist and Colorist 30(5), 19-23.
86.Sheng, G.-P., Yu, H.-Q., and Li, X.-Y. (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advances* **28**(6), 882-894.
87.Shin, H.-S., Kang, S.-T., and Nam, S.-Y. (2001) Effect of carbohydrate and protein in the EPS on sludge settling characteristics. *Water Science & Technology* **43**(6), 193-196.
88.Siegrist, H., Salzgeber, D., Eugster, J., and Joss, A. (2008) Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Science & Technology* **57**(3), 383-388.
89.Sobeck, D.C., and Higgins, M.J. (2002) Examination of three theories for mechanisms of cation-induced bio-flocculation. *Water Research* **36**(3), 527-538.
90.Stewart, M.J. (1964) Activated sludge process variations: The complete spectrum. *Water and Sewage Works Journal* **111**, 241-262.
91.Sutherland, I.W. (1977) Bacterial exopolysaccharides- their nature and production. In *Surface Carbohydrates of the Prokaryotic Cell*, Academic Press. 27-96.
92.Tchobanoglous, G., Burton, F.L., Sensel, H.D. (2003) Wastewater Engineering: Treatment and Reuse. McGraw-Hill, Boston.
93. Urbain, V., Block, J.C., and Manem, J. (1993) Bio-flocculation in activated sludge: An analytic approach. *Water Research* **27**(5), 829-838.

94.USEPA (2010) Evaluation of energy conservation measures for wastewater treatment facilities. US Environmental Protection Agency, Washington, DC. 20460.
95.Van Dierdonck, J., Van den Broeck, R., Vervoort, E., D'Haeninck, P., Springael, D., Van Impe, J., and Smets, I. (2012) Does a change in reactor loading rate affect activated sludge bio-flocculation? *Process Biochemistry* **47**(12), 2227-2233.
96.Van loodstredtch, M.C.M., and Heijnen, J.J. (2002) Modelling of activated sludge processes with structured biomass. *Water Science Technology* **45**(6), 13-22.
97.Van loodstredtch, M., Norde, W., Lyklema, L., and Zehnder, J. (1990) Hrdrophobic abd electrostatic parameters in bacterial adhesion. *Aquatic Sciences* **51**, 103-114.
98.Walker, L.F. (1971) Hydraulically controlling solids retention time in the activated sludge process. *Water Pollution Control Federation* **43**(1), 30-39.
99.Wang, C., Zheng, Y., Lou, J., and Wu, P. (2007) Dynamic simulation of a WWTP operated at low dissolved oxygen condition by integrating activated sludge model and a floc model. *Biochemical Engineering* **33**, 217-227.
100.Wahlberg, E.J., Keinath, T.M., and Parker, D.S. (1994) Influence of activated sludge flocculation time on secondary clarification. *Water Environment Research* **66**(6), 779-786.
101.Wilén, B.-M., and Balmér, P. (1998) Short term effects of dissolved oxygen concentration on the turbidity of the supernatant of activated sludge. *Water Science & Technology* **38**(3), 25-33.
102.Wilén, B.-M., Jin, B., and Lant, P. (2003a) The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water Research* **37**, 2127-2139.
103.Wingender, J., Jager, H.-C., and Flemming (1999) Interaction between extracellular polysaccharides and enzymes, in : Wingender, J., Neu, T.R (Eds.). Microbial Extracellular Polymeric Substances, Springer, Heidelberg. pp. 1-19.
104.Winkler, H.K., and Widmann, W. (1994) Comparison of single-stage and two-stage activated sludge processes for the expansion of the Innsbruck WWTP. *Water Science & Technology* **29**(12), 69-79.
105.Witzig, R., Manz, W., Rosenberger, S., Kruger, U., Kraume, M., and Szewzyk, E. (2002) Microbiological aspects of a bioreactor with submerged membranes for aerobic treatment of municipal wastewater. *Water Research* **36**, 394-402.
106.WRF and EPRI, 2013. Electricity use and management in the municipal water supply and wastewater industries. Final report.
107.Xie, B., and Yang, S.-F. (2009) Analyses of bio-flocculation and bacterial communities in sequencing batch reactors. *Environmental Engineering Science* **26**(3), 481-487.
108.Xie, B., Gu, J., and Lu, J. (2010) Surface properties of bacteria from activated sludge in relation to bio-flocculation. *Environmental Sciences* **22**(12), 1840-1845.
109.Yang, S.-F., and Li, X.-Y. (2009) Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady state conditions. *Process Biochemistry* **44**, 91-96.
110.Zhang, X., Li, X., Zhang, Q., Peng, Q., Zhang, W., and Gao, F. (2014) New insight into the biological treatment by activated sludge: The role of adsorption process. *Bioresource Technology* **153**, 160-164.

