Utilization of ultraviolet-visible spectroscopy and rheology for sludge characterization and monitoring

by

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Abstract

Operation of sludge treatment processes mainly relies on manual control, which is far from ideal. There is a need for new approaches to optimize the operation of sludge treatment processes and wastewater plants. This research aims to identify new tools and methods that can be used for in-line and real-time characterization and monitoring of sludge. Two methods that were examined in this thesis that have potential to be used as monitoring technologies were ultraviolet/visible spectrophotometry and torque rheology. Effluent and filtrate absorbance measurements in the ultraviolet/visible range were successful in monitoring the progress of aerobic digestion. Torque rheology was not found to be sensitive enough for monitoring aerobic digestion of sludge, however it was able to detect changes in the total solids content of anaerobically digested sludge. Torque rheology detected significant changes in anaerobically digested sludge when trivalent cations were added, but not when divalent cations were added.

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List of Abbreviations

BSA (Bovine Serum Albumin)

COD (Chemical Oxygen Demand)

D.I. (Deionized Water)

DO (Dissolved Oxygen)

EIS (Electrical Impedance Spectroscopy)

ESP (Extracellular Polymers)

HRT (Hydraulic Retention Time)

IBA (Image Based Analysis)

LR (Linear Regression)

MLR (Multivariate Regression)

MS-PUFM (Pressurized Ultra Filtration Membrane with a Mesh Screen)

NIS (Near-Infrared Spectroscopy)

OM (Organic Matter)

PCR (Principal Components Regression)

PLS (Partial Least Squares)

qPCR (Quantitative Polymerase Chain Reaction)

R^2 (Coefficient of Determination)

RMSE (Residual Mean Square Error)

ROPEC (Robert O. Pickard Environmental Centre)

sCOD (Soluble Chemical Oxygen Demand)

SOUR (Specific Oxygen Uptake Rate)

TS (Total Solids)

TSS (Total Suspended Solids)

TTQ (Totalized Torque)

UV (Ultraviolet)

Vis (Visible)

VS (Total Volatile Solids)

VSR (Volatile Solids Reduction)

WAS (Waste Activated Sludge)

Chapter 1 – Introduction

Cities around the world view sludge treatment and management as an increasing issue that must be dealt with. Sludge treatment and management costs can account for approximately half of the operational budget of a wastewater treatment plant and there is a reliance on manual control which is far from ideal for optimization (Nowak, 2006). Results to determine the level of treatment of sludge can be delayed which limits process control from being optimized. Lastly, as the population continues to increase so will sludge quantities which means new wastewater facilities must be constructed or existing ones must be upgraded to keep up with the growing population and stricter regulations.

Sludge generated from wastewater treatment plants can be treated using biological, chemical, and thermal treatment. Conventional aerobic digestion of sludge, where the air is provided in a basin containing sludge, is one of the most commonly used biological treatment processes. In the presence of oxygen, microorganisms can break down the biodegradable organic matter within the sludge. This produces new cellular material, carbon dioxide (CO_2), water (H_2O) and ammonia (NH_3) among other products, with the NH_3 being further oxidized to nitrate (NO_3) under extended aeration (Tchobanoglous & Burton, 1991).

The amount of organic matter (OM) present in the sludge can be used to monitor the level of treatment of the sludge within an aerobic reactor. The OM content can be measured by determining the total volatile solids (VS) of the sludge, which is related to the total solids (TS). As the aerobic process progresses, the VS should decrease as OM is broken down and then stabilize upon the completion of the treatment with no more OM being consumed. The amount of OM in the sludge can also be determined by measuring the chemical oxygen demand (COD). COD is a measurement

of the amount of oxygen required to chemically oxidize the organic substances within the sludge (Tchobanoglous & Burton, 1991).

Even though these measurements are effective at assessing the quality of sludge, the duration for which it takes these tests to be completed is not ideal as they are lab-based measurements. In order to determine the VS of a sample, it can take over 24 hours depending on the organic matter and moisture content of the sample. The COD can be determined in a timelier manner as it takes 3-4 hours if the HACH method is used. This still limits process control and optimization as there is a time lag between when the sludge treatment is complete and when the results are known.

Therefore, there is a need for new approaches to optimize the operation of sludge treatment processes as there are currently very few real-time monitoring tools and technologies for sludge treatment. This research aims to determine tools and methods that can be used for in-line and real-time characterization and monitoring of sludge. This would allow determining, modeling, and predicting sludge behaviour and flow characteristics through treatment processes, thereby optimizing process control based on this knowledge. Two methods that have the potential to be used as monitoring technologies are ultraviolet/visible spectrophotometry and rheology.

When electromagnetic radiation in the ultraviolet (UV) and visible (Vis) spectrum interacts with matter in a sample, the matter absorbs light (Owen, 1996). The measured absorbance can then be used in quantitative analysis of a sample by correlating the absorbance of the sample to the desired parameter, such as concentration, with statistical methods. Two common statistical methods that are used to do this are multivariate regression (MLR) and principal components regression (PCR). Both methods use the absorbance data for a specified wavelength range and the measured value of the parameter of interest which is calculated using a different method. Equations with regression coefficients are developed which can then be used to predict the parameter of interest using

absorbance data instead of measuring the parameter in a more time-consuming fashion (Mark & Workman, 2007).

There are multiple types of spectrophotometers, including lab spectrophotometers and real-time spectrophotometers. For lab spectrophotometers, a sample is placed within the machine and the absorbance is measured. Real-time spectrophotometers can measure absorbance in real-time in-line at treatment plants, thereby allowing real-time optimization.

In the water and wastewater industry, UV/Vis spectrophotometers have been used to monitor the quality of water in fresh lakes, wastewater treatment plants and sewers (Lepot et al., 2016). Lepot et al. (2016) used the UV/Vis spectrum to correlate the absorbance of samples from sewers, rivers, and wastewater treatment plants to water quality parameters such as total suspended solids (TSS), COD and sCOD (soluble COD). They did this by applying statistical methods to the absorbance data sets to correlate the data to the TSS and COD concentrations. They then used statistical criteria to determine if the methods were accurate. In their study, they found that linear regression was more effective at estimating TSS and COD concentrations compared to partial least squares using the UV/Vis spectrophotometer.

An inline UV/Vis spectrophotometer has the potential to be used for sludge treatment monitoring as there potentially may be a correlation between the absorbance of the sludge, supernatant, or filtrate and the level of treatment of the sludge. It would most likely be too difficult to measure the absorbance of the sludge as too much matter would absorb light, however measuring the light of the supernatant or filtrate could potentially be used in a treatment plant setting. There could be a side stream from the sludge treatment process where a vortex would separate the solids from the liquid (supernatant) portion of the sludge and then an in-line and real-time spectrophotometer

could measure the absorbance of the supernatant which potentially could be correlated to the level of treatment of the sludge. A schematic of this potential setup can be seen in Figure 1 below.

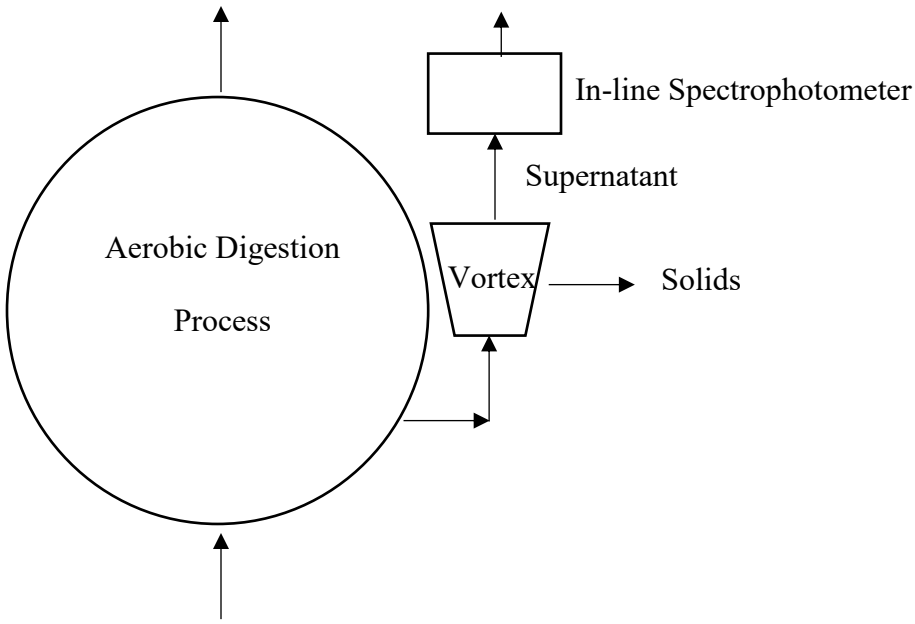

Figure 1. Schematic of Potential Treatment Monitoring Process using UV/Vis Spectrophotometry.

The next method that has the potential to be used as a monitoring technology is rheology. Rheology is a holistic descriptor encompassing the physical, biological and chemical changes that occur in sludge and is superior to single descriptive parameters such as TS, VS or COD. Rheology provides information on the overall sludge matrix and can be used to predict sludge behaviour and achieve real-time optimization. It studies the deformation and flow of matter and includes measuring the flow characteristics (shear rate, shear stress, yield stress, rheogram peaks, etc.) of fluids (Ormeci, 2007). A torque rheometer is a device which can be used to determine the rheological properties of a fluid such as sludge. It consists of a shaft connected to an impeller that rotates at a desired

speed within a cup in which the sludge is placed. As the impeller rotates it measures the sludge's torque by measuring the sludge's resistance to shear over a period of time. From this a torque rheogram can be produced.

There are two different torque rheometry methods that can be used to determine the rheological properties of sludge. The first method is known as the totalized torque (TTQ) method. TTQ is a term that is used to describe the area under a torque-time curve, representing the total energy dissipation which can be used to estimate the overall network strength of the sludge (Ormeci et al., 2004, Ormeci & Abu-Orf, 2005). This method was used to determine the optimum polymer dose during dewatering in lab-scale and full-scale tests at treatment plants (Abu-Orf & Ormeci, 2005; Ormeci & Abu-Orf, 2006; Ormeci, 2007). A decrease in TTQ values with increasing polymer dose corresponds to the optimum polymer dose. Comparing the TTQ of conditioned sludge samples is advantageous over using other rheological parameters such as the yield stress or yield point as these parameters are not able to monitor changes that the sludge can undergo during conditioning (Ormeci et al., 2004).

The second method that can be used to establish the rheological properties of sludge is known as the direct injection method. The direct injection method uses unconditioned sludge and only utilizes the peaks observed after direct injection of a chemical, such as a polymer, into the sludge sample to assess the overall network strength of the sludge. This method has also been used to determine the optimum polymer dose during dewatering in lab-scale and full-scale tests at treatment plans (Ormeci, 2007; Murray & Ormeci, 2008). Peak heights from the rheogram increase with increasing polymer dose until the optimum dose is reached at the maximum peak height. After this, peak heights gradually decrease with increasing polymer doses in the overdose region. This method is advantageous compared to rheological methods using concentric cylinder rheometers as

it allows direct injection of chemicals during measurements which is not allowed by concentric cylinder rheometers.

The overall goal of this study was to determine tools and methods that can be used for in-line and real-time characterization and monitoring of sludge and evaluate whether this information can be used for optimization of treatment processes. If these tools and methods are developed, it will decrease operational costs, the time it takes to obtain results and the environmental impact while increasing the efficiency of the overall process. The first part of the research investigated the use of UV/Vis spectrophotometry and rheology to monitor the level of treatment and performance during aerobic digestion of sludge. Aerobic digestion was chosen as a representative biological treatment process. The second part of the research aimed to explore the use of rheology to optimize process control of sludge treatment. This was done by varying sludge characteristics such as TS, metal ions and extracellular polymers concentration and determining their effect on the rheological properties of the sludge. Microscopy analyses was also used for both parts to examine the effect aerobic digestion, metal ions and extracellular polymers have on sludge structure and floc matrix.

Chapter 2 – Literature Review

2.1 Types of sludge and sludge treatment

When wastewater is treated at a wastewater treatment plant a mixture of undesired solids and liquids are removed from the wastewater to ensure the treated effluent satisfies certain regulatory effluent guidelines for discharge into a water body. The mixture of solids and liquids removed is commonly known as sludge and must be further treated prior to disposal. The sludge characteristics are dependent upon where within the wastewater treatment train the sludge is produced as seen in Figure 2 below.

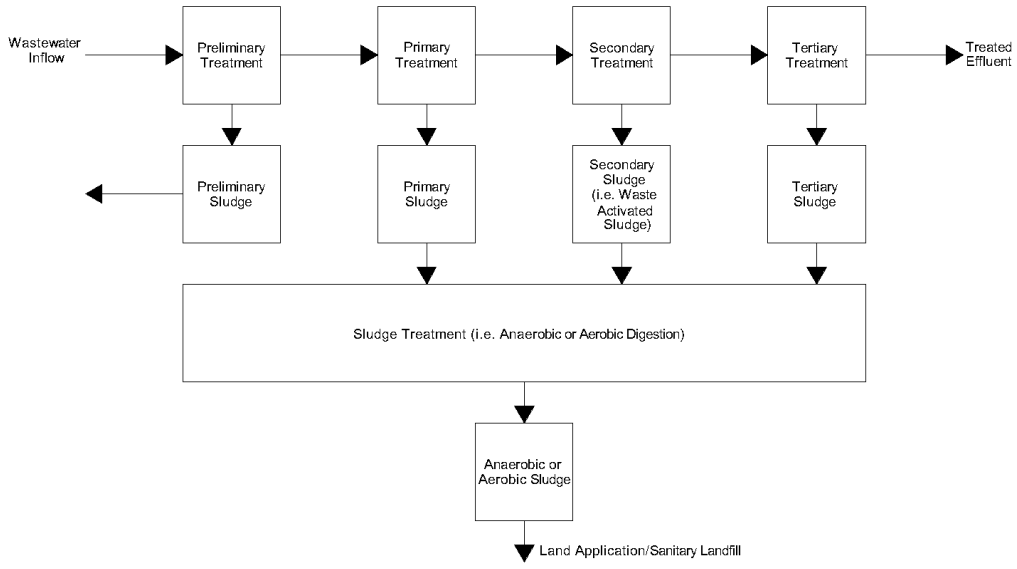

Figure 2. Typical treatment train for wastewater treatment plants.

2.1.1 Preliminary Sludge

Preliminary sludge usually consists of coarse solids such as rocks, branches, rags and plastics (Tchobanoglous & Burton, 1991). These materials are collected on the bar racks and screens as

the wastewater enters the treatment plant. Preliminary sludge is treated separately from the other sludges due to its characteristics differing significantly from the other sludges and its large inorganic component.

2.1.2 Primary Sludge

Primary sludge comes from primary clarifiers or settling tanks and has a very strong odour associated with it (Tchobanoglous & Burton, 1991). It is commonly grey and slimy, containing a significant number of pathogens which means precautions must be taken when dealing with it. Primary sludge can be stabilized using common sludge treatment methods.

2.1.3 Waste Activated Sludge (WAS)

One method of secondary wastewater treatment is known as the activated-sludge process. In this process, a mass of active microorganisms is introduced into a tank which contains wastewater. The microorganisms consume the organics within the wastewater, decreasing the biological oxygen demand (Tchobanoglous & Burton, 1991).

To maintain an optimum food-to-microorganism ratio during the activated-sludge process, the excess activated sludge produced each day must be removed from the basin (Tchobanoglous & Burton, 1991). The excess activated sludge that is withdrawn from the basin and not recycled back in is known as waste activated sludge (WAS).

WAS has a brownish, flocculant appearance and does not have as strong an odour as primary sludge. It has an “earthy” smell when it is in good condition and contains significantly less pathogens compared to primary sludge. However, if WAS is withdrawn and not aerated, microorganisms will begin to die and odours will be produced (Tchobanoglous & Burton, 1991).

Also, due to the small microorganisms having large surface areas, dewatering WAS is very difficult as water is attached by physical and chemical means. WAS can be digested by itself or when it is mixed with primary sludge using methods such as aerobic or anaerobic digestion.

2.1.4 Aerobic Digestion

Aerobic digestion of sludge is similar to the activated-sludge process used for wastewater treatment. Sludge is aerated in an open basin where the biodegradable matter is oxidized by the microorganisms resulting in production of new cellular. As the amount of organic matter (OM) decreases within the tank there is less food available for the microorganisms to consume, resulting in the microorganisms being oxidized as well. When these extracellular materials are oxidized carbon dioxide (CO_2), water (H_2O) and ammonia (NH_3) are produced. Throughout the digestion process NH_3 oxidizes to nitrate (NO_3^-) and the overall reaction can be seen in Equation 1 below:

$$C_5H_7NO_2 + 7O_2 \rightarrow 5CO_2 + NO_3^- + 3H_2O + H^+ \qquad \text{Equation 1}$$

Once the aerobic digestion is complete, the aerobically digested sludge will have lower amounts of volatile solids and pathogens compared to the sludge prior treatment (Tchobanoglous & Burton, 1991). Its odours will have significantly been reduced as well.

One positive from this process is that it achieves stabilization much faster than anaerobic digestion because oxygen is a much better electron acceptor than the electron acceptors used during anaerobic digestion. However, negatives associated with this method are the high costs due to the pumping and aeration in addition to the larger volume of sludge generated by the process (Tchobanoglous & Burton, 1991).

2.1.5 Anaerobic Digestion

The second sludge treatment method previously mentioned is anaerobic digestion. Anaerobic treatment, the most common method of sludge stabilization, is a biological treatment method that is carried out in the absence of oxygen (Tchobanoglous & Burton, 1991). In this process, the organic matter within the sludge is converted to a collection of end products including methane (CH_4) and CO_2.

The decomposition of the OM within the sludge during anaerobic digestion is carried out in three steps: hydrolysis, acidogenesis and methanogenesis. In hydrolysis, complex organics such as proteins, carbohydrates and lipids are broken down into soluble organics by extracellular enzymes. These soluble organics, including glucose, amino acids and fatty acids, are converted into organic acids by acid producers during the acidogenesis stage (Tchobanoglous & Burton, 1991). Lastly, in the methanogenesis stage, methanogens kick in and convert the organic acids into CH_4 and CO_2. Once the treatment is complete, the anaerobically digested sludge will no longer have strong odours associated with it and the organic and pathogen content will have been reduced significantly (Tchobanoglous & Burton, 1991).

When compared to aerobic digestion, one advantage anaerobic digestion has is it requires less energy and produces less sludge during operation (Tchobanoglous & Burton, 1991). Another positive of anaerobic digestion is that it produces CH_4 which can be burned on site to produce electricity and provide heat to the treatment plant.

2.2 Regression Analysis Methods

2.2.1 Multivariate Regression (MLR)

Multivariate regression (MLR) is the method of using a single regression model with more than one outcome model. The principle of MLR is based on least squares and the best MLR model will have the lowest sum of error between observed and predicted parameters. Abyaneh (2014) used MLR to predict the BOD and COD of wastewater in a timelier manner using parameters which could be measured more rapid.

2.2.2 Principle Components Regression (PCR)

Principle components regression (PCR) involves carrying out singular value decomposition (SVD) for the absorbance. During SVD the absorbance matrix is factorized and the result is three matrices, known as the *U* matrix, *S* matrix, and *V* matrix. These three developed matrices are then used to predict values. This method has been used to monitor the anaerobic treatment of sludge using near-infrared spectroscopy. Reed et al. (2011) created predictive models using PCR to predict the volatile fatty acids, bicarbonate alkalinity, and TS and VS throughout the anaerobic digestion process.

2.3 Lab-based tools and methods for sludge monitoring

Depending on the method of disposal or reuse for sludge, the level of treatment of the sludge, also known as the level of stabilization of the sludge, that is required varies (Sanin et al., 2011). Due to this variation, there is no one definition to describe stabilization processes, however, Sanin et al. (2011) define them as “those that reduce detrimental effects, including the reduction of pathogens,

elimination of nuisances such as offensive odours, and the reduction of the potential for further biodegradation that could cause environmental damage."

There are many parameters that are currently used as indicators of the stability of sludge during the stabilization process and they all include lab-based measurements. These lab-based stability parameters include volatile solids reduction (VSR), chemical oxygen demand (COD), pathogen reduction, odour, specific oxygen uptake rate (SOUR) and gas production (Sanin et al. 2011).

2.3.1 Volatile Solids Reduction (VSR)

The amount of organic matter present within the sludge is important to measure because it can be used as an indication of the stability of the sludge (Sanin et al., 2011). Sludges with a greater amount of OM are expected to require more treatment and are less stable. One way OM can be measured is by determining the total volatile solids content (VS) of the sludge which is related to the total solids content (TS) of the sludge (Tchobanoglous & Burton, 1991).

The TS of sludge can be determined by placing a specified volume of sludge in an oven in a laboratory at 100 °C until constant weight is obtained. The residue from the oven can then be placed in a furnace at 550 °C until constant weight is obtained to determine the VS of the sample (Peces et al., 2014).

As a treatment process proceeds, the VS of the sludge is expected to decrease as OM within the sludge gets broken down and the sludge becomes stabilized (Tchobanoglous & Burton, 1991). Therefore, a common method to determine how effective a treatment process can be is to determine the volatile solids reduction that occurs throughout the process (Sanin et al., 2011).

Bhattacharya et al. (1996) used VSR as one of the parameters to monitor the performance of conventional anaerobic sludge digestion to two-phase anaerobic sludge digestion. The VS of the

sludge samples was periodically measured using the lab-based method previously described. Using a 50% primary sludge/50% waste activated sludge feed and a 100% WAS feed, it was found that a greater VSR reduction was observed when the two-phase anaerobic sludge digestion was used (Bhattacharya et al., 1996). Since the two-phase anaerobic sludge digestion had a greater VSR reduction, it was concluded that two-phase anaerobic sludge digestion was a more effective treatment method for the sludge feeds.

To evaluate the effectiveness of sequential anaerobic/aerobic digestion to sludge, Tomei et al. (2011) measured VS over time, among other parameters. They did this to determine the VSR throughout the sequential digestion process, which was used to monitor the level of treatment of the sludge. They found that the anaerobic digestion achieved 32% VSR while the aerobic digestion achieved an additional 17%, indicating an improvement in the level of treatment from the conventional anaerobic digestion process (Tomei et al., 2011).

Ju et al. (2016) also used VSR as one of the parameters to monitor the performance of the anaerobic digestion of chemically enhanced primary treatment sludge compared to secondary sludge, consisting of mixed thickened primary sludge and thickened secondary activated sludge. From their study, they could conclude that anaerobic digestion of chemically enhanced primary treatment sludge was more effective than that of combined sludge as the VSR was found to be 54% and 36%, respectively (Ju et al., 2016). Once this was demonstrated, they then used VSR as a parameter to evaluate variations in key operational parameters such as ferric chloride dose, temperature, salinity and hydraulic retention time (HRT) during the anaerobic digestion of the chemically enhanced primary treatment sludge.

Currently in the US, the vector attraction reduction requirement for biosolids land application includes that the sludge must undergo a VSR of 38% or more during aerobic or anaerobic digestion

to be deemed a Class A or B sludge. If a VSR of 38% cannot be achieved, other methods must be used to ensure the biosolids can safely be land applied. The reason VSR is used as a measurement for vector attraction reduction requirements is because vectors such as flies, rodents and birds are thought to be attracted to the volatile component in sludge (Sanin et al., 2011). If the volatile component in the sludge is reduced, the vectors are not expected to be attracted to the sludge which then limits the spread of infectious diseases from these flies, rodents and birds to humans (USEPA, 1995).

The problem with this requirement is there is no data which indicates that there is a certain VS at which vectors are no longer attracted to the sludge as all that is required is a VSR of 38%. Therefore, a VSR of 38% does not give an indication of the exact stability of the sludge or if vectors will be attracted to it, it is just a measure of stability from its initial characteristics (Sanin et al., 2011).

Another problem with using VSR as a parameter to indicate the level of treatment of sludge is that the duration for which it takes to measure VS in a laboratory is not ideal. To determine the VS of a sample, it can take over 24 hours depending on the organic matter and moisture content of the sample. This could result in a stabilization process continuing longer than required which results in unnecessary operational costs.

2.3.2 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is another parameter that can be used to measure the amount of OM in the sludge. COD is a measurement of the amount of oxygen required to chemically oxidize the organic substances within the sludge (Tchobanoglous & Burton, 1991). It is similar to measuring the total organic carbon and the biochemical oxygen demand of the sludge where a

decrease in these three parameters are expected throughout the stabilization process as the OM content decreases (Sanin et al., 2011). Once COD values become constant, this can indicate that the sludge has been treated and the remaining non-degraded COD is due to highly resistant OM to biodegradation or to non-biodegradable organic compounds (Barragan Sanchez et al., 2006).

One of the current methods to determine the COD is known as the HACH method. It is a lab-based method that takes 3-4 hours for the reaction to reach completion. The absorbance is then measured and correlated to the COD.

Barragan Sanchez et al. (2006) studied variations in microbial activity parameters during aerobic digestion of sludge and compared them to more traditional parameters to indicate sludge stabilization such as COD and TS. They compared the ratio of COD/TS, which represents the amount of substrate available per gram of biomass, to the ratio of dehydrogenase activity/esterase activity, which provides information on the primary and secondary metabolism (Barragan Sanchez et al., 2006). Initially, both ratios were high when the sludge was not stabilized, but as stabilization process progressed both ratios decreased and eventually equilibrated. They found a correlation coefficient of 0.7 when comparing the curves of these two ratios over the course of the experiment, indicating the potential to use the relationship between dehydrogenase activity and esterase activity as a tool to monitor the aerobