Investigation of the Impacts of Thermal Activated Sludge Pretreatment and Development of a Pretreatment Model

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Waste activated sludge (WAS) pretreatment technologies are typically evaluated in terms of the associated improvement in biogas and sludge production during digestion and post-digestion dewaterability. However, WAS properties, and hence the impact of pretreatment on WAS properties, are dependent upon the raw wastewater composition and configuration of the wastewater treatment plant (WWTP). A generally accepted means of characterizing and comparing all pretreatment processes does not exist. The motivation for this project was to evaluate the impact of pretreatment on WAS properties in terms of changes in COD fractionation. The first objective of this study was to fractionate the COD of the WAS before and after pretreatment to show how pretreatment may increase the rate and extent of aerobic digestion. The second objective was to develop a COD-based stoichiometric pretreatment model that may be integrated into WWTP simulations.

A bench-scale biological reactor (BR) with a solids retention time (SRT) of 5 days was started up with WAS from the Waterloo WWTP. The BR was fed daily with a completely biodegradable synthetic substrate so that the BR WAS contained only biomass and decay products after 3 SRTs of operation. In the first phase of the study, an aerobic digester (AD) with a SRT of 10 d was fed daily with BR WAS. The BR-AD system was operated at steady state for one month. A range of physical and biochemical properties were regularly measured in each process stream. Offline respirometric tests were regularly conducted to determine the aerobic degradability and fractionate the COD of the BR and AD WAS. The oxygen uptake rate (OUR) associated with the daily addition of BR WAS to the AD was determined as an additional measurement of the aerobic degradability of the BR WAS.

In the second phase of the study, the BR WAS was pretreated prior to being fed daily to the AD. High pressure thermal hydrolysis (HPTH) pretreatment was selected for this project since it is one of the most popular and promising pretreatment techniques. A sealed volume of BR WAS was heated to 150°C at 3 bars for 30 minutes. The same physical, biochemical and biological tests used to characterize the process streams in Phase 1 were employed to characterize those in Phase 2. The Phase 2 system was operated for two months at steady-state.

The results of several independent tests showed that the COD of the BR WAS was comprised of storage products (X_{STO}) in addition to active heterotrophs (Z_{bh}) and decay products (Z_e). However, it was shown that the AD WAS only contained Z_{bh} and Z_e as X_{STO} was depleted in the AD.

HPTH pretreatment did not reduce the TCOD concentration of the WAS however it did solubilize 56 ± 7% of COD, 49% ± 11% of organic nitrogen, 56 ± 10% of VSS and did not solubilize ISS. Furthermore, pretreatment did not generate soluble non-biodegradable COD. These findings were consistent with prior research on HPTH WAS pretreatment.

Pretreatment increased the rate at which the BR WAS was aerobically degraded. The offline respirometric tests showed that the pretreated BR WAS contained a substantial amount of readily biodegradable COD (S_{bsc}). However, pretreatment did not increase the extent of biodegradation. The results of several independent tests showed that the non-biodegradable COD component of the BR WAS, i.e. Z_e, was not converted to biodegradable COD by pretreatment.

A COD-based stoichiometric pretreatment model was developed for the dose of HPTH pretreatment employed in this study. When this model was integrated into BioWin®, it was able to accurately simulate both the steady state performance of the overall system employed in this study as well as dynamic respirometry results. The experimental results showed that the TCOD of the BR WAS consisted of 51% Z_{bh}, 12% Z_e and 37% X_{STO} and the pretreated BR WAS consisted of 12% Z_e and a negligible amount of Z_{bh}. The pretreatment model verified these fractions and predicted that the pretreated BR WAS also contained 54% S_{bsc} and 32% slowly biodegradable COD (X_{sp}). The approach described in this study may be followed to determine the impacts of pretreatment on Z_{bh}, Z_e and X_{STO} when other doses of HPTH pretreatment and other pretreatment techniques are employed.

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List of Abbreviations

AD	Aerobic Digester
ADM1	Anaerobic Digestion Model Number 1
ASM3	IWA Activated Sludge Model Number 3
AS	Activated Sludge
ASM	IWA Activated Sludge Model
ASM1	IWA Activated Sludge Model Number 1
bCOD	Biodegradable COD
b_h	Aerobic Decay Rate of Z_{bh}
BR	Biological Reactor
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substances
ffCOD	Filtered and Flocculated COD
HPTH	High Pressure Thermal Hydrolysis
HRT	Hydraulic Retention Time
ISS	Inorganic Settleable Solids
IWA	International Water Association
LCFA	Long Chain Fatty Acid
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
MOE	Ontario Ministry of the Environment
nbCOD	Non-Biodegradable COD
NH_3**-N**	Ammonia Nitrogen
NO_3**-N**	Nitrate Nitrogen
NOUR	Nitrogenous Oxygen Uptake Rate
ΣOU	Cumulative Oxygen Uptake
OUR	Oxygen Uptake Rate
PAA	Peracetic acid
PAO	Phosphate Accumulating Organism
PCOD	Particulate Chemical Oxygen Demand
PHA	Poly-hydroxy-alkanoates
PS	Primary Sludge
PT	Pretreatment
RAS	Return Activated Sludge
rbCOD	Readily Biodegradable COD

sbCOD	Slowly Biodegradable COD
SBR	Sequencing Batch Reactor
S_{bsa}	Readily Biodegradable COD (acetate)
S_{bsc}	Readily Biodegradable COD (complex)
SCa	Dissolved Calcium Concentration
sCOD	Soluble COD
SMg	Dissolved Magnesium Concentration
S_{phb}	Stored COD
SRT	Solids Residence Time
sON	Soluble Organic Nitrogen
SRT	Solids Residence Time
SST	Secondary Settling Tank
sTKN	Soluble Total Kjeldahl Nitrogen
S_{us}	Soluble Inert COD
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphorous
TSS	Total Settleable Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Settleable Solids
WAS	Waste Activated Sludge
WW	Wastewater
X_i	Particulate Inert COD
X_{sc}	Slowly Biodegradable COD (colloidal)
X_{sp}	Slowly Biodegradable COD (particulate)
X_{STO}	Stored COD
Y_h	Aerobic Yield of Z_{bh}
Z_{bh}	Heterotrophic Microorganisms
Z_e	Endogenous Products
μ_{max}	Specific Maximum Growth Rate

1. Introduction

The main by-product of biological wastewater treatment is waste activated sludge (WAS). The use of biological wastewater treatment and hence the generation of WAS is increasing with population growth and the need to sustain water resources (Reynolds and Richards, 1996). At the same time, sludge disposal to agricultural lands is becoming increasingly limited as sanitation standards become more stringent (Henze et al., 2008). The sludge must be adequately stabilized and sterilized and its production minimized.

WAS is typically stabilized by biological digestion to reduce pathogens, eliminate offensive odours and reduce the potential for putrefaction (Tchobanoglous et al., 2003). In a time of continually rising energy costs, anaerobic digestion is preferred over aerobic digestion because it requires substantially less energy and generates methane which can be used as fuel. Furthermore, anaerobic digestion produces a lower biomass yield than aerobic digestion, thereby reducing the energy demand associated with further processing or transporting the sludge. However the significant disadvantage of anaerobic digestion is that it requires a long digestion time. The anaerobic digestion process is composed of four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Henze et al., 2008). The hydrolytic reactions are considered to be the rate limiting step in the overall process (Li and Noike, 1992; Shimizu et al., 1993).

Recent research has shown that various WAS pretreatment technologies have the potential to enhance the digestibility of the sludge, thereby reducing the required anaerobic digestion time. In general, pretreatment causes the bacterial membranes in the WAS to rupture. As a result, organic substances and nutrients are released. These organics are more easily hydrolyzed (Wang et al., 1999). A wide range of promising WAS pretreatment methods are currently being developed and a growing number of full-scale installations exist. These technologies can be broadly categorized as thermal (Camacho et al., 2002; Donoso-Bravo et al., 2010 b; Tattersall et al., 2011), chemical (Deleris and Rouston, 2000; Saby et al., 2002; Tanaka and Kamiyama, 2002; Appels et al., 2010), biological (Neyens and Baeyens, 2003) or mechanical (Tiehm et al., 2001; Chu et al., 2002; Braguglia et al., 2008) disintegration, with some technologies falling under more than one category (Musser, 2009).

In order to characterize the impact of pretreatment on WAS, a variety of indicators have been employed. Many of these indicators are based on the associated improvement in plant performance. For example, pretreatment has been shown to enhance methane generation during anaerobic digestion

(Wang et al., 1995). These plant-performance indicators are however, heavily dependent on the raw sludge composition and configuration of WWTPs. Hence it is difficult to apply the results of existing pretreatment studies to waste streams different from the stream used in the original study. Furthermore, it has been shown that some pretreatment doses such as ozonation and sonication are challenging to quantify (Musser, 2009). A generally accepted means of characterizing and comparing all pretreatment processes does not exist.

An essential design tool of biological wastewater treatment is the activated sludge model. The International Water Association (IWA) has sponsored the development of standardized activated sludge model platforms. The first activated sludge model, the IWA Activated Sludge Model Number 1 (ASM1), was developed in 1987. Since then increasingly more complex activated sludge models have been designed (Henze et al., 2008). The concentrations of all organic components in the IWA activated sludge models are given in units of chemical oxygen demand (COD). The COD is a consistent measure of the energy that is present in the wastewater. In the ASMs, the organic wastewater constituents are separated into biodegradable versus non-biodegradable and soluble versus particulate COD fractions. The development of a COD-based model that could describe WAS pretreatment would provide a means of comparing all types and doses of WAS pretreatment.

1.1 Motivation

WAS pretreatment is typically evaluated in terms of the associated improvement in sludge digestion and biogas production. However, WAS properties and hence the impact of pretreatment on WAS properties depend on the raw sludge composition and configuration of WWTPs. A generally accepted means of characterizing and comparing all pretreatment processes does not exist. The motivation for this project was to show the potential of using COD fractionation as a universal characterizer of WAS pretreatment.

The most extensively researched and widely used WAS pretreatment technique is thermal pretreatment, in particular high pressure thermal hydrolysis (HPTH). Full-scale installations of this type have been successfully used for more than a decade (Tattersall et al., 2011). This type of pretreatment has the potential to produce Class A biosolids as defined by United States CFR 40 Part 503.32 (USEPA, 1999). Class A biosolids contain no detectable levels of pathogens. In general, Class A biosolids may be used in small quantities by the public without buffer requirements or

restrictions on crop type, crop harvesting or site access. HPTH pretreatment is of great interest to the wastewater industry because it holds the combined benefits of decreasing the biosolids production while improving the acceptability of biosolids (Wilson and Novak, 2009). Based on its popularity and proven potential, HPTH pretreatment was selected to be used in this project.

1.2 Objectives

This project aimed to:

- Fractionate the COD of raw and pretreated WAS using analytical and bioassay methods to show how pretreatment may increase the rate and extent of aerobic digestion.
- Develop a COD-based pretreatment model.
- Identify any limitations of the pretreatment model and recommend ways to improve the model

1.3 Scope

This project investigated the impacts of thermal waste activated sludge (WAS) pretreatment in a bench-scale system and the experimental results were used to develop a pretreatment model. The scope of this project included:

- Operation of a bench-scale biological reactor (BR) initially seeded with activated sludge from the Waterloo WWTP, and then fed daily with synthetic wastewater to generate a stable source of WAS
- Operation of a bench-scale aerobic digester (AD) fed daily with BR WAS in Phase 1, and then fed daily with thermally pretreated BR WAS in Phase 2
- Characterization of the raw and pretreated BR WAS and the Phase 1 and 2 AD WAS with respect to pH, suspended solids and COD and nitrogen species
- Assessment of the biodegradability and concentrations of active biomass and endogenous decay products of the various sludge streams by online and offline respirometric methods
- Simulation of the Phase 1 and 2 systems using an activated sludge model calibrated with measured data

- Development of a COD-based thermal pretreatment model and integration of the model into a wastewater treatment plant (WWTP) simulator

2. Background

2.1 Thermal Pretreatment

2.1.1 Introduction

Thermal pretreatment has been studied using a wide range of temperatures ranging from 60°C to 270°C (Climent et al., 2007). Pretreatment at temperatures under 100°C is considered low temperature (LT) pretreatment. Pretreatment at temperatures above 100°C is accompanied by elevated pressure and is referred to as high pressure thermal hydrolysis (HPTH).

Research suggests that LT pretreatment enhances the bioactivity of some thermophilic bacterial populations with an optimum activity at temperatures around 70°C (Nielsen et al., 2004). Although LT pretreatment requires considerably less energy input than HPTH, previous studies have shown that LT pretreatment is ineffective in improving sludge digestion. Nielsen et al. (2011) reported that pretreatment at 80°C had no effect on methane yield in an anaerobic digester. In a study carried out by Prorot et al. (2011), samples were tested at a constant heating time of 20 minutes while the heating temperature was varied from 20°C to 95°C. These researchers found that the cumulative biogas production in anaerobic conditions was very similar for both untreated and heat treated samples. Therefore, low temperature heat treatment did not seem to improve the anaerobic biodegradability of the sludge.

HPTH pretreatment is much more promising than LT pretreatment. HPTH disintegrates cells which releases intracellular matter that is more accessible to anaerobic microorganisms (Gurieff et al., 2011). This in turn reduces the sludge viscosity which eases the mixing of the digester. Without good mixing, the solids settle in the digester and hence reduce the active volume. Reducing the sludge viscosity therefore allows digesters to be operated at substantially higher organic loading rates (Morgan-Sagasume et al., 2010). Furthermore HPTH produces volatile fatty acids (VFAs) and it has been found that the ratio of acetic to propionic acid in fermented HPTH is very similar to that needed for biological phosphorous removal. Thus the dewatered digested sludge could potentially be recycled to the anaerobic reactor to promote the growth of polyphosphate accumulating organisms (Morgan-Sagasume et al., 2010).

HPTH is becoming established worldwide as an effective sludge pretreatment technique due to its capacity to deliver Class A biosolids and enhance VSS reduction and methane production (Tattersall et al., 2011). Producing Class A biosolids, as defined by the USEPA, provides more diverse disposal options (USEPA, 1999). Several studies have shown that the increase in biogas production could be sufficient to preheat the WAS and heat the anaerobic digesters (Bougrier et al., 2007; Phothilangka et al., 2008; Gurieff et al., 2011). The high temperatures are typically applied by heat exchangers or steam injection (Climent et al., 2007). All these listed benefits provide significant operational cost savings. The remainder of this chapter will focus on HPTH pretreatment, the selected type of pretreatment for this project.

2.1.2 Doses

Most HPTH pretreatment methods involve heating the sludge to temperatures in the range of 140 to 200°C under a corresponding pressure of 3 to 25 bars. Numerous studies have shown that within this temperature range, the duration of heating has less effect on the sludge properties than the treatment temperature (Bougrier et al., 2007; Climent et al., 2007). The common heating time for HPTH pretreatment is 30 to 60 minutes. Dwyer et al. (2008) showed that the organic matter became more solubilized when the reaction temperature was increased from 140°C to 165°C. However, they monitored the soluble residuals after digestion, overall methane formation and hydrolysate organic acid concentration and showed that the additional solubilized materials were not degradable. Furthermore, the degradation rate did not change substantially over the range 140°C to 165°C (Dwyer et al., 2008). Several reports have shown that HPTH pretreatment polymerizes low molecular weight intermediate compounds such as carbohydrates and amino acids to produce coloured recalcitrant refractory compounds (Bougrier et al., 2007; Climent et al., 2007; Dwyer et al., 2008; Donoso-Bravo et al., 2010 b). Studies have reported that refractory compounds begin to form at temperatures as low as 150°C to as high as 190°C. These refractory compounds are undesirable because they will contribute soluble non-biodegradable COD that could add to the treatment plant effluent stream if dewatering streams are recycled.

HPTH pretreatment techniques have been commercialized and are in full-scale operation (Morgan-Sagasume et al., 2010). The most well-known is CAMBI^{TM} which has more than 25 installations worldwide (Abu-Orf and Goss, 2011). CAMBI^{TM} reactors are operated in batch mode whereby the sludge temperature is initially heated to 80°C, followed by thermal hydrolysis at 165°C at 7 bars for

30 minutes. The sludge then enters a flash tank where the steam is released and used to preheat the raw sludge (Abu-Orf and Goss, 2011). Besides CAMBI™, the only other commercial HPTH pretreatment technique that has been proven to produce Class A biosolids is Exelys™. Up to now, only one full-scale Exelys™ pilot plant is in operation (at Hillerod, Denmark). The Exelys™ process operates at the same temperature, pressure and retention time as CAMBI™. However unlike CAMBI™, Exelys™ is a continuous plug flow system that uses a series of batch tanks and does not include a flash period (Gurieff et al., 2011). It has been suggested that the flash period assists in disintegrating the sludge however Gurieff et al. (2011) showed that eliminating the flash period did not affect the COD solubilization.

Based on the results of existing HPTH pretreatment research, a conservative temperature of 150°C was selected for this study with a corresponding pressure of 3 bars. Heating the sludge at this temperature was expected to improve the degradability while minimizing the generation of soluble non-biodegradable COD. The selected heating duration was 30 minutes which is similar to the CAMBI™ and Exelys™ processes.

2.1.3 Physical Properties

In the current project, the impact of HPTH pretreatment on the physical properties of WAS was assessed by monitoring the TSS and VSS. This section presents a brief literature review of the impact of HPTH pretreatment on these parameters.

Bougrier et al. (2008) pretreated five different WAS samples for 30 minutes at temperatures ranging from 90 to 210°C in a laboratory autoclave. The authors showed that the VSS/TSS ratio decreased with the treatment temperature indicating that the particulates became more mineral in nature. At 150°C, the average VSS/TSS ratio was 72% whereas the ratio was 81% for untreated sludge. In another study, the performance of the CAMBI™ process was evaluated at three full-scale WWTPs (Morgan-Sagasume et al., 2010). The authors showed that the CAMBI™ process caused a decrease in TSS of 20 to 30% demonstrating that HPTH pretreatment solubilizes suspended matter. In a third study, Gurieff et al. (2011) operated an Exexlys™ pilot plant over a 9 month period and showed that the average VSS solubilization was 31%. In this study, the VSS solubilization was calculated by equation 2.1:

$$VSS\ solubilization\ \% = \frac{VSS_i - VSS_f}{TS} \times 100\% \quad (2.1)$$

In equation 2.1, VSS_i and VSS_f were the respective VSS concentrations before and after pretreatment and TS was the total solids concentration.

2.1.4 Biochemical Properties

In the current project, pH, COD and nitrogen species were measured in order to assist in fractionating the COD of the WAS before and after pretreatment. Recently published related studies have considered the impact of HPTH pretreatment on these particular biochemical indicators. The extent of solubilization of COD and nitrogen species may be used to assess the impact of pretreatment on the biodegradability of WAS (Kianmehr, 2010). Solubilized materials are potentially more easily hydrolyzed than particulate materials. The results of four HPTH studies are compared here.

The desired pH range for anaerobic digestion is 6.6 to 8.2 (Parker, 2010). Therefore it is important to assess the impact of HPTH pretreatment on the pH of WAS to determine whether pH adjustment is required before feeding the WAS to the anaerobic digester. Bougrier et al. (2008) showed that the pH of the pretreated WAS increased from 6.9 at 90°C to 7.3 at 150°C then decreased to 6.8 at 170°C. The authors postulated that the pH increase was due to protein desorption or acidic compound volatilization. They suggested that the pH decrease could be linked to the degradation of macromolecules into acidic compounds. Morgan-Sagasume et al. (2010) showed that the CAMBI™ process slightly decreased the pH of the WAS from an average of 6.7 to 6.2. The results of these two studies indicate that HPTH at temperatures around 165 to 170°C slightly decreases the pH of the WAS.

By far the most common indicator employed by the HPTH pretreatment studies referenced in this project is the COD solubilization caused by pretreatment. In the four studies presented below, COD solubilization was calculated with equation 2.2:

$$COD\ solubilization\ \% = \frac{SCOD_f - SCOD_i}{PCOD_i} \times 100\% \quad (2.2)$$

In this formula, $SCOD_i$ and $SCOD_f$ were the respective SCOD concentrations measured before and after pretreatment and $PCOD_i$ was the initial particulate COD. Bougrier et al. (2008) showed that COD solubilization increased linearly with temperature from 90°C up to 200°C. The reported average COD solubilization at 150°C was 40%. Donoso-Bravo et al. (2010 b) carried out lab-scale and pilot-

scale HPTH pretreatment experiments on WAS in which the temperature and pressure were held constant at 170°C and 8 bars respectively while the contact time was varied. This study showed that the SCOD concentration increased with contact time up to 15 minutes but there were no additional changes for a contact time of 30 minutes. The COD solubilization was 45% at 30 minutes. In a CAMBI^{TM} pretreatment study, Morgan-Sagasume et al. (2010) reported that the average COD solubilization was 39%. These authors also reported that both the TCOD and TN concentrations remained unchanged by pretreatment, indicating that no significant degradation or removal of organic matter occurred. Therefore, the HPTH process did not diminish the available resource for methane production. In an Exexlys^{TM} pretreatment study, Gurieff et al. (2011) reported that the average COD solubilization was 28%. Hence it can be observed that the COD solubilization results of these four studies were comparable and ranged from 28 to 45%.

In terms of changes in the fractionation of nitrogen compounds, Bougrier et al. (2008) reported that the ammonia concentration increased with temperature up to 90°C, then remained constant at higher temperatures. During thermal pretreatment, proteins may be broken down to polypeptides which may in turn be broken down to amino acids. The amino acids may then be mineralized, i.e. cleaved to release ammonia. The results of Bougrier et al. (2008) indicate LT pretreatment mineralized proteins. It is highly likely that further protein degradation occurred at the higher temperatures tested however mineralization did not occur above 90°C. Donoso-Bravo et al. (2010 b) reported that HPTH only slightly increased the ammonia concentration, demonstrating marginal protein mineralization. Morgan-Sagasume et al. (2010) found that the mass of total nitrogen per mass of total solids in the sludge remained constant during thermal pretreatment. Because the mass of TCOD per mass of total solids and mass of total phosphorous per mass of total solids also remained constant during pretreatment, these authors concluded that no significant degradation of organic matter occurred. As previously discussed, these same studies showed that HPTH solubilized COD. It can therefore be concluded that proteins were solubilized rather than mineralized by HPTH.

2.1.5 Biological Properties

The biological properties of WAS such as the activity of the biomass and biodegradability of the sludge are expected to be impacted by pretreatment. In this part of the literature review, the biological impacts of HPTH pretreatment are discussed and the indicators that describe such impacts are introduced.

2.1.5.1 Activity of Bacteria

The inactivation of bacteria in WAS caused by HPTH may be indicative of the conversion of biomass into a more readily biodegradable form (Kianmehr, 2010). Disrupted, dead and inactive biomass may be disintegrated and hydrolyzed easier than active biomass. The inactivation of biomass may also change the nature of the flocs and facilitate their disintegration. Donoso-Bravo et al. (2010 b) measured the total coliforms in the sludge before and after pretreatment at 170°C and 8 bars over a range of contact periods. They found undetectable levels in every pretreated sludge sample. Gurieff et al. (2011) tested samples before and after pretreatment by the Exelys™ process for fecal coliforms, *Enterococci* and helminth eggs. Unlike the CAMBI™ process that is operated in batch mode, the Exelys™ process is continuous and it is possible that some organisms may short-circuit the process and remain viable. However they showed that the remaining organisms were below the detection limit. It must be noted that the measurement of viable microorganisms in these studies was employed to determine whether the sludge could be considered sterile as defined by the USEPA for Class A biosolids. The culturable microorganisms measured in these studies probably only comprised a small fraction of the total active heterotrophs present.

A much more accurate method to measure the concentration of active heterotrophic bacteria is to use batch-mode respirometry. In this method, the oxygen uptake rate (OUR) is determined over a period of time by measuring the consumption of dissolved oxygen in the WAS sample in an airtight vessel. In the current project, the active fraction is defined as the ratio of initial active biomass (mg COD/L) measured by respirometry to TCOD (mg COD/L). Two approaches will be presented to determine the active fraction from OUR time-series data.

In the first approach, a small volume of WAS is combined with a comparably large volume of substrate in the batch respirometric test such that the food to microorganism (F/M) ratio is greater than 10. The measured OUR will increase exponentially with time, indicating that the bacteria are reproducing. Once the substrate is depleted, the measured OUR will decrease with time. Both Kianmehr (2010) and Musser (2009) successfully employed this approach to measure the concentration of active bacteria in WAS before and after pretreatment by sonication and ozonation. Wentzel et al. (1998) used equation 2.3 to determine the initial active biomass concentration (Z_{bh0}) in mg COD/L on the basis of respirometry data:

$$Z_{bh0} = \frac{e^{y-intercept}}{\frac{1-Y_h}{Y_h} \times slope \times b_h} \tag{2.3}$$

In this formula, the slope and y-intercept are of the plot of ln(OUR) versus time (d) for the portion of the measured OUR data that exponentially increases with time. The measured OUR is in units of mg COD/L/d. The aerobic decay rate is represented as b_h (d^{-1}) and the aerobic yield coefficient is represented as Y_h. Kianmehr (2010) reported that estimates of Z_{bh0} were not significantly sensitive to the value of b_h and so a value of 0.24 d^{-1} at 20°C was used in the current project unless otherwise stated. This is the value of b_h recommended by Henze et al. (2008). The typical value of Y_h in real activated sludge systems is 0.67 (Henze et al., 2008). In activated sludge systems fed by sodium acetate as the sole carbon source Y_h values of 0.6 have been reported (Ramdani et al., 2012). Unless otherwise stated, the Y_h value used in the current project was 0.6.

The second approach uses only WAS in a batch respirometric test. The measured OUR will decrease exponentially with time, indicating that endogenous decay is the only oxygen-consuming process in the vessel. A nonlinear regression fit of equation 2.4 to the measured OUR data will yield an estimate of Z_{bh0} in a sample (Jones et al., 2009).

$$\mathrm{OUR} = (4.33 f_N + 1)(1 - f) b_h Z_{bh0} e^{-b_h t} \left(\frac{1}{24}\right) \quad (2.4)$$

Where OUR = measured oxygen uptake rate (mg O_2/L/h)

f_N = nitrogen content of heterotrophic organisms

f = endogenous decay product fraction of organisms (0.2)

t = time (days)

In systems where nitrification is suppressed, the $4.33f_N$ term is omitted to eliminate the nitrogenous OUR (NOUR); the measured OUR is entirely carbonaceous OUR (COUR).

2.1.5.2 Biodegradability

HPTH pretreatment may impact the rate and extent of biodegradability of the WAS. As demonstrated by Kianmehr (2010), the anaerobic biodegradability of the WAS may be measured using the biochemical methane potential (BMP) test or the biochemical acidogenic potential (BAP) test however these tests were outside the scope of this study. This project focused on measuring the impact of HPTH on the aerobic degradability of the WAS using respirometric methods. Several WAS pretreatment studies have employed respirometry to estimate the readily and slowly biodegradable COD concentrations in raw and pretreated WAS (Spanjers and Vanrolleghem, 1995; Mathieu and Etienne, 2000; Musser, 2009; Kianmehr, 2010). Readily biodegradable COD is typically soluble whereas slowly biodegradable COD is particulate. Since HPTH pretreatment has

been shown to solubilize organic materials, an increase in the level of readily biodegradable COD (rbCOD) or rate of aerobic biodegradability is expected when HPTH pretreatment is applied.

Musser (2009) analyzed the effects of sonication and ozonation on the aerobic biodegradability of authentic WAS using respirometry. F/M ratios between 0.1 and 10 were employed where neither the growth nor the decay processes were dominant. Musser (2009) estimated the readily and slowly biodegradable COD concentrations by fitting OUR responses predicted by ASM1 to measured respirometric data. OUR curves generated using ASM1 showed three distinct, successive phases that were defined by the dominant process in each phase: growth on rbCOD, growth on slowly biodegradable COD (sbCOD), followed by decay. In this study typical values were assumed for all the kinetic and stoichiometric parameters, and hence the simulated respirometry responses were determined by the initial Z_{bh}, rbCOD and sbCOD concentrations. The active fraction was determined using the first approach described in the previous section. Musser (2009) then used linear regression to fit the modeled response to the measured respirometric data to yield estimates of rbCOD and sbCOD.

Similar to Musser (2009), Kianmehr (2010) analyzed the effects of sonication and ozonation on the aerobic biodegradability of WAS by employing respirometry to quantify the generation of rbCOD by pretreatment. In this study samples with low F/M ratios were employed such that the substrate was depleted quickly in the respirometry test. Kianmehr (2010) then developed an equation to estimate the concentration of rbCOD in the respirometry test. In this equation, the rbCOD was calculated on the basis of the oxygen consumed during the rapid uptake portion of the test less the oxygen uptake that was attributed to endogenous decay.

2.2 Existing Pretreatment Models

Sludge pretreatment modeling is currently in the early stages of development. A few research groups have proposed approaches for modeling WAS pretreatment technologies and an even fewer number have published stand-alone pretreatment models. Lei et al. (2010) integrated the ASM2d and ADM1 models into a proprietary whole-plant simulator that was employed to simulate a number of WWTPs employing sludge reduction technologies. At one plant, RAS was treated with ozone. The simulated data was fit to the measured data by testing a number of modifications to the ASM2d model that

could characterize the ozone treatment such as a COD reduction due to oxidation and mineralization, conversion of X_i to X_{sp} and conversion of Z_{bh} to X_{sp} and Z_e. The results of this exercise were inconclusive because the sludge was not well characterized. At another plant, WAS was pretreated by HPTH. Lei et al. (2010) fit the simulations to the measured data by testing a number of modifications to ADM1 that were intended to characterize the impact of HPTH pretreatment such an increased digester hydrolysis rate and a partial conversion of inert decay products to carbohydrates, lipids and proteins. The results of this study were also inconclusive. Lei et al. (2010) recommended that further site-specific studies be carried out that focus on fractionating the COD in the sludge upstream and downstream of the sludge reduction technology.

Phothilangka et al. (2008) developed and calibrated a plant-wide model of the Zirl WWTP by integrating the ASM1 and ADM1 models in a Matlab/SIMBA simulation environment. At this plant, WAS was anaerobically digested then dewatered and the dewatering stream was recycled to the aeration basin. A continuous HPTH process was later implemented at the plant whereby WAS was pretreated at 180°C under 19 to 21 bars prior to anaerobic digestion. Similar to the approach taken by Lei et al. (2010), the original plant-wide model was adjusted to fit the data measured after pretreatment implementation by increasing the anaerobic disintegration rate and transforming inert decay products to biodegradable COD. The authors demonstrated that the measured ammonia increase after digestion due to the HPTH pretreatment could only be explained by a complete degradation of decay products. The measured COD concentration in the plant effluent increased after the HPTH process was implemented, indicating that the HPTH pretreatment process generated soluble non-biodegradable COD. The calibrated model successfully predicted this increase.

Dhar et al. (2012) monitored the performance of bench-scale anaerobic digesters that were fed with raw municipal WAS and thermo-chemical pretreated WAS respectively. The impact of pretreatment was simulated using the BioWin 3.0 Activated Sludge Digestion Model. Similar to the previously mentioned studies, these authors varied the digester hydrolysis rate to fit the simulations to the measured responses. Dhar et al. (2012) concluded that pretreatment increased the hydrolysis rate by 30% but did not enhance the digestibility of the sludge.

Frigon and Isazadeh (2010) and Musser (2009) developed COD-based pretreatment models that were rate dependent and could be integrated into whole-plant simulators. Frigon and Isazadeh (2010) developed and compared three hypotheses of the changes in COD fractionation that were caused by pretreating return activated sludge (RAS) with ozone. An extension of the IWA-ASM3 model was

developed for each hypothesis and the model simulations were compared to the results of pilot-scale experiments and the findings of other researchers. The first model extension hypothesized that ozonation transformed biomass to substrate COD and non-biodegradable COD with a small fraction of the COD being oxidized. Storage products were similarly transformed except that non-biodegradable COD was not generated from these materials. In addition to these transformations, the second model extension assumed that inert and substrate solids were also transformed. The third model extension contained the transformations of the second model extension and also included biomass inactivation processes. This model extension was found to provide the best fit of test data. The inactivation of biomass and associated release of stored COD was shown to occur at a higher rate than the transformation rate of the solids COD fractions. Frigon and Isazadeh (2010) found that the third model extension could be further improved by lowering the heterotrophic biomass level without further lowering the nitrifying biomass. This could be achieved by assuming lower inactivation rates for nitrifiers than for ordinary heterotrophs or assuming nitrifying biomass metabolically adapted to the ozone treatment which would have changed parameters such as the maximum specific growth rate.

Musser (2009) designed two separate COD-based stoichiometric pretreatment models, one for sonication and one for ozonation. These models were based on experiments using WAS from the New Hamburg WWTP in Ontario. Each pretreatment model was integrated into a WWTP simulation using BioWin version 3.0. This simulator did not permit a simple stoichiometric conversion of COD species hence a rate-based approach was required to implement the pretreatment model into the simulator. For each pretreatment model, Musser used the model builder reactor in BioWin. In order to model different pretreatment doses, Musser set the HRT of the pretreatment reactor using equation 2.5:

$$HRT = e^{\frac{w}{k}} - 1 \tag{2.5}$$

In equation 2.5, w is the dose and k is the dose constant. For simplicity, Musser converted only one biomass fraction, the heterotrophic biomass, in both his pretreatment models.

During sonication, biomass was observed to be inactivated according to equation 2.6:

$$\frac{Z_{bh} - Z_{bh0}}{Z_{bh0}} = e^{-\frac{w}{k}} - 1 \tag{2.6}$$

In equation 2.6, Z_{bh} and Z_{bh0} were the final and initial heterotroph concentrations. The experimental data showed that 45% of the inactivated biomass was converted to readily degradable substrate and

12% to slowly degradable substrate, which was assumed to be colloidal. Musser (2009) assumed that 8% of the inactivated biomass was cell residue and the remaining 35% was converted to slowly degradable particulate COD. Musser's experiments also showed that 57% of slowly degradable COD was converted to readily degradable COD and the remainder was converted to colloidal biodegradable COD. This model therefore also included a separate process for this conversion. Each conversion proceeded at a rate dependent on the ultrasound dose. In Musser's study and the current study, colloidal matter was defined as that which passed through a filter with a pore size of 1.5 μm but was retained by a filter with a pore size of 0.45 μm.

Musser (2009) designed a model for the ozonation pretreatment process based on experimental findings of COD solubilization, heterotroph inactivation and nitrate production. The ozone pretreatment data showed a rapid inactivation of heterotrophs and a much slower production of readily biodegradable COD (rbCOD); hence the process was modeled using two stages, both of which depended on the ozone dose. In the first stage, heterotrophs were converted to slowly biodegradable COD (sbCOD) and in the second stage the sbCOD was converted to rbCOD. These two stages were expressed by equations 2.7 and 2.8:

$$(1)\ Z_{bh} = Z_{bh0} \times e^{-53k} \tag{2.7}$$

$$(2)\ rbCOD = Z_{bh0} \times 43.8\%(1 - e^{-18.3k}) \tag{2.8}$$

Similar to sonication, a cell residue fraction of 8% was assumed to remain after ozonation. Musser's ozone pretreatment model also included a process to convert nitrite to nitrate. Each conversion process depended on the ozone dose.

The simpler model proposed for sonication was based on the respirometric data whereas the more complex ozonation model was based on a combination of respirometric and nitrogen fraction data. In both models the extent of heterotroph conversion increased with the pretreatment dose however the rate and maximum conversion differed (Musser, 2009).

The sludge pretreatment modeling efforts discussed in this section were based on simulating real activated sludge systems before and after the application of a pretreatment technology. Various COD transformations associated with pretreatment have been proposed. However, these transformations were not solely empirical as the concentrations of some COD species were assumed. It is challenging to accurately fractionate the COD of real activated sludge because it is comprised of a complex mixture of components such as rbCOD, sbCOD, S_{us}, X_i, biomass and decay and storage products and some of these components are not readily isolated. For example, X_i cannot be distinguished and

separately measured from Z_e when both are present in wastewater. Therefore a change in the measured concentration of non-biodegradable particulate COD could be due to a change in the concentration of X_i or Z_e or both. Phothilangka et al. (2008) considered the transformation of Z_e to bCOD but did not consider the transformation of X_i to bCOD. Ramdani et al. (2010) have shown that the COD fractionation of activated sludge is substantially simplified when the system is fed with a synthetic soluble biodegradable substrate such as acetate. The MLSS composition of real and synthetically-fed activated sludge systems is depicted in Figure 2.1 (Ramdani et al., 2010).

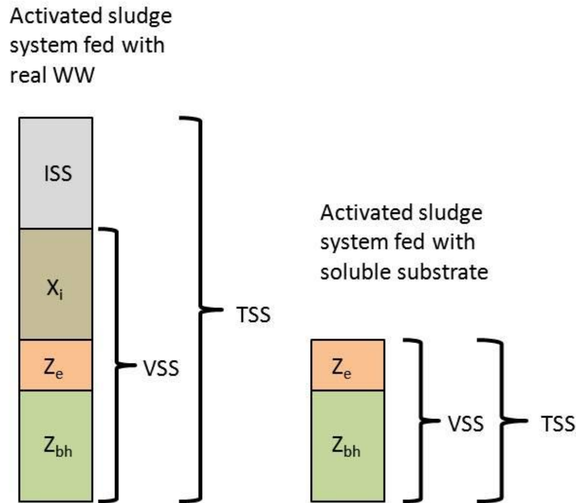

Figure 2.1 MLSS Composition of Activated Sludge Systems (Ramdani et al., 2010)

The COD concentrations of active biomass and endogenous decay products can be accurately measured using respirometric methods. In this way, WAS generated from a synthetically-fed biological reactor may be completely fractionated in terms of COD. Correctly characterizing the raw WAS would allow the fractionation of the pretreated WAS to be characterized more accurately. For example, any soluble COD present in the pretreated WAS would be a direct result of pretreatment since soluble COD would be absent in the raw WAS from a synthetically-fed biological reactor. In this project, WAS generated from a synthetically-fed biological reactor was fed to an aerobic digester for a period of time (Phase 1). The WAS was then pretreated prior to being fed to the aerobic digester and the system was operated for an additional period of time (Phase 2). The Phase 1 and 2 systems were each modeled and the models were calibrated using the measured COD fractions in the various WAS streams. The models were then used to estimate any missing COD fractions. In this way, it was possible to accurately determine the COD transformations caused by pretreatment.

The stand-alone pretreatment models proposed by Musser (2009) and Frigon and Isazadeh (2010) were rate-based. It is proposed that the pretreatment models may be simplified to stoichiometric

COD transformations, without compromising the robustness of the simulations. The pretreatment models presented in this section were based on ozonation and sonication hence there is a need to develop an accurate model for the HPTH pretreatment process.

3. Materials and Methods

3.1 Reactor Design and Materials

The overall process flow diagrams for the reactors used in this project are shown in Figure 3.1 and 3.2.

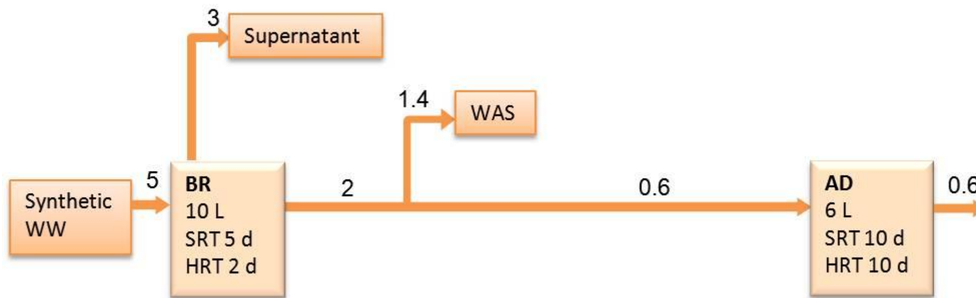

Figure 3.1 Phase 1 Process Flow Diagram (Flow in L/d)

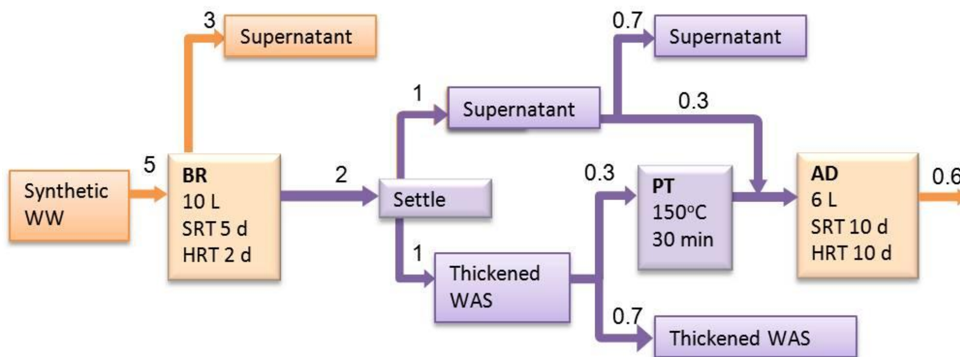

Figure 3.2 Phase 2 Process Flow Diagram (Flow in L/d)

A bench-scale synthetically fed biological reactor (BR) was used in this project to provide a source of stable WAS that consisted of active biomass and decay products. This WAS was used to start up and feed, on a daily basis, a bench-scale aerobic digester (AD). Once the AD had reached steady-state and was sufficiently characterized, a high temperature and pressure batch reactor was used to pretreat the BR WAS before it was fed to the AD.

Two identical transparent acrylic watertight containers were used for the bench-scale BR and AD reactors. These reactors were operated on a lab bench at a temperature of 20 ±1°C over the duration of the project. The containers were cylindrical with an internal diameter of 20 cm, depth of 38 cm and total volume of 12 L. Both reactors were fit with a transparent acrylic lid held in place by bolts.

A hole was drilled into the center of each lid and fitted with a bearing. A stainless steel mixing shaft fitted with two propellers was inserted through this hole into the reactor. Mixing in each reactor was provided by a Bodine® Model 0158 DC Gearmotor that was connected to a power converter. Each mixer had a rotational speed of 200 rpm. A photograph of the biological reactor and aerobic digester are shown in Figure 3.3.

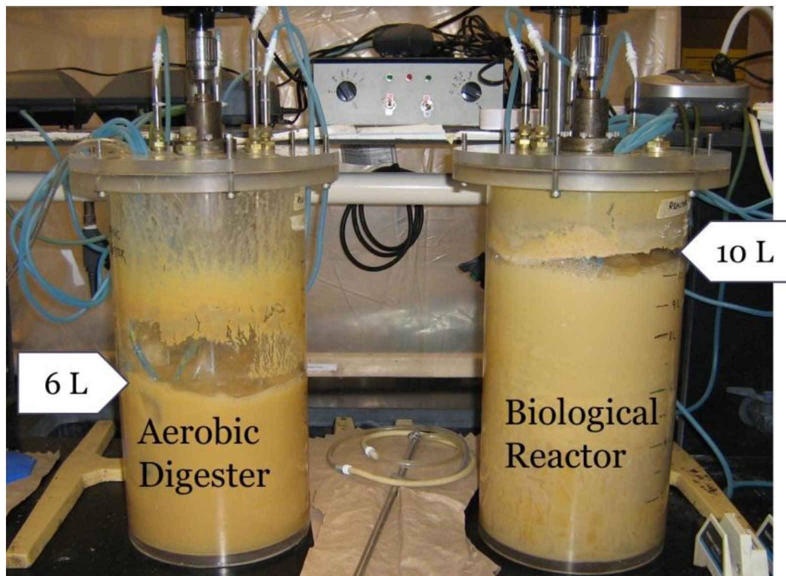

Figure 3.3 Biological Reactor and Aerobic Digester

Each reactor was provided with two TetraTec® Whisper AP300 Deep Water Air Pumps. In addition, the BR was equipped with two TopFin® AIR-3000 air pumps as well as the bench air supply. The air was directed from the pumps and bench air valve through Top Fin® 6 mm diameter silicone airline tubing. For the air supply from the TetraTec® pumps, the silicone tubes were connected to four 6 mm diameter stainless steel tubes inserted into holes in the lid of each reactor. The silicone tubing connected to the TopFin® pumps and bench air valve were inserted directly into the BR through a 3 cm diameter opening in the lid.

Liquid was removed from each reactor through a 6 mm stainless steel tube that was inserted into a hole in the lid. This tube was connected to flexible tubing and a peristaltic pump. Liquid was added to each reactor through a funnel inserted into a 3 cm diameter opening in the lid.

The high temperature and pressure pretreatment of WAS was performed using a Parr® Model 4563 Mini Pressure Reactor, shown in Figure 3.4. This reactor treated a maximum liquid volume of 400

mL in batch mode. Continuous stirring was provided during operation by a variable speed motor. This reactor can operate up to a maximum pressure of 68 bars and a maximum temperature of 225°C. Heating was provided by a mantle heater assembly connected to a programmable temperature control. Cooling was achieved by connecting the cold water tap on the lab bench to the cooling loop on the head of the reactor vessel using flexible tubing. The vessel was fit with a thermocouple that was connected to a display so the temperature of the reactor contents could be monitored.

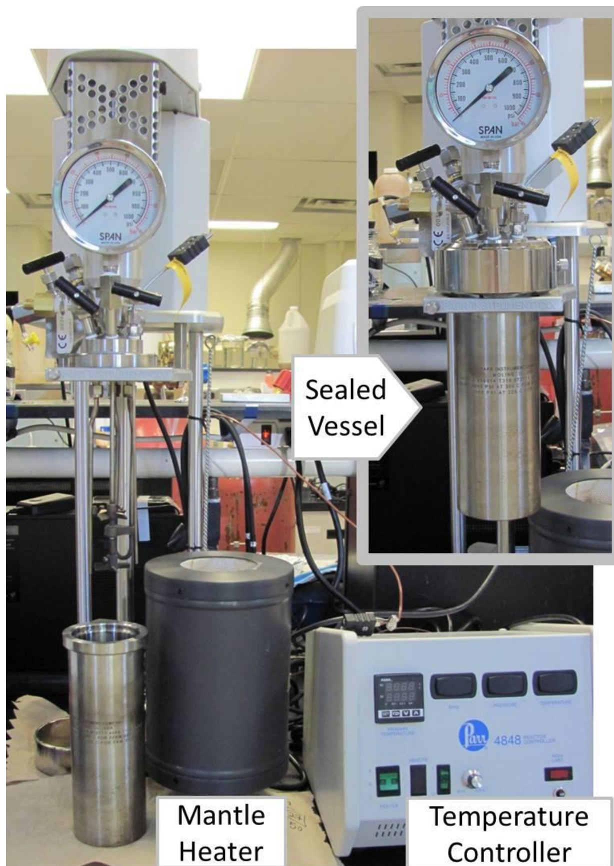

Figure 3.4 Parr® 4563 Mini Pressure Reactor for HPTH Pretreatment

3.2 Synthetic Wastewater

A synthetic wastewater was used to provide the organics, trace metals, nitrogen, phosphorous and alkalinity required for bacterial cell maintenance and growth in the BR. The selected synthetic wastewater recipe was adopted from Mohammadali and Hall (2011). This recipe was modified by increasing the concentration of every compound by a factor of 12.5 except sodium bicarbonate and ammonium chloride which were increased by factors of 1.5 and 6.5 respectively. This modification

resulted in a theoretical total COD (TCOD) of the synthetic wastewater of 4,000 mg COD/L. It should be noted that from day 0 to 179 of the project, the synthetic wastewater concentrations were twice as high, i.e. theoretical TCOD of 8,000 mg COD/L. The concentration was halved on day 179, at the beginning of Phase 1, because it was found that the aeration requirements in the BR could not be satisfied with the higher strength feed. The minimum DO concentration in the reactor for uninhibited activity of aerobic heterotrophs is 2 mg O_2/L. The synthetic feed was prepared in stock solutions every 6 to 12 days and stored in the fridge. The inorganic chemicals (Table 3.2) were kept in a separate solution from the organic chemicals (Table 3.1) to prohibit the growth of microorganisms in the stock solution that would have depleted the COD of the organic feed. The yeast extract is listed with the organics to show its contribution to the TCOD however it was included with the inorganic stock solution because it also contained inorganic nutrients. The compound concentrations in the synthetic wastewater fed to the BR are summarized in Tables 3.1 and 3.2.

Table 3.1 Organic Synthetic Wastewater

Organic Synthetic Feed	**COD Fraction**	**Concentration (mg/L)**
yeast extract	0.12	350
glucose ($C_6H_{12}O_6$)	0.14	525
starch ($(C_6H_{10}O_5)_n$)	0.23	775
sodium acetate ($NaCH_3COO$)	0.26	1315
acetic acid ($C_2H_4O_2$)	0.25	1100
Column Total =	1	

Table 3.2 Inorganic Synthetic Wastewater

Inorganic Synthetic Feed	**Concentration (mg/L)**
monopotassium phosphate (KH_2PO_4)	265
sodium bicarbonate ($NaHCO_3$)	167
ammonium chloride (NH_4Cl)	665
magnesium chloride ($MgCl_2*6H_20$)	300
calcium chloride ($CaCl_2*2H_20$)	450
zinc sulphate ($ZnSO_4*7H_2O$)	5

In order to enhance the flocculation process in the BR, a ferric chloride solution was added to the BR at the same time as the synthetic feed. The influent concentration was 125 mg/L $FeCl_3*6H_2O$. This was made using a stock solution of 17.54 g/L $FeCl_3*6H_2O$ that was prepared every 28 days.

Measurements of the COD, SS and pH of the synthetic feed were taken at least once per month throughout the project to monitor any fluctuations in the feed concentrations. Both the fresh and two

week old stock solutions were measured and it was found that the COD did not change significantly during two weeks of storage in the fridge. The COD fractionation of the synthetic feed will be presented in section 4.2.1.

3.3 Operation of Reactors

3.3.1 Biological Reactor

The BR was operated from startup until the end of the project on day 283. This reactor was operated as an SBR as shown in Figure 3.5. At start-up, 5 liters of AS were collected from the aerated biological reactor at the Waterloo WWTP and added to the bench-top BR. This provided the BR with a diverse spectrum of microorganisms. The reactor was mixed continuously and aeration was provided such that the DO concentration remained above 2 mg/L. After 5 hours, one liter of synthetic feed solution was added to the BR every hour until the total volume in the BR reached 10 L.

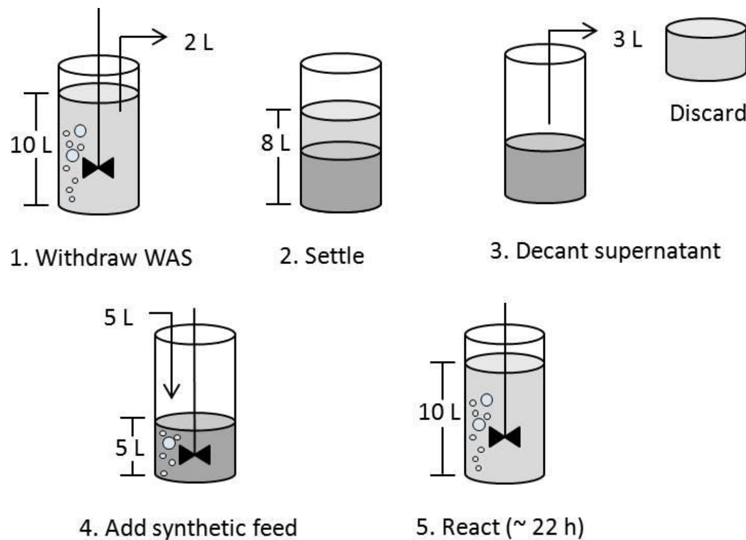

Figure 3.5 Daily Operation of the Biological Reactor

From Figure 3.5 it can be seen that WAS was removed from the BR while the reactor was being mixed and aerated. Mixing and aeration were then ceased to allow the contents to settle. Once the sludge blanket settled below the 5 L graduation mark on the reactor, 3 L of supernatant was siphoned off. Mixing and aeration were then resumed and 5 L of synthetic feed was added to the reactor. The

DO concentration in the reactor dropped to almost zero immediately after feeding however it was ensured that the DO concentration rose above 2 ppm within two hours after feeding and remained at that level for the remainder of the reaction period. Steps 1 through 4 in Figure 3.5 typically required a total of 2 hours leaving approximately 22 hours for the reaction period. These steps were repeated daily, 7 days per week. The hydraulic residence time (HRT) of the BR was 2 d and the SRT was 5 d.

The BR was cleaned twice per week throughout its operation. This was necessary to remove any biofilm that collected on the inside walls and lid of the reactor as well as on the propeller blades and steel tubes inside the reactor. The reactor was cleaned after the supernatant had been removed since this was the time when the reactor contained the smallest volume (5L). The mixer and aerators were turned off and the lid was opened. Tissues were used to wipe off the sludge collected on the inside lid and walls above the liquid level. The propeller, shaft and steel tubes were removed from the liquid and wiped clean using tissues. A clean handheld brush was used to scrape the biofilm from the walls below the liquid level. During the third week of operation of the reactor, worms became visible on the inside walls and lid. These worms were removed by hand and did not appear again.

3.3.2 Aerobic Digester Fed with Raw BR WAS

The aerobic digester was started up with BR WAS on day 20 and operated until the end of the project, day 283. As will be shown in section 4.1, the BR was predicted to reach steady state conditions by day 15 hence it was assumed that the AD was started up with a stable WAS source. On day 20, 2 L of WAS from the BR was poured into the AD and the mixer and aerators were turned on. This was repeated for the following two days so that the AD contained 6 L of AS on day 22. From day 23 until day 77, the AD was operated as a SBR with an HRT of 3 d and SRT of 10 d. The BR-AD system was modeled in BioWin 3.1 and it was predicted that the AD reached steady state conditions within 3 SRTs or 30 days from the day it was started up. On day 77, the operation of the AD was simplified so that the HRT and SRT were both 10 d, as depicted in Figure 3.6. This operation was continued until the end of the project.

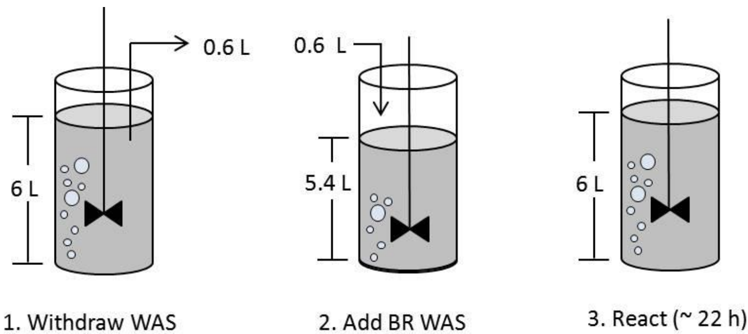

Figure 3.6 Daily Operation of the Aerobic Digester (day 77 to 283)

The main reason for changing the operation of the AD was to decrease the TCOD of the AD WAS so that the aeration requirements in the AD could be satisfied. As mentioned in section 3.2, the minimum DO concentration in the reactor for the uninhibited activity of aerobic heterotrophs is 2 mg O_2/L. The second reason was to make available a portion of the BR WAS for running tests. The entire daily volume of BR WAS was fed to the AD when the AD was operated as a SBR. In the modified operating regime, only 30% of the daily BR WAS volume was fed to the AD. This change in the operation of the AD was simulated using BioWin 3.1 and it was predicted that the AD reached steady state conditions after 3 SRTs, on day 107.

Similar to the BR, the AD was cleaned twice per week throughout its operation. The digester was cleaned when it contained the smallest volume of mixed liquor. The AD was cleaned in the same way as the BR. Unlike the BR, worms did not appear at any time during the operation of the AD.

3.3.3 Aerobic Digester Fed with Pretreated BR WAS

On day 203, pretreatment of the BR WAS that was fed to the AD was initiated on a daily basis. The pretreatment reactor was always operated at its maximum capacity of 400 mL. The mantle heater and programmable temperature controller were used to increase the temperature of the sludge to 150°C. This took approximately 20 minutes. The Parr® 4563 reactor is a pressure vessel designed to prevent any gas or liquid from escaping. At 150°C, the internal pressure was 3 bars. No additional pressure was applied to the vessel. The temperature was held at 150°C for 30 minutes. The mantle heater was then turned off and removed from the reaction vessel and the cold water tap connected to the cooling coil was turned on. It took approximately 45 minutes to decrease the internal temperature from 150°C to 20°C.

Once empty, the inside of the pressure vessel was washed clean in the sink using a brush with soap and water. The lid and attached mixing shaft, cooling coil and sampling ports were also cleaned. These components were carefully inspected to insure no sludge remained before the next pretreatment batch was started.

The AD required a daily feed of 600 mL. In addition, up to 1 L of pretreated WAS was required to run tests. To meet these volume requirements, the 2 L of WAS that was withdrawn from the BR was allowed to settle in a 2 L graduated cylinder until the sludge blanket level was below the 1 L graduation mark. The supernatant was then siphoned off and retained. The remaining 1 L of thickened sludge was then stirred and 400 mL was poured into the pretreatment reactor. Following pretreatment, 300 mL of pretreated sludge was then diluted to 600 mL using the BR supernatant that had been removed from the graduated cylinder. This sludge was then fed to the AD. This sequence is illustrated in Figure 3.7.

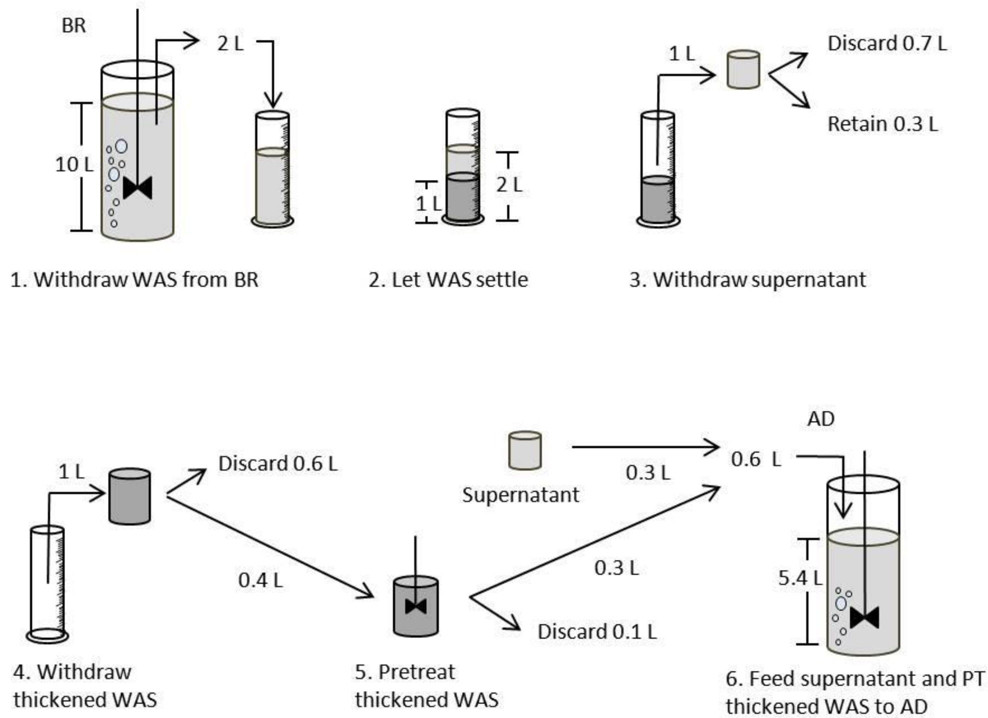

Figure 3.7 Daily Pretreatment Procedure (day 203 to 283)

Under this operation, 100 mL of pretreated thickened sludge and 700 mL of BR supernatant were available daily for running tests. There was also an excess volume of 600 mL of BR WAS that had

been thickened in the graduated cylinder. On days when greater volumes of pretreated WAS were required for running tests, a second batch of thickened BR WAS was pretreated.

3.4 Project Timeline and Sampling Schedule

An overview of the monitoring and modeling conducted to address the objectives of the project is presented in Figure 3.8.

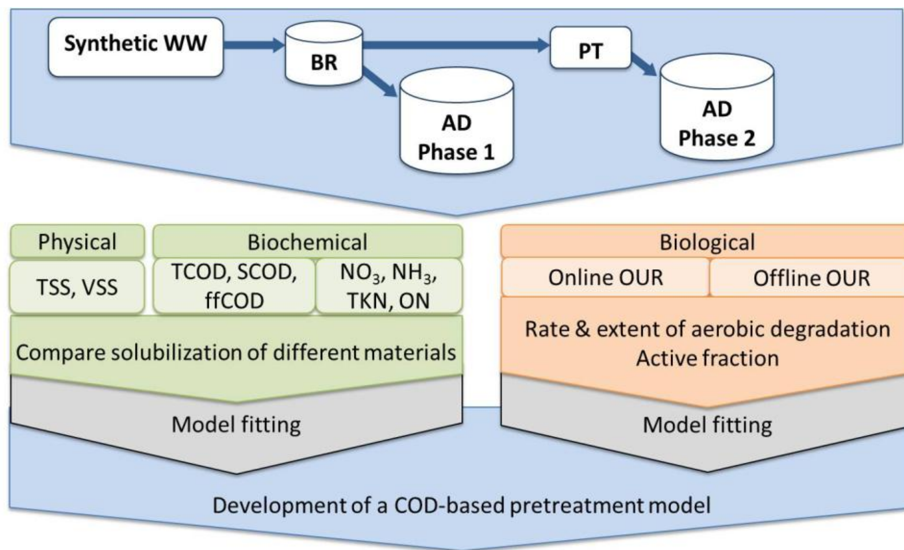

Figure 3.8 Overall Framework for Characterization of Impacts of HPTH Pretreatment on WAS

The project timeline is shown in Figure 3.9. The focus of the project is the period from day 179 to 283, when Phases 1 and 2 took place.

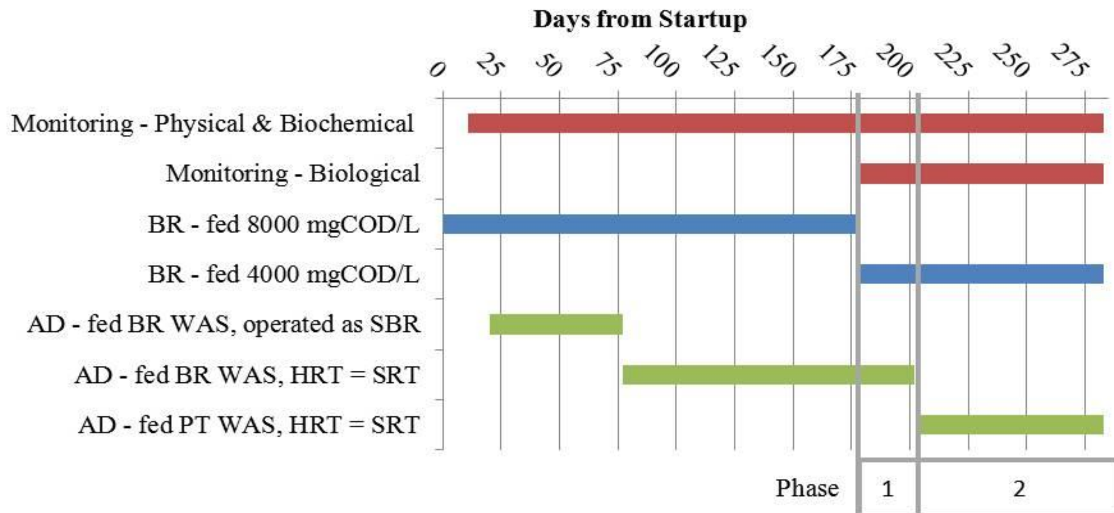

Figure 3.9 Project Timeline

When needed for characterization, samples of AS were taken from the BR and AD at the end of the reaction period, approximately 22 hours after the reactors had been fed. While the reactors were being mixed and aerated, samples were withdrawn by inserting the sampling tube well below the liquid level and at least 5 cm away from the floor and walls of the reactor. The sampling schedule is presented in Table 3.3.

Table 3.3 Sampling Schedule for Physical and Biochemical Tests

Period	**pH**	**TCOD & sCOD**	**ffCOD**	**TSS & VSS**	**NO3**	**NH3**	**sTKN & sON**
WAS from BR							
Day 0 to 20	Daily	Once		Once			
WAS from AD and BR							
Day 20 to 92	Daily	Bimonthly	Monthly	Bimonthly	Monthly		
WAS from AD, Pretreated and Raw WAS from BR							
Day 92 to 112	Daily	Weekly	Weekly	Weekly			
Day 112 to 134	Daily	Weekly	Weekly	Weekly	Weekly	Weekly	
Day 112 to 160	Daily						
Day 160 to 236	Daily	Weekly	Weekly	Weekly	Weekly	Weekly	
Day 236 to 283	Daily	Weekly	Weekly	Weekly	Weekly	Weekly	Weekly

Two types of biological analyses were carried out on the activated sludge. Offline respirometric tests were performed using a Challenge® AER-208 Respirometer. The test was run until the endogenous OUR curve was clearly detectable which was typically between 100 to 160 hours. Online respirometric tests were conducted by allowing the DO in the reactor to fluctuate by automatic

aeration control and then measuring the rate of decline of DO. The online respirometric tests were conducted over three consecutive days. The purpose of this measurement was to determine the OUR in the reactor throughout the 22 hour reaction period. The offline respirometric tests were conducted bimonthly from day 83 to 173 and weekly from day 173 to 283. The online respirometric tests were conducted monthly from day 173 to 204 and weekly from day 204 to 283.

3.5 Analytical Methods

The physical and biochemical indicators listed in Figure 3.8 were determined by conventional analyses. These analyses were conducted according to the relevant sections of Standard Methods for the Examination of Water and Wastewater (Eaton et al., 2005). Every sample was measured in duplicate. Blank and standard samples were prepared and measured for all of the biochemical tests.

3.5.1 Suspended Solids

A volume of 5 mL was filtered through a prepared 1.5 μm pore size filter. The residue and paper were dried to a constant weight in an oven at 105°C. The increase in weight of the filter represented the total suspended solids (TSS). The residue and paper were then placed in a furnace at 550°C for 45 minutes. The remaining solids represented the inorganic suspended solids (ISS) and the weight lost on ignition was the volatile suspended solids (VSS).

3.5.2 COD

The total, soluble (sCOD) and flocculated and filtered COD (ffCOD) were measured at 600 nm using a HACH DR/2000 Spectrophotometer. A range of standard samples were prepared and measured to generate a calibration curve. For the total COD (TCOD) analysis, the samples were first homogenized for 30 seconds then diluted by an appropriate factor. A volume of 2.5 mL of the emulsified, diluted sample was then added to the COD vial containing the reagents. The vial was then mixed by being inverted several times and then placed in the preheated HACH COD Reactor for 3 hours at 150°C.

For the soluble COD (sCOD) analysis, 50 mL of the sample was centrifuged for 30 minutes. The supernatant was then filtered through a Whatman Glass Microfibre filter (934-AH) with a pore size of 1.5 μm. The filtrate was then diluted if necessary, added to the COD vial and heated in the COD reactor identically to the TCOD sample. It should be noted that both soluble and colloidal matter were measured in this sCOD procedure. In this study, colloidal matter was defined as that which passed through a filter with a pore size of 1.5 μm but was retained by a filter with a pore size of 0.45 μm.

The flocculated and filtered COD (ffCOD) analysis is designed to measure the truly soluble COD. The sample was taken from the filtrate that had been collected for the SCOD sample. A stock solution of 25 g/L of alum was prepared. Using this stock solution, 2.5 mg of alum was added to a 50 mL of the sample filtrate. The sample was mixed vigorously for 30 seconds to begin flocculation, allowed to stand for 10 minutes, and then centrifuged for 15 minutes. Finally the sample was filtered using a 0.45 μm pore size filter. The filtrate was diluted if necessary, added to a COD vial, mixed and then heated in an identical manner to the TCOD and sCOD samples. As mentioned, blank and standard samples were also subjected to this procedure. This made it possible to detect whether the COD was significantly altered by the ffCOD procedure.

3.5.3 Ammonia

The concentration of ammonia in samples was measured using a Thermo Scientific Orion 9512HPBNWP High Performance Ammonia Electrode. The sample was taken from the filtrate that had been collected for the sCOD sample. The ammonia probe was inserted into the sample while it was mixed continuously. The pH of the sample was then raised to above 11 using a 5 M NaOH solution. The ammonia reading was taken once the ammonia concentration had stabilized. A calibration curve was prepared using ammonia standards every time a set of samples was measured.

3.5.4 Total Kjeldahl Nitrogen

Both total Kjeldahl nitrogen (TKN) and soluble TKN (sTKN) concentrations were measured. Samples analyzed for TKN measurements were first homogenized for 30 seconds. The soluble

sample was taken from the filtrate that had been collected for sCOD analysis. The digestion solution was prepared by dissolving 40 g potassium sulfate and 2 mL selenium oxychoride in 250 mL concentrated sulfuric acid and then diluting the solution to 500 mL with deionized water. A volume of 1 mL of sample and 1.5 mL of digestion solution were added to a TKN digestion tube. The tube was heated uncovered in a Bran & Lubbe BD-40 block digester first at 220°C for 1.5 h and then at 380 °C for 2.5 h in order to convert organic nitrogen to ammonia. This method was developed in the Environment Canada Wastewater Technology Center in Burlington, Ontario. The ammonia was measured using the ammonia probe following the procedure described previously.

3.5.5 Organic Nitrogen

The organic nitrogen (ON) was estimated by subtracting the ammonia concentration from the TKN concentration for a particular sample. Both the total ON and soluble ON (sON) concentrations were calculated.

3.5.6 Nitrate

Nitrate was measured in the soluble sample taken from the filtrate that had been collected for sCOD analysis. The sample was first diluted if necessary and then 25 mL of sample was poured into a sample cell and one HACH NitraVer® 5 Nitrate Reagent powder pillow was added. Once sufficient time had elapsed for the cadmium reduction to complete, the sample was analyzed at 400 nm using the HACH DR/2000 Spectrophotometer.

3.5.7 pH

The pH of the pretreated sludge and the activated sludge in the BR and AD was measured daily using an Omega PHB-600R pH Benchtop Meter.

3.6 Respirometric Methods

Two respirometric methods were used in this project: online and offline. Both methods were used to determine the oxygen uptake rate (OUR) and cumulative oxygen uptake (ΣOU) associated with the WAS from the BR. The oxygen uptake data from both respirometric methods was used to assess the impact of pretreatment on the aerobic degradability of the WAS. In addition, the oxygen uptake data from the offline respirometric test was used to determine the activity of the microorganisms in the WAS. The overall goal of the respirometry measurements was to assist in fractionating the COD of the raw and pretreated WAS samples and hence only the carbonaceous oxygen demand was desired. The nitrogenous oxygen demand was inhibited by adding HACH 2533 nitrification inhibitor to the WAS. The collection and analysis of respirometric data is not standardized hence the two approaches are described here in detail.

3.6.1 Online Respirometry

Online respirometry was used to measure the OUR and ΣOU in the aerobic digester over the reaction period. Throughout the project, the temperature in the aerobic digester was 20 ±1°C and the pH was 8.3 ± 0.2. A Jenco© model LD-900-5-DO Industrial Inline DO Probe was used to continuously measure the DO concentration in the reactor. This probe and the digester aerators were connected to a Jenco© model 6309-PDT Advanced Multi-Parameter Analyzer. The probe, analyzer, aerators and AD are shown in Figure 3.10. The analyzer was programmed to turn on the aerators when the DO concentration in the reactor declined to 3 mg O_2/L and turn off the aerators when the concentration reached 5 mg O_2/L. An EasyLog© Model EL-USB-4 Data Logger was connected to the analyzer to record the DO concentration in the reactor every 10 seconds. This logger could store up to 3.7 days of data; hence each online respirometry test was run for 3 days. The rate of DO decline was calculated for every time period between the high and low DO set points to generate OUR values.

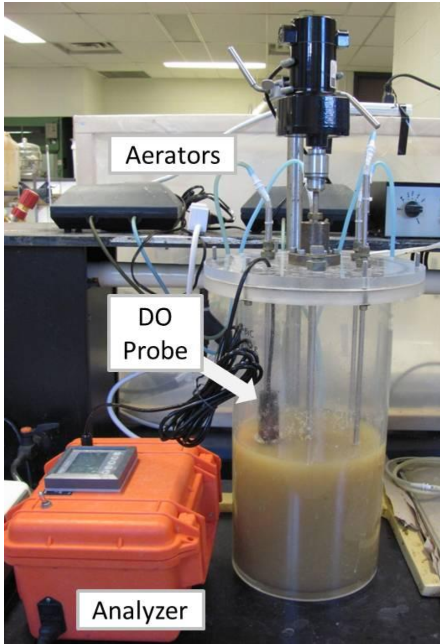

Figure 3.10 DO Probe and Analyzer Connected to Aerators in the AD

The nitrate levels in the aerobic digester were routinely measured as described in the previous section. Two grams of HACH 2533 nitrification inhibitor were added to the digester every two weeks to keep the nitrate level below 0.5 mg N/L. This essentially eliminated the nitrogenous oxygen demand in the digester.

3.6.2 Offline Respirometry

Offline respirometry was performed using a Challenge Technology© AER-208 Respirometer. The temperature was set at 25°C for all respirometry tests and the pH was in the range of 8.1 ± 0.5. This direct input respirometer replenished the oxygen consumed by the microorganisms in the sample by injecting oxygen bubbles into the sample headspace using fluid action (Young and Cowan, 2004). The pressure drop caused by the consumption of oxygen initiated the injection of the oxygen bubbles. Each flow cell was carefully calibrated to determine the mass of oxygen in one bubble. The bubbles were counted by the respirometer and the ΣOU in each sample was recorded every 10 minutes. The change in oxygen consumption for each time step was calculated to provide an estimate of the OUR in the sample bottle throughout the test. Each offline respirometry test was run until a distinct decay

curve was observed, indicating that most of the aerobically biodegradable material had been consumed. The test duration was typically 100 to 160 hours.

The carbon dioxide gas produced by the aerobic heterotrophs was removed from the headspace by suspending a vial containing 30% w/w potassium hydroxide in the sample vessel. In order to ensure that heterotrophic processes dominated all responses, 300 mg of HACH 2533 Nitrification Inhibitor was added to each sample bottle. The total volume of each sample bottle was 250 mL and a maximum of eight sample bottles could be tested simultaneously. The contents of the sample bottles were mixed using magnetic stir bars and the stirring rate was adjusted to produce a vortex that touched the stir bar. The sample bottles were contained in a water bath so that the temperature could be held constant at 25°C. The sample bottles, water bath, oxygen flow measurement cells and oxygen supply manifold of the respirometer are pictured in Figure 3.11. The contents of the sample bottles for the offline respirometric tests are summarized in Table 3.4.

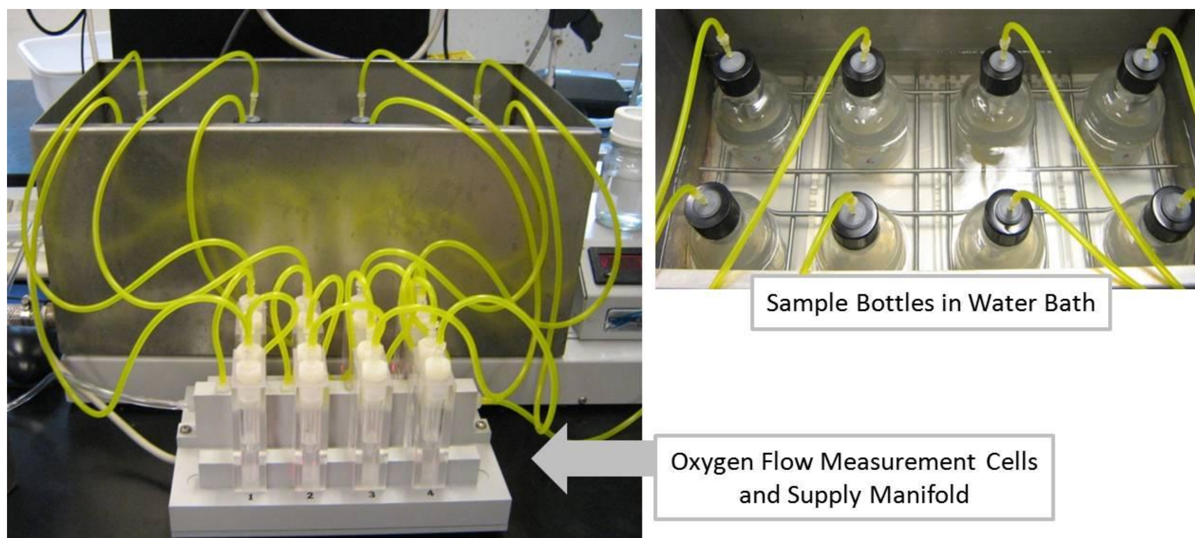

Figure 3.11 Challenge Technology AER-208 Respirometer

Table 3.4 Sample Bottle Contents for Offline Respirometric Tests

Sample Bottle	Whole Substrate	Filtered Substrate	Inoculum
1, 2	150 mL		50 mL
3, 4			50 mL
5, 6	150 mL		
7, 8		150 mL	50 mL

As shown in Table 3.4, every sample was tested in duplicate. The WAS samples used in offline respirometry were taken from the reactors at the end of the reaction period. In Table 3.4, the substrate was BR WAS in Phase 1 and pretreated WAS in Phase 2. The filtered substrate was prepared in the same way that samples were prepared for the SCOD test, i.e. centrifuged then filtered through a 1.5 μm glass fiber filter. In Table 3.4, the inoculum was the AD WAS. The F/M ratio in the inoculated bottles was 5 to 10 which was high enough to allow clear identification of the growth OUR response if present in the sample bottle.

Once a month during Phase 2, offline respirometry was carried out on sample bottles containing only 150 mL of BR WAS to verify that the active heterotroph concentration remained constant throughout the project. On day 278, an alternative offline respirometric test was used to measure the active fraction of the BR WAS. In this test, the sample bottles contained BR WAS and synthetic feed to yield F/M ratios in the range of 10 to 40. The four pairs of bottles contained 0.5, 1, 2 and 4 mL of BR WAS, respectively, each topped up to 200 mL with synthetic feed. Thus the BR WAS was diluted 400, 200, 100 and 50 times, respectively, in the four pairs of bottles. Respirometry carried out on control bottles consisting of water or synthetic feed showed zero oxygen uptake hence it was concluded that the observed oxygen uptake in the sample bottles was due to the metabolism of the microorganisms.

4. Results

4.1 Startup of Reactors

At the beginning of the project, the operation of the BR was simulated using the BioWin 3.1 Integrated Model to predict the changes in concentration of the various COD components during the startup of the BR and estimate the time required for the BR to reach steady state. This simulation was carried out for a number of reasons:

- It was used to support the assumption that the COD components present in the seed sludge, which were absent in the synthetic feed, would be washed out of the BR.
- It was used to show that at steady state, the BR WAS consisted of only active biomass and decay products.
- It was used to estimate the date when the BR reached steady state. This was required to select the startup date of the AD. The AD needed to be started up with a stable BR WAS in order to minimize the time required for the AD to reach steady state.
- A stable source of BR WAS was required for pretreatment in order to accurately characterize the impacts of pretreatment.

As mentioned in section 3.3.1, the BR was initially seeded with 5 L of activated sludge from the Waterloo WWTP. On the date the sample was taken from the WWTP, the TCOD of the activated sludge reported by the plant was 3280 mg/L. The SRT of the aeration basin from which the sample was withdrawn was reported to be approximately 8 d. Using these values, the aeration basin of the Waterloo WWTP was simulated in BioWin 3.1 to estimate the concentration of the major COD contributors in the activated sludge. It was assumed that the plant received a continuous flow of typical settled medium-strength municipal wastewater that contained 450 mg/L TCOD, 15 mg/L ISS and 60 mg/L non-biodegradable particulate COD (X_i). The MLSS of the AS therefore included a mixture of ISS and VSS. The simulation was run until steady state conditions were reached. The major COD contributors in the AS were predicted to be particulate inert COD (X_i), endogenous products (Z_e), and ordinary heterotrophic biomass that does not accumulate phosphorus (Z_{bh}).

In a separate simulation, the bench scale BR was then modeled as an SBR with a 5 d SRT. The activated sludge from the Waterloo WWTP predicted in the previous simulation was used as the seed sludge in the BR. The influent parameters were specified according to the theoretical composition of

the synthetic feed used from day 0 to day 179 of the project, as shown in Table 4.1. The COD:N:P ratio of the synthetic wastewater was 100: 4.4: 1.5. This is very close to the conventionally recommended ratio of 100: 5: 1 for aerated activated sludge systems.

Table 4.1 Influent Synthetic Wastewater Parameters

Parameter	Based on Synthetic Wastewater Recipe		Converted to Required Units for BioWin 3.1®	
	Concentration	Units	Concentration	Units
COD	8000	mg/L COD	8000	mg/L COD
TKN	1330	mg/L NH_4Cl	350	mgN/L
TP	530	mg/L KH_2PO_4	120	mgP/L
SCa	900	mg/L $CaCl_2*2H_2O$	245	mgCa^{2+}/L
SMg	600	mg/L $MgCl_2*6H_2O$	72	mgMg^{2+}/L
Alkalinity	333	mg/L $NaHCO_3$	4	mmol/L

Figure 4.1 shows the simulated COD fractionation in the activated sludge in the BR during the first 3 days of operation. In a given day, the biodegradable COD fractions sharply increased when the reactor was fed, corresponding to a sharp increase in TCOD. The active organisms (Z_{bh}) underwent rapid growth, and then began to decline when the substrate was depleted. The endogenous products (Z_e) steadily increased during the reaction period and began to accumulate in the reactor. When the reactor was allowed to settle, the particulate COD concentrations dropped to zero because the model assumed perfect settling.

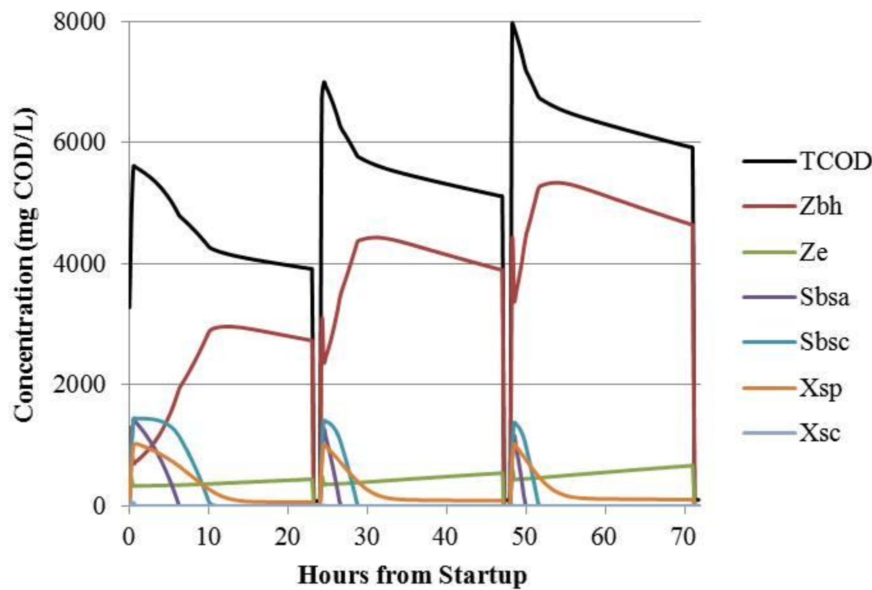

Figure 4.1 Simulated COD Fractionation of WAS in BR during Startup

The active heterotroph concentration in the BR at the end of the reaction period was predicted to drastically increase during the first three days of operation, as shown in Figure 4.1. This was due to the relatively high strength of the synthetic feed compared to the assumed strength of the municipal wastewater at the Waterloo WWTP. Consequently, the predicted Z_e concentration also steadily increased in the BR. The model predicted that X_i would be washed out of the BR within 3 SRTs or 15 days. As shown in Figure 4.2, the TCOD of the WAS was predicted to be almost entirely comprised of Z_{bh} and Z_e at steady state conditions which also occurred within 3 SRTs. The TCOD of the BR WAS was measured on day 15 and was found to be 7700 ± 150 mg/L. In this report, a number preceded by the symbol ± indicates one standard deviation above and below the mean of a sample set. The predicted TCOD of the BR WAS at the end of the reaction period on this day was 7790 mg/L which matched the measured value.

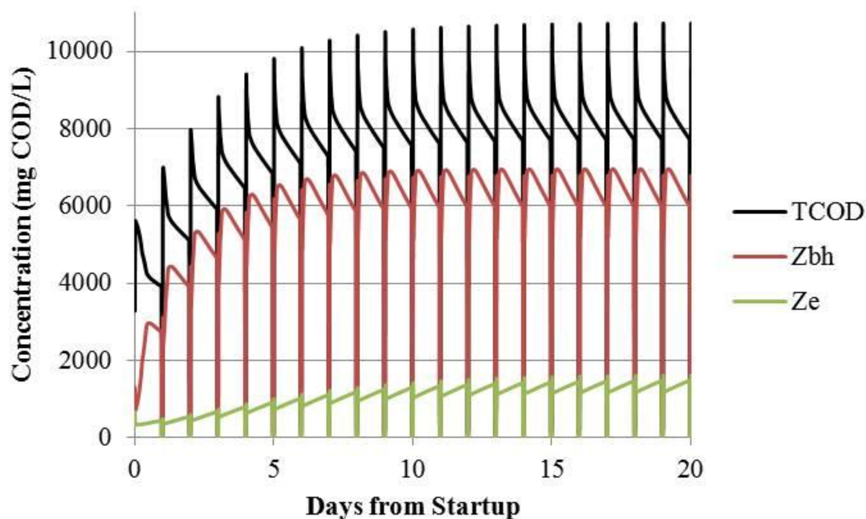

Figure 4.2 COD Fractionation of WAS in BR Over 20 Days

As mentioned in section 3.2, the synthetic feed concentration was halved on day 179. It was predicted using another BioWin simulation that a new steady state condition would be reached within 3 SRTs following this feeding change. After day 194, the predicted steady-state TCOD, Z_{bh} and Z_e concentrations in the BR were half of the corresponding steady-state values shown in Figure 4.2.

In conclusion, the simulation work presented in this section corroborated the findings of Ramdani et al (2010) that showed the activated sludge fed with synthetic substrate was comprised of only active biomass and decay products. The BR was predicted to reach steady state within 3 SRTs from startup. It was therefore believed that when the AD was started up with BR WAS on day 20, it was fed with a stable WAS source. Based on the simulations, the BR WAS was predicted to be changing in

composition from day 0 to day 15 and from day 179 until day 194. Pretreatment experiments were therefore not carried out during these periods.

4.2 Physical and Biochemical Characterization of Process Streams

4.2.1 Synthetic Wastewater

The concentration of conventional parameters in the synthetic feed obtained from the physical and biochemical analyses are presented in Table 4.2. These values represent the concentrations observed throughout Phases 1 and 2 of the project, i.e. day 179 until day 283.

Table 4.2 Concentration of Conventional Parameters in Synthetic Wastewater

Parameter	Avg. Concentration (mg/L)	Std. Dev. (mg/L)
TCOD	4006	183
SCOD	2983	73
ffCOD	2751	117
ISS	100	61
VSS	833	57
pH	4.5	0.1

The COD of the synthetic feed was fractionated into the components employed by the BioWin 3.1 Integrated Model, as shown in Figure 4.3. This allowed the synthetic substrate to be accurately represented in the Phase 1 and 2 system simulations which will be presented in section 5. As shown in Table 4.2, suspended solids measurements of the synthetic feed revealed the presence of 100 ± 61 mg/L of ISS. Hence the AS in the BR contained some ISS at steady-state operation and thus deviated slightly from the ideal composition presented in Figure 2.1. This ISS probably originated from the yeast extract. The average measured ffCOD concentration in the BR WAS was 53 ± 12 mg COD/L and this was assumed to be soluble microbial products (SMPs) generated by the microorganisms in the reactor. As explained by Kianmehr (2010), SMPs are soluble non-biodegradable COD. Therefore the net COD that bacteria converted in the BR was the measured TCOD of the synthetic feed less the measured ffCOD of the BR WAS which was 98.7 ± 6.1% of the TCOD of the synthetic substrate. The activated sludge models used in this project are not capable of predicting the

channeling of COD into SMPs. Therefore, for the purpose of modeling, the remaining 1.3% was assigned as S_{us} in the synthetic feed, as shown in Figure 4.3. The slowly biodegradable COD fraction (25.5%) was obtained by dividing the average measured particulate COD by the TCOD. It was assumed that 75% of the slowly biodegradable COD was particulate (X_{sp}) and the remainder was colloidal (X_{sc}). The readily biodegradable COD fraction was obtained by subtracting the nbCOD and sbCOD from the TCOD. As was shown in Table 3.1, half of the synthetic feed TCOD was from acetate hence the S_{bsa} fraction was assigned to be half the rbCOD. The remaining rbCOD was assigned as complex (S_{bsc}).

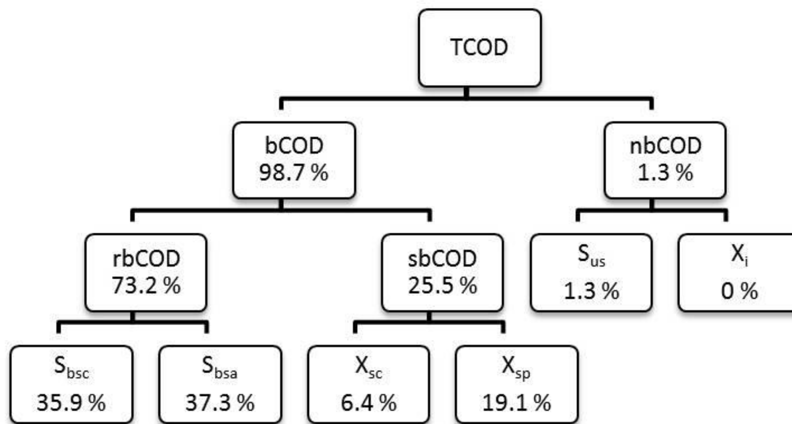

Figure 4.3 COD Fractionation of Synthetic Wastewater

4.2.2 Biological Reactor

As mentioned in section 4.1, the synthetic feed concentration was halved on day 179 and the BR reached steady state within 3 SRTs following this feeding change. As shown in Figure 4.4, the concentrations of the COD and SS components measured by conventional analyses remained relatively stable from day 197 until the end of the project, day 283. It was therefore concluded that the BR was at steady state during this period

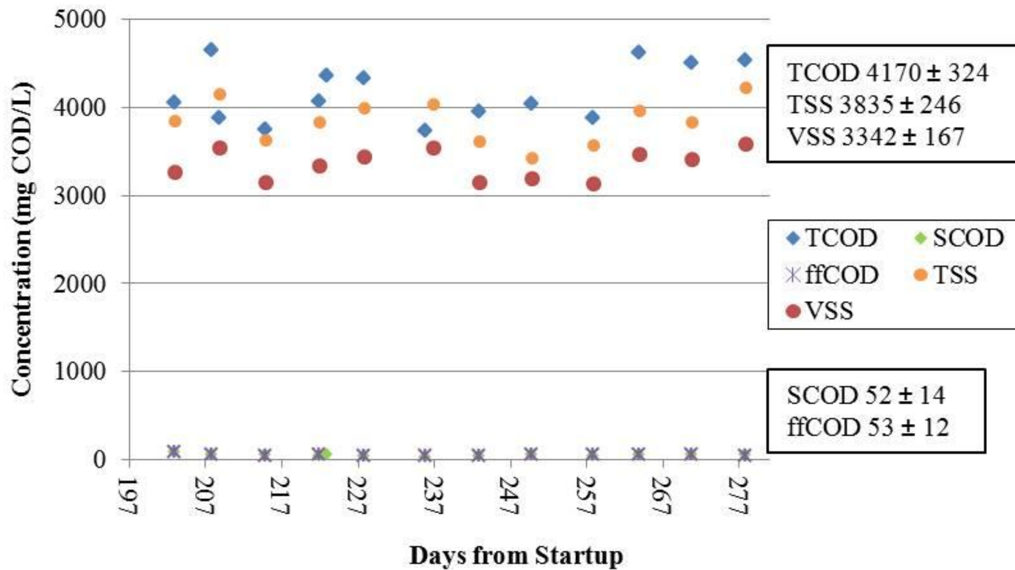

Figure 4.4 COD and SS Concentrations in BR WAS

The COD of the BR WAS was expected to be entirely comprised of biomass and associated products because the synthetic substrate was believed to be entirely oxidized within the reaction period in the BR. This was shown in the reactor simulation presented in section 4.1. Furthermore, the DO concentration in the BR dropped to almost zero immediately after the reactor was fed and then increased above 2 ppm within two hours, indicating that most of the substrate was depleted within this time.

The data presented in Figure 4.4 was used to estimate several properties of the BR WAS. The BR WAS contained an average ffCOD concentration of 53 ± 12 mg/L and this was assumed to be S_{us} from SMPs generated in the reactor. In addition, colloidal COD was not present in the BR WAS because there was no significant difference between the measured SCOD and ffCOD concentrations. As explained by Kianmehr (2010), colloidal COD in WAS is typically comprised of extra cellular polymeric substances (EPS). Therefore the BR WAS did not contain EPS that was in the colloidal range. Besides the relatively small concentration of SMPs, the COD of the BR WAS was therefore entirely particulate.

The average measured COD/VSS ratio of the BR WAS was calculated to be 1.23 ± 0.08. This was less than the typical value of 1.42 (Henze et al., 2008) for active heterotrophs and endogenous residue. The biomass in the BR was therefore more oxidized than typical biomass and it was hypothesized that this suggested the presence of stored COD such as glycogen or poly-hydroxy-

alkanoates (PHA). Although stored COD is not directly identifiable chemically, it may be recovered in COD analysis and must satisfy COD conservation. The COD/VSS ratio of glycogen, a polysaccharide, has been reported to be 1.1 gCOD/gVSS (Ramdani et al., 2012). Therefore the presence of glycogen in addition to active heterotrophs and decay products in the BR WAS would lower the average COD/VSS ratio below 1.42.

The following section presents the derivation of a mass balance that was conducted to estimate the fraction of the biomass COD which was contributed by storage products. In equations 4.1, 4.2 and 4.3 below, the COD/VSS ratio of biomass is described by Υ_Z, the COD/VSS ratio of stored COD is defined as Υ_{STO} and the average measured COD/VSS ratio of the BR WAS is defined as Υ_{OBS}. Assuming that stored COD (X_{STO}) was present in the BR WAS and had an associated Υ_{STO} ratio and that the only other COD components in the BR WAS were active heterotrophs (Z_{bh}) and endogenous residue (Z_e), Υ_{OBS} may be expressed by equation 4.1.

$$\Upsilon_{OBS} = \frac{X_{STO}+Z_{bh}+Z_e}{\frac{X_{STO}}{\Upsilon_{STO}}+\frac{Z_{bh}+Z_e}{\Upsilon_Z}} \quad (4.1)$$

The fraction of the TCOD that was present as storage products (f_{STO}) was equal to the ratio $\frac{X_{STO}}{TCOD}$ and the TCOD was the sum of X_{STO}, Z_{bh} and Z_e. These terms were substituted into equation 4.1 and it was rearranged to yield equation 4.2.

$$\Upsilon_{OBS} = \frac{\Upsilon_Z \Upsilon_{STO}}{\Upsilon_Z f_{STO} + \Upsilon_{STO}(1 - f_{STO})} \quad (4.2)$$

Rearranging equation 4.2 to solve for f_{STO} yielded equation 4.3.

$$f_{STO} = \frac{\Upsilon_{STO}\Upsilon_Z - \Upsilon_{OBS}\Upsilon_{STO}}{\Upsilon_{OBS}\Upsilon_Z - \Upsilon_{OBS}\Upsilon_{STO}} \quad (4.3)$$

Substituting the range of Υ_{OBS} values and an assumed Υ_{STO} value of 1.1 into equation 4.3 yielded an average f_{STO} value of 54 ± 28%. The results of this calculation indicated that more than half of the TCOD of the BR WAS was contributed by storage products. Therefore, the heterotrophs in the BR did not use all of the synthetic biodegradable substrate for growth within the 5 d SRT and the concentration of active biomass in the BR was less than expected. In a similar experiment, Ramdani et al. (2012) used an MBR with a 5.2 d SRT to treat a completely biodegradable synthetic influent comprised of sodium acetate as the sole carbon source. These authors reported that the Z_{bh} and Z_e

fractions were 68 and 32% respectively. Assuming an f_{STO} value of 54%, the sum of Z_{bh} and Z_e in the BR WAS was only 46% of the biomass COD. The composition of the BR WAS therefore deviated from the composition reported by Ramdani et al. (2010) in Figure 2.1. It is important to note that the calculated standard deviation of the average f_{STO} value was ± 28%, which was high. The calculated f_{STO} ratio was found to be very sensitive to the Y_{OBS} value.

Because the AD was fed with BR WAS, some properties of the AD WAS could be estimated based on the properties of the BR WAS. First, the COD of the BR WAS consisted of Z_{bh}, Z_e and X_{STO} hence the AD WAS was expected to be comprised of biomass and associated products. Furthermore, it was expected that the X_{STO} present in the BR WAS would be assimilated to AD biomass because the digester had an SRT twice as long as that in the BR. Thus the AD WAS was expected to be mainly comprised of Z_{bh} and Z_e. In addition the AD WAS was expected to contain concentrations of SCOD and ffCOD similar to those measured in the BR WAS. It was hypothesized that the small concentration of SMPs believed to be present in the BR WAS were generated by biomass growth. Because biomass growth was not expected to occur in the AD, additional SMPs were therefore not expected to be generated in the AD. The properties of the WAS from the AD will be discussed further in section 4.2.4.

4.2.3 Effects of Pretreatment on BR WAS

The pH was measured in the BR throughout the project as one way of assessing the stability of the reactor. The pH of the BR WAS remained relatively constant throughout Phases 1 and 2 of the project, as shown in Figure 4.5, indicating reactor stability. Pretreatment slightly decreased the pH of the WAS from an average of 8.5 ± 0.1 to 7.8 ± 0.2. Both the original and lower pH values were within the range reported by Tchobanoglous et al. (2003) for uninhibited heterotroph activity hence pH adjustment was not required before the pretreated WAS was fed to the AD. This pH decrease was expected as it has been shown that HPTH pretreatment produces volatile fatty acids (Morgan-Sagasume et al., 2010).

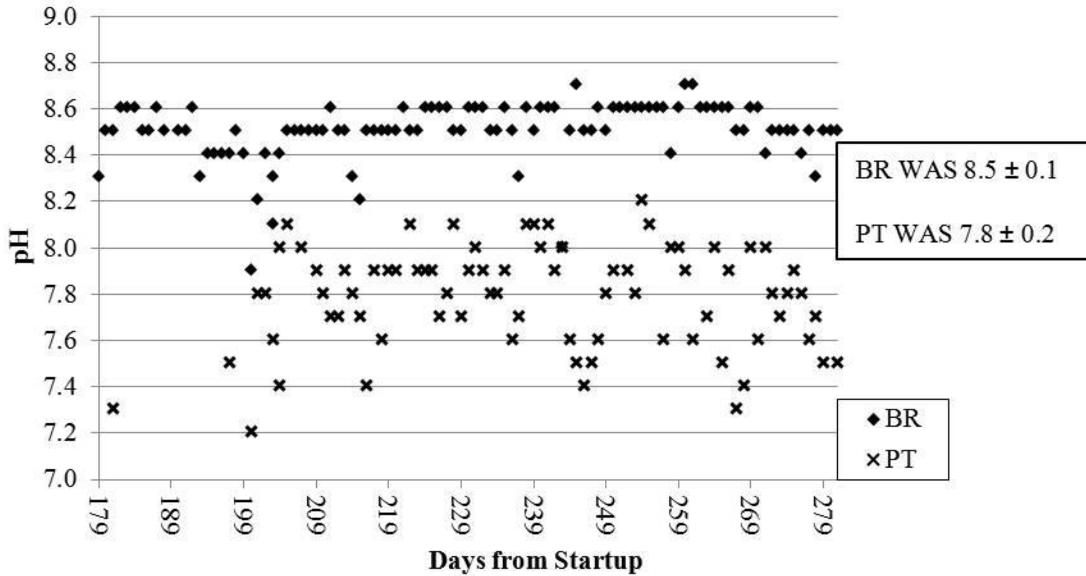

Figure 4.5 Measured pH in Raw and Pretreated BR WAS

Pretreatment substantially solubilized COD, as was expected based on the results of published HPTH pretreatment studies. The average concentration of each COD species in the raw and pretreated WAS was calculated over the period from day 194 to 283, i.e. while the BR was operating at steady state. For each measurement, the PCOD was calculated by subtracting the SCOD from the TCOD. As shown in Figure 4.6, the sCOD and ffCOD concentrations calculated using equation 2.2 increased by 56 ± 7% and 41 ± 5% respectively due to pretreatment. By comparison, the range of COD solubilization reported in the HPTH pretreatment studies referenced in section 2.1.4 was 28 to 45%. In these studies, the pretreatment temperature ranged from 150 to 170°C and the soluble COD was defined as that passing through filters with pore diameters ranging from 0.45 to 1.6 μm. The pressure, duration of heating and mode of operation also differed among the referenced studies. Therefore a direct comparison between the COD solubilization values measured in this project and those measured in the referenced research could not be made. The BR WAS did not contain colloidal COD whereas the difference between the SCOD and ffCOD measurements in the pretreated WAS showed that it contained a colloidal COD concentration of 644 ± 285 mg/L. Kianmehr (2010) suggested that the increase in colloidal COD due to pretreatment could result from the solubilization of extra cellular polymeric substances (EPS).

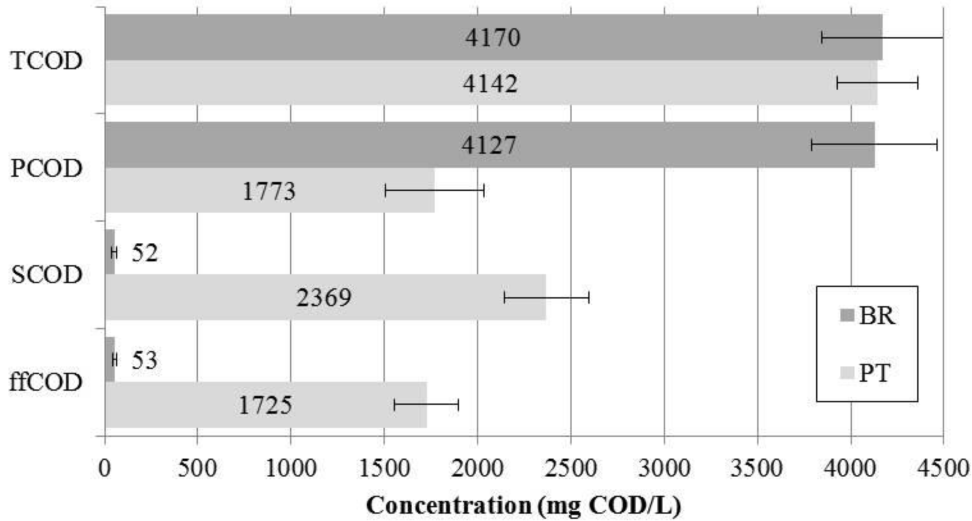

Figure 4.6 Average COD Concentrations in Raw and Pretreated BR WAS

As shown in Figure 4.6, the TCOD concentration remained unchanged by pretreatment, indicating no significant removal of organic matter occurred. This was consistent with the results of Morgan-Sagasume et al. (2010) where TCOD was observed to be conserved during CAMBI^{TM} pretreatment. Pretreatment caused a noticeable colour change in the WAS in both the supernatant and the settled sludge. The colour changed from light brown to dark brownish purple. Because the COD was measured by spectrophotometry, it was necessary to determine whether this colour change affected the COD measurement. Samples of raw and pretreated WAS were prepared at the same dilution factor. Four replicate bottles of each sample were prepared and a potassium hydrogen phthalate solution of a known COD concentration was added to two of the bottles. Four replicate bottles containing only the COD standard were also prepared. The bottles were then analyzed for COD according to the method described in 3.5.2. For each bottle containing dosed COD and WAS, the COD of the standard was calculated as the measured TCOD of this bottle less the measured COD of the bottle containing WAS only. The results showed no significant difference in the measured COD of the standard among all the bottles, including the bottles containing only standard. This test was repeated for filtered WAS samples and again no significant difference in the measured COD of the standard was observed among all the bottles. Therefore the colour change imparted by pretreatment to the supernatant and sludge did not interfere with the COD measurement.

Suspended solids measurements were carried out on the raw and pretreated BR WAS on 12 separate days during Phases 1 and 2. On the basis of these measurements it was concluded that the organic suspended solids were preferentially solubilized by pretreatment as compared to the inorganic

suspended solids. As shown in Figure 4.7, the average VSS concentrations before and after pretreatment were 3342 ± 167 mg/L and 1518 ± 230 mg/L, respectively. A t-test at the 95% confidence level revealed that pretreatment caused the VSS concentration to decrease by 1824 ± 284 mg VSS/L. The average ISS concentrations before and after pretreatment were 489 ± 103 mg/L and 412 ± 131 mg/L, respectively, and a t-test at the 95% confidence level showed no significant difference between these average values.

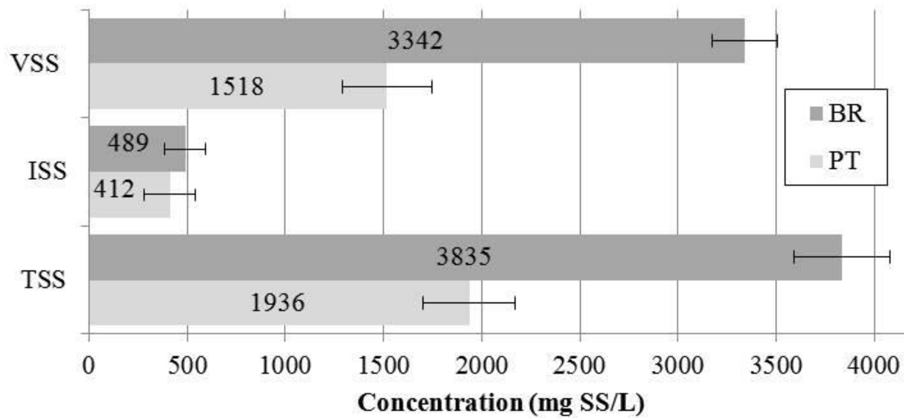

Figure 4.7 Average SS Concentrations in Raw and Pretreated BR WAS

In a study of the CAMBI^{TM} pretreatment technology, Morgan-Sagasume et al. (2010) reported that pretreatment caused the TSS concentration to decrease between 20 and 30%, according to equation 4.4:

$$TSS\ decrease\ \%\ = \frac{TSS_{BR} - TSS_{PT}}{TSS_{BR}} \times 100\% \qquad (4.4)$$

To compare the results of this study to those reported by these authors, the decrease in the TSS concentration due to pretreatment was calculated for each of the 12 sampling days. The average decrease in TSS concentration due to pretreatment was 49 ± 6% which was higher than that reported by Morgan-Sagasume et al. (2010). A t-test at the 95% confidence level showed that the average TSS concentration decreased 1899 ± 205 mg/L due to pretreatment in the current study.

Bougrier et al. (2008) reported a 9% decrease in the VSS/TSS ratio of a sludge that was pretreated at 150°C. In order to compare the results of this study to those reported by these authors, the VSS/TSS ratio was calculated for the raw and pretreated WAS on each day the suspended solids were measured. The average VSS/TSS ratio before and after pretreatment was 87 ± 15% and 77 ± 8%, respectively. A t-test at the 95% confidence level showed no significant difference between these two averages. However, at the 90% confidence level, the VSS/TSS ratio decreased 10 ± 9% due to pretreatment which is comparable to the findings of Bougrier et al. (2008).

On each sampling day, the VSS destruction was calculated according to equation 4.5 using the measured VSS concentration of the BR WAS (VSS_{BR}) and pretreated BR WAS (VSS_{PT}). The average VSS destruction due to pretreatment was found to be 56 ± 10%. In summary, the bench-scale pretreatment used in this project was deemed to be representative of full-scale processes because the measured effects of pretreatment on pH, SS and COD species were found to be comparable to similar published research.

$$VSS\ destruction\ \% = \frac{VSS_{BR} - VSS_{PT}}{VSS_{BR}} \times 100\% \tag{4.5}$$

The concentrations of various nitrogen species before and after pretreatment are presented in Figure 4.8. The concentrations of these species were measured in the raw and pretreated WAS on 7 separate days during Phases 1 and 2. The concentrations of ammonia and nitrate in both types of WAS were measured on 5 additional days.

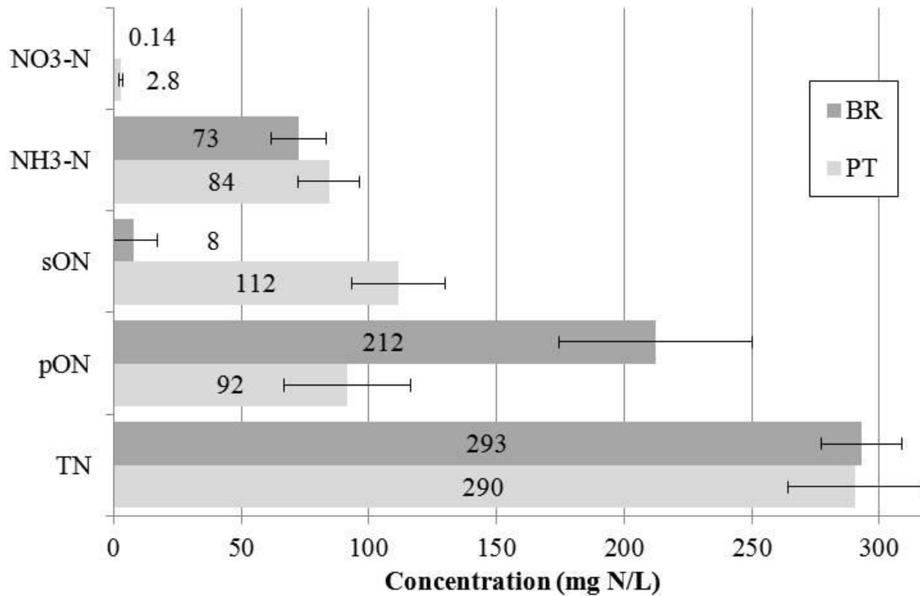

Figure 4.8 Average Concentration of Nitrogen Species in Raw and Pretreated BR WAS

A t-test at the 95% confidence level showed that the total nitrogen (TN) which included nitrate, ammonia and soluble and particulate organic nitrogen was not significantly altered by pretreatment. Another t-test at the 95% confidence level showed that the ammonia concentration was not significantly changed by pretreatment. This indicates that significant mineralization of organic nitrogen did not occur. By comparison, Bougrier et al. (2008) and Donoso-Bravo et al. (2010 b) both reported that HPTH pretreatment slightly increased the ammonia concentration. Because both the total nitrogen and ammonia concentrations were unaltered by pretreatment, the organic nitrogen (ON)

concentration also remained unchanged. However, as shown in Figure 4.8, ON was solubilized. The average ON solubilization was 49% ± 11%, as calculated according to equation 4.6.

$$ON\ solubilization\ \% = \frac{sON_{PT} - sON_{BR}}{pON_{BR}} \times 100\% \quad (4.6)$$

The nitrogen content of the organics was calculated by dividing the measured particulate ON (pON) by the measured particulate COD (PCOD). The average pON/PCOD ratios before and after pretreatment were found to equal 0.053 ± 0.006 mgN/mgCOD and 0.052 ± 0.016 mgN/mgCOD, respectively. A t-test revealed that there was no significant different difference between these means at the 95% confidence level. This indicates that all types of particulate organics were solubilized to the same extent by pretreatment.

The measured pON/PCOD values were less than the typical value of 0.07 mgN/mgCOD reported by Henze et al. (2008) for active biomass and decay products which suggested the presence of storage products such as glycogen or PHA that do not contain nitrogen. A mass balance approach on the nitrogen species was therefore employed to estimate the contribution of storage products to the biomass COD. In equations 4.7 and 4.8 below, the measured pON/PCOD ratio was defined as fN_{OBS}, the typical pON/PCOD ratio for biomass was defined as fN_Z and the pON/PCOD ratio for stored COD was defined as fN_{STO}. Assuming that X_{STO} was present and that the PCOD consisted of X_{STO}, Z_{bh} and Z_e, the fN_{OBS} value may be expressed by equation 4.7.

$$fN_{OBS} = \frac{fN_{STO} + fN_Z\,(Z_{bh} + Z_e)}{X_{STO} + Z_{bh} + Z_e} \quad (4.7)$$

The value of fN_{STO} was assumed to be zero and the fraction of the PCOD that was present as storage products (f_{STO}) was equal to the ratio $\frac{X_{STO}}{pCOD}$. Substituting these values into equation 4.7 and solving for f_{STO} yielded equation 4.8.

$$f_{STO} = 1 - \left(\frac{fN_{OBS}}{fN_Z}\right) \quad (4.8)$$

Substituting the range of fN_{OBS} values measured in the BR into equation 4.8 yielded an average f_{STO} value of 25 ± 9%. As mentioned in section 4.2.2, the average f_{STO} values calculated using equation 4.3 were 54 ± 28%. Thus the difference between the f_{STO} values calculated using equations 4.3 and 4.8 was 29% and a t-test at the 95% confidence level revealed that this difference was significant. It is believed that the f_{STO} values estimated using equation 4.8 were more reliable that those estimated using equation 4.3 because only one value (fN_Z) was assumed in this approach whereas two values

(Y_Z and Y_{STO}) were assumed in equation 4.3. The f_{STO} values were shown to be sensitive to the assumed values in both equations. From a practical standpoint, it seems unlikely that the mass of COD stored within the cells could be much higher than the mass of the cells.

The soluble nitrogen (sN) was calculated as the sum of the nitrate, ammonia and soluble organic nitrogen. Based on the calculated decrease in VSS concentration caused by pretreatment ($VSS_{destroyed}$) and the pON/PCOD and COD/VSS ratios measured in the BR WAS, the expected increase in soluble nitrogen due to pretreatment was 119 ± 24 mgN/L, as determined by equation 4.9.

$$Increase\ in\ sN\ \left(\frac{mgN}{L}\right) = \frac{COD}{VSS}_{measured} \times \frac{pON}{pCOD}_{measured} \times VSS_{destroyed} \qquad (4.9)$$

Based on seven independent measurements of the nitrogen species in the raw and pretreated WAS, the average soluble nitrogen increase due to pretreatment was 118 ± 26 mgN/L. A t-test at the 95% confidence level showed no significant difference between the measured values and the expected values calculated by equation 4.9.

In summary, the HPTH pretreatment employed in this study substantially solubilized the COD of the WAS while conserving the TCOD. Furthermore, organics were preferentially solubilized over inorganics. These findings are supported by the results of similar published research. Lastly, the analyses of the nitrogen species in this study indicated that pretreatment solubilized rather than mineralized proteins and that all types of particulate organics were solubilized to the same extent by pretreatment.

4.2.4 Aerobic Digester

The pH in the AD was measured throughout the project as one way of assessing the stability of the digester. The pH remained relatively constant during Phases 1 and 2 despite the fact that it was receiving feed with a lower pH in Phase 2 than in Phase 1. The measured pH of the AD WAS from day 179 until the end of the project is shown in Figure 4.9. The pH remained within the range reported by Tchobanoglous et al. (2003) for uninhibited heterotroph activity. During Phase 1, the average pH of the AD WAS was 8.43 ± 0.09 compared to 8.33 ± 0.17 during Phase 2. A t-test at the 95% confidence level revealed no significant difference between the Phase 1 and 2 average values. During Phases 1 and 2, the average pH of the AD WAS was 8.30 ± 0.20. As mentioned in section

2.1.4, HPTH has been linked to the degradation of macromolecules into acidic compounds such as volatile fatty acids. The insignificant pH decrease in the AD WAS from Phase 1 to 2 suggests that the acidic compounds believed to be generated by the pretreatment process were biodegraded in the AD.

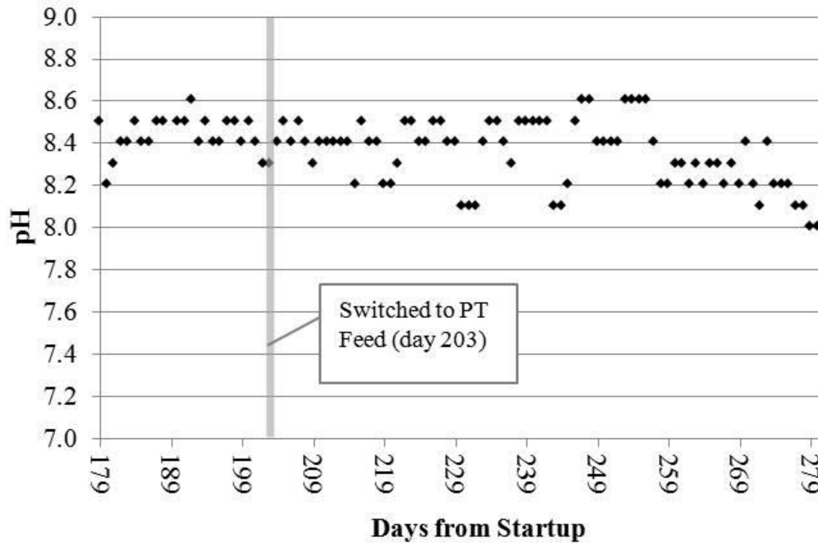

Figure 4.9 pH in AD WAS

The COD and SS species in the AD were measured by conventional analyses throughout Phases 1 and 2 of the project to assess the performance of the digester. As previously mentioned, the concentration of the synthetic substrate fed to the BR was halved on day 179. Consequently, the concentrations of the COD and SS species in both the BR and AD steadily decreased following this change. From day 197 until the end of the project the measured TCOD, SCOD, ffCOD and VSS concentrations in the AD remained relatively stable, despite the fact that the AD feed was switched from raw to pretreated BR WAS on day 203, i.e. at the beginning of Phase 2.

The average concentrations of the COD species were calculated for the stable period of Phase 1 (day 197 to 203) and Phase 2 (day 203 to day 283) and plotted in Figure 4.10. The average PCOD, SCOD and ffCOD concentrations decreased 12%, 23% and 7%, respectively, from Phase 1 to 2. T-tests at the 95% confidence level showed that the decreases in PCOD and SCOD were significant whereas the decrease in ffCOD was insignificant. However, the decreases in PCOD and SCOD were considered relatively small. Because the measured ffCOD concentrations in the AD WAS were equivalent in Phases 1 and 2, it was deduced that pretreatment did not generate S_{us} at the pretreatment temperature of 150°C that was employed in this study. This finding is in agreement with previous research which showed that HPTH pretreatment only produced refractory compounds that were

essentially S_{us} when the temperature was above 150°C (Bougrier et al., 2007; Climent et al., 2007; Dwyer et al., 2008; Donoso-Bravo et al., 2010 b).

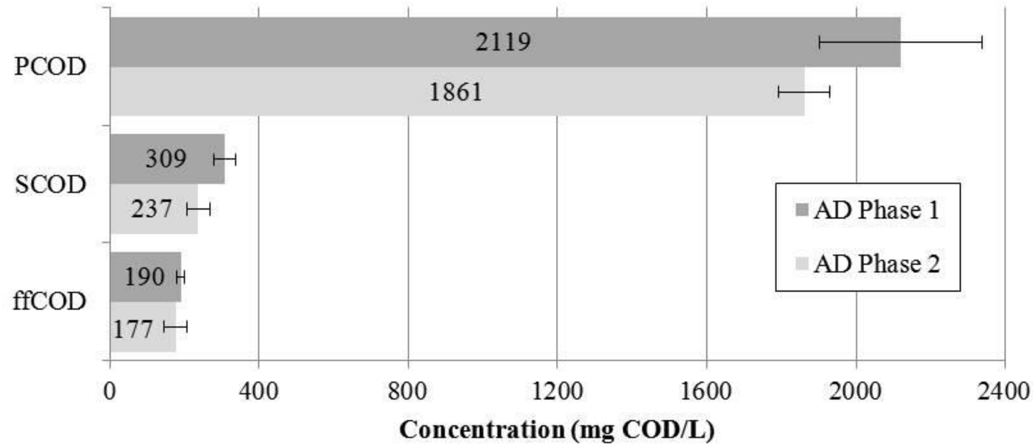

Figure 4.10 Average COD Concentrations in Phase 1 and 2 AD WAS

The observed stability of the measured TCOD, SCOD, ffCOD and VSS concentrations in the AD from day 197 until the end of the project is shown in Figure 4.11. During this period the average measured COD/VSS ratio of the AD WAS was calculated to be 1.44 ± 0.11. A t-test hypothesis at the 95% confidence level showed that this value was equivalent to the typical value of 1.42 for active biomass and decay products. This validated the assumption that the AD WAS consisted entirely of these two components during Phases 1 and 2. Hence, it was concluded that any storage products that may have been present in the BR WAS were depleted in the AD.

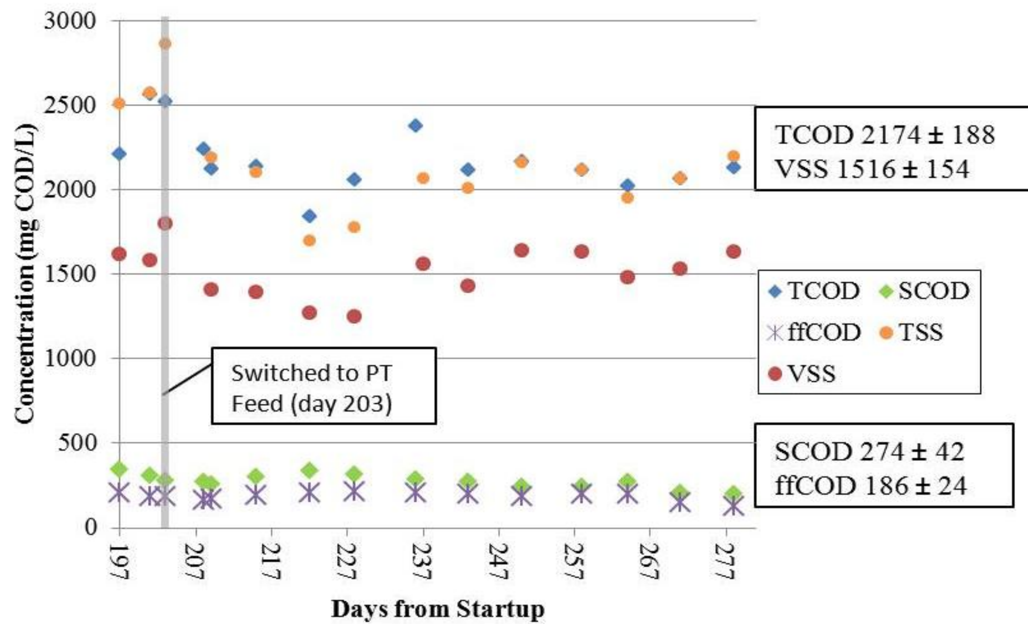

Figure 4.11 COD and SS Concentrations in AD WAS

The AD WAS contained some colloidal COD based on the data presented in Figure 4.11. A t-test at the 95% confidence level showed a significant difference between the average measured SCOD and ffCOD concentrations in the AD WAS. This difference, calculated to be 88 mg/L, was the colloidal COD concentration and it comprised 4 ± 1% of the TCOD of the AD WAS. As reported in section 4.2.3, the BR WAS did not contain significant concentrations of colloidal COD hence it would appear that a small amount was generated in the AD during Phase 1. The pretreated BR WAS contained an average colloidal COD concentration of 644 ± 285 mg/L hence most of this was removed in the AD during Phase 2.

Based on the average measured ffCOD concentrations in the BR and AD WAS, it would appear that S_{us} was generated in the digester in addition to that generated in the BR. The average ffCOD concentration in the AD WAS was 133 mg/L higher than that in the BR WAS and a t-test at the 95% confidence level showed that this difference was significant. This difference was attributed to additional S_{us} that was generated in the digester which suggested that the S_{us} was generated by endogenous decay rather than by growth. Similar to the S_{us} generated in the BR, the S_{us} generated in the AD was probably due to the production of SMPs. Many studies have shown that the majority of soluble organic matter in WWTP effluent is of microbial origin (Namkung and Rittmann, 1986). A portion of the SMPs generated in the AD may have been due to the decay of bacteria that stored

COD. For example, S_{us} generation upon the decay of polyphosphate accumulating organisms (PAOs) has been included in the BioWin Integrated Model®.

Figure 4.12 shows that switching from raw to pretreated feed did not alter the VSS concentration of the AD WAS. The average VSS concentration of the AD WAS was 1667 ± 117 mg/L in Phase 1 and 1557 ± 90 mg/L in Phase 2 and a t-test at the 95% confidence level revealed no significant difference between these two average values. This indicates that pretreatment did not change the fraction of the BR WAS that could be biodegraded by the AD biomass, despite the fact that the organics in the pretreated WAS were substantially more solubilized than the organics in the raw WAS. With the HRT employed in the AD it was expected that the VSS concentration in the AD would have decreased had pretreatment increased the extent of aerobic biodegradability of the digester feed.

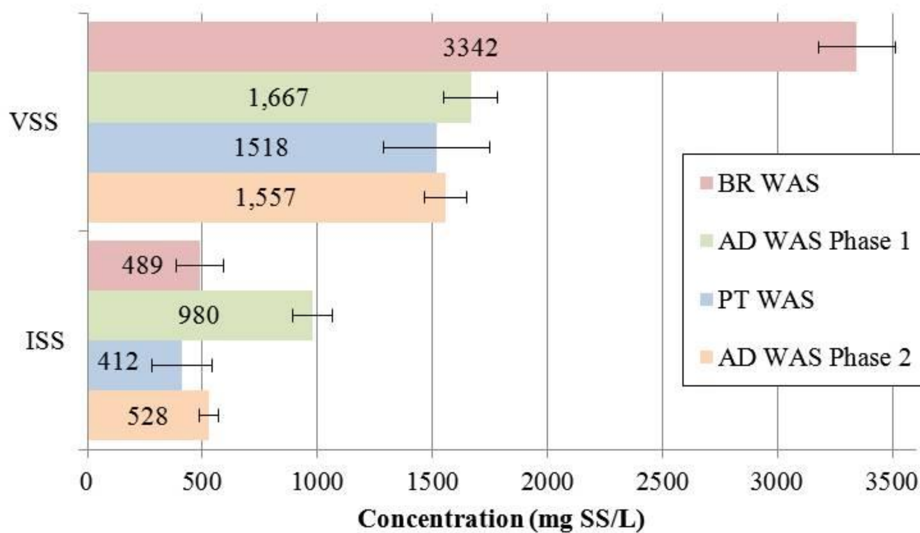

Figure 4.12 Average SS Concentrations in Sludge Streams in Phases 1 and 2

The VSS removal in the AD was calculated according to equation 4.10. The average VSS removal in the AD was 50 ± 7% in Phase 1. According to Tchobanoglous et al. (2003), VSS reductions ranging from 35 to 50% are achievable by aerobic digestion with an SRT of 40 d at 20°C. Thus the VSS reduction in the Phase 1 AD was at the upper end of the reported range despite the fact that the SRT was only 10 d. It is important to note that the reported range was for aerobic digesters treating WAS from WWTPs with primary treatment where 20 to 35% of the WAS is assumed to be non-biodegradable. The high observed VSS reduction in the Phase 1 AD was likely due to two reasons. First, the non-biodegradable fraction of the WAS was expected to be lower than typical because the system in this study was fed with synthetic wastewater that did not contain X_i. Therefore, the non-

biodegradable particulate COD fraction of both the BR and AD WAS consisted only of Z_e. Second, a substantial fraction of stored COD was present in the BR WAS and this was likely more biodegradable than Z_{bh}.

$$VSS\ removal\ \% = \frac{VSS_{AD\ Feed} - VSS_{AD\ WAS}}{VSS_{AD\ Feed}} \quad (4.10)$$

Using equation 4.10, it was shown that VSS removal did not occur in the AD in Phase 2. A t-test at the 95% confidence level showed that the average VSS concentration of the pretreated BR WAS was equivalent to that of the Phase 2 AD WAS. Thus the digester did not remove VSS beyond that which had been removed by pretreatment. This was expected because pretreatment substantially solubilized organic matter, as was shown in section 4.2.3.

Although the TCOD, SCOD, ffCOD and VSS concentrations remained relatively stable in the AD during Phases 1 and 2, the average ISS concentration decreased 46% in Phase 2, as shown in Figure 4.12. A t-test at the 95% confidence level showed that this decrease was significant. Ramdani et al. (2012) showed that the ISS/TSS ratio for active heterotrophs is almost four times greater than that for endogenous decay products. Thus the measured ISS concentration may have been lower in the AD in Phase 2 than in Phase 1 because the active fraction in the AD was lower in Phase 2. As will be presented in section 4.3.2.3, the active fraction in the AD determined from the offline respirometric data was 55 ± 13% in Phase 1 and 32 ± 7% in Phase 2. The difference between these two averages was 23% and a t-test at the 95% confidence level showed that this difference was significant.

In summary, the results presented in this section suggested that the AD WAS was entirely comprised of Z_{bh} and Z_e in Phases 1 and 2. It appeared that any X_{STO} present in the WAS fed to the AD was depleted in the AD. The VSS concentration in the AD remained constant during Phases 1 and 2 which indicated that pretreatment did not alter the biodegradable fraction of the BR WAS. Furthermore, the ffCOD concentration in the AD remained constant during Phases 1 and 2 which suggested that pretreatment did not generate S_{us}. These findings will be used in section 4.3.2.4 and chapter 5 to help fractionate the COD of the raw and pretreated BR WAS and AD WAS.

4.3 Biological Analyses of Process Streams

4.3.1 Online Respirometry

In the online respirometry in the AD, automatic aeration control was used to allow the DO concentration to fluctuate between 3 and 5 ppm, as described in section 3.6.1. A typical DO concentration profile measured in the AD over 3 days is shown in Figure 4.13. The rate of DO decline was calculated for every time period between the high and low DO set points to generate OUR values.

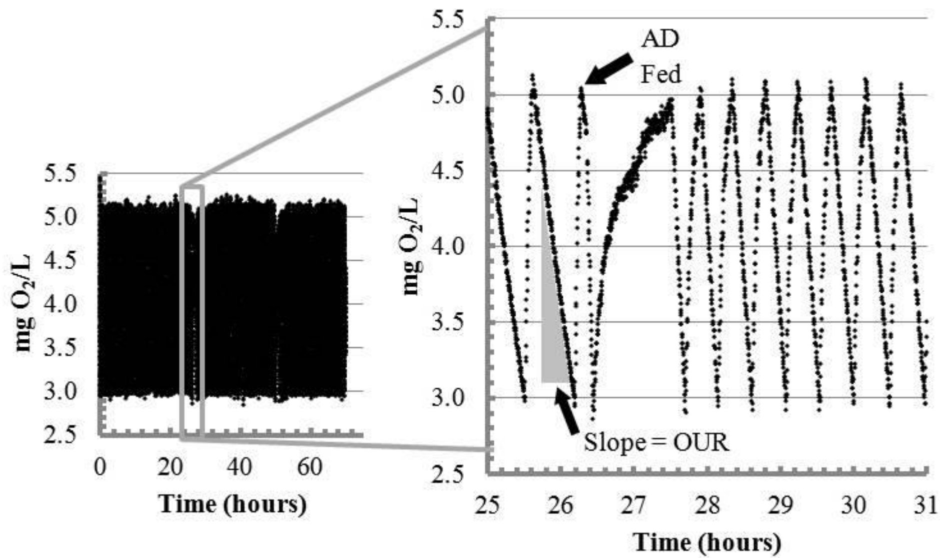

Figure 4.13 Typical DO Concentration in AD and OUR Calculation from Rate of DO Decline

The OUR values were plotted with respect to time as shown in Figure 4.14. Three complete reaction periods are visible in Figure 4.14. The highest OUR values occurred immediately following substrate addition when the substrate utilization rate in the digester was highest. It can also be observed that the OUR values became effectively constant at the end of each reaction period, implying that most of the substrate was consumed. The area under the OUR curve during the entire reaction period was calculated as the cumulative oxygen uptake (ΣOU_t) in the reactor. The measured OU was entirely carbonaceous because the nitrogenous OU was suppressed throughout Phases 1 and 2. Since the length of the reaction periods varied by 2 to 3 hours between days, the average ΣOU_t per reaction period was determined over the three consecutive days of the online respirometry test.

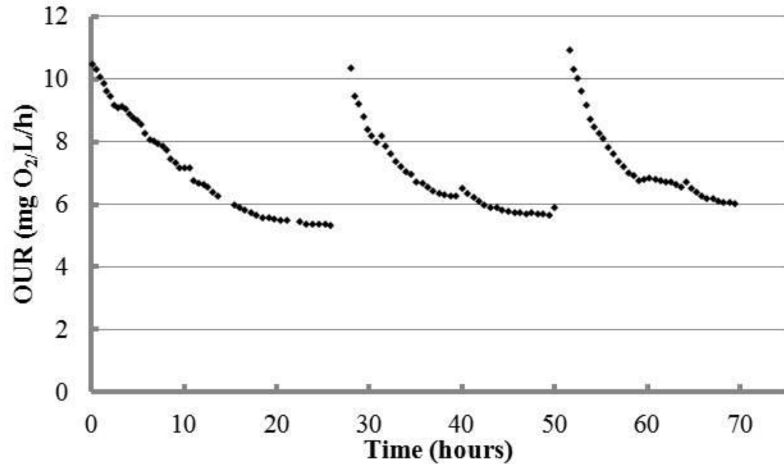

Figure 4.14 Typical OUR in AD

Only one successful online respirometry measurement was made during Phase 1 whereas 12 successful measurements were obtained during Phase 2. The limited number of measurements in phase 1 was due to challenges associated with the experimental apparatus. The Jenco© model LD-900-5-DO Industrial Inline DO Probe used in this project consisted of a gold cathode and silver anode submersed in an electrolyte solution. Oxygen entered the probe through a permeable membrane by diffusion and was reduced at the cathode, creating a measurable electrical current. The Bodine® Model 0158 DC Gearmotor used to mix the AD contents imparted a current to the liquid via the shaft and this interfered with the DO measurement and control. Several unsuccessful attempts were made to isolate the DC gearmotor. Hence, accurate ΣOU_t measurements were only obtained when this DC gearmotor was replaced by an AC motor.

In addition, it was during Phase 1 that it was observed that the anode and cathode of the DO probe underwent rapid and extensive fouling while the probe was immersed in the AD and this negatively impacted the DO readings. This fouling may have been caused by the relatively high chloride concentration in the AD, originating from the ammonium chloride, ferric chloride, magnesium chloride and calcium chloride used in the synthetic wastewater. A special reconditioning kit was ordered from the supplier and used after every respirometry run to restore the gold cathode. The silver anode was cleaned after every test by soaking the probe overnight in 3% ammonium hydroxide and then sanding it to remove the tarnish. Accurate ΣOU_t measurements were only obtained when these cleaning procedures were carefully followed prior to use.

The ΣOU_t in the AD over the reaction period was assumed to be comprised of two parts: the ΣOU due to the endogenous respiration of the AD biomass (ΣOU_e) and the ΣOU attributed to the WAS fed to the AD (ΣOU_s). The ΣOU_s is discussed further here. In Phase 1, the COD of the BR WAS fed to the AD was expected to be essentially comprised of Z_{bh}, Z_e and X_{STO}. Therefore, the ΣOU_s was expected to result from growth on X_{STO} and decay of Z_{bh}. In Phase 2, the COD of the pretreated BR WAS was expected to be comprised of S_{bsc}, X_{sp} and Z_e. It was hypothesized that pretreatment would not change Z_e but would convert X_{STO} to S_{bsc} and Z_{bh} to portions of S_{bsc} and X_{sp}. Therefore the ΣOU_s in Phase 2 was expected to result from growth on S_{bsc} and X_{sp} and decay of Z_{bh}.

An analysis of the COD and VSS measurements in the AD showed that the average food to microorganism (F/M) ratio was 0.30 ± 0.05 mgCOD/mgVSS throughout Phases 1 and 2. This was low enough to cause a decay-dominated OUR response (Musser, 2009). Therefore, it was assumed that any growth OUR response would be masked by the decay response. This assumption was validated by the results. The OUR response in the AD shown in Figure 4.14 was measured during Phase 2. However, these curves were typical of the OUR measured in the AD throughout Phases 1 and 2. As shown in Figure 4.14, the OUR decreased over the reaction period. The shape of these decreasing OUR curves shows substrate depletion and endogenous respiration but not growth.

To determine the ΣOU_s value (mg O_2/L) for each online respirometric test, the ΣOU_e value was first estimated from the offline respirometric tests on the AD WAS. The endogenous OUR equation 2.4 proposed by Jones et al. (2009) was modified to eliminate the nitrogenous OUR, producing equation 4.11.

$$\mathrm{OUR} = 1 - f\ (b_h) Z_{bh0} e^{-(b_h)t} \tag{4.11}$$

A nonlinear regression fit of equation 4.11 to the endogenous OUR response measured by offline respirometry was used to yield the initial active heterotroph concentration in the AD WAS, as described previously in section 2.1.5.1. Offline respirometry was carried out at 25°C hence the value of $b_{h,25C}$ was determined using equation 4.12. The assumed value of $b_{h,20C}$ was 0.24 d^{-1} (Henze et al., 2008).

$$b_{h,T} = b_{h,20C}\ (1.029)^{T-20} \tag{4.12}$$

Using equation 4.12, the value of $b_{h,25C}$ was calculated to be 0.28 d^{-1}. The results of the offline respirometric tests will be presented in the next section however the measured and predicted endogenous OUR (OUR_e) curves obtained on day 264 are shown as an example in Figure 4.15. This curve fit was representative of those obtained for the other measurement events.

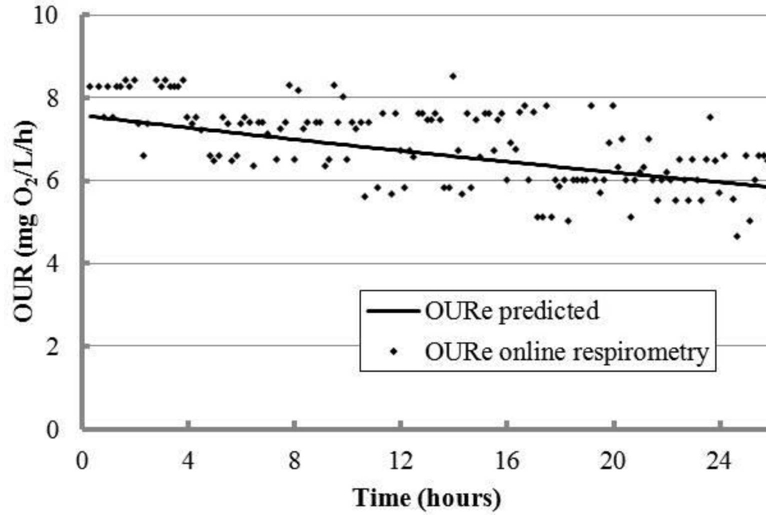

Figure 4.15 Measured and Predicted Endogenous OUR in AD WAS at 25°C

Using the predicted initial active heterotroph concentration, equation 4.11 with $b_{h,20C}$ was then used to estimate the endogenous OUR response at 20°C in the AD since online respirometry was carried out at 20°C. This endogenous OUR response was then plotted together with the total OUR response measured by online respirometry on day 264, as shown in Figure 4.16. These curves are representative of those obtained for the other measurement events. The area under this endogenous OUR curve was then used to estimate the ΣOU_e value which was then subtracted from the area under the total OUR curve (ΣOU_t) to yield the ΣOU_s value.

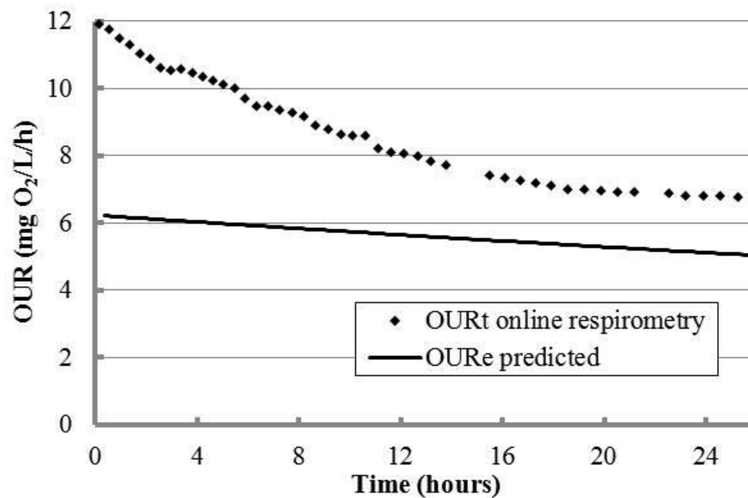

Figure 4.16 Total and Endogenous OUR in AD at 20°C

The online respirometric data was used to determine whether pretreatment changed the overall aerobic degradability of the BR WAS. The ΣOU_s value was divided by the measured TCOD concentration of the substrate, i.e. the WAS fed to the AD, for every successful test result. Calculation of the ΣOU_s/TCOD ratio was based upon the mass associated with each response for one cycle of operation. As mentioned in section 3.3.2, 0.6 L of BR WAS was fed to the AD each cycle and the AD was 6 L. Thus the calculated ΣOU_s values (mg O_2/L) were multiplied by 6 L and the corresponding measured TCOD concentrations (mg COD/L) of the substrate were multiplied by 0.6 L.

The average value of the ΣOU_s/TCOD ratio was calculated for Phases 1 and 2. A significant increase in these values between Phase 1 and 2 average would indicate that pretreatment changed the overall aerobic biodegradability of the WAS. The average value of the ΣOU_s/TCOD ratio was calculated to be 16% in Phase 1 and 15 ± 4% in Phase 2. A t-test at the 95% confidence level showed that the difference between these two averages was insignificant which suggested that pretreatment did not change the aerobic degradability of the WAS over the AD reaction period.

4.3.2 Offline Respirometry

4.3.2.1 Shape and Magnitude of OUR Curves

Unlike online respirometry, it was expected that the OUR curves generated from offline respirometry would show growth responses. This was because the F/M ratio in the offline respirometric tests on bottles containing AD WAS and raw or pretreated BR WAS ranged from 5 to 10 mgCOD/mgVSS which was sufficiently high to show a growth response if substrate were present in the bottle.

In Phase 1 it was assumed that the BR WAS consisted of Z_{bh}, Z_e and X_{STO} hence the OUR response in bottles containing whole BR WAS was expected to reflect growth on X_{STO}, depletion of X_{STO} and decay of Z_{bh}. The SRT in the BR was 5 days whereas it was 10 days in the AD hence the BR WAS was expected to have a higher ratio of active organisms to endogenous decay products as compared to the AD. Correspondingly, the OUR (mg O_2/L/h) in the BR WAS was expected to be greater than that in the AD WAS at any given time during the offline respirometric test.

Three offline respirometric tests were run during the first phase of the project, from day 179 to 203, with the bottles containing BR WAS only, AD WAS only and BR WAS that was inoculated with AD WAS. Only the second and third tests produced useful data as residual rbCOD from the synthetic wastewater was present in the first test that was run on day 175. This test was conducted just before the synthetic feed concentration was halved. Figure 4.17 shows a typical OUR response in the bottles of the two successful tests.

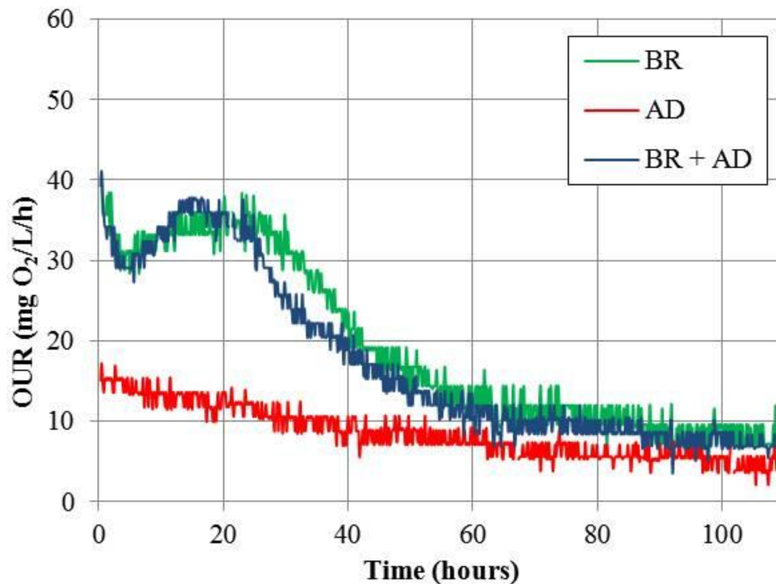

Figure 4.17 Typical Phase 1 OUR in BR WAS, AD WAS and Inoculated BR WAS

In the bottle containing AD WAS, the OUR decreased exponentially with time. This was the expected response for a sludge that consumes oxygen through endogenous respiration alone. In the bottles containing BR WAS only and inoculated BR WAS, the OUR decreased for the first 5 hours of the test, then increased, peaked and finally decreased exponentially with time. The magnitude of these two curves during the first 5 hours was more than twice that of the AD WAS curve. This was expected because the BR was assumed to contain a higher active fraction than the AD. The peak in the OUR curves for BR WAS and inoculated BR WAS suggested biomass growth. Furthermore, because the peaks were observed in these two responses but absent in the AD WAS response, the substrate for this biomass growth was assumed to be present in the BR WAS alone. As previously mentioned, the BR WAS used in the offline respirometry test was taken from the BR at the end of the reaction period, long after the synthetic substrate had been consumed. Hence the apparent growth was not due to the synthetic substrate. The observed OUR responses were therefore consistent with the previously stated hypothesis that the BR WAS contained X_{STO} in addition to Z_{bh} and Z_e. This hypothesis was supported by the previously described low average COD/VSS ratio of 1.25 in the BR

WAS; a low average pON/PCOD ratio of 0.05 in the BR WAS; and the observed generation of S_{us} in the AD. It appeared that X_{STO} was consumed during the batch respirometry and exerted an oxygen demand that was different than that which would be attributed to endogenous respiration.

In Phase 2, offline respirometric tests were run on bottles containing pretreated BR WAS only, AD WAS only and pretreated BR WAS inoculated with AD WAS. Once the feed to the AD was switched from raw to pretreated BR WAS on day 203, one offline respirometric test was carried out every week for 9 weeks. Due to power failures, two of these tests did not run until the endogenous OUR response was clearly visible and thus the data could not be used. Three tests were run when the AD WAS was not acclimatized to the pretreated BR WAS. Only one of these three tests produced usable data as there were various sources of error in the other tests. These error sources included restricted oxygen flow through the tubing and needles, leaks in the bottle caps and water collection in the oxygen supply lines. The set of OUR curves generated in the successful offline respirometric tests on pretreated BR WAS and the associated AD WAS are presented in Appendix C.

Overall, there was no distinguishable difference in the shape of the OUR curves from the inoculated bottles in either the non-acclimatized test or the progressively acclimatized tests. The differences in the areas under these curves will be discussed in section 4.3.2.2.

In Phase 2 it was expected that the OUR response in the bottles containing pretreated BR WAS and AD WAS would result from both growth and decay since pretreatment was expected to convert portions of the TCOD of the BR WAS to S_{bsc} and X_{sp}. As shown in section 4.2.3, pretreatment increased the SCOD concentration by 56%. Since it was also shown in section 4.2.4 that pretreatment did not generate S_{us}, the SCOD generated by pretreatment was expected to be S_{bsc}. Similar to Phase 1, the OUR responses in bottles containing AD WAS were expected to show only decay.

Figure 4.18 shows typical OUR responses in the bottles during Phase 2 of the project. The labels PT, AD and PT + AD represent the respective OUR curves for pretreated BR WAS, AD WAS and pretreated BR WAS inoculated with AD WAS. This graph is representative of the eight successful offline respirometric tests involving pretreated BR WAS and the associated AD WAS. In the bottles containing only AD WAS, the OUR decreased exponentially with time as expected. At any given time during the respirometry test, the height of this curve was always less than that of the Phase 1 AD WAS, indicating that the active fraction was lower in the AD in Phase 2 than in Phase 1. The bottles containing only pretreated BR WAS exerted oxygen uptake, indicating viable cells remained in the

sludge after pretreatment. Estimates of the heterotroph concentrations will be presented in section 4.3.2.3. The OUR curve for pretreated BR WAS showed a peak between 60 and 70 hours, suggesting delayed biomass growth. However, the area under this peak comprised only 5% of the total area under the curve. Therefore, this delayed peak was considered negligible.

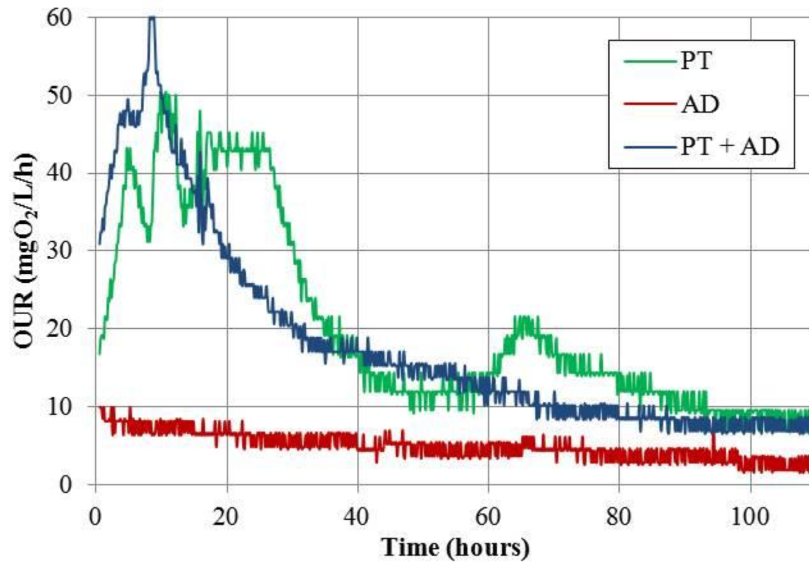

Figure 4.18 Typical Phase 2 OUR in Pretreated BR WAS, AD WAS and Inoculated Pretreated BR WAS

Comparing Figures 4.17 and 4.18, the OUR curve for the inoculated pretreated BR WAS peaked earlier and to a higher value than the OUR curve for the inoculated raw BR WAS. This response is characteristic of growth on rbCOD.

For each successful respirometry run, the mass OUR curves (mg O_2/h) were plotted by multiplying the measured OUR concentration (mg O_2/L/h) by the volume of WAS in the bottle. The endogenous OUR curve was subtracted from the OUR curve of the inoculated bottle to yield the OUR response of the substrate only. This approach was carried out for both the whole and filtered substrate. The substrate OUR responses from the offline respirometric tests on pretreated BR WAS and associated AD WAS were compared to the substrate OUR responses from the tests on BR WAS and associated AD WAS. The OUR responses of the whole and filtered pretreated BR WAS substrates were found to be reproducible as were those of the whole and filtered BR WAS substrates. Therefore, the OUR responses of the whole and filtered pretreated BR WAS substrates (Phase 2) were compared to the OUR responses of the whole and filtered BR WAS substrates (Phase 1), as shown in Figure 4.19.

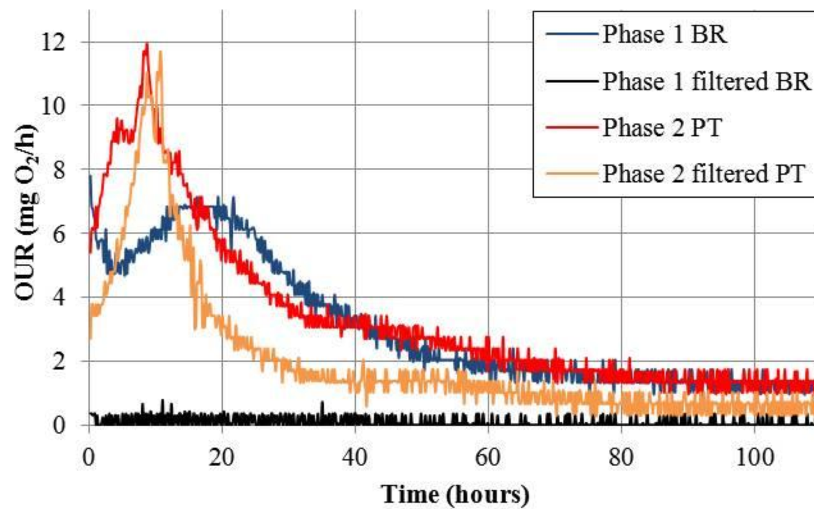

Figure 4.19 Comparison of Typical Substrate OUR in Phases 1 and 2

From Figure 4.19 it can be seen that the OUR in the filtered BR WAS was negligible, indicating the absence of soluble biodegradable COD. The biodegradable COD of the BR WAS was thus entirely particulate. This was expected because filtration removed the only two components in the BR WAS that were assumed to exert an oxygen demand, i.e. Z_{bh} and X_{STO}. In contrast, the area under the OUR curve for the filtered pretreated WAS contributed more than 60% of the area under the whole pretreated WAS curve which demonstrates that pretreatment substantially solubilized biodegradable COD.

4.3.2.2 Extent of Aerobic Biodegradability

Similar to online respirometry, offline respirometry was used to determine whether pretreatment changed the overall aerobic degradability of the BR WAS. The cumulative oxygen uptake (mg O_2) associated with the substrate during every successful offline respirometric test was divided by the measured TCOD (mg COD) of the substrate at the beginning of the test. The average value of this ratio was calculated for the raw BR WAS and pretreated BR WAS. A significant difference between these average ratios would indicate that pretreatment changed the overall aerobic biodegradability of the WAS. This ratio was only determined for the whole substrate because it was shown that the SCOD concentration in the BR WAS was negligible. Hence the cumulative oxygen uptake and TCOD of the filtered BR WAS was negligible.

The cumulative oxygen uptake (mg O_2) associated with the substrate was calculated in two separate ways using the offline respirometric data. The first approach relied on the measured mass of TCOD in the respirometry bottle at the beginning and end of the test whereas the second approach employed the mass of gas phase oxygen measured by the respirometer during the test.

In the first approach, the difference between the initial and final mass of TCOD in each bottle ($TCOD_i - TCOD_f$) was calculated for each offline respirometric test. The mass of TCOD in the bottle was determined by multiplying the measured TCOD concentration (mg COD/L) in the bottle by the volume of liquid in the bottle. The ($TCOD_i - TCOD_f$) value for the substrate was then determined by subtracting the ($TCOD_i - TCOD_f$) value in the bottle containing only AD WAS from the ($TCOD_i - TCOD_f$) value in the bottle containing AD WAS and raw or pretreated BR WAS.

The biodegradability of the substrate was then assessed by dividing the ($TCOD_i - TCOD_f$) value for the substrate by the initial TCOD of the substrate in the bottle for each respirometry run, as shown in Figure 4.20. A significant difference between the average ($TCOD_i - TCOD_f$)/$TCOD_i$ ratios for raw BR WAS and pretreated BR WAS would indicate that pretreatment changed the overall aerobic biodegradability of the WAS. Because the BR WAS was shown to remain stable throughout Phases 1 and 2, the only factor that could cause a change in the overall aerobic biodegradability of the BR WAS would be pretreatment.

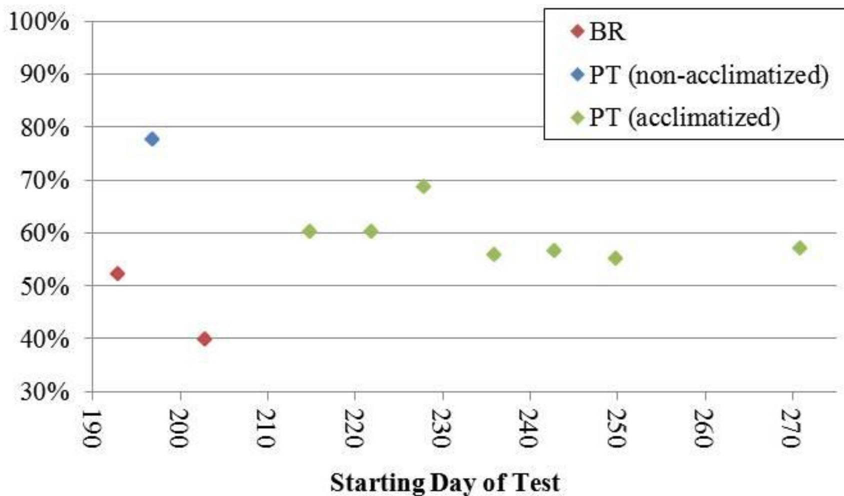

Figure 4.20 ($TCOD_i - TCOD_f$)/$TCOD_i$ Ratio from Offline Respirometry on Substrate

Using the data presented in Figure 4.20, the average ($TCOD_i - TCOD_f$)/$TCOD_i$ values were 46 ± 15% for the BR WAS, 78% for the pretreated BR WAS with non-acclimatized inoculum and 59 ± 11% for

the pretreated BR WAS with acclimatized inoculum. A t-test at the 95% confidence level showed that the average ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratio for BR WAS was equivalent to the average ratio for pretreated BR WAS with acclimatized inoculum, indicating that pretreatment did not change the fraction of the BR WAS that could be degraded by acclimatized inoculum. This finding corroborated the results of the online respirometric tests and supported the finding that the VSS concentration in the AD remained constant during Phases 1 and 2. Considering the seven data points for the acclimatized pretreated ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratio in Figure 4.20, the last four data points had an average and standard deviation of 56 ± 1%, which showed very low variability. The stability of these last four measurements indicated that the AD biomass had acclimatized to the pretreated feed.

Based on the data presented in Figure 4.20, the ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratio for pretreated BR WAS with non-acclimatized inoculum was 32% higher than the average ratio for BR WAS and a t-test at the 95% confidence level showed that this difference was significant. This suggested that pretreatment increased the fraction of the BR WAS that could be degraded by non-acclimatized inoculum. However, because there was only one valid measurement of pretreated BR WAS with non-acclimatized inoculum, additional experiments should be carried out to further investigate this trend. By comparison, Kianmehr (2010) found that with WAS pretreated with ozone, the biodegradable fraction determined by offline respirometry was higher using an acclimatized inoculum than a non-acclimatized inoculum. However, Kianmehr (2010) also demonstrated that it was unnecessary to acclimatize the inoculum when using sonication pretreatment. Further studies should be carried out to determine the impact of biomass adaptation on the degradation of WAS pretreated by HPTH.

The second approach to estimate the aerobic biodegradability from the offline respirometry employed gas phase oxygen consumption to estimate the cumulative oxygen uptake (mg O_2) associated with the substrate. In these cases, the ΣOU_s value (mg O_2) for each offline respirometric test was determined by subtracting the measured ΣOU in the sample bottles containing only AD WAS (ΣOU_e) from the ΣOU value in the inoculated bottle (ΣOU_t). The ΣOU_s value was then corrected for the mass of oxygen required to reach liquid saturation in the bottle since the DO concentration was effectively zero at the beginning of the respirometry test. In addition, the ΣOU_s value was corrected for the mass of oxygen manually drawn into the bottle using a syringe in the air-tightness test performed at the start of the run. The biodegradability of the substrate was then assessed by dividing the ΣOU_s value for the substrate by the measured initial mass of TCOD of the substrate for each respirometry run, as shown in Figure 4.21.

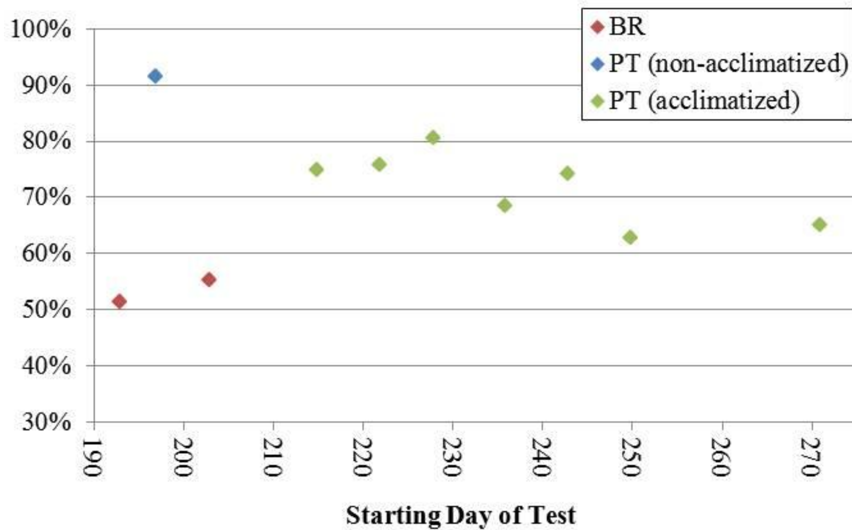

Figure 4.21 $\Sigma OU_s/TCOD_i$ Ratio from Offline Respirometry on Substrate

Overall, the trends in Figure 4.21 were similar to those in Figure 4.20 in terms of the relative magnitude of the ratios among the different types of substrate. The average $\Sigma OU_s/TCOD_i$ values were calculated for the BR WAS, pretreated WAS with non-acclimatized inoculum and pretreated WAS with acclimatized inoculum. It was found that each of these average values was higher than the respective average value calculated using the measured initial and final TCOD concentrations in the sample bottle. Because the $(TCOD_i - TCOD_f)/TCOD_i$ ratio in the first approach relied on only one measurement technique whereas the $\Sigma OU_s/TCOD_i$ ratio in the second approach involved two techniques, there may have been a higher level of error associated with the second approach than with the first. The potential sources of error are discussed subsequently.

The accuracy of the TCOD measurements was verified in several ways. Every day the TCOD concentrations in the respirometry bottle were measured, blank and standard samples were also tested. The measured concentrations of the blanks and standards were significantly close to the expected values. In addition, on day 203 and 250, four replicate samples were taken from a randomly selected respirometry bottle and a COD standard was added to half of these replicates. The average measured TCOD of the two replicates containing only sample was subtracted from that of the replicates containing standard and sample in order to calculate the TCOD of the standard. There was no significant difference between the calculated value of the standard and the directly measured value. Therefore, the measured TCOD concentrations in the offline respirometry bottles at the beginning and end of the test were believed to be accurate.

The TCOD closure error was calculated according to equation 4.13 for the bottles containing AD WAS only, substrate only and inoculated substrate in every successful batch respirometry test. In this equation, the cumulative oxygen uptake (mg O_2) in the bottle calculated by the first approach ($TCOD_i$ – $TCOD_f$) was subtracted from that calculated by the second approach that employed the gas phase oxygen consumption (ΣOU). This difference was then divided by the measured mass of TCOD in the bottle at the end of the test ($TCOD_f$) and expressed as a percentage.

$$TCOD\ Closure\ Error\ \ \% \ = \frac{\Sigma OU - (TCOD_i - TCOD_f)}{TCOD_f} \qquad (4.13)$$

The cumulative oxygen uptake calculated on the basis of oxygen consumption was consistently higher than that calculated by COD removal; hence the TCOD closure error was consistently positive. The average TCOD closure error for bottles containing only AD WAS was found to be 5 ± 2%, which was relatively low. By comparison, the TCOD closure error ranged from 10 to 35% in the other bottles. As expected, the cumulative oxygen uptake in bottles containing AD WAS was consistently lower than the cumulative oxygen uptake in bottles containing either substrate or inoculated substrate. The higher TCOD closure errors would therefore appear to be associated with the higher oxygen demands. Since the TCOD measurements were believed to be accurate, this positive closure error indicated that the offline respirometric tests overestimated the oxygen uptake in the bottles. As a result, the $\Sigma OU_s/TCOD_i$ ratio was probably less accurate than the ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratio. Therefore, the assessment of the impact of pretreatment on the overall aerobic biodegradability of the BR WAS was based on the comparison of the average ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratios rather than a comparison of the average $\Sigma OU_s/TCOD_i$ ratios.

As presented in section 4.3.1, the average $\Sigma OU_s/TCOD_i$ ratio determined by online respirometry was 16% for BR WAS and 15 ± 4% for pretreated BR WAS. By comparison, the average ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratio determined by offline respirometry was 46 ± 15% for BR WAS and 59 ± 11% for pretreated BR WAS with acclimatized inoculum. Thus the average ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratios determined by offline respirometry for BR WAS and pretreated BR WAS with acclimatized inoculum were 30% and 44% higher, respectively, than the corresponding ratios determined by online respirometry. T-tests at the 95% confidence level showed that both of these differences were significant. It was hypothesized that the online respirometric measurements underestimated the cumulative oxygen uptake over the reaction period because these measurements failed to capture rapid and high oxygen uptake. In Phases 1 and 2, delays of 30 to 60 minutes were observed in raising the DO concentration from the low to high set-points during the period immediately after the AD was fed. These delays were presumably due to insufficient aeration in the AD. In addition, the differing

methodologies of the two respirometric tests may have further contributed to generating ($TCOD_i$ – $TCOD_f$)/$TCOD_i$ ratios from offline respirometry that were significantly higher than the magnitudes of the $\Sigma OU_s/TCOD_i$ ratios from online respirometry. The offline respirometric tests were run for 4 to 5 days at an F/M ratio of 5 to 10 mgCOD/mgVSS. By contrast, the online respirometric tests measured the ΣOU in the AD over the reaction period of 22 hours and the average F/M ratio in the AD was 0.30 ± 0.05 mgCOD/mgVSS.

In summary, the offline respirometric results showed that pretreatment did not change the overall aerobic biodegradability of the BR WAS. It was previously shown in section 4.2.3 that pretreatment also did not change the TCOD of the BR WAS. Therefore, it was hypothesized that the concentration of non-biodegradable COD in the pretreated BR WAS was equivalent to that in the raw BR WAS. This non-biodegradable COD was assumed to be comprised of only Z_e since the S_{us} concentration in the BR WAS was negligible and S_{us} was not generated by pretreatment. Thus the concentration of bCOD in the BR WAS was assumed to be equivalent to that in the pretreated BR WAS. The bCOD of the BR WAS was shown to be comprised of Z_{bh} and X_{STO}. It was hypothesized that the bCOD of the pretreated BR WAS was comprised of S_{bsc} and X_{sp}. This hypothesis was based on the shape and magnitude of the OUR curves generated by offline respirometry and the measured increase in SCOD concentration caused by pretreatment. It was not possible to use the approach proposed by Kianmehr (2010) to quantify the S_{bsc} concentration in the pretreated BR WAS using the offline respirometric data. The F/M ratio was high in the tests employed in the current study hence significant growth occurred in bottles believed to contain S_{bsc}. The peaks due to growth were high and extended and multiple successive peaks were typically observed. The area under the OUR curve was thus a complex function of cell growth, decay and the oxidation of S_{bsc} and X_{sp}. Instead, the S_{bsc} and X_{sp} concentrations in the pretreated BR WAS were estimated by fitting OUR responses predicted by activated sludge models to the measured offline respirometric data. This approach will be presented in section 5.

4.3.2.3 Active and Endogenous COD Fractions

This section presents the results of an analysis of the offline respirometric data that estimated the concentrations of Z_{bh} and Z_e in the WAS samples. These estimated concentrations were compared to the measured TCOD concentration of the WAS to determine the active and endogenous COD fractions. The estimation of Z_{bh} and Z_e in the BR WAS is presented first. The X_{STO} concentration

was then estimated by subtracting the sum of Z_{bh} and Z_e from the TCOD of the BR WAS. Estimates of the active and endogenous COD fractions in the pretreated BR WAS are subsequently presented. Lastly, the estimation of the active fractions in the AD and corresponding endogenous fractions during Phases 1 and 2 are presented.

The literature review presented in section 2.1.5.1 discussed two methods to estimate the active fraction of the BR WAS. However, in this study, the OUR response measured in batch respirometry bottles containing only BR WAS showed both decay and growth and hence the active fraction could not be determined by fitting the endogenous OUR equation 2.4. The results suggested that in these batch tests, the stored COD in the BR WAS was used to generate new biomass. Hence, the concentration of active heterotrophs in the BR WAS was determined by the offline respirometric test carried out on day 278 on sample bottles containing BR WAS and synthetic feed in high F/M ratios.

Figure 4.22a presents a typical respirogram obtained from this test and Figure 4.22b shows the exponential growth portion of the curve. Using the data from Figure 4.22b, ln(OUR) versus time was plotted in Figure 4.22c. The active heterotroph concentration in the sample bottles (Z_{bh0}) was determined by equation 2.3 using the slope and y-intercept from the plot of Figure 4.22c and assuming typical values for Y_h and b_h. These three graphs were plotted for each sample bottle and the active heterotroph concentration in each bottle was calculated. For each bottle, the concentration obtained using equation 2.3 was then multiplied by the dilution factor in the bottle to estimate the concentration of active heterotrophs in the BR WAS ($Z_{bh,BR}$).

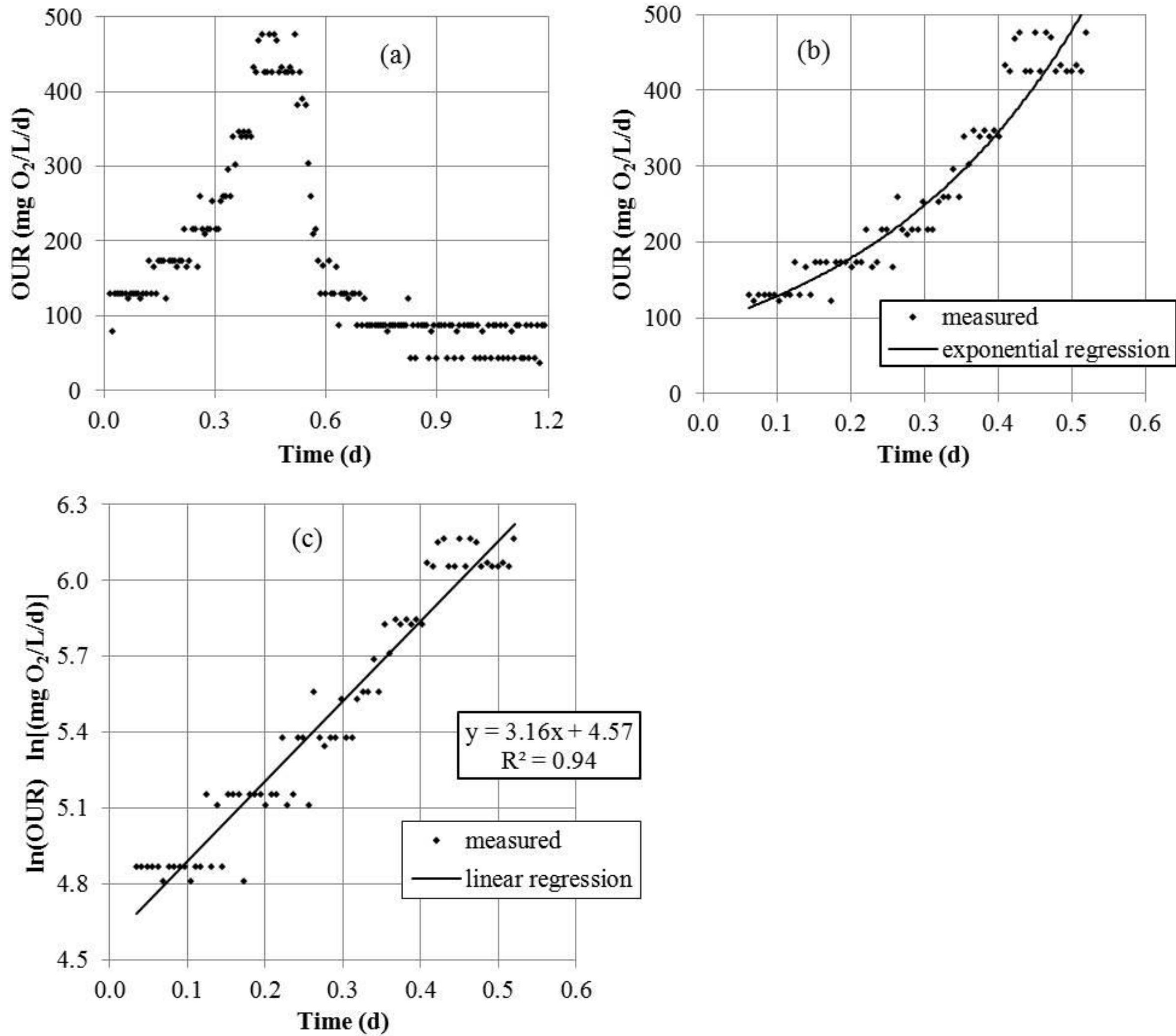

Figure 4.22 Typical Respirometry Data Used to Estimate $Z_{bh,0}$ (a) Total Respirogram (b) Exponentially Increasing Portion of Respirogram (c) Log of Exponentially Increasing Portion

The average concentration of active heterotrophs in the BR WAS was estimated to be 2133 ± 73 mg COD/L. As reported in section 4.2.2, the average TCOD concentration in the BR WAS was 4170 ± 324 mg/L. Therefore the average active fraction of the BR WAS was 51 ± 4%. This was 17% lower than the active fraction reported by Ramdani et al. (2012) who operated an MBR with a 5.2 day SRT fed with sodium acetate as the sole carbon source. The active fraction was expected to be lower in the BR WAS because a portion of the biodegradable synthetic substrate was believed to be stored inside the cells in the BR WAS whereas the WAS employed by Ramdani et al. (2012) did not contain stored COD.

The concentration of endogenous decay products was determined by the endogenous respiration approach:

$$Z_{e,BR} = f b_h Z_{bh,BR}(SRT_{BR}) \quad \text{(Parker, 2010)} \quad (4.14)$$

Where b_h = 0.28 d^{-1} at 25°C and f = 0.2. The dataset of concentrations estimated for the active heterotrophs as per equation 2.3 was substituted into equation 4.14 to generate a dataset of concentrations for the endogenous decay products. The average COD concentration of endogenous products in the BR WAS was 512 ± 17 mg/L and the average endogenous COD fraction was 12 ± 1%. This was 20% lower than the endogenous fraction reported by Ramdani et al. (2012) who operated a similar system. The lower fraction was expected because the BR WAS contained stored COD whereas the WAS used by these authors did not. The presence of stored COD would result in less grown biomass and hence less decay of biomass to produce Z_e. The measured ffCOD in the BR WAS was 53 ± 12 mg COD/L which was only 1% of the TCOD. Thus the soluble non-biodegradable COD fraction was considered negligible. The sum of the active and endogenous COD fractions was 63 ± 4% hence the remaining 37 ± 4% was assumed to be stored COD. This estimate of f_{STO} was comparable to the estimate derived from the measured pON/PCOD value (f_{STO} = 25 ± 9%) and the estimate derived from the measured COD/VSS ratio (f_{STO} = 54 ± 28%).

Based on the literature review of HPTH pretreatment studies, it was expected that pretreatment would substantially if not completely inactivate the heterotroph population in the WAS. In this study, the active fraction was determined from the OUR response in the sample bottles containing only pretreated BR WAS. The Z_{bh0} concentration in the sample bottles containing pretreated BR WAS was determined by plotting ln(OUR) versus time for the exponentially increasing portion of the measured OUR curve and fitting equation 2.3 with typical values for Y_h and b_h. The Z_{bh0} concentration was then multiplied by the dilution factor in the bottle to determine the concentration of active heterotrophs in the pretreated BR WAS ($Z_{bh,PT}$). The active fraction in the pretreated BR WAS was consistently found to be less than 5%. For the purpose of fractionating the COD, it was therefore assumed that pretreatment fully inactivated the biomass.

The constant measured VSS concentration in the AD during Phases 1 and 2 and the results of the online and offline respirometric tests showed that the biodegradable COD fraction (bCOD) of the WAS was unchanged by pretreatment with an acclimatized biomass. It was therefore assumed that none of the endogenous residue was converted to biodegradable COD by pretreatment. Since the TCOD of the WAS was unchanged by pretreatment, the endogenous fraction of the pretreated WAS was assumed to be equivalent to that of the BR WAS, i.e. 12 ± 1%.

The concentration of active heterotrophs in the bottles containing AD WAS was determined by fitting equation 2.4 by nonlinear regression to the measured OUR in these bottles. This concentration was then multiplied by the dilution factor in the bottle to estimate the concentration of active heterotrophs in the AD WAS ($Z_{bh,AD}$). It is believed that this approach was valid because the measured OUR response was typical of that associated with endogenous decay. The concentration of active heterotrophs in the steady-state AD was found to be 1200 ± 260 mg COD/L in Phase 1 and 700 ± 150 mg COD/L in Phase 2. The average TCOD of the AD WAS throughout the project was 2174 ± 188 mg COD/L hence the average active fraction was 55 ± 13% in Phase 1 and 32 ± 7% in Phase 2. A t-test at the 95% confidence level showed no significant difference between the average active fraction in the BR WAS and that in the Phase 1 AD WAS. It was expected that the active fraction of the AD WAS would be less than that of the BR WAS in Phase 1 without pretreatment. However, unlike the BR WAS, the AD WAS did not contain stored COD as: the measured COD/VSS ratio of the AD WAS was typical of that for active biomass and decay products; and the OUR response in the AD WAS showed only an endogenous decay response. Therefore, it is believed that the biomass in the AD oxidized the storage products present in the BR WAS for energy production and cell synthesis. The active fraction in the AD WAS was therefore comprised of active biomass from the BR WAS that was undergoing endogenous decay in the digester and new biomass that grew in the digester on the storage products contained in the BR WAS. This would explain the higher than expected active fraction of the AD WAS in Phase 1.

The endogenous residue in the AD was assumed to consist of the sum of that originating from the BR WAS and that generated in the AD and was estimated using equation 4.15:

$$Z_{e,AD} = f b_h Z_{bh,AD} \; SRT_{AD} \; + Z_{e,BR} \quad (4.15)$$

Where $b_h = 0.28\ d^{-1}$ at 25°C and f = 0.2. The concentrations estimated for the active heterotrophs in the AD in Phases 1 and 2 were substituted into equation 4.15 to generate corresponding values for the concentrations of the endogenous decay products. The average $Z_{e,AD}$ value in Phase 1 was estimated to be 1090 ± 350 mg COD/L and hence the endogenous COD fraction was estimated to be 50 ± 17%. In Phase 2, the $Z_{e,AD}$ value was estimated to be 850 ± 270 mg COD/L and the endogenous COD fraction was estimated to be 39 ± 13%. The active and endogenous COD fractions in the BR, pretreated and AD WAS as well as the stored COD fraction in the BR WAS will be summarized in section 4.3.2.4.

4.3.2.4 COD Fractionation of the WAS

Figure 4.23 summarizes the COD fractions in each sludge stream that could be estimated from the compiled results of the conventional analyses and offline respirometric tests. Only significant COD fractions are shown. The sludge streams included the BR WAS, pretreated BR WAS, AD WAS treating raw BR WAS (Phase 1) and AD WAS treating pretreated BR WAS (Phase 2). The COD fractions presented in Figure 4.23 were also estimated by simulating the entire system with calibrated activated sludge models and this will be discussed further in chapter 5.

BR WAS

37 ± 4%	X_{STO}
51 ± 4%	Z_{bh}
12 ± 1%	Z_e

PT WAS

S_{bsc}	?
X_{sp}	?
Z_e	12 ± 1%

Phase 1 AD WAS

9 ± 1%	S_{US}
55 ± 13%	Z_{bh}
50 ± 17%	Z_e

Phase 2 AD WAS

8 ± 2%	S_{US}
32 ± 7%	Z_{bh}
39 ± 13%	Z_e

Figure 4.23 COD Fractionation of Sludge Streams

The BR WAS was comprised of Z_{bh}, Z_e and X_{STO}. As described in section 4.3.2.3, the COD fractions attributed to Z_{bh} and Z_e were estimated to be 51 ± 4% and 12 ± 1%, respectively, based on analyses of the offline respirometric data. The stored COD fraction, f_{STO}, was estimated in three different ways: using the COD/VSS ratios (f_{STO} = 54 ± 28%); using the pON/PCOD ratios (f_{STO} = 25 ± 9%); and using the COD not attributed to active and endogenous fractions determined by offline respirometry (f_{STO} = 37 ± 4%). As discussed in section 4.2.3, the first approach was probably less accurate than the second because only one value was assumed in the first whereas two values were assumed in the second. A t-test at the 95% confidence level showed that the f_{STO} estimates from the second and third approaches differed significantly. However, this difference was only 12% which was considered relatively small. Hence, for the purpose of COD closure, the COD fraction attributed to X_{STO} was assumed to be 37 ± 4% in Figure 4.23. The BR WAS contained an average measured ffCOD concentration of 53 ± 12 mg/L which was assumed to be S_{us} from SMPs. This fraction comprised only 1.3 ± 0.3% of the TCOD of the BR WAS hence it was considered negligible in the overall COD fractionation presented in Figure 4.23.

The pretreated BR WAS was assumed to be comprised of S_{bsc}, X_{sp} and Z_e. It was shown that pretreatment did not change the TCOD of the BR WAS nor did it change the biodegradable fraction. Therefore the endogenous fraction in the pretreated BR WAS was assumed to be equivalent to that in

the BR WAS, i.e. 12 ± 1%. It was assumed that X_{STO} and Z_{bh} in the BR WAS were converted to a mixture of S_{bsc} and X_{sp} in the pretreated BR WAS. As shown in section 4.2.3, pretreatment increased the average SCOD concentration in the BR WAS from 1% to 57%. Since it was shown in section 4.2.4 that pretreatment did not generate S_{us}, the SCOD generated by pretreatment was expected to be S_{bsc}. Because this S_{bsc} generated by pretreatment was significantly higher than the X_{STO} estimated to be present in the BR WAS, it was assumed that X_{STO} had been fully converted to S_{bsc}. The storage products were assumed to be released when the cells were inactivated by pretreatment. The balance of S_{bsc} not originating from X_{STO} was assumed to originate from Z_{bh}. The portion of Z_{bh} not converted to S_{bsc} was assumed to be converted to X_{sp}. The S_{bsc} and X_{sp} concentrations in the pretreated BR WAS were estimated by fitting OUR responses predicted by activated sludge models to the measured offline respirometric data. This work will be presented in chapter 5.

The AD WAS was comprised of S_{us}, Z_{bh} and Z_e in Phases 1 and 2. The S_{us} was believed to be generated by the decay of cells that stored COD. As shown in section 4.2.4, the average measured ffCOD concentration in the AD WAS was 190 ± 11 mg COD/L in Phase 1 and 177 ± 31 mg COD/L in Phase 2. Thus the average S_{us} fraction was 9 ± 1% in Phase 1 and 8 ± 2% in Phase 2. Both of these fractions are substantial and were therefore included in the overall fractionation of the AD WAS presented in Figure 4.23. The sum of the average calculated S_{us}, Z_{bh} and Z_e concentrations in the Phase 1 AD WAS was 2480 ± 436 mg/L and a t-test at the 95% confidence level showed no significant difference between this sum and the average measured TCOD of the Phase 1 AD WAS. This mass balance closure suggested that the active and endogenous fractions were well estimated. However, the sum of the average calculated S_{us}, Z_{bh} and Z_e concentrations in the Phase 2 AD WAS was 1727 ± 310 mg/L which was 447 mg/L less than the average measured TCOD of the Phase 2 AD WAS. A t-test at the 95% confidence level showed that this difference was significant. This lack of mass balance closure may have been due to inaccuracies in the respirometric measurements, error in the nonlinear regression fit of equation 2.4 to the respirometric data or inaccuracies in the parameter estimates for b_h, f and Y_h. As will be discussed in sections 5.2 and 5.3, the COD fractions in the various sludge streams were also estimated using calibrated activated sludge models thus the COD fractionation of the Phase 2 AD WAS will be revisited.

5. Development of the Pretreatment Model

The results of the conventional analyses and respirometric tests were previously used to fractionate the COD of the various sludge streams in order to identify the COD transformations caused by pretreatment. It was concluded that pretreatment did not alter the aerobically biodegradable fraction of the BR WAS. However, the results of the batch respirometric tests indicated that pretreatment increased the rate of aerobic degradation. The experimental results presented in section 4 could not be directly used to quantify the concentrations of S_{bsc} and X_{sp} in the pretreated BR WAS. In this chapter these concentrations will be estimated using activated sludge models. Furthermore, modeling was employed to verify the COD fractions in the various sludge streams that were estimated from the results presented in section 4. An overview of the modeling procedure is discussed first.

The Phase 1 system was simulated using two different models, the BioWin 3.1® Integrated Sludge Model and the ASM3 Model. The models were initially calibrated by systematically adjusting key kinetic and stoichiometric parameters within their typical ranges such that the predicted concentrations of particulate COD species in the BR and AD WAS were statistically equivalent to the respective average measured concentrations. The accuracy of the parameter estimates was then improved by minimizing the sum of squared differences between the predicted and measured OUR responses from offline respirometry on BR WAS. The calibrated model that best fit the measured data was then employed to simulate the Phase 2 system. The concentrations of S_{bsc} and X_{sp} in the pretreated BR WAS were adjusted such that the predicted concentrations of PCOD and SCOD in the pretreated BR WAS and PCOD in the Phase 2 AD WAS were statistically equivalent to the respective average measured concentrations. The accuracy of the estimated S_{bsc} and X_{sp} concentrations was then improved using a least squares regression between the predicted and measured OUR responses from offline respirometry on inoculated pretreated BR WAS. Once the S_{bsc} and X_{sp} concentrations had been estimated, the COD fractionation of the pretreated BR WAS was complete. A COD-based stoichiometric pretreatment model was then developed for the dose of HPTH pretreatment employed in this study. This model was evaluated and its limitations were identified.

5.1 Modeling Approach

This section describes the approach taken to simulate the startup and operation of the BR-AD system using two different activated sludge models. An overview of the operation of the reactors throughout the project is first discussed. As described in section 4.1, the BR was operated as an SBR with an SRT of 5 d. The BR was initially seeded with activated sludge and then fed daily with a synthetic wastewater. The concentrations of alkalinity and inorganic nutrients in the synthetic wastewater were specified according to the theoretical composition. The TCOD and COD fractionation of the synthetic wastewater were estimated based on COD measurements and required some assumptions. On day 20, the AD was started up by being filled with 2 L of BR WAS per day until it contained 6 L. The AD continued to be fed daily with BR WAS until the end of the project. The AD was initially operated as an SBR with an HRT of 3 d and an SRT of 10 d. On day 77, the operation of the AD was simplified so that the HRT and SRT were both 10 d. The concentration of the synthetic wastewater fed to the BR was halved on day 179 and this feed concentration was maintained until the end of the project on day 283. The focus period of the project consisted of two phases: Phase 1 from day 179 to 203 when the AD was fed with raw BR WAS; and Phase 2 from day 203 to 283 when the AD was fed with pretreated BR WAS.

The startup and operation of the BR-AD system was simulated using the BioWin 3.1® Integrated Model in the BioWin® platform. The system was simulated exactly as it was operated from day 0 to 283. The change in the operation of the AD implemented on day 77 and the change in the concentration of the synthetic wastewater implemented on 179 were included in the simulation and it was predicted that a new steady state condition would be reached following each change. The results of the conventional analyses confirmed this prediction. As discussed in section 4.2.2, the concentrations of COD and SS species measured by conventional analyses remained relatively stable from day 197 until day 283, indicating that the BR was at steady state during this period. The concentrations of TCOD, SCOD, ffCOD and VSS measured in the AD during this period also remained relatively constant, showing that the AD was at steady state as well.

It was hypothesized that the steady-state conditions of the Phase 1 BR-AD system only depended on the operating conditions and synthetic wastewater recipe employed during this phase. This assumption was tested using a separate, simplified simulation in which the BR was seeded with a typical activated sludge, fed synthetic wastewater with the composition used from day 179 onwards

and then allowed to reach steady-state. The AD was then started up and operated as it had been during Phase 1, until it stabilized. The steady-state compositions of the sludge streams in this simplified simulation were found to be equivalent to the compositions of the respective sludge streams in the Phase 1 steady-state system achieved by modeling the actual operation from day 0. The simplified approach was therefore deemed adequate to model the Phase 1 BR-AD system.

In addition to the BioWin 3.1® Integrated Model, the Phase 1 BR-AD system was also modeled with the ASM3 Model in the BioWin® platform. Although the BioWin 3.1® Integrated Model is more complex and more widely used than the ASM3 Model, it does not include processes for the storage of COD by ordinary heterotrophic biomass that does not accumulate phosphorus (Z_{bh}). The results presented in section 4 showed that the BR WAS contained stored COD in addition to active heterotrophs and decay products. In the ASM3 Model, all readily biodegradable substrate is first stored inside cells before it is used for growth and endogenous respiration. It was therefore hypothesized that the ASM3 Model would be well suited for this project.

The configuration of the Phase 1 BR-AD system used in the BioWin® platform is shown in Figure 5.1. The overall system was simulated at 20°C as this was the average temperature at which the BR and AD were operated. In the simulations, the concentrations of the COD, SS and nitrogen species were monitored in each sludge stream so that the predicted steady-state concentrations could be compared with the average measured concentrations. In addition, the OUR in the simulated AD was monitored so that it could be compared to the online respirometric results.

In order to simulate the batch respirometric tests, a variable volume reactor labeled "OUR Test" was included in the configuration in Figure 5.1. This reactor was simulated at 25°C because the offline respirometric tests were operated at this temperature. Once the BR-AD system was at steady-state, 150 mL of WAS from the BR was fed to the OUR Test reactor and the simulation was continued for a number of days. The DO concentration in the simulated batch reactor was held constant at 2 mg/L and the OUR in this reactor was monitored over time. Offline respirometry simulations were also carried out using 50 mL AD WAS only and a mixture of 150 mL of BR WAS and 50 mL of AD WAS.

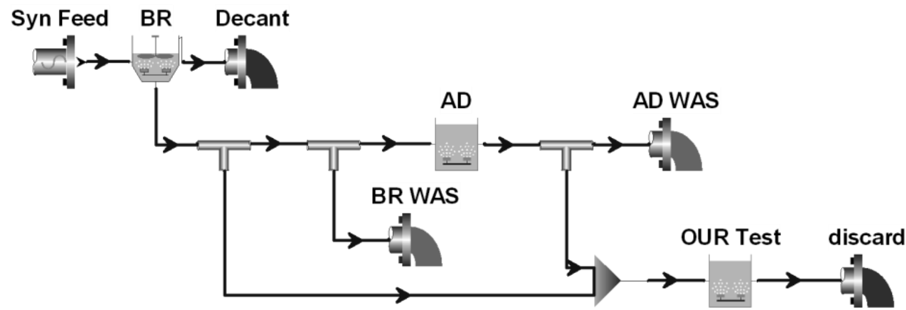

Figure 5.1 Configuration of Phase 1 BR-AD System in BioWin® 3.1 Platform

It was shown in sections 4.2.2 and 4.2.4 that the WAS from the BR and AD contained concentrations of ffCOD and this was assumed to be S_{us} from SMPs generated in the reactors. A significantly higher average concentration of S_{us} was measured in AD WAS than in the BR WAS which suggested that the SMPs were generated by endogenous decay rather than by growth. Neither the BioWin 3.1® Integrated Model nor the ASM3 Model predicts the channeling of COD into SMPs for processes involving Z_{bh}. Therefore, the simulated synthetic wastewater was assigned a S_{us} concentration equivalent to the average measured ffCOD concentration in the BR WAS. No provision was made to simulate the additional S_{us} shown to be generated in the AD hence it was expected that both models would underestimate the ffCOD and SCOD concentrations in the AD.

During the focus period of the project, the nitrate concentrations measured in the BR and AD were consistently below 1 mg N/L, indicating that nitrification was negligible in both reactors. In order to simulate this response, the value of the maximum specific growth rate of the ammonia oxidizing biomass (μ_a) in both the BioWin 3.1® Integrated Model and ASM3 Model was set to zero. Besides this parameter, the kinetic and stoichiometric parameters employed in each model differed considerably and will therefore be discussed separately in the next two sections. An overview of the capabilities of each model will also be presented.

5.1.1 BioWin 3.1® Integrated Model

The BioWin 3.1® Integrated Model is a biochemical mass balance model that characterizes organic wastewater components in terms of COD. The model describes biological processes, chemical precipitation reactions and gas-liquid mass transfers using fifty state variables and sixty process expressions. Because it incorporates many different process units, the model is well suited for

simulating entire WWTPs. This work focused on the processes within the overall model that address the behavior of heterotrophic biomass in aerobic systems and the associated consumption of substrates and generation of byproducts. The selection of the appropriate parameter values for these processes was an important element of the model implementation.

The values of f and b_h for heterotrophs that were employed in chapters 3 and 4 were 0.2 and 0.24 d^{-1}, respectively, at 20°C (Henze et al., 2008). A value of 0.6 was assumed for Y_h since this was the value reported by Ramdani et al. (2012) for acetate-fed systems. These values are based on the endogenous respiration approach to describe the kinetics of cell maintenance, endogenous metabolism, decay, lysis and death. However, the BioWin 3.1® Integrated Model employs the death-regeneration approach. The corresponding values of f and b_h using this approach were 0.09 and 0.53 d^{-1} at 20°C, respectively. The value of b_h was calculated to be 0.61 d^{-1} at 25°C using equation 4.12. The f value is considered to be independent of temperature.

Besides μ_a, b_h, Y_h and f, the default values in the BioWin® 3.1 Integrated Model were used for the other kinetic and stoichiometric parameters. As mentioned, the model was used to simulate the Phase 1 BR-AD system. The initial simulation employed Y_h, f and b_h values of 0.6, 0.09 and 0.53 d^{-1} at 20°C, respectively, and then the model was calibrated by systematically adjusting Y_h, f and b_h within their respective typical ranges. This will be described in section 5.2.

5.1.2 ASM3 Model

The ASM3 Model simulates oxygen consumption, sludge production, nitrification and denitrification in activated sludge systems treating wastewater of primarily domestic origin. Compared to the BioWin 3.1® Integrated Model, the ASM3 Model is much simpler as it has only thirteen state variables and twelve reactions. The ASM3 Model is designed to be the core of many different models. Although it does not include modules for biological phosphorus removal, chemical precipitation, growth of filamentous organisms or pH calculations, they can easily be connected as add-on modules (Henze et al., 2000).

The matrix representation of the ASM3 Model in the BioWin platform is presented in Table 5.1. This matrix shows only the processes that were believed to be involved in the Phase 1 BR-AD system. Therefore, anoxic processes and the growth and endogenous respiration of autotrophs were omitted

because these processes were determined to be negligible. The BR and AD were always operated aerobically and the measured nitrate levels in the various sludge streams were consistently below 1 mg N/L. Both the stoichiometric matrix and kinetic rate expressions shown in Table 5.1 were adapted to the BioWin® platform from the ASM3 Model proposed by Henze et al. (2000). The matrix employs symbols specific to the BioWin® platform for compounds and kinetic and stoichiometric parameters. The ASM3 Model does not include a variable for acetate (S_{bsa}) thus all rbCOD was represented as S_{bsc}. Furthermore, the ASM3 Model does not include a variable for slowly biodegradable colloidal COD (X_{sc}) thus all slowly biodegradable COD was represented as X_{sp}. The ASM3 Model does not differentiate endogenous residue from other non-biodegradable particulate COD and therefore represented both as X_i. Stored COD in the ASM3 Model is represented by the symbol S_{phb} in the BioWin® platform. Therefore, S_{phb} will be used to denote stored COD in this chapter. The ASM3 Model matrix presented by Henze et al. (2000) included independent variables for alkalinity and suspended solids however each of these variables is a combined variable in the BioWin® platform. Therefore, these variables were calculated according to the BioWin method when the BioWin® platform was used to run the ASM3 Model.

Table 5.1 ASM3 Model in BioWin® Platform Showing Processes in Phase 1 BR-AD System

Process	DO	S_{bsc}	NH_3-N	X_i	X_{sp}	Z_{bh}	S_{phb}	Kinetic Rate Expressions
	mg O_2/L	mg COD/L	mg N/L	mg COD/L				
Hydrolysis		1	$-i_{NSS} + i_{NXS}$		-1			$k_H \times \frac{X_{sp}}{0.0001 + Z_{bh}} \times \frac{1}{K_X + \frac{X_{sp}}{0.0001 + Z_{bh}}} \times Z_{bh}$
Aerobic storage of S_{bsc} by Z_{bh}	$Y_{STO,O2} - 1$	-1	i_{NSS}				$Y_{STO,O2}$	$k_{STO} \times \frac{DO}{K_O + DO} \times \frac{S_{bsc}}{K_S + S_{bsc}} \times Z_{bh}$
Aerobic growth of Z_{bh} on S_{phb}	$1 - \frac{1}{Y_{H,O2}}$		$-i_{NBM}$			1	$-\frac{1}{Y_{H,O2}}$	$\mu_H \times \frac{DO}{K_O + DO} \times \frac{NH_3N}{K_{NH} + NH_3N} \times \frac{S_{phb}}{0.0001 + Z_{bh}} \times \frac{1}{k_{STO} + \frac{S_{phb}}{0.0001 + Z_{bh}}} \times Z_{bh}$
Aerobic endogenous respiration of Z_{bh}	$f_i - 1$		$i_{NBM} - (f_i \times i_{NXI})$	f_i		-1		$b_{H,O2} \times \frac{DO}{K_O + DO} \times Z_{bh}$
Aerobic respiration of S_{phb}	-1						-1	$b_{H,O2} \times \frac{DO}{K_O + DO} \times S_{phb}$

The values of the kinetic and stoichiometric parameters that were described in Table 5.1 are presented in Table 5.2. These values were determined by Koch et al. (2000) for the calibrated ASM3 Model at 20°C. The values of temperature-dependent kinetic parameters were calculated at 25°C using the corresponding coefficient θ_T reported by Koch et al. (2000) and the ASM3 temperature equation presented by Henze et al. (2000). When the ASM3 Model was run in the BioWin® platform, the COD/VSS ratio of storage products was specified as 1.1 gCOD/gVSS because this is the typical value for glycogen which was believed to be stored inside the cells. The particulate inert COD/VSS ratio was specified as 1.42 gCOD/gVSS because the inert fraction was expected to be solely comprised of endogenous residue.

Table 5.2 Kinetic and Stoichiometric Parameters in ASM3 Model for Phase 1 BR-AD System

Kinetic Parameter	Symbol	Unit	Value (20°C)	Value (25°C)
Hydrolysis rate constant	k_H	d^{-1}	9	11
Hydrolysis saturation constant	K_X	$\frac{g\ X_{sp}}{g\ Z_{bh}}$	1	
Aerobic storage rate constant	k_{STO}	$\frac{g\ S_{bsc}}{g\ Z_{bh}\ d}$	12	17
Inhibition constant for oxygen (DO)	K_O	$\frac{g\ O_2}{m^3}$	0.2	
Saturation constant for substrate (S_{bsc})	K_S	$\frac{g\ COD}{m^3}$	10	
Saturation constant for storage	K_{STO}	$\frac{g\ S_{phb}}{g\ Z_{bh}}$	0.1	
Heterotrophic maximum aerobic growth rate	μ_H	d^{-1}	3	4.3
Saturation constant for ammonia (NH_3-N)	K_{NH}	$\frac{g\ N}{m^3}$	0.1	
Aerobic endogenous respiration rate of Z_{bh}	$b_{H,O2}$	d^{-1}	0.3	0.4
Production of X_i in endogenous biomass respiration	f_i	$\frac{g\ X_i}{g\ Z_{bh}}$	0.2	
Aerobic yield of stored products per S_{bsc}	$Y_{STO,O2}$	$\frac{g\ S_{phb}}{g\ S_{bsc}}$	0.80	
Aerobic yield of heterotrophic biomass growth on S_{phb}	$Y_{H,O2}$	$\frac{g\ Z_{bh}}{g\ S_{phb}}$	0.80	
Nitrogen content of S_{bsc}	i_{NSS}	$\frac{g\ N}{g\ COD}$	0.03	
Nitrogen content of X_i	i_{NXI}	$\frac{g\ N}{g\ COD}$	0.04	
Nitrogen content of X_{sp}	i_{NXS}	$\frac{g\ N}{g\ COD}$	0.03	
Nitrogen content of Z_{bh}	i_{NBM}	$\frac{g\ N}{g\ COD}$	0.07	

The ASM3 Model with the values presented in Table 5.2 was used in the BioWin® platform to simulate the Phase 1 BR-AD system. The predicted responses were then matched to the measured data by systematically adjusting μ_H and $b_{H,O2}$, as will be described in section 5.2.

5.2 Phase 1 System Modeling

The BioWin 3.1® Integrated Model and the ASM3 Model were each used to simulate the Phase 1 BR-AD system. A trial and error approach was used to initially estimate the values of the key kinetic and stoichiometric parameters such that the predicted concentrations of particulate COD species in the BR and AD WAS were statistically equivalent to the corresponding average measured concentrations using t-tests at the 95% confidence level. Following this, a least squares regression between the predicted and measured OUR responses from offline respirometry on BR WAS was employed to improve the accuracy of the estimates of the parameter values.

Using a trial and error approach, the value of Y_h was adjusted while f and b_h were held constant at their initially assumed values, i.e. 0.09 and 0.53 d^{-1}, respectively. For each new value of Y_h, the system was simulated using the BioWin 3.1® Integrated Model. The values of Y_h that yielded simulated particulate COD concentrations in the reactors that were statistically equivalent to respective measured concentrations were recorded. Using these Y_h values, the offline respirometric test on BR WAS was then simulated. The Y_h value that minimized the sum of squared differences between predicted and measured OUR responses was thus determined. This approach was repeated a second time by adjusting f while keeping b_h and Y_h constant at 0.53 d^{-1} and 0.6, respectively. This approach was repeated a third time by adjusting b_h while keeping f and Y_h constant at 0.09 and 0.6, respectively. It was found that the model best fit the measured data when the values of Y_h, f and b_h were 0.67, 0.09, 0.53 d^{-1} at 20^oC, respectively. Thus the calibrated model employed the initially assumed values of f and b_h whereas the Y_h value was changed from the initially assumed value of 0.6 that was developed from systems fed by acetate. This Y_h of 0.67 was believed to be reasonable as it is the standard value reported by Henze et al. (2008) for ordinary heterotrophic organisms.

The same trial and error approach described above for the BioWin Model was used to calibrate the ASM3 Model except that the adjusted parameters were $b_{H,O2}$ and μ_h. The initially assumed values of $b_{H,O2}$ and μ_h were 0.3 d^{-1} and 3 d^{-1}, respectively. It was found that the best fit was achieved using $b_{H,O2}$ and μ_h values of 0.3 d^{-1} and 0.56 d^{-1} at 20^oC, respectively. Thus the calibrated model employed the initially assumed value of $b_{H,O2}$. However, the calibrated model employed a μ_h value that was much lower than the initially assumed value of 3 d^{-1}, i.e. the value reported by Koch et al. (2000). This indicated that the heterotrophic growth rate was lower in the current study. This difference may have

been because the current study employed synthetic wastewater whereas Koch et al. (2000) used municipal wastewater.

Tables 5.3 and 5.4 present the measured concentrations of COD, SS and nitrogen species in the Phase 1 BR-AD system and the corresponding values predicted by the calibrated BioWin 3.1® Integrated Model and the calibrated ASM3 Model. Percent differences between corresponding simulated and measured values are shown and significant differences, as defined by t-test hypotheses at the 95% confidence level, are shaded grey.

Table 5.3 Measured and Simulated COD, SS and N Species in BR WAS

Parameter	Measured		BioWin		ASM3 Model	
	Avg.	Std. dev.	Predicted	Difference from Measured (%)	Predicted	Difference from Measured (%)
PCOD	4127	334	4315	5	4218	2
SCOD	52	14	53	2	53	2
ffCOD	53	12	53	0	53	0
VSS	3342	167	3039	9	3374	1
ISS	489	103	320	35	358	27
NO_3-N	0.1	0.1	0	100	0	100
NH_3-N	73	11	71	3	70	4

Concentrations in mg/L

Table 5.4 Measured and Simulated COD, SS and N Species in Phase 1 AD WAS

Parameter	Measured		BioWin		ASM3 Model	
	Avg.	Std. dev.	Predicted	Difference from Measured (%)	Predicted	Difference from Measured (%)
PCOD	2119	218	2208	4	2142	1
SCOD	309	30	53	83	53	83
ffCOD	190	11	53	72	53	72
VSS	1667	117	1577	5	1648	1
ISS	980	85	745	24	732	25
NO_3-N	0.3	0.4	0	100	0	100
NH_3-N	112	9	108	4	113	1

Concentrations in mg/L

As shown in Tables 5.3 and 5.4, both models were successfully calibrated in terms of predicting PCOD concentrations in the BR and AD WAS that were statistically equivalent to the respective average measured concentrations. As expected, the predicted SCOD and ffCOD concentrations in the

BR and AD WAS were equivalent to the S_{us} concentration in the synthetic feed, i.e. 53 mg/L, because neither model addresses the generation of S_{us}.

The predicted NH_3-N concentrations in the WAS from the BR and AD processes were statistically equivalent to the respective average measured concentrations. This finding validated the model calibration as the NH_3-N response was independent of the calibration. The predicted nitrate (NO_3-N) concentrations significantly differed from measured levels. However, both predicted and measured concentrations were very close to zero and therefore considered negligible.

Neither model accurately predicted the ISS concentration in the BR or AD WAS. This was expected because the processes involving ISS were not well developed in either model. The predicted ISS response was not expected to significantly impact the behaviour of particulate COD throughout the system.

Compared to the measured VSS concentrations, the BioWin 3.1® Integrated Model predicted a 9% lower concentration in the BR WAS and a 5% lower concentration in the AD WAS. Both these differences were significant however the 5% difference was considered minimal. The 9% difference may have been due to the fact that the model employed the typical COD/VSS ratio of 1.42 whereas the average measured COD/VSS ratio of the BR WAS was 1.23 ± 0.08. Compared to the measured VSS concentrations, the ASM3 Model predicted a 1% higher concentration in the BR WAS and a 1% lower concentration in the AD WAS however both these differences were insignificant. This finding validated the ASM3 Model calibration as the VSS response was independent of the calibration.

In addition to the PCOD concentrations, the calibration of the models was based on predicting Z_{bh}, Z_e and S_{phb} concentrations in the BR and AD WAS that were statistically equivalent to the corresponding average measured concentrations. Tables 5.5 and 5.6 present the concentrations of Z_{bh}, Z_e and S_{phb} in the Phase 1 BR-AD system estimated using the calibrated models. Percent differences between corresponding simulated and measured values are shown and significant differences, as defined by t-test hypotheses at the 95% confidence level, are shaded grey.

Table 5.5 Measured and Simulated Z_{bh}, Z_e and S_{phb} in BR WAS

Parameter	Measured				BioWin Model			ASM3 Model		
	Avg.	Std. dev.	% of PCOD		Avg.	% of PCOD	Difference from Measured (%)	Avg.	% of PCOD	Difference from Measured (%)
			Avg.	Std. dev.						
PCOD	4127	334			4315		5	4218		2
Z_{bh}	2133	73	52	5	3452	80	62	2109	50	1
Z_e	512	17	12	1	863	20	69	548	13	7
S_{phb}	1543	205	37	6	0	0	100	1561	37	1

Concentrations in mg/L

Table 5.6 Measured and Simulated Z_{bh}, Z_e and S_{phb} in Phase 1 AD WAS

Parameter	Measured				BioWin Model			ASM3 Model		
	Avg.	Std. dev.	% of PCOD		Avg.	% of PCOD	Difference from Measured (%)	Avg.	% of PCOD	Difference from Measured (%)
			Avg.	Std. dev.						
PCOD	2119	218			2208		4	2142		5
Z_{bh}	1200	260	57	11	1281	58	7	1116	52	7
Z_e	1090	350	51	17	927	42	15	982	46	10
S_{phb}	0	0	0	0	0	0	0	44	2	100

Concentrations in mg/L

The BioWin model could not be calibrated to predict Z_{bh}, Z_e and S_{phb} concentrations in the BR WAS that were statistically equivalent to the respective average measured concentrations while also satisfying the condition that the predicted PCOD concentrations in the BR and AD WAS be statistically equivalent to the respective average measured concentrations. The offline respirometric measurements indicated that a portion of the synthetic feed remained stored inside the BR biomass. The presence of stored COD resulted in less grown biomass and hence less decay of biomass to produce Z_e. However, the BioWin model assumed that the COD of the synthetic feed was completely oxidized by the BR biomass with the SRT employed. Therefore, the model overestimated the Z_{bh} and Z_e concentrations in the BR WAS when compared to the respective measured concentrations. The BioWin model was successfully calibrated in terms of predicting Z_{bh}, Z_e and S_{phb} concentrations in the AD WAS that were statistically equivalent to the corresponding measured concentrations and this was because the measured S_{phb} concentration in the AD WAS was negligible.

The ASM3 Model was successfully calibrated in terms of predicting Z_{bh} and S_{phb} concentrations in the BR WAS that were statistically equivalent to the respective measured concentrations. This model therefore successfully accounted for the presence of stored COD in the BR WAS. The Z_e

concentration in the BR WAS predicted by this model was significantly higher than the measured concentration although the difference was 7% which was considered to be relatively small. The ASM3 Model was also successfully calibrated in terms of predicting Z_{bh} and Z_e concentrations in the Phase 1 AD WAS that were statistically equivalent to the respective measured concentrations. The calibrated ASM3 Model predicted that 44 mg/L of S_{phb} remained in the AD WAS whereas the measurements indicated that S_{phb} was fully depleted in the AD. However, this predicted S_{phb} concentration was only 2% of the PCOD and hence was considered negligible.

Overall, the Z_{bh}, S_{phb} and Z_e concentrations in the BR and AD WAS predicted by the calibrated ASM3 Model were deemed to be comparable to those estimated from the offline respirometric measurements despite the fact that the modeling and respirometric approaches employed different values of Y_h, b_h and μ_h. In the calibrated ASM3 Model, the values of $Y_{STO,O2}$, $Y_{H,O2}$, $b_{H,O2}$ and μ_h were 0.8, 0.8, 0.3 d^{-1} and 0.56 d^{-1} at 20°C, respectively. The net yield of heterotrophic biomass (Y_h) was the product of $Y_{STO,O2}$ and $Y_{H,O2}$, i.e. 0.64. By comparison, equations 2.3 and 4.15 used to estimate Z_{bh} and Z_e from respirometry employed Y_h, b_h and muh values of 0.6, 0.24 d^{-1} and 3 d^{-1}, respectively.

In addition to predicting particulate COD concentrations in the reactors that were statistically equivalent to the respective measured concentrations, the calibration of the models was also based on minimizing the sum of squared differences between the measured and predicted OUR responses from offline respirometry on the BR WAS. Figure 5.2 shows the OUR responses in the BR WAS measured by offline respirometry and simulated by the calibrated models. The measured OUR curve in Figure 5.2 was typical of that obtained throughout Phases 1 and 2.

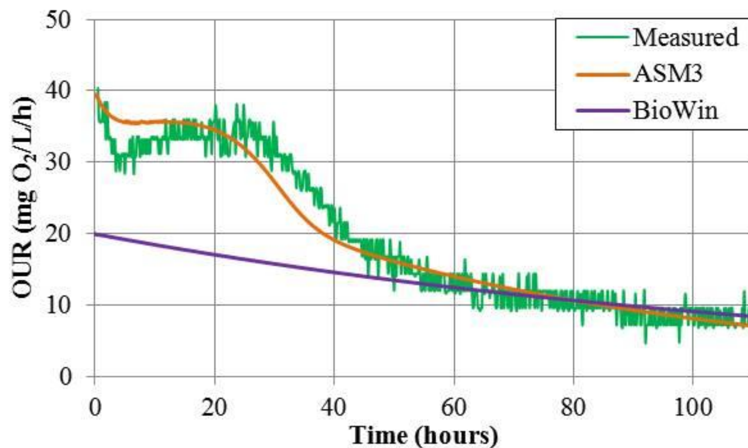

Figure 5.2 Measured and Simulated OUR from Offline Respirometry on BR WAS

From Figure 5.2 it can be seen that the measured OUR curve initially declined, peaked and then decreased exponentially which suggested that biomass growth, COD oxidation and endogenous respiration occurred during the test. The calibrated BioWin Model successfully predicted only the endogenous response, i.e. from 55 hours onwards. It was not possible to adjust the kinetic or stoichiometric parameters in the BioWin Model to simulate the growth or COD oxidation observed in the batch test because this model did not include processes for COD storage by Z_{bh}. The BioWin Model was therefore deficient in this regard. Although the OUR curve predicted by the calibrated ASM3 Model did not demonstrate a peak, it did plateau and then decrease exponentially which suggested that COD oxidation and endogenous respiration occurred during the test. These results and the predicted and measured Z_{bh}, Z_e and S_{phb} concentrations in the BR WAS, previously presented in Table 5.5, indicate that the calibrated ASM3 Model was better suited to simulate the BR-AD system than the calibrated BioWin Model. Therefore, the calibrated ASM3 Model was selected to subsequently simulate the Phase 1 and 2 systems.

Using the calibrated ASM3 Model, the measured and simulated OUR responses in the batch respirometric tests of inoculated BR WAS and AD WAS were subsequently compared, as shown in Figure 5.3. The model successfully predicted the OUR response due to oxidation of stored COD and endogenous respiration in the inoculated BR WAS. The model also correctly predicted the endogenous OUR response in the AD WAS. The apparent success of these two predicted responses was considered to validate the calibration of the ASM3 Model since these responses were independent from the calibration.

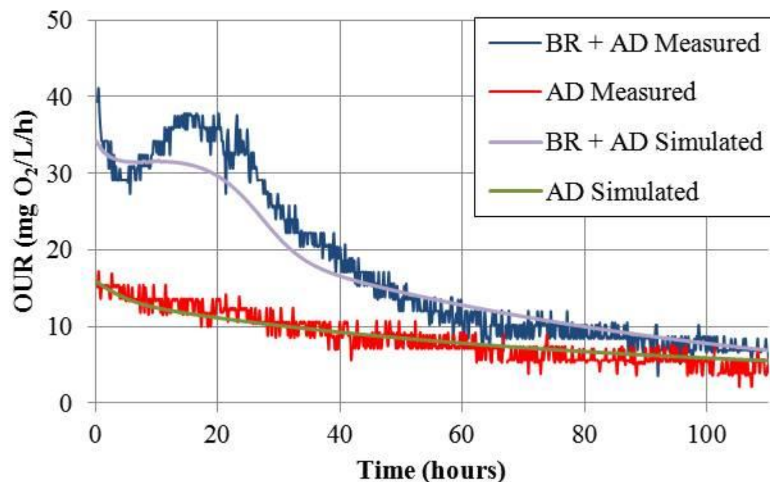

Figure 5.3 Measured and Simulated OUR from Offline Respirometry on Inoculated BR WAS and AD WAS

The OUR response in the AD simulated by the calibrated ASM3 Model was compared to the OUR response measured by online respirometry during Phase 1, as shown in Figure 5.4. As mentioned in section 4.3.1, there was only one viable online respirometric test in Phase 1.

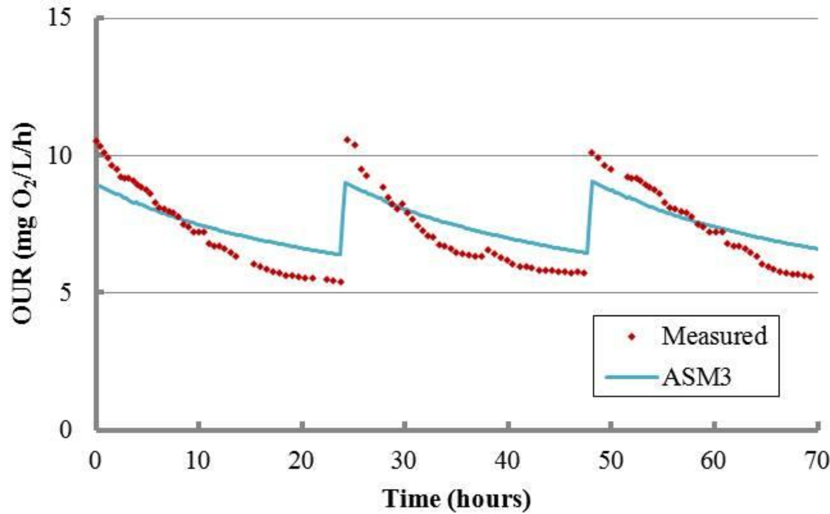

Figure 5.4 Measured and Simulated OUR from Online Respirometry in Phase 1 AD

Similar to the measured OUR curve in each reaction period, the simulated curves in Figure 5.4 appeared to be decay-dominated. The areas under the predicted and measured OUR curves in each reaction period were comparable which indicated that the predicted and measured total oxygen uptake in each reaction period were comparable. However, the simulated OUR curve somewhat underestimated the OUR immediately after feeding and somewhat overestimated the OUR over the second half of the reaction period. As shown in Figure 5.4, the observed OUR in the Phase 1 AD immediately after feeding was 10.5 mgO_2/L/h, and this decreased over the reaction period to 5.5 mgO_2/L/h. By comparison, the OUR in the AD predicted by the calibrated ASM3 Model was 8.9 mgO_2/L/h immediately feeding, and this decreased over the reaction period to 6.4 mgO_2/L/h. Since there was only one viable measured response, additional experiments should be carried out to further assess the extent to which the OUR response predicted by the calibrated ASM3 Model may deviate from the measured OUR response and investigate the possible causes of this deviation.

5.3 Phase 2 System Modeling

The calibrated ASM3 Model that was used to simulate the Phase 1 system was subsequently employed to simulate the Phase 2 system with the same kinetic and stoichiometric parameter values. Once the simulated Phase 1 system was at steady state, the feed to the AD was switched from BR WAS to an influent intended to represent the pretreated BR WAS, labeled "PT" in Figure 5.5. The simulation was continued for a number of days until a new steady state condition was reached. The configuration of this switchover from Phase 1 to 2 in the BioWin 3.1® platform is shown in Figure 5.5.

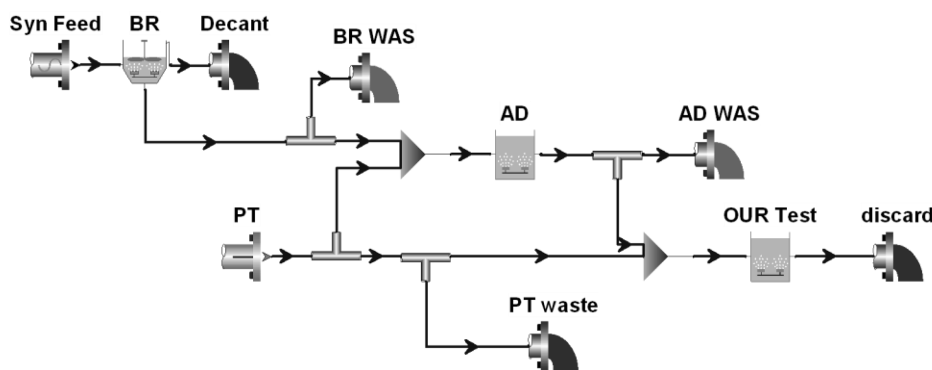

Figure 5.5 Configuration of Switchover from Phase 1 to 2 in BioWin® 3.1 Platform

As discussed in section 5.2, the predicted steady-state concentrations of the COD species in the BR WAS were found to be statistically equivalent to the respective average measured concentrations except for Z_e which was 7% higher than the measured concentration. However, this 7% difference was considered relatively small hence the predicted composition of the BR WAS was deemed representative of the measured composition. The simulated BR WAS contained 53 mg/L S_{us}, 2109 mg/L Z_{bh}, 548 mg/L Z_e and 1561 mg/L S_{phb}. Thus the simulated TCOD concentration was 4271 mg/L.

The simulated composition of the BR WAS was used to estimate several properties of the pretreated BR WAS, as follows:

- The simulated pretreated BR WAS was assigned the same concentrations of ISS, phosphate, magnesium, calcium, alkalinity, cations and anions as were in the simulated BR WAS.
- The TCOD concentration in the pretreated BR WAS was assumed to be equivalent to that in the BR WAS because it was demonstrated in section 4.2.3 that TCOD was conserved during pretreatment.

- The S_{us} concentration in the simulated pretreated BR WAS was assumed to be equivalent to that in the BR WAS because it was shown in section 4.2.4 that pretreatment did not generate this type of COD.
- The X_i concentration in the pretreated BR WAS was assumed to be equivalent to that in the BR WAS, i.e. the concentration of endogenous residue in the BR WAS, because it was shown in section 4.2.4 that pretreatment did not alter the biodegradable fraction of the WAS.
- The S_{phb} concentration in the BR WAS was assumed to be fully converted to S_{bsc} by pretreatment because it was assumed that the storage products in the BR WAS were released upon cell inactivation by pretreatment.
- The Z_{bh} in the BR WAS was assumed to be converted to a mixture of S_{bsc} and X_{sp} by pretreatment.

Therefore, the simulated pretreated BR WAS contained 53 mg/L S_{us}, 548 mg/L Z_e and the remaining 3670 mg COD/L was assumed to be bCOD comprised of a mixture of S_{bsc} and X_{sp}. Thus the bCOD comprised 86% of the TCOD of the pretreated BR WAS.

The measured concentrations of nitrogen species presented in chapter 4 were used to estimate the nitrogen species in the simulated pretreated WAS. As shown in section 4.2.3, pretreatment solubilized organic nitrogen but did not change the ammonia concentration in the WAS. Therefore the same ammonia concentration predicted in the BR WAS was assumed to be present in the pretreated BR WAS, i.e. 70 mg N/L. The particulate organic nitrogen (X_{on}) concentration in the pretreated BR WAS was assumed to be the product of the typical nitrogen content of biomass (0.07 mg N/mg COD) and the sum of X_{sp} and X_i since X_{sp} originated from biomass and X_i was comprised of endogenous residue. Similarly, the soluble organic nitrogen (N_{os}) concentration in the pretreated BR WAS was assumed to be the product of the typical nitrogen content of biomass (0.07 mg N/mg COD) and the S_{bsc} concentration in the pretreated BR WAS that originated from Z_{bh} in the BR WAS. The S_{bsc} in the pretreated BR WAS that originated from S_{phb} in the BR WAS was assumed to be absent of nitrogen since glycogen, the likely storage product, does not contain nitrogen.

The Phase 2 system was simulated as described above for various concentrations of S_{bsc} and X_{sp} in the pretreated BR WAS while maintaining a bCOD concentration of 3670 mg/L and a minimum S_{bsc} concentration of 1561 mg/L, i.e. the concentration of S_{bsc} originating from S_{phb} in the BR WAS. A trial and error approach was used to initially estimate the S_{bsc} and X_{sp} concentrations such that the predicted concentrations of PCOD and SCOD in the pretreated BR WAS and PCOD in the Phase 2 AD WAS were statistically equivalent to the respective measured concentrations using t-tests at the

95% confidence level. Following this, a least squares regression between the predicted and measured OUR responses from offline respirometry on the inoculated pretreated BR WAS was employed to improve the accuracy of the S_{bsc} and X_{sp} estimates. Based on this approach the concentrations of S_{bsc} and X_{sp} in the pretreated BR WAS were estimated to be 2316 mg/L and 1354 mg/L. Table 5.7 presents the simulated and measured PCOD and SCOD concentrations in the pretreated BR WAS.

Table 5.7 Measured and Simulated COD Species in Pretreated BR WAS

Parameter	Measured		ASM3 Model			
	Avg.	Std. dev.	Parameter	Predicted		Difference from Measured (%)
PCOD	1773	264	X_{sp}	1354	1902	7
			Z_e	548		
SCOD	2369	228	S_{bsc}	2316	2369	0
			S_{us}	53		

Concentrations in mg/L

In Table 5.7 the predicted PCOD concentration was the sum of the predicted X_{sp} and Z_e concentrations. Although the predicted PCOD concentration was 7% higher than the average measured concentration, this difference was insignificant at the 95% confidence level. The predicted SCOD concentration, which was the sum of the predicted S_{bsc} and S_{us} concentrations, was identical to the average measured SCOD concentration.

Employing S_{bsc} and X_{sp} estimates of 2316 mg/L and 1354 mg/L, respectively, in the pretreated BR WAS also resulted in the predicted PCOD concentration in the Phase 2 AD WAS being statistically equivalent to the average measured concentration. The predicted and measured PCOD concentrations in the Phase 2 AD WAS are shown in Table 5.8. In addition, Table 5.8 compares the predicted and measured SCOD, ffCOD, VSS, ISS, NO_3-N and NH_3-N concentrations. Percent differences between corresponding simulated and measured values were calculated and significant differences, determined by t-test hypotheses at the 95% confidence level, are shaded grey in Table 5.8.

Table 5.8 Measured and Simulated COD, SS and N Species in Phase 2 AD WAS

Parameter	Measured		ASM3 Model	
	Avg.	Std. dev.	Predicted	Difference from Measured (%)
PCOD	1861	67	1898	2
SCOD	237	31	53	78
ffCOD	177	31	53	70
VSS	1557	90	1518	3
ISS	528	44	732	39
NO3-N	0.9	0.9	0	100
NH3-N	112	9	113	1

Concentrations in mg/L

As shown in Table 5.8, the model predicted VSS and NH_3-N concentrations in the Phase 2 AD that were statistically equivalent to the respective measured concentrations. It is believed that this finding validated the estimation of the S_{bsc} and X_{sp} concentrations in the pretreated BR WAS since the VSS and NH_3-N responses were independent of the calibration responses. The predicted NO_3-N concentration significantly differed from the measured values however both the predicted and measured concentrations were very close to zero and therefore considered negligible. As expected, the model did not predict the generation of S_{us} in the AD hence the simulated SCOD and ffCOD concentrations were significantly lower than measured. Also as expected, the model did not accurately predict the concentration of ISS in the AD.

Table 5.9 compares the steady-state concentrations of Z_{bh}, Z_e and S_{phb} in the Phase 2 AD WAS estimated from offline respirometric measurements to the respective simulated concentrations. The simulated concentrations were determined using the calibrated ASM3 Model with S_{bsc} and X_{sp} estimates of 2316 mg/L and 1354 mg/L, respectively, in the pretreated BR WAS. Percent differences between corresponding simulated and measured values were calculated. T-test hypotheses at the 95% confidence level were used to determine significant differences and these are shaded grey in Table 5.9.

Table 5.9 Measured and Simulated Z_{bh}, Z_e and S_{phb} in Phase 2 AD WAS

Parameter	Measured				ASM3 Model		
	Avg.	Std. dev.	% of PCOD		Avg.	% of PCOD	Difference from Measured (%)
			Avg.	Std. dev.			
PCOD	1861	67			1898		2
Z_{bh}	700	150	38	8	833	44	19
Z_e	850	270	46	15	1017	54	20
S_{phb}	0	0	0	0	48	2	100

Concentrations in mg/L

The model predicted that 2% of the PCOD of the Phase 2 AD WAS consisted of S_{phb} whereas the batch respirometric measurements indicated that S_{phb} was absent. However, this predicted level was relatively small when compared to the uncertainty associated with the test method and therefore considered negligible. The predicted concentrations of Z_{bh} and Z_e were significantly different from the respective measured concentrations. As mentioned in section 4.3.2.4, the sum of the measured COD fractions in the Phase 2 AD WAS was significantly less than the measured TCOD of the WAS, resulting in a significant COD closure error. This indicated that there were inaccuracies in the estimates of Z_{bh} and Z_e in the Phase 2 AD WAS based on the batch respirometric measurements. The predicted Z_{bh} and Z_e concentrations were believed to be more accurate because the predicted PCOD and VSS concentrations were statistically equivalent to the respective average measured levels. Although the Z_{bh} concentration in the Phase 2 AD WAS predicted by the calibrated ASM3 Model was 19% higher than the measured concentration, it was still significantly less than either the measured or predicted Z_{bh} concentration in the Phase 1 AD WAS. This finding was consistent with the observed OUR responses in batch respirometry bottles containing AD WAS.

In addition to matching predicted and measured COD concentrations in the pretreated BR WAS and Phase 2 AD WAS, the estimation of the S_{bsc} and X_{sp} concentrations in the pretreated BR WAS was also based on minimizing the sum of squared differences between the predicted and measured OUR responses from offline respirometry on inoculated pretreated BR WAS. These predicted and measured OUR responses are shown in Figure 5.6.

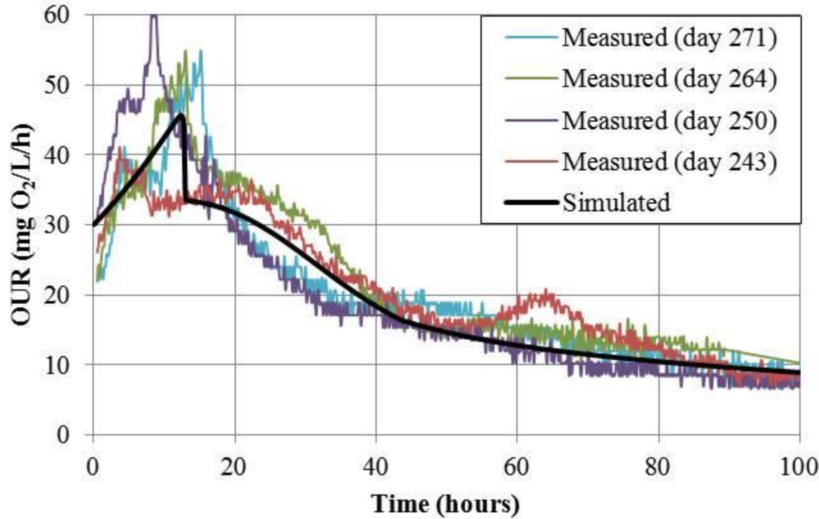

Figure 5.6 Measured and Simulated OUR from Offline Respirometry on Inoculated Pretreated BR WAS

As mentioned in section 4.3.2.2, seven valid offline respirometric measurements were carried out during Phase 2 and an analysis of the results showed four of the tests were conducted with AD biomass that was acclimatized to the pretreated BR WAS. The OUR responses measured during these tests (days 243, 250, 264 and 271) are shown in Figure 5.6. Although there was considerable variability among the four measured responses, each curve showed growth, substrate oxidation and endogenous respiration. Each measured curve showed a sharp peak within the first 20 hours of the test, suggesting growth on S_{bsc}. Following this, substrate oxidation appeared to dominate the four measured OUR responses until approximately 60 hours. In the tests started on days 250, 264 and 271, the endogenous response became visible after approximately 60 hours. The measured OUR response from the sample taken on day 243 showed a peak between 60 and 70 hours, suggesting delayed biomass growth. However, the area under this peak comprised only 5% of the total area under the curve. Therefore, this delayed peak was considered negligible.

A variable volume reactor labeled "OUR Test" was included in the configuration in Figure 5.5 to simulate the offline batch respirometric tests with BR WAS pretreatment. Once the Phase 2 system was at steady-state, 150 mL of pretreated BR WAS and 50 mL of AD WAS was fed to the OUR Test reactor and the simulation was continued for a number of days.

In the initial round of calibrations it was found that when the S_{bsc} to X_{sp} ratio in the pretreated BR WAS ranged from 1.6 to 1.8 and the bCOD concentration of the pretreated BR WAS was equal to 3670 mg/L, the predicted PCOD and SCOD concentrations in the pretreated BR WAS and predicted

PCOD concentration in the AD WAS were statistically equivalent to the corresponding measured concentrations. Hence, in the refined calibration, the OUR Test reactor was simulated five times using S_{bsc} to X_{sp} ratios of 1.60, 1.65, 1.70, 1.75 and 1.80. The sum of squared differences was calculated between each of the four measured OUR responses and each of the five simulated OUR responses. For each of the four measured responses, the simulated response that yielded the minimum sum of squared differences was determined. It was found that the best fit estimates of S_{bsc} to X_{sp} ratios varied over the full extent of the tested range, i.e. 1.6 to 1.8. Therefore, the accuracy of the estimated S_{bsc} and X_{sp} concentrations could not be further improved using this least squares regression approach. The median value of the S_{bsc} to X_{sp} ratio (1.7) was selected and the simulated OUR response associated with this ratio is shown in Figure 5.6. The S_{bsc} to X_{sp} ratio of 1.7 corresponded to the previously mentioned estimated S_{bsc} and X_{sp} concentrations of 2316 mg/L and 1354 mg/L, respectively. The simulated OUR response appeared to be comprised of two overlapping peaks. An analysis of the modeling results showed that the higher and sharper peak was associated with biomass growth on S_{bsc} whereas the lower and more gradual peak was associated with biomass growth on X_{sp}.

The OUR response in the Phase 2 AD was simulated by the calibrated ASM3 Model with pretreated BR WAS containing S_{bsc} and X_{sp} concentrations of 2316 mg/L and 1354 mg/L. The simulated response was compared to the OUR response measured by online respirometry, as shown in Figure 5.7. The measured response in Figure 5.7 was typical of the OUR responses measured by online respirometry throughout Phase 2.

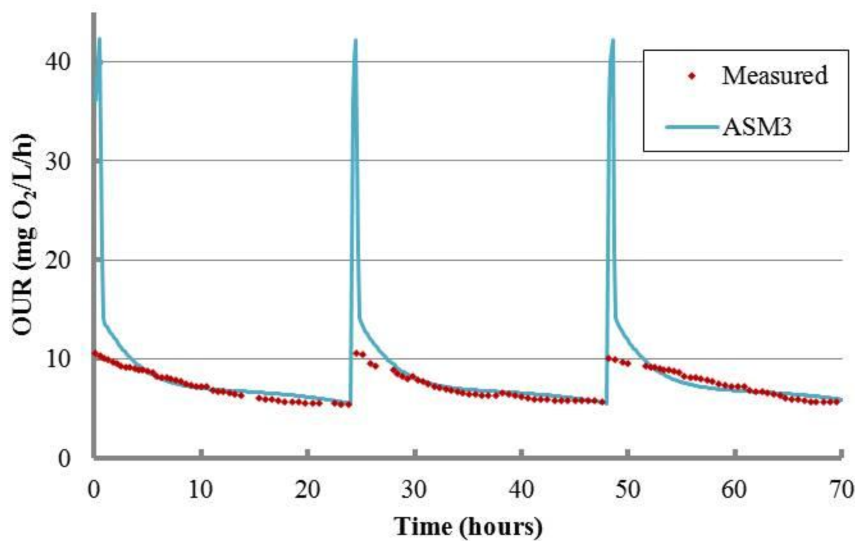

Figure 5.7 Measured and Simulated OUR from Online Respirometry in Phase 2 AD

As shown in Figure 5.7, the measured OUR in the Phase 2 AD appeared to be decay-dominated as it decreased relatively slowly from 10.5 $mgO_2/L/h$ immediately after feeding to 5.5 $mgO_2/L/h$ at the end of the reaction period. By comparison, the simulated OUR in the AD spiked to 42 $mgO_2/L/h$ immediately after feeding, decreased to 13.5 $mgO_2/L/h$ within approximately 45 minutes, decreased to 9 $mgO_2/L/h$ within approximately 3 hours, and then gradually decreased over the reaction period to approximately 5.5 $mgO_2/L/h$. Although the simulated curve appeared to fit the measured curve from approximately 4 hours after feeding until the end of the reaction period, the simulated curve showed a relatively high spike immediately after feeding that was not observed in the measured response. As explained in section 4.3.2.2, it was hypothesized that the online respirometric measurements failed to capture rapid and high oxygen uptake. Typically 1 hour was required to increase the DO concentration from 3 to 5 ppm immediately after the AD was fed with pretreated BR WAS. For the remainder of the reaction period, the DO concentration was typically increased from 3 to 5 ppm within 5 minutes. This delay was presumably due to insufficient aeration in the AD. An analysis of the simulated OUR response showed that the spike immediately after feeding was due to the aerobic growth on S_{bsc}. Apart from this, both the simulated and measured OUR curves appeared to show the oxidation of X_{sp}, followed by endogenous respiration.

5.4 Pretreatment Model

This section describes the approach taken to develop the pretreatment model. The COD fractionation of the WAS before and after pretreatment predicted by the calibrated Phase 2 ASM3 Model was summarized in Table 5.10. The data in Table 5.10 was used to develop a COD-based stoichiometric pretreatment model that is compatible with the Petersen matrix-based models employed in most wastewater treatment simulators, as shown in Table 5.11. Based on the data in Table 5.10, 36% of Z_{bh} in the BR WAS was converted to S_{bsc} and the remainder was converted to X_{sp}. Thus the fraction of Z_{bh} converted to S_{bsc} ($fS_{bsc}Z_{bh}$) in Table 5.11 was 0.36. As existing simulators do not have the functionality to accommodate instantaneous transformation processes, kinetic processes were employed and the rate constants, $k_{d}_Z_{bh}$ and $k_{d}_S_{phb}$, were assigned very high values, i.e. 1000 d^{-1}, so that the rates were effectively instantaneous. In accordance with the experimental results, the pretreatment model conserved TCOD and did not generate ammonia.

Table 5.10 Simulated COD Fractionation of BR WAS and Pretreated BR WAS

BR WAS	COD (mg/L)	COD Fraction (%)	Pretreated WAS	COD (mg/L)	COD Fraction (%)
S_{phb}	1561	37	S_{bsc} (from S_{phb})	1561	36
Z_{bh}	2109	49	S_{bsc} (from Z_{bh})	755	18
			X_{sp}	1354	32
Z_e	548	13	Z_e	548	13
S_{us}	53	1	S_{us}	53	1
TCOD	4271	100	TCOD	4271	100

Table 5.11 Pretreatment Model

Process	S_{bsc}	X_{sp}	Z_{bh}	S_{phb}	Kinetic Rate Expressions
	mg COD/L				
Conversion of Heterotrophs	$fS_{bsc}Z_{bh}$	$1 - fS_{bsc}Z_{bh}$	-1		$k_{d_}Z_{bh} \times Z_{bh}$
Release of Stored COD	1			-1	$k_{d_}S_{phb} \times S_{phb}$

To verify the model, a reactor was added directly upstream of the aerobic digester in the configuration presented in Figure 5.1. This reactor was specified as a BioWin model builder unit with the pretreatment model presented in Table 5.11 to simulate the pretreatment dose employed in this study. The overall system was simulated with the calibrated ASM3 Model that was fit in Phase 1 and the simulations were continued until the AD reached steady state. The configuration of this Phase 2 system is presented in Figure 5.8.

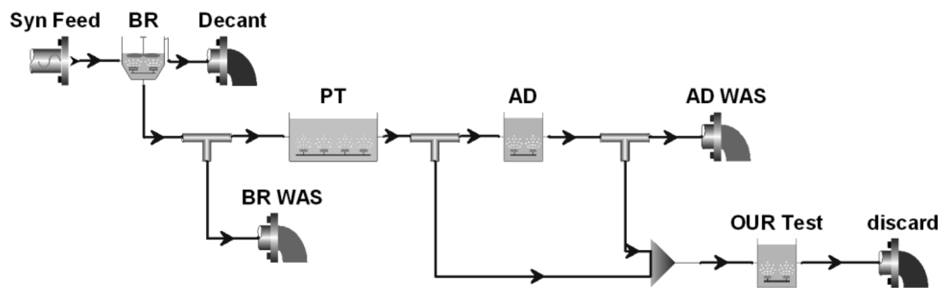

Figure 5.8 Configuration of Phase 2 System in BioWin® 3.1 Platform

The steady-state concentrations of COD, SS, ammonia and nitrate in the Phase 2 AD using the system configuration in Figure 5.8 were identical to the respective concentrations in the Phase 2 AD using the configuration presented previously in Figure 5.5. Likewise, the OUR responses in the online and offline tests simulated using the configuration in Figure 5.8 were identical to those simulated using

the configuration in Figure 5.5. It was therefore concluded that the pretreatment model presented in Table 5.11 accurately described the COD transformations associated with the HPTH pretreatment dose employed in this study.

The COD-based stoichiometric pretreatment model proposed in Table 5.11 was somewhat specific to the system in this study. The model was based on using the ASM3 Model in the BioWin platform for a non-nitrifying aerobic system treating WAS comprised of Z_{bh}, Z_e and S_{phb}. However, the approach described in this study may be followed to determine the impacts of pretreatment on Z_{bh}, Z_e and S_{phb} when other doses of HPTH pretreatment and other pretreatment techniques are employed. It would seem to be reasonable to assume that other types of biomass typically occurring in WWTPs would be undergo similar conversions through pretreatment as was observed for Z_{bh} in this study. However, tests should be carried out to verify this. The proposed pretreatment model did not generate ammonia which was in accordance with the experimental results. The model did not account for the possible impacts of pretreatment on phosphorous species since the measurement of these species was outside the scope of this study.

6. Conclusions

This study sought to characterize and develop a COD based model for WAS pretreatment by HPTH and it is believed that all of the objectives were met. The COD of a raw and pretreated synthetically generated WAS was fractionated using analytical and bioassay methods and activated sludge modeling. It was found that HPTH WAS pretreatment at 150°C and 3 bars for 30 minutes increased the rate at which the WAS was aerobically biodegraded but did not increase the extent of biodegradation. A COD-based pretreatment model was then developed and the applicability of the model was assessed. The following summarizes the specific conclusions that were made.

In the study it was found that stored COD was present in the synthetically generated (BR) WAS. This conclusion was based on the following independent results:

- The average measured COD/VSS ratio of the BR WAS was 1.23 ± 0.08 which was significantly lower than the typical value of 1.42 for active biomass and decay products. This suggested that stored COD such as glycogen was present since it has a relatively low COD/VSS ratio of 1.1.
- The average measured pON/PCOD ratio of the BR WAS was 0.053 ± 0.006 mg N/mg COD which was significantly lower than the typical value of 0.07 mg N/mg COD for active biomass and decay products. Because typical storage products such as glycogen or PHA do not contain nitrogen, this lower measured ratio suggested that storage products were present.
- The average measured ffCOD concentration in the AD WAS was significantly higher than that in the BR WAS which suggested that S_{us} was generated in the AD. Prior research has shown that the decay of cells storing COD generated S_{us}. Therefore, the production of S_{us} in the AD supported the hypothesis that storage products were present in the biomass fed to the AD.
- The OUR responses from offline respirometry on BR WAS showed both growth and decay. It appeared that stored COD was consumed during the batch respirometry and exerted an oxygen demand that was greater than that which would have been attributed to endogenous respiration.
- The BR WAS was estimated to contain 51 ± 4% Z_{bh} and 12 ± 1% Z_e based on an analysis of the data from the offline respirometric test on mixtures of BR WAS and synthetic feed in high F/M ratios. The calibrated ASM3 Model employed to simulate the Phase 1 BR-AD

system verified these Z_{bh} and Z_e COD fractions in the BR WAS and predicted that the remaining 37% was essentially comprised of X_{STO}.

It was concluded that stored COD was not present in the AD WAS. This conclusion was based on two findings: the average measured COD/VSS ratio in the AD WAS was statistically equivalent to the typical ratio for Z_{bh} and Z_e; and the OUR curves from offline respirometry on AD WAS showed only decay and no growth. Therefore, the stored COD present in the BR WAS was depleted in the AD.

This study showed that HPTH pretreatment at 150^oC and 3 bars for 30 minutes did not reduce the TCOD concentration of the WAS. This conclusion was based on the finding that the average TCOD concentrations of the raw and pretreated BR WAS were statistically equivalent.

The dose of pretreatment employed in this project did not mineralize organic nitrogen as the average ammonia concentration in the BR WAS did not significantly increase after pretreatment.

HPTH pretreatment at 150^oC and 3 bars for 30 minutes solubilized $56 \pm 7\%$ of COD, $49\% \pm 11\%$ of organic nitrogen, $56 \pm 10\%$ of VSS and did not solubilize ISS. All types of particulate organics were solubilized to the same extent by pretreatment as the average measured pON/PCOD ratio of the BR WAS was statistically equivalent to that of the pretreated BR WAS. The observed solubilization was consistent with prior research on HPTH WAS pretreatment.

The soluble COD generated by pretreatment was characterized as S_{bsc} hence pretreatment increased the rate at which the BR WAS was aerobically degraded. This conclusion was based on three results:

- Pretreatment did not generate S_{us} because it was shown that the average ffCOD concentrations in the AD WAS in Phases 1 and 2 were statistically equivalent. This finding was consistent with literature reports that have shown that refractory compounds, i.e. S_{us}, were only generated at pretreatment temperatures above 150^oC.
- The offline respirometric tests showed that the OUR curve for inoculated pretreated BR WAS peaked earlier and to a higher value than the OUR curve for the inoculated raw BR WAS. This response was characteristic of growth on S_{bsc}.
- The offline respirometric tests showed that the OUR in the filtered BR WAS was negligible, indicating the absence of S_{bsc}. By comparison, the area under the OUR curve for filtered

pretreated BR WAS contributed more than 60% of the area under the curve for whole pretreated BR WAS, demonstrating that pretreatment generated a substantial amount of S_{bsc}.

Although pretreatment increased the rate at which the BR WAS was aerobically biodegraded, it did not increase the extent of biodegradation. The non-biodegradable COD component of the BR WAS, i.e. Z_e, was not converted to biodegradable COD under the pretreatment conditions employed in this study. This conclusion was based on the results of three independent tests:

- The online respirometric data showed that the average ΣOU_s/TCOD ratios for raw and pretreated BR WAS were statistically equivalent, demonstrating that the extent to which the BR WAS was degraded over the AD reaction period was not increased by pretreating the BR WAS.
- Using the TCOD measurements from the sample bottles of the offline respirometric tests, it was shown that the average $(TCOD_i - TCOD_f)/TCOD_i$ ratio for BR WAS was statistically equivalent to that for pretreated BR WAS. This indicated that pretreatment did not change the fraction of the BR WAS that could be aerobically degraded by acclimatized inoculum.
- The average measured VSS concentration in the AD WAS was statistically equivalent in Phases 1 and 2. Had pretreatment increased the aerobically biodegradable fraction of the BR WAS, it was expected that the VSS concentration in the AD would have decreased.

A COD-based stoichiometric pretreatment model was developed for HPTH pretreatment at 150°C and 3 bars for 30 minutes. When this model was integrated into BioWin, it was able to accurately simulate both the steady state performance of the overall system employed in this study as well as dynamic respirometry results. The experimental results showed that the TCOD of the BR WAS consisted of 51% Z_{bh}, 12% Z_e and 37% X_{STO} and the pretreated BR WAS consisted of 12% Z_e and a negligible amount of Z_{bh}. The pretreatment model verified these fractions and predicted that the pretreated BR WAS also contained 54% S_{bsc} and 32% X_{sp}. Two kinetic processes with effectively instantaneous rates were included in the pretreatment model: X_{STO} was fully converted to S_{bsc}; and 36% of Z_{bh} was converted S_{bsc} and the remaining Z_{bh} was converted to X_{sp}. The approach described in this study may be followed to determine the impacts of pretreatment on Z_{bh}, Z_e and X_{STO} when other doses of HPTH pretreatment and other pretreatment techniques are employed.

7. Recommendations

The experiments and modeling procedures carried out in this study were successfully used to characterize the impacts of HPTH pretreatment on WAS containing Z_{bh}, Z_e and X_{STO}. Several recommendations are provided to improve the approach in future studies:

- The rate of oxygen transfer in the AD should be increased such that the time to raise the DO concentration from the low to high set point is less than one minute, especially after the AD is fed. This will allow the online respirometric test to capture rapid and high oxygen uptake. To achieve this, the current aerators should be replaced by aerators with much higher oxygen transfer efficiencies. Alternatively, the OUR in the AD could be reduced by adding the BR WAS over one or two hours instead of all at once.
- Phase 2 should only be commenced after at least two valid offline respirometric tests with reproducible results have been obtained for pretreated BR WAS with non-acclimatized inoculum. This will allow the impacts of biomass adaptation on the aerobic biodegradability of WAS by HPTH pretreatment to be more reliably determined.
- Phosphorous species should be measured in the various sludge streams during Phases 1 and 2 to determine the associated impacts of HPTH pretreatment. The COD: N: P ratio of the synthetic feed employed by the current study was 100: 4.4: 1.5 hence the concentration of monopotassium phosphate in the synthetic wastewater may have been excessively high. Using the measurements of the phosphorous species, a phosphorous balance could be used to determine whether the monopotassium phosphate concentration in the synthetic wastewater should be decreased.
- Storage products in the BR WAS should be eliminated to simplify the COD fractionation of the sludge streams and the modeling of the overall system. Without significant stored COD, it is believed that the system could be successfully simulated with the BioWin 3.1® Integrated Model. It was hypothesized that the BR biomass stored COD such as glycogen or PHA due to the alternating anaerobic and aerobic conditions in the reactor. Although the measured DO concentration was above 2 ppm for most of the reaction period, it was close to zero for up to two hours after feeding. Thus the operation of the BR should be changed to eliminate any anaerobic conditions. For example, the synthetic wastewater could be fed to the BR over an extended period of time. If storage products are still detected after improving the BR operation, the cells could be microbiologically examined using a suitable staining procedure to gain a better understanding of the nature of the storage granules.

The approach described in this project with the improvements recommended above should be applied to the following future studies:

- Investigation of the impacts of HPTH pretreatment on X_i. Instead of synthetic wastewater, the BR-AD system could be fed with screened raw municipal wastewater which would contain X_i. Thus the non-biodegradable particulate COD in each sludge stream would be the sum of X_i and Z_e. Since the constituent concentrations in municipal wastewater vary with time, Phases 1 and 2 would each need to be operated long enough to accurately estimate the COD fractions. Using the VSS measurements and analyses of the online and offline respirometric data, the impact of pretreatment on the extent of aerobic biodegradability of the BR WAS could be determined. A significant increase would suggest that X_i was partially converted to bCOD by pretreatment since it was shown that Z_e is not affected.
- Investigation of the impacts of HPTH pretreatment at temperatures above and below 150°C and the development of corresponding pretreatment models. Prior research suggests that the relative proportion of S_{bsc} to X_{sp} in the pretreated WAS may increase with pretreatment temperature however it has also been shown that refractory compounds, i.e. S_{us}, may be generated at temperatures above 150°C. It should be determined whether other pretreatment temperatures can change the aerobically biodegradable fraction of the WAS.
- Investigation of the impacts of other pretreatment techniques such as sonication and ozonation and the development of COD-based stoichiometric pretreatment models that accurately characterize each technique.

The approach in the current project should be combined with investigations of the impacts of HPTH pretreatment on other biomass fractions besides Z_{bh} so that appropriate processes may be added to the pretreatment model. In WWTPs, heterotrophs that accumulate phosphorus and bacteria that oxidize ammonia and nitrite are present in significant quantities in the activated sludge and would likely be affected by HPTH pretreatment. Frigon and Isazadeh (2010) showed that pretreatment with ozone did not reduce the level of each type of biomass to the same degree. Further research is required to determine whether such a trend exists for HPTH pretreatment.

It is recommended that the approach described in this project be paired with an analysis of the impacts of HPTH pretreatment on the rate and extent of anaerobic biodegradability. This is an important consideration since promising HPTH techniques such as CAMBI™ or Exexlys™ are used in conjunction with anaerobic digestion. Biochemical methane potential tests could be carried out on samples from the various sludge streams in the system.

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Appendix A Physical and Biochemical Data

Table A.1 Synthetic Wastewater

Date Sampled	COD (mg/L)				SS (mg/L)			pH
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS	
13	2508	1082	1426					
19	2018							8.0
19	2182							8.0
34	7557							
36	7201				1320	1320	0	6.0
41	4664				1250	930	320	
43	5225							
48	5761							3.9
69	7715							
76	7806							
83	7108							
85	7432							
117								4.6
119	7379							6.2
172								4.5
175	8754	6223	2531	5836	2210	1940	270	4.5
181	4153	2918	1235	2718	1090	900	190	4.6
193	3903	3080	823	2768	1020	860	160	4.6
225	3654	2862	792	2619	810	750	60	4.5
273	4103	2993	1110					4.5
273	4028	2980	1047		920	880	40	4.5
273	4190	3030	1160		880	820	60	4.5
278	4015	3018	998	2899	880	790	90	4.5
*Mean	4006	2983	1023	2751	933	833	100	4.53
*Std. dev.	183	73	166	117	103	57	61	0.05

*Mean and standard deviation of data over Phases 1 and 2 from day 179 to 283

Table A.2 BR WAS

Date Sampled	COD (mg/L)				SS (mg/L)			pH	N (mg/L)	
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS		NO_3	NH_3
11	3283	52	3230		3780	3210	570			
21	4150	54	4096					9.3		
27	4988	180	4808							
34	9326	245	9081		8350	6900	1450	9.4		
36	8218	200	8018		7950	6540	1410	7.0		
40								9.2	0.5	
41	7328	176	7152		7470	6050	1420	9.2	0.5	
48	7258	121	7137					8.9		
63								8.6	1.05	
69	8068	145	7923	267				8.1		
70	8611	202	8409					8.7		
76	10451	202	10249	160				8.3	0.8	
78	8829									
83	11871	140	11732					8.5	0.5	
85	8554							8.6		
90								8.6	0.45	
91	8554	142	8412					8.6		
92	8953	151	8803	113	8180	7120	1060	8.3		
103	7519	928	6592		11630	5330	6300	6.5		
105	7108	922	6186		6200	5580	620	7.8		
105	6603	955	5648					7.8		
112								7.5	0.45	231
113	4664	688	3975	708				7.9		
116	8916	828	8088					6.4	0.85	
118	9851	1465	8386					8.3		
120								8.6	1	160
124	9178	1665	7513	1634				7.7		
124	8863	1771	7092	1702				7.7		
127	9053	1334	7719					8.2	0.8	202
132								8.4	1.15	150
133								8.6	0.8	
134	9203	1646	7557					8.0	0.6	
159								8.2	0.7	
160	7806	1913	5893					4.8	0.9	294
162	6989	280	6709					8.1	1.3	184
163	8280							7.7		
168								8.5	0.7	
174								7.9	0.6	
175	9502	1877	7625	1833	7520	6640	880	7.6	0.5	236
179								8.3	0.4	
180	8679	1113	7566	780	7305	6320	985	8.5	0.9	

Table A.3 BR WAS

Date Sampled	COD (mg/L)				SS (mg/L)			TCOD/ VSS
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS	
193	4489	165	4325	47	3990	3330	660	1.35
196	5761	165	5597	81	6590	2765	3825	2.08
203	4053	90	3963	85	3840	3260	580	1.24
208	4651	54	4598	59				
209	3878				4150	3530	620	1.10
215	3753	59	3695	46	3620	3140	480	1.20
222	4078	35	4043	49	3820	3330	490	1.22
223	4365	54	4311					
228	4327	44	4283	44	3990	3430	560	1.26
232								
233								
236	3741	34	3707	41			440	
237					4030	3530	500	0.00
243	3953	46	3907	47	3600	3140	460	1.26
250	4040	49	3992	50	3420	3180	240	1.27
258	3878	46	3832	59	3560	3130	430	1.24
264	4626	56	4570	59	3960	3460	500	1.34
271	4502	56	4446	51	3820	3400	420	1.32
278	4539	51	4488	45	4210	3570	640	1.27
283								
*Mean	4170	52	4127	53	3835	3342	489	1.23
*Std. dev.	324	14	334	12	246	167	103	0.08

*Mean and standard deviation of data from day 193 to 283 when the BR was at steady-state

Table A.4 BR WAS

Date Sampled	pH	N (mg/L)				pON/ pCOD
		NO_3	NH_3	TKN	sTKN	
193	8.3	1.40	74			
196	8.4	1.00	86			
203	8.1	0.10	85			
208	8.5	0.10	74			
209	8.5					
215	8.2	0.10	69			
222	8.5	0.10	54			
223	8.5					
228	8.5	0.10	48			
232	8.6			266	99	
233	8.5	0.10	70			
236	8.5	0.10	76	258	85	0.046
237	8.3					
243	8.0	0.10	78	277	79	0.051
250	8.6	0.10	79	301	87	0.053
258	8.4	0.20	74	285	81	0.053
264	8.6	0.20	73	284	78	0.045
271	8.4	0.10	84	348	90	0.058
278	8.3	0.40	80	358	80	0.062
283	8.5					
*Mean	8.52	0.14	73	297	85	0.053
*Std. dev.	0.12	0.09	11	36.9	7	0.006

*Mean and standard deviation of data from day 193 to 283 when the BR was at steady-state

Table A.5 Pretreated BR WAS

Date Sampled	COD (mg/L)				SS (mg/L)			TCOD/ VSS
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS	
193	4228	2470	1758	1853	2098	1657	441	2.55
196	4305	2080	2225	1560	1917	1514	403	2.84
197	4285	2005	2280	1504	2100	1659	441	2.58
200	4105	2503	1602	1877				
201	4366	2494	1872	1871				
202	4315	2510	1805	1883				
203	4100	2320	1780	1740	2320	1833	487	2.24
209	4215	2606	1609	1846	2090	1590	500	2.65
215	4165	2506	1659	1746	1970	1440	530	2.89
222	4153	2669	1484	1802	1660	1270	390	3.27
223	4290	2494	1796					
228	4402	2694	1708	2001	1740	1240	500	3.55
236	4090	2475	1615	1908				
237					1840	1440	400	
243	3778	2213	1565	1715	1720	1280	440	2.95
250	3965	2213	1752	1546	1830	1780	50	2.23
254					1610	1210	400	0.00
258	3741	2176	1565	1727	2280	1730	550	2.16
264	4227	2226	2001	1659	2200	1710	490	2.47
271	4365	2076	2288	1652	2020	1710	310	2.55
278	4315	2082	2232	1378	2270	1810	460	2.38
283								
*Mean	4142	2369	1773	1725	1936	1518	412	2.73
*Std. dev.	215	228	264	171	237	230	131	0.79

*Mean and standard deviation of data from day 193 to 283 when the BR was at steady-state

Table A.6 Pretreated BR WAS

Date Sampled	pH	N (mg/L)				pON/ pCOD
		NO_3	NH_3	TKN	sTKN	
193	7.3	2.1				
196	6.1	2.2	108			
197	7.5	3.8	88			
200	7.2	4.5	97			
201	7.8	1.2	75			
202	7.8	5	79			
203	7.6	3.0	81			
209	7.9	2.1	84			
215	7.7	1.8	83			
222	8.1	1.8	59			
223	7.9					
228	8.1	2.4	62			
232	7.9			309	231	
233	7.8	2.1	81			
236	7.6	2.5	95	281	198	0.051
237	7.7					
243	8.0	2.2	92	262	184	0.050
250	7.9	3.1	92	276	200	0.043
254	8.2					
258	8.0	4.1	87	314	205	0.069
264	8.0	3.9	96	286	193	0.047
271	8.0	3.9	91	306	196	0.048
278	7.7	3.9	92	317	210	0.048
283	7.5					
*Mean	7.8	2.9	84	294	202	0.052
*Std. dev.	0.2	1.1	12	20	14	0.016

*Mean and standard deviation of data from day 193 to 283 when the BR was at steady-state

Table A.7 AD WAS

Date Sampled	COD (mg/L)				SS (mg/L)			pH	N (mg/L)	
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS		NO_3	NH_3
27	6709	185	6524							
34	11809	429	11380		11880	9140	2740	9.3		
36	11491	370	11121		12590	9540	3050	9.3		
41	13037	379	12658		15000	10950	4050	9.3	1.5	
63								8.4	3.9	
69	16909							8.1		
70	16305	409	15896					8.0		
78	14590	625		352					415	
83	10924	549	13965					6.7	340	
90			10375					6.3	335	
91	9527	481						6.1		
92	8380	384	9046	237	8030	6630	1400	6.8	290	
95			7996					7.9	198	
103	6385	269			6270	4970	1300	8.1	56	420
104			6115					8.1		348
105	5836	248			5580	4530	1050	8.3		
112			5588					8.3	12	213
116	3965	251						8.3	34	
118	5237	364	3715					8.0		
120			4873					6.5	305	29
124	6609	451		408				6.9		
127	6011	414	6158					7.8	340	1
132			5597					8.4	160	0
134	5138	448						8.6	92	
159			4690					8.7	0.9	
160	4053	434						8.7	0.8	173
162			3619					8.6	0.7	176
163	3816	424						8.5	0.6	159
168			3392					8.5	0.8	
174								8.3	50	
175	4788	426		342	5240	3550	1690	8.4	36	150
179			4362					8.5	8.5	
181	4639	416		267	5120	3460	1660	8.3	3.4	
186								8.4	2.2	
193	2868	463	2405	291	3190	1990	1200	8.4	0.7	136

Table A.8 AD WAS Phase 1

Date Sampled	COD (mg/L)				SS (mg/L)			TCOD / VSS	pH	N (mg/L)	
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS			NO_3	NH_3
197	2207	340	1.36	203	2510	1620	890	1.36	8.5	1.0	122
201	2556	304	1.62	183	2570	1580	990	1.62	8.4	0.7	106
203	2519	282	1.40	185	2860	1800	1060	1.40	8.3	0.6	107
*Mean	2427	309	1.46	190	2647	1667	980	1.46	8.40	0.8	112
*Std dev	192	30	0.14	11	187	117	85	0.14	0.10	0.2	9

*Mean and standard deviation of data from day 197 to 203 when the AD was at steady-state during Phase 1

Table A.9 AD WAS Phase 2

Date Sampled	COD (mg/L)				SS (mg/L)			TCOD /VSS
	TCOD	SCOD	PCOD	ffCOD	TSS	VSS	ISS	
208	2234	269	1965	165				
209	2120	258	1862	167	2190	1410	780	1.50
215	2132	301	1832	190	2100	1390	710	1.53
222	1833	337	1496	207	1700	1270	430	1.44
228	2051	315	1736	213	1780	1250	530	1.64
236	2369	283	2086	208			440	
237					2070	1560	510	
243	2114	271	1843	197	2010	1430	580	1.48
250	2164	239	1924	188	2160	1640	520	1.32
258	2114	241	1873	201	2120	1633	487	1.29
264	2014	269	1745	200	1950	1480	470	1.36
271	2058	205	1853	151	2070	1530	540	1.34
278	2126	197	1929	126	2200	1630	570	1.30
283								
*Mean	2098	237	1861	177	2085	1557	528	1.39
*Std. dev.	53	31	67	31	94	90	44	0.08

*Mean and standard deviation of data from day 243 to 283 when the AD was at steady-state during Phase 2

Table A.10 AD WAS Phase 2

Date Sampled	pH	N (mg/L)				pON/ pCOD
		NO_3	NH_3	TKN	sTKN	
208	8.4	0.6	103			
209	8.3	0.6	104			
214	8.4	1.5				
215	8.2	1.6	99			
222	8.5	1.1	87			
228	8.4	1.2	62			
232	8.1			273	188	
233	8.4	1.2	86			
236	8.4	1.1	97	231	108	0.059
237	8.3					
243	8.1	0.9	90	222	97	0.068
250	8.4	0.8	89	263	108	0.081
258	8.2	0.9	90	253	104	0.079
264	8.2	1.0	113	251	121	0.075
271	8.2	0.7	103	297	119	0.096
278	8.1	1.0	100	299	112	0.097
283	8.0					
*Mean	8.32	0.9	98	264	110	0.075
*Std. dev.	0.16	0.1	10	29	9	0.012

*Mean and standard deviation of data from day 243 to 283 when the AD was at steady-state during Phase 2

Appendix B Online Respirometry Data

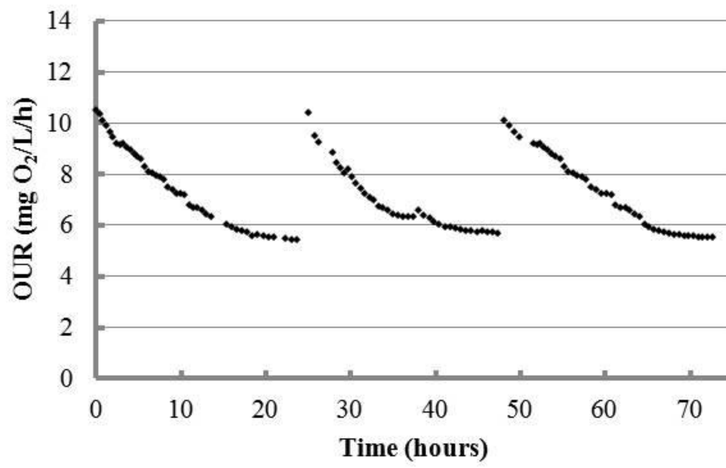

Figure B.1 Phase 2 Day 197 to 199

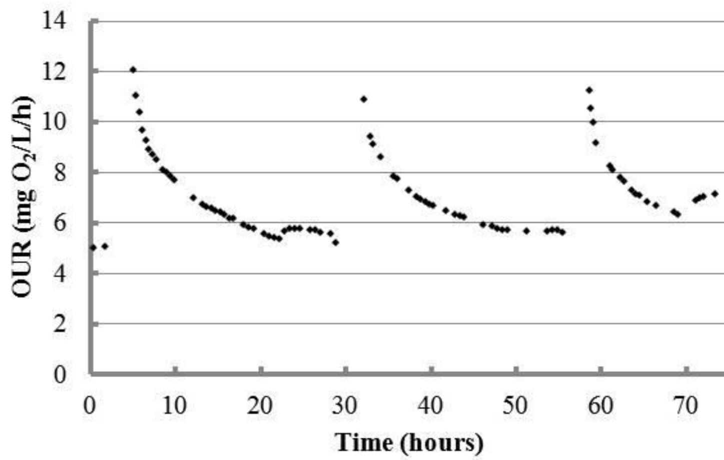

Figure B.2 Phase 2 Day 206 to 208

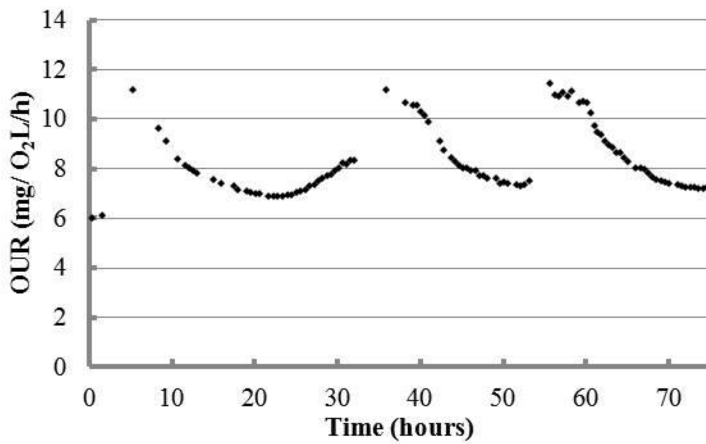

Figure B.3 Phase 2 Day 212 to 214

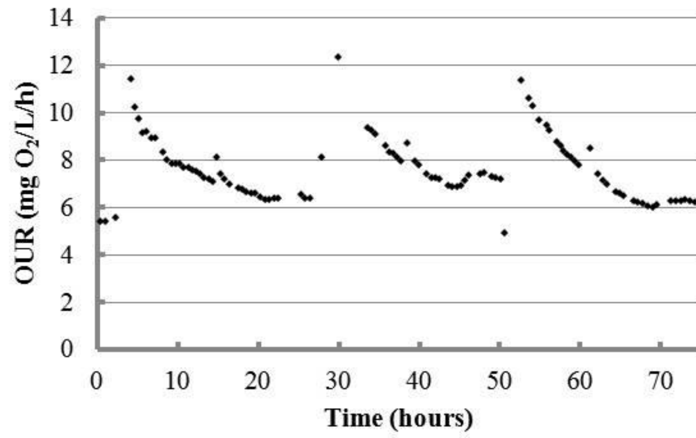

Figure B.4 Phase 2 Day 228 to 230

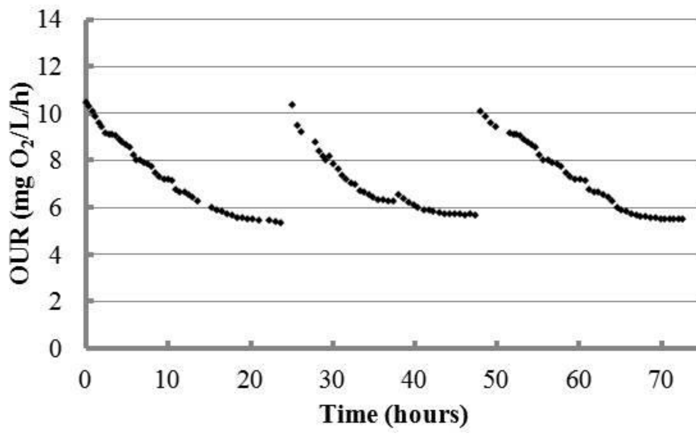

Figure B.5 Phase 2 Day 234 to 236

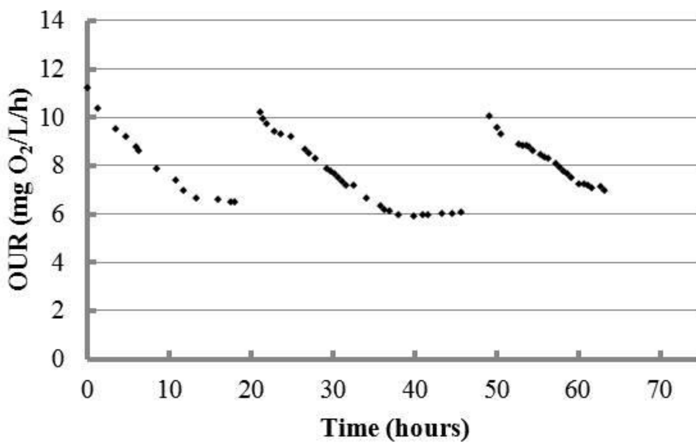

Figure B.6 Phase 2 Day 240 to 242

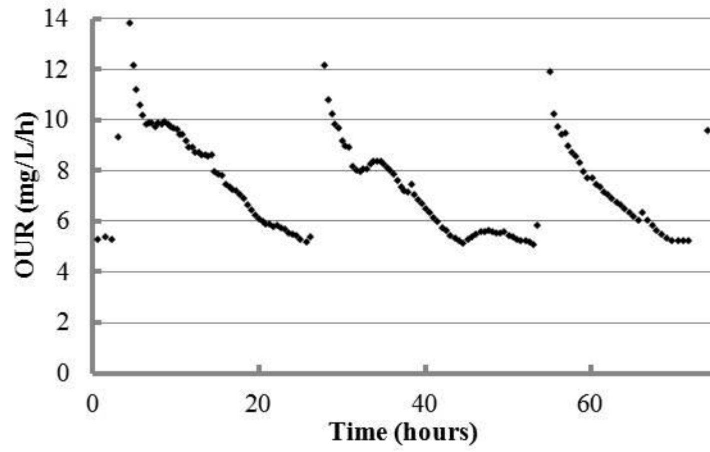

Figure B.7 Phase 2 Day 246 to 248

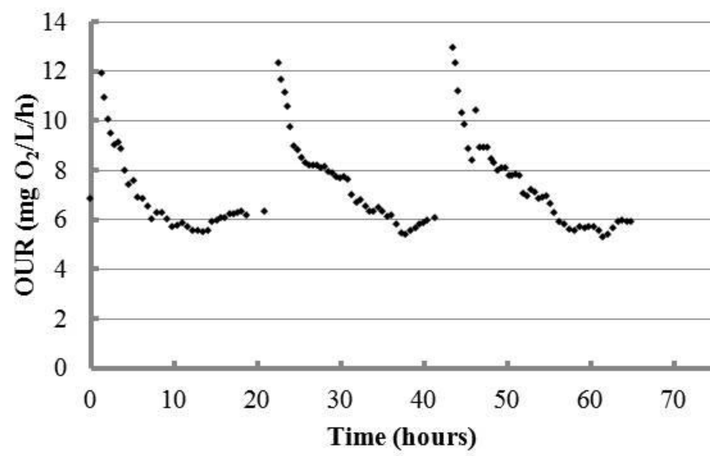

Figure B.8 Phase 2 Day 252 to 254

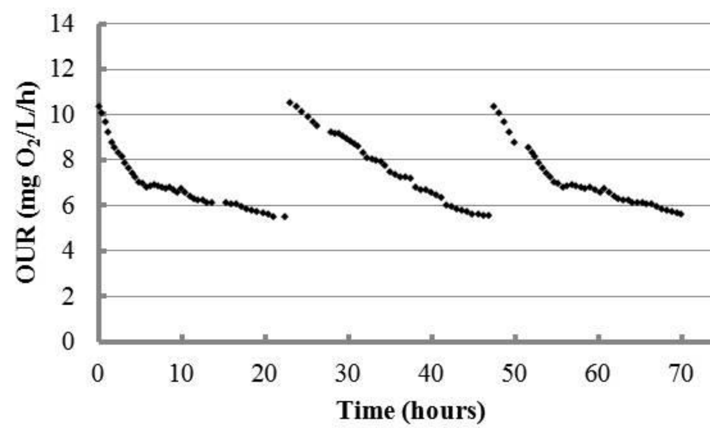

Figure B.9 Phase 2 Day 258 to 260

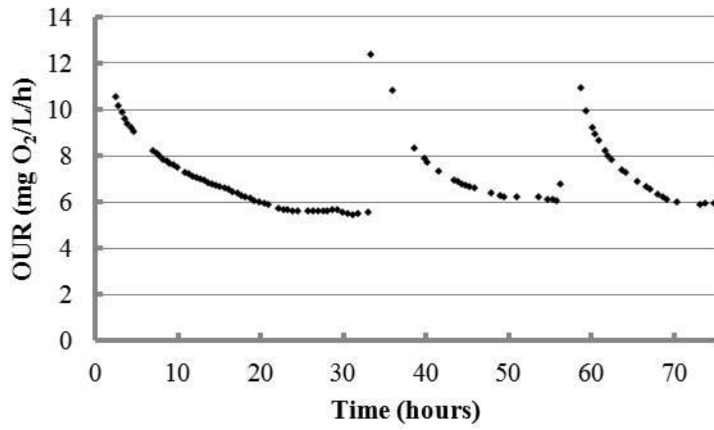

Figure B.10 Phase 2 Day 264 to 266

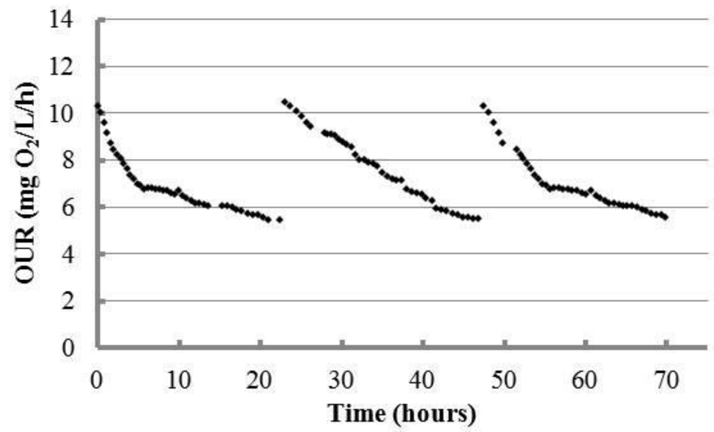

Figure B.11 Phase 2 Day 270 to 272

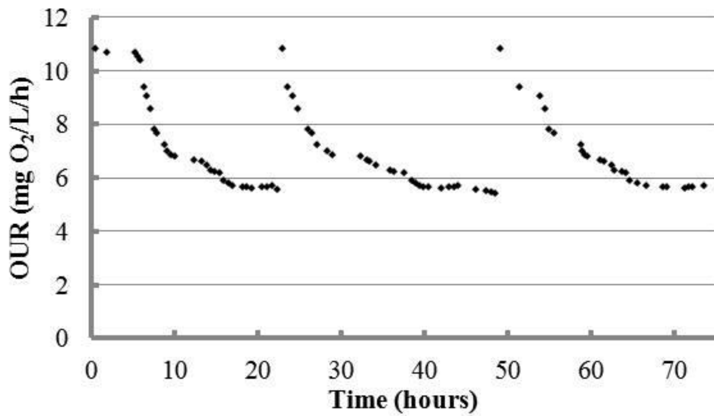

Figure B.12 Phase 2 Day 275 to 277

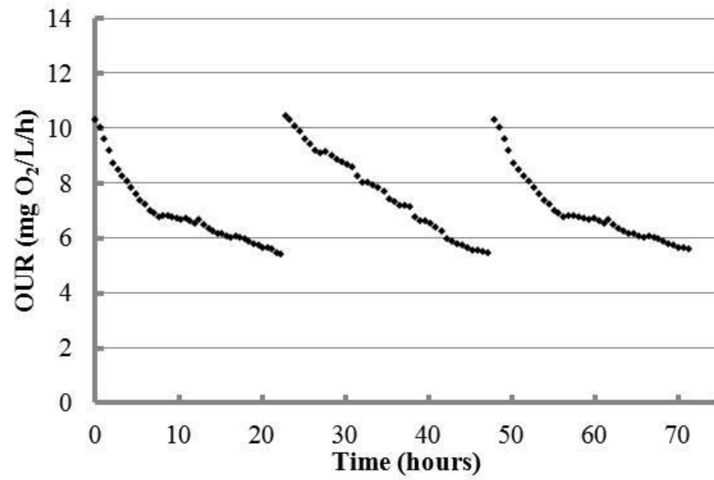

Figure B.13 Phase 2 Day 280 to 282

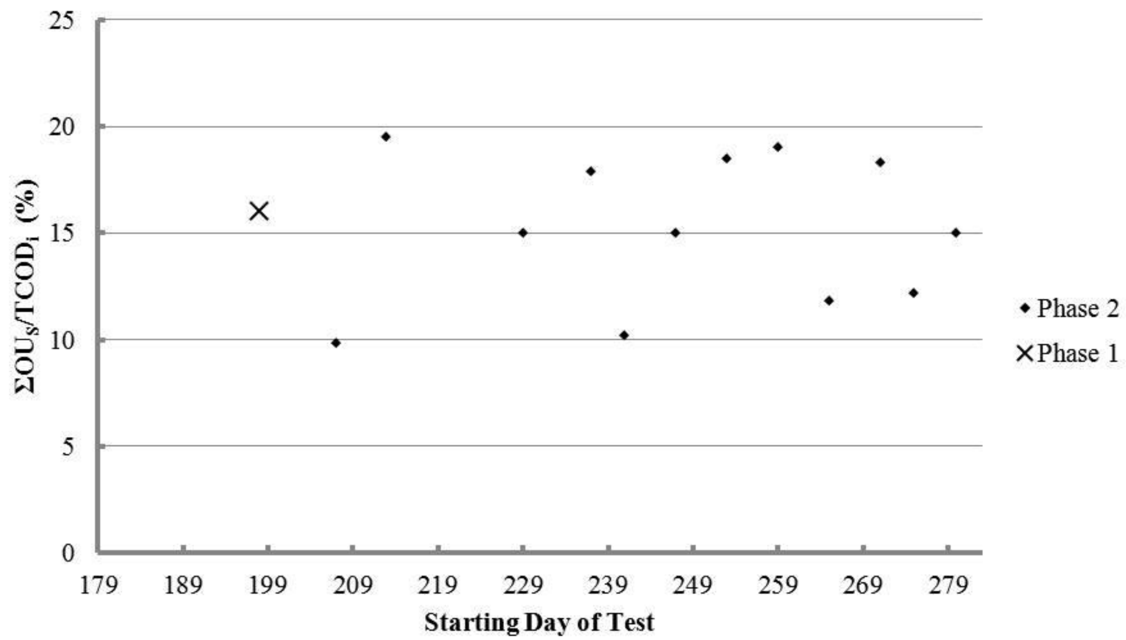

Figure B.14 Measured $\Sigma OU_s/TCOD_i$ Ratios of BR WAS Fed to AD

Appendix C Offline Respirometry Data

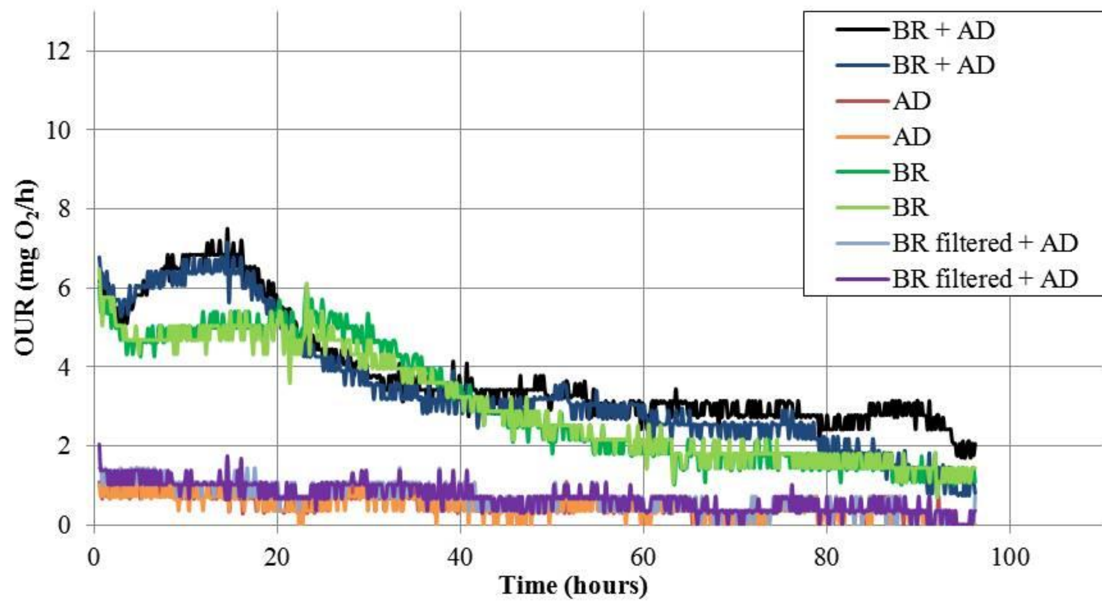

Figure C.1 BR WAS and AD WAS - Test Started on Day 193

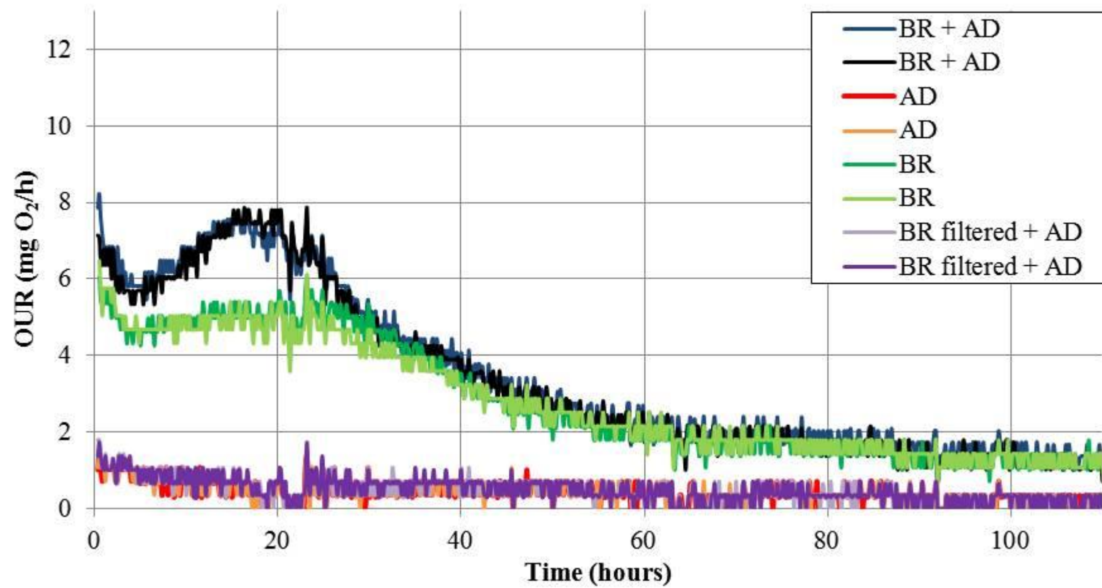

Figure C.2 BR WAS and AD WAS – Test Started on Day 203

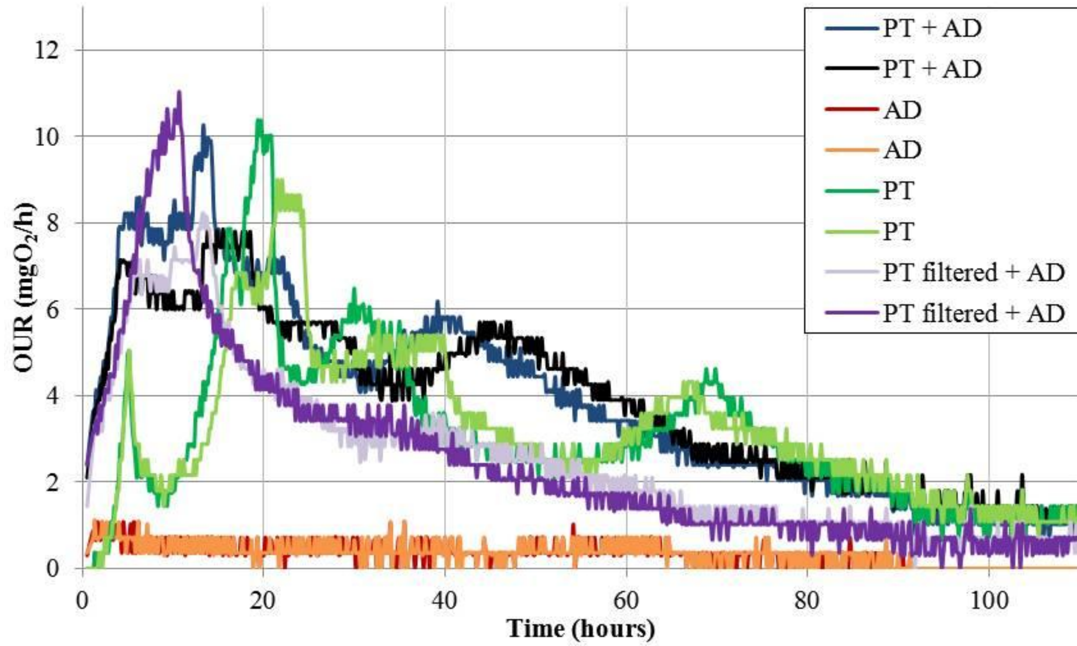

Figure C.3 Pretreated BR WAS and Non-acclimatized AD WAS – Test Started on Day 197

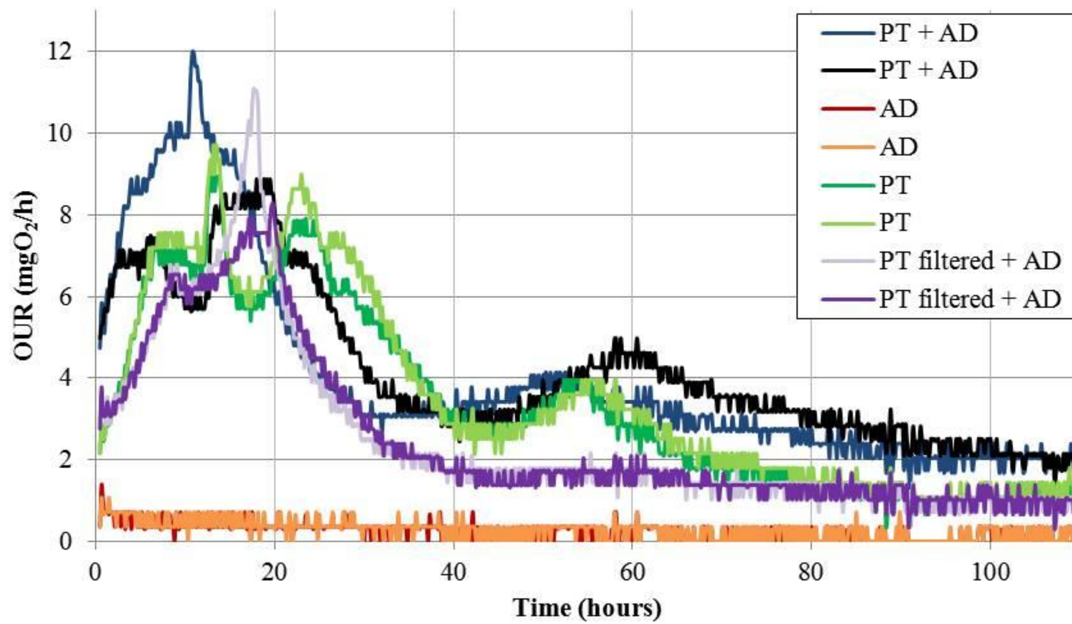

Figure C.4 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 215

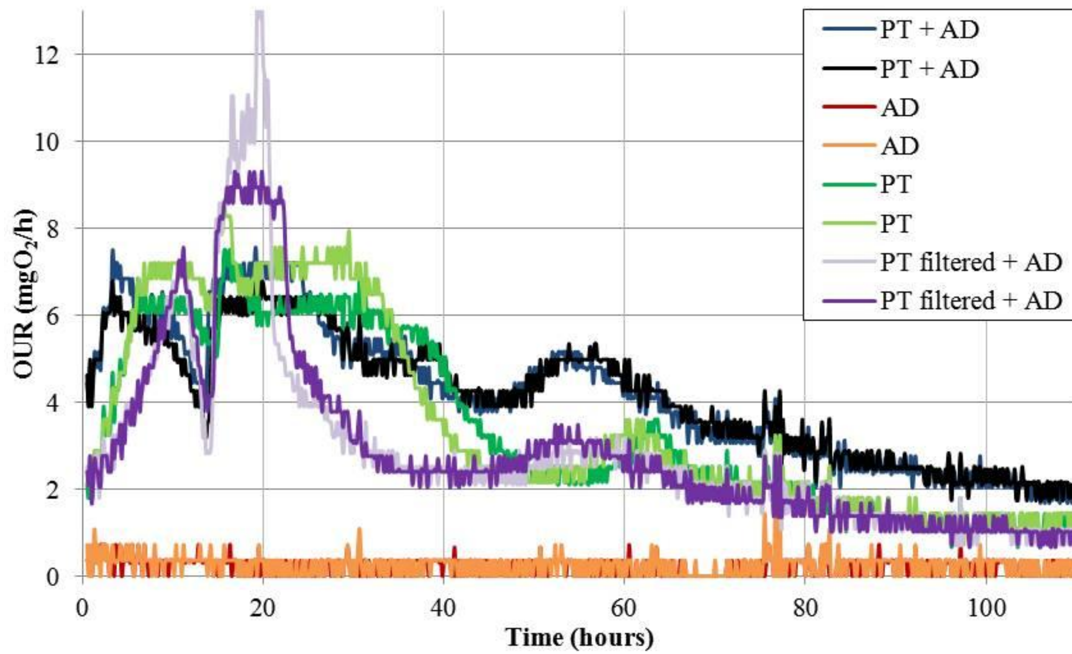

Figure C.5 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 222

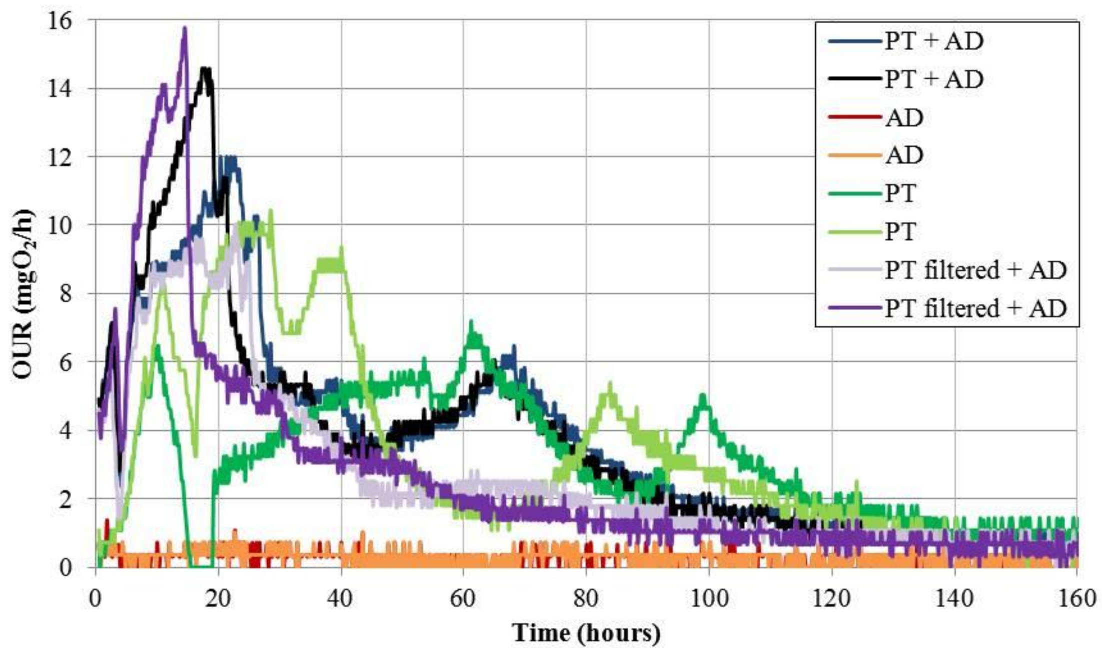

Figure C.6 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 228

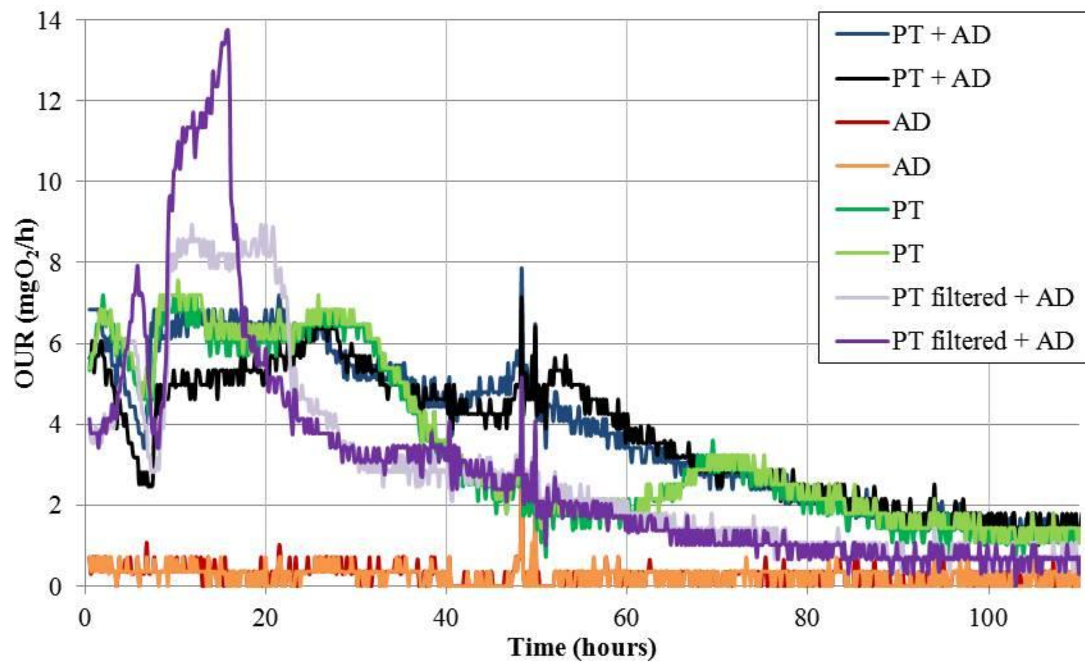

Figure C.7 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 236

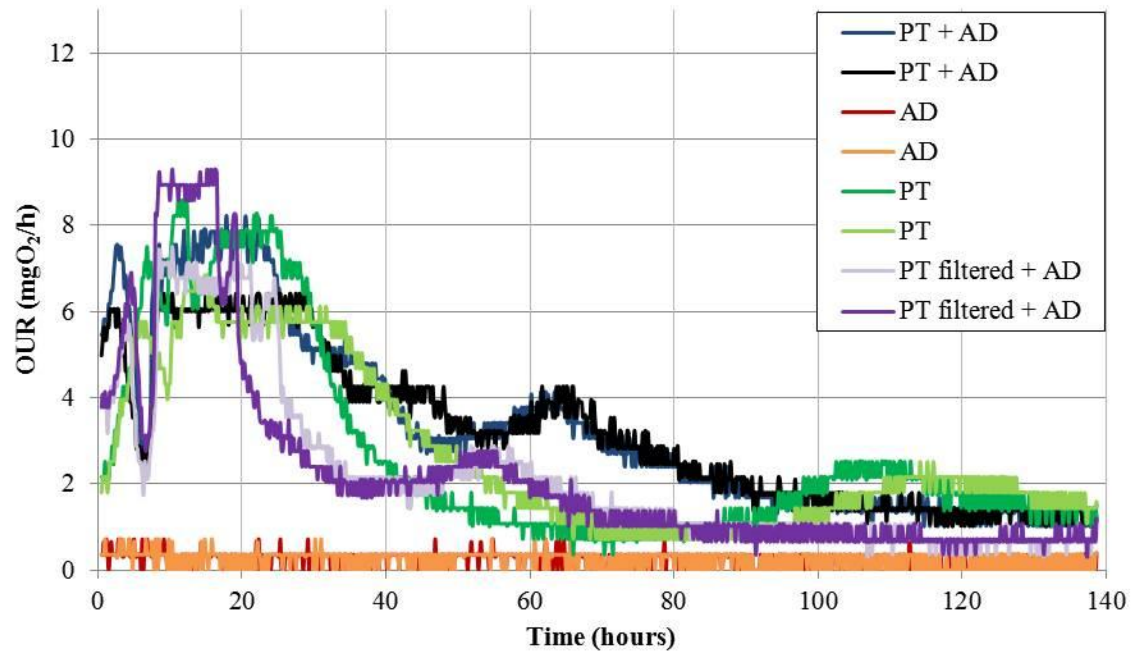

Figure C.8 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 243

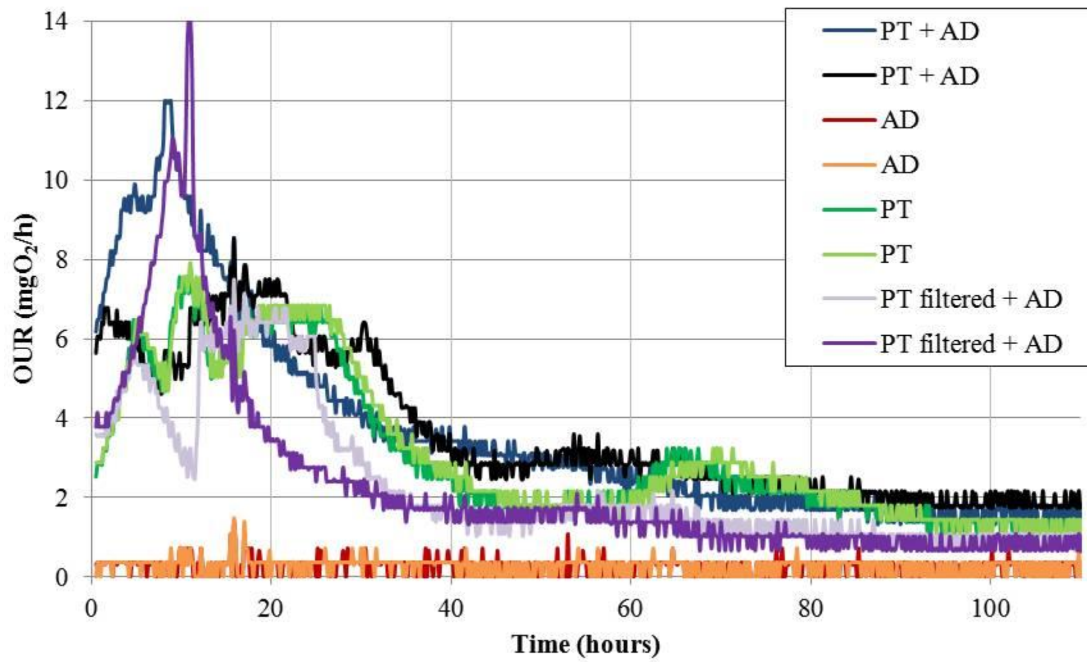

Figure C.9 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 250

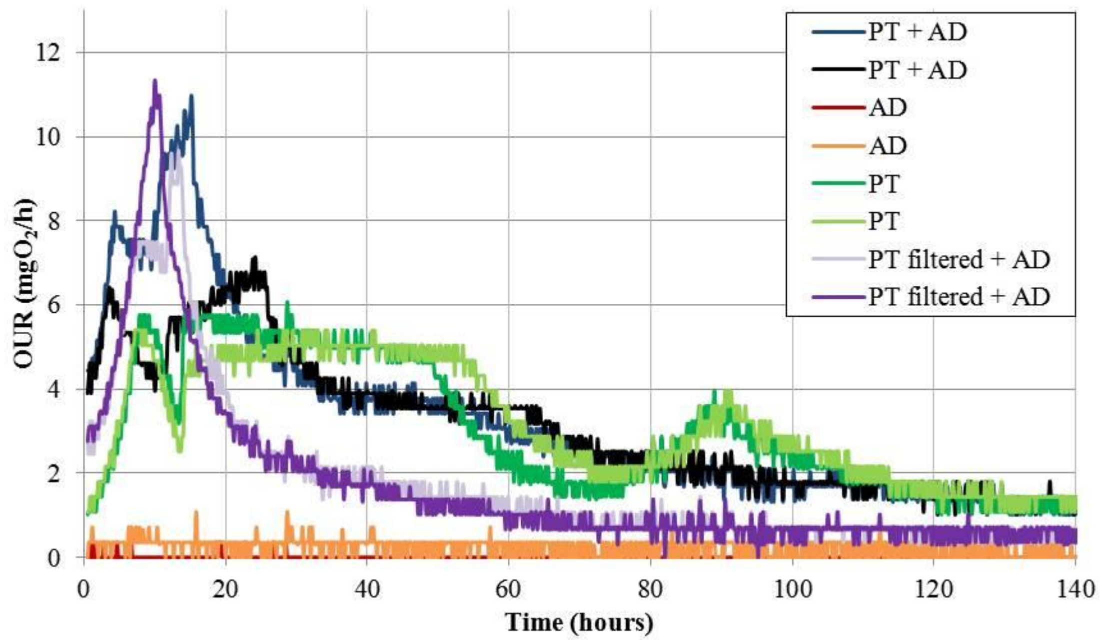

Figure C.10 Pretreated BR WAS and Acclimatized AD WAS – Test Started on Day 271