111.Zhao, W., Ting, Y.P., Chen, J.P., Xing, C.H., and Shi, S.Q. (2000) Advanced primary treatment of waste water using a bio-flocculation-adsorption sedimentation process. *Acta Biotechnologica* **20**(1), 53-64.

112.Zielinska, M., Bernat, K., Cydzik-Kwiatkowska, A., and Wojnowska-Baryla, I. (2012) Respirometric Activity of activated sludge in sequencing batch reactor depending on substrate and dissolved oxygen concentration. *Environmental Protection* Engineering **38(**2), 41-49.

113.Zita, A., and Hermansson, M. (1997a) Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system. *Applied Environmental Microbiology* **60**, 3041-3048.

114.Zita, A., and Hermansson, M. (1997b) Effects of bacterial cell surface sturctures and hydrophobicity on attachment to activated sludge flocs. *Applied Environmental Microbiology* **63**(1), 1168-1170.

APPENDIX: I

Pearson Correlation Coefficients (Correlations: Weak < ± 0.399; Mild = ± 0.400-0.799; Strong > ± 0.800)

A.1.1: Correlations between EPS component concentrations (mgCOD/gVSS) and operating conditions/system performance with an average SRT of 0.26 days (STRONG = Highlighted; MILD = Bold).

Parameter	Units	S Pn	LB Pn	TB Pn	Total Pn	LB/TB Pn	S Ps	LB Ps	TB Ps	Total Ps	LB/TB Ps
SRT	Day	-0.306	**-0.481**	**-0.550**	**-0.462**	-0.324	**-0.513**	**-0.510**	**-0.400**	**-0.522**	-0.193
DO	mg/L	-0.428	-0.371	**-0.515**	**-0.447**	-0.156	-0.822	**-0.612**	-0.883	-0.825	0.104
MLSS	mg/L	-0.822	**-0.775**	**-0.775**	-0.870	-0.493	-0.880	-0.857	**-0.722**	-0.900	-0.349
Airflow	SLPM	-0.224	0.240	0.240	0.023	-0.053	-0.053	0.055	0.055	0.043	-0.007
TSS (%)	%	-0.875	-0.923	-0.871	-0.933	**-0.778**	**-0.733**	-0.864	-0.338	**-0.724**	**-0.709**
tCOD (%)	%	-0.834	-0.938	-0.833	-0.915	-0.823	-0.851	-0.973	-0.427	-0.838	**-0.733**
cCOD (%)	%	-0.339	-0.171	-0.231	-0.252	-0.102	**-0.490**	-0.338	**-0.510**	**-0.472**	0.050
pCOD (%)	%	**-0.707**	-0.901	**-0.734**	-0.829	-0.848	**-0.612**	-0.828	-0.133	**-0.601**	-0.830
SVI	mL/g	0.875	0.915	**0.764**	0.900	0.865	**0.727**	0.800	0.241	**0.652**	**0.715**
Parameter	Units	S COD	LB COD	TB COD	Total COD	LB/TB COD	S Pn/Ps	LB Pn/Ps	TB Pn/Ps	Total Pn/Ps	
SRT	Day	**-0.432**	**-0.482**	-0.142	-0.434	-0.399	-0.078	-0.024	0.079	0.115	
DO	mg/L	**-0.644**	-0.388	**-0.503**	**-0.608**	-0.073	**0.657**	**0.484**	**0.661**	**0.585**	
MLSS	mg/L	-0.898	-0.827	**-0.573**	-0.938	**-0.487**	0.282	-0.007	0.090	0.029	
Airflow	SLPM	0.039	0.131	0.131	0.146	0.038	**0.496**	0.081	0.081	-0.059	
TSS (%)	%	**-0.742**	-0.989	**-0.418**	-0.910	**-0.747**	0.022	-0.346	-0.304	-0.324	
tCOD (%)	%	-0.871	-0.892	-0.109	**-0.750**	-0.844	-0.001	-0.155	0.084	-0.122	
cCOD (%)	%	0.221	-0.027	-0.124	-0.186	0.025	0.221	0.279	**0.418**	0.315	
pCOD (%)	%	**-0.653**	-0.883	-0.014	**-0.644**	-0.889	-0.256	-0.376	**-0.408**	-0.341	
SVI	mL/g	**0.597**	0.839	0.124	**0.657**	0.808	0.204	**0.496**	0.309	0.371	

A.1.2: Correlations between EPS component concentrations (mgCOD/gVSS) and operating conditions/system performance with an average SRT of 0.54 days (STRONG = Highlighted; MILD = Bold).

Parameter	Units	S Pn	LB Pn	TB Pn	Total Pn	LB/TB Pn	S Ps	LB Ps	TB Ps	Total Ps	LB/TB Ps
SRT	Day	-0.897	-0.818	-0.910	-0.886	**-0.514**	-0.930	**-0.750**	-0.909	-0.904	-0.195
DO	mg/L	-0.860	-0.874	-0.907	-0.901	**-0.643**	-0.866	-0.845	-0.902	-0.942	-0.356
MLSS	mg/L	-0.854	-0.872	-0.872	-0.909	**-0.612**	**-0.795**	-0.805	-0.887	-0.904	-0.297
Airflow	SLPM	**-0.781**	-0.802	-0.802	**-0.795**	**-0.532**	**-0.753**	**-0.755**	**-0.755**	**-0.761**	-0.195
TSS rem.	%	**-0.579**	**-0.628**	**-0.600**	**-0.621**	**-0.478**	-0.385	**-0.676**	-0.369	**-0.562**	**-0.573**
tCOD rem.	%	**-0.459**	-0.328	-0.344	-0.372	-0.135	-0.301	**-0.428**	-0.098	-0.308	**-0.484**
cCOD rem.	%	**-0.481**	-0.136	**-0.426**	-0.318	0.295	-0.356	0.010	-0.355	-0.194	0.347
pCOD rem.	%	0.025	0.075	0.146	0.089	0.057	0.114	-0.077	0.336	0.116	-0.392
SVI	mL/g	0.365	**0.488**	0.353	**0.425**	**0.534**	0.153	0.395	0.131	0.283	**0.443**
Parameter	Units	S COD	LB COD	TB COD	Total COD	LB/TB COD	S Pn/Ps	LB Pn/Ps	TB Pn/Ps	Total Pn/Ps	
SRT	Day	-0.854	**-0.740**	-0.813	-0.857	-0.361	-0.058	-0.316	0.109	-0.263	
DO	mg/L	-0.882	-0.830	-0.911	-0.949	**-0.412**	-0.050	-0.235	0.132	-0.176	
MLSS	mg/L	-0.901	-0.824	-0.921	-0.953	-0.398	-0.253	-0.360	0.015	-0.346	
Airflow	SLPM	**-0.756**	**-0.694**	**-0.694**	**-0.751**	-0.362	-0.165	-0.004	-0.004	-0.379	
TSS rem.	%	**-0.695**	**-0.683**	**-0.487**	**-0.667**	**-0.489**	**-0.567**	0.079	-0.506	**-0.419**	
tCOD rem.	%	**-0.517**	-0.362	-0.197	-0.350	-0.243	**-0.425**	0.329	-0.597	-0.300	
cCOD rem.	%	**-0.478**	0.038	**-0.501**	-0.261	**0.458**	-0.343	**-0.481**	-0.165	**-0.466**	
pCOD rem.	%	0.000	-0.024	0.318	0.120	-0.195	-0.235	**0.554**	**-0.525**	-0.046	
SVI	mL/g	**0.435**	**0.418**	0.276	**0.400**	0.361	**0.423**	0.231	**0.434**	**0.501**	

A.1.3. Correlations between EPS component concentrations (mgCOD/gVSS) and operating conditions/system performance using all data from both SRTs combined (STRONG = Highlighted; MILD = Bold).

Parameter	Unit	S Pn	LB Pn	TB Pn	total Pn	LB/TB Pn	S Ps	LB Ps	TB Ps	total Ps	LB/TB Ps
SRT	Day	**-0.690**	**-0.449**	**-0.797**	**-0.663**	**0.049**	**-0.578**	**-0.663**	**-0.665**	**-0.704**	-0.353
DO	mg/L	**-0.563**	**-0.651**	**-0.630**	**-0.652**	**-0.403**	**-0.794**	**-0.655**	-0.823	**-0.797**	-0.118
Airflow	SLPM	0.046	-0.249	0.100	-0.056	**-0.459**	-0.025	0.113	0.011	0.059	0.168
MLSS	mg/L	-0.819	-0.829	-0.920	-0.901	**-0.424**	**-0.785**	-0.817	-0.833	-0.884	-0.369
TSS rem.	%	**-0.792**	**-0.704**	**-0.732**	**-0.776**	**-0.414**	**-0.648**	-0.807	**-0.444**	**-0.702**	**-0.685**
tCOD rem.	%	**-0.483**	-0.169	**-0.449**	-0.365	0.223	**-0.450**	-0.230	**-0.471**	-0.380	0.116
cCOD rem.	%	**-0.750**	**-0.562**	**-0.641**	**-0.673**	-0.258	**-0.697**	**-0.780**	**-0.407**	**-0.682**	**-0.665**
pCOD rem.	%	**-0.502**	-0.385	-0.374	**-0.435**	-0.248	**-0.419**	**-0.567**	-0.071	-0.393	**-0.675**
SVI	mL/g	-0.083	0.287	-0.145	0.046	0.587	-0.047	-0.058	-0.192	-0.114	0.052
Parameter	Unit	S COD	LB COD	TB COD	Total COD	LB/TB COD	S Pn/Ps	LB Pn/Ps	TB Pn/Ps	total Pn/Ps	
SRT	Day	**-0.692**	**-0.502**	**-0.703**	**-0.710**	-0.052	-0.256	**0.455**	-0.132	0.012	
DO	mg/L	**-0.617**	**-0.608**	**-0.606**	**-0.701**	-0.247	0.360	0.016	**0.446**	0.279	
Airflow	SLPM	0.211	-0.075	0.185	0.093	-0.240	0.157	**-0.642**	0.172	-0.186	
MLSS	mg/L	-0.810	-0.820	**-0.791**	-0.929	-0.354	-0.148	-0.009	-0.016	-0.123	
TSS rem.	%	**-0.778**	-0.820	**-0.555**	-0.814	**-0.507**	-0.380	0.194	**-0.405**	-0.292	
tCOD rem.	%	**-0.494**	-0.048	**-0.436**	-0.327	0.311	-0.110	-0.009	0.072	-0.035	
cCOD rem.	%	-0.824	**-0.641**	-0.361	**-0.649**	**-0.429**	-0.214	0.381	-0.326	-0.114	
pCOD rem.	%	**-0.540**	**-0.503**	-0.089	-0.398	**-0.475**	-0.245	0.334	**-0.474**	-0.199	
SVI	mL/g	-0.169	0.145	-0.266	-0.087	0.384	-0.117	**0.624**	0.042	0.293	

A.1.4. Correlations between influent wastewater characteristics and operating conditions on overall system performance classified by the applied DO concentration at a 0.54-day SRT (STRONG = Highlighted; MILD = Bold).

Category	Parameter	Unit	Effluent			Removals				
			SVI (mL/g)	C/N (mg/mg)	TSSsd (mg/L)	TSS (%)	tCOD (%)	cCOD (%)	pCOD (%)	sCOD (%)
All data for HRT = 60 min	Inf. TSS	mg/L	0.085	0.033	-0.114	**0.408**	**0.561**	**0.632**	**0.411**	-0.087
	Inf. tCOD	mg/L	0.243	-0.243	-0.190	**0.564**	**0.546**	**0.407**	**0.485**	-0.044
	Airflow	SLPM	-0.628	0.104	-0.119	0.007	-0.140	**0.497**	**-0.417**	0.057
	DO	mg/L	-0.382	-0.200	**-0.485**	0.255	0.165	**0.519**	-0.170	0.261
	MLSS	mg/L	-0.169	-0.240	**-0.467**	**0.407**	**0.400**	0.810	0.142	-0.213
	SRT	Day	-0.267	-0.025	-0.193	-0.042	-0.167	0.296	-0.270	-0.289
High_0.5	Inf. TSS	mg/L	**-0.494**	**0.554**	0.322	0.103	0.269	0.922	0.077	**-0.720**
	Inf. tCOD	mg/L	**-0.630**	**0.418**	**0.614**	0.100	0.291	0.355	0.161	-0.117
	Airflow	SLPM	**-0.591**	**0.423**	-0.109	0.083	-0.089	**0.795**	-0.131	**-0.536**
	DO	mg/L	-	-	-	-	-	-	-	-
	MLSS	mg/L	0.215	0.307	0.282	-0.171	0.043	**0.453**	-0.126	**-0.687**
	SRT	Day	**0.662**	-0.226	**-0.492**	0.060	0.296	0.175	0.158	-0.317
High_1.0	Inf. TSS	mg/L	**0.514**	-0.218	**-0.585**	**0.632**	**0.683**	**0.673**	**0.640**	**0.542**
	Inf. tCOD	mg/L	**0.514**	-0.363	**-0.614**	**0.697**	**0.635**	**0.625**	**0.525**	**0.563**
	Airflow	SLPM	**-0.661**	**0.642**	**0.593**	-0.287	**-0.468**	**0.453**	**-0.653**	-0.878
	DO	mg/L	-	-	-	-	-	-	-	-
	MLSS	mg/L	**-0.432**	0.032	-0.062	0.332	0.103	0.828	-0.179	-0.267
	SRT	Day	**-0.771**	**0.476**	**0.520**	-0.289	**-0.567**	-0.051	**-0.585**	**-0.781**
High_1.5	Inf. TSS	mg/L	**0.564**	0.364	0.382	-0.194	0.238	0.045	0.182	0.063
	Inf. tCOD	mg/L	-0.265	-0.240	**0.503**	0.378	-0.317	-0.056	-0.272	-0.059
	Airflow	SLPM	-0.133	-0.111	**0.541**	-0.070	-0.049	0.242	-0.100	-0.184
	DO	mg/L	-	-	-	-	-	-	-	-
	MLSS	mg/L	-0.309	-0.012	-0.135	**-0.470**	-0.158	0.955	-0.248	**-0.559**
	SRT	Day	-0.209	**-0.440**	-0.362	0.100	-0.111	**0.549**	-0.094	**-0.786**

A.1.5. Correlations between influent wastewater characteristics and operating conditions on overall system performance classified by the applied DO concentration at a 0.26-day SRT (STRONG = Highlighted; MILD = Bold).

Category	Parameter	Unit	Effluent			Removals				
			SVI (mL/g)	C/N (mg/mg)	TSSsd (mg/L)	TSS (%)	tCOD (%)	cCOD (%)	pCOD (%)	sCOD (%)
All data for HRT = 30 min	Inf. TSS	mg/L	-0.235	-0.069	0.226	**0.456**	**0.531**	**0.414**	**0.460**	0.286
	Inf. tCOD	mg/L	-0.067	-0.207	0.028	**0.567**	0.385	0.380	**0.416**	-0.249
	Airflow	SLPM	-0.298	0.102	0.010	0.028	-0.060	0.134	-0.060	**0.480**
	DO	mg/L	**-0.425**	**-0.649**	**-0.484**	**0.443**	**0.507**	**0.422**	0.338	**0.614**
	MLSS	mg/L	**-0.556**	**-0.697**	**-0.595**	**0.622**	**0.579**	0.063	**0.553**	0.202
	SRT	Day	-0.318	-0.136	-0.164	0.190	0.159	-0.168	0.209	0.112
Low_0.5	Inf. TSS	mg/L	**-0.645**	**0.566**	**0.694**	**0.427**	0.258	-0.277	0.251	**-0.573**
	Inf. tCOD	mg/L	-0.348	**0.607**	0.884	0.168	-0.025	-0.813	-0.008	0.118
	Airflow	SLPM	-0.370	**0.687**	**0.425**	0.135	-0.271	-0.316	-0.268	-0.261
	DO	mg/L	-	-	-	-	-	-	-	-
	MLSS	mg/L	-0.151	**-0.760**	-0.361	**0.426**	**0.485**	0.945	**0.541**	-0.375
	SRT	Day	**-0.632**	**-0.491**	-0.134	**0.599**	0.823	0.962	**0.716**	-0.271
Low_1.0	Inf. TSS	mg/L	**0.575**	-0.150	-0.036	0.144	**0.558**	0.811	0.293	0.047
	Inf. tCOD	mg/L	**0.428**	**-0.503**	-0.312	**0.790**	**0.600**	0.800	0.195	0.156
	Airflow	SLPM	**-0.419**	**0.698**	-0.148	-0.218	**-0.526**	**-0.445**	-0.376	**-0.688**
	DO	mg/L	-	-	-	-	-	-	-	-
	MLSS	mg/L	-0.007	0.204	**-0.537**	-0.195	-0.415	**-0.711**	-0.355	**-0.439**
	SRT	Day	**-0.530**	0.252	0.090	-0.202	-0.331	**-0.767**	0.082	-0.073
Low_1.5	Inf. TSS	mg/L	-0.163	-0.262	0.266	0.280	**0.630**	-0.133	0.385	**0.682**
	Inf. tCOD	mg/L	0.304	**-0.567**	-0.218	**0.642**	0.197	-0.289	**0.580**	-0.317
	Airflow	SLPM	-0.355	-0.201	0.100	0.169	0.329	0.137	0.222	0.321
	DO	mg/L	-	-	-	-	-	-	-	-
	MLSS	mg/L	-0.297	-0.319	-0.325	**0.408**	-0.059	-0.819	0.199	0.383
	SRT	Day	-0.024	-0.044	**-0.607**	0.257	-0.209	**-0.719**	-0.070	0.156

VITA

Matthew S. Elliott

Department of Civil and
Environmental Engineering
Old Dominion University
Norfolk, VA 23529

Employment

HRSD Graduate Student Research Intern (May 2013 – June 2016)
HRSD Research Intern (Sept. 2012 – May 2013)
Norfolk Yacht and Country Club Swim Coach (May 2007 – Aug. 2016)
Norfolk Collegiate School Swim Coach (Oct. 2012 – March 2014)
Norfolk Collegiate School Baseball Coach (March 2013 – June 2015)
1-800-GOT-JUNK? Sales Associate and Customer Service (June 2006 – Oct. 2008)
VIRTEXCO Construction Laborer (June 2005 – August 2005)

Education

M.S., Old Dominion University, Environmental Engineering, Graduate GPA: 3.42
Tentative Completion: August, 2016
Co-Directors: Charles Bott, Gary Schafran, and Peter Pommerenk
Topic: Impacts of Operating Parameters on Extracellular Polymeric Substances Production in a High-Rate Activated Sludge System with Low Solids Retention Times.

B.S., Virginia Military Institute, Biology, Undergraduate GPA: 3.56

Honors and Awards

Beta Beta Beta, Biological Honors Society
Honor Court Prosecutor, VMI (2011 - 2012)
Honor Court Assistant Prosecutor, VMI (2010 - 2011)

Publications and Presentations

Elliott, M.S., Kinyua, M.N., Wett, B., Murthy, S., Chandran, K., Bott, C.B. (2016) **Effect of Operating Parameters on the Production of Extracellular Polymeric Substances in a High Rate Activated Sludge System.** Abstract accepted for the 89th Annual Water and Environment Federation Technical Exposition and Conference (WEFTEC) 2016 to be held in New Orleans, LA September 24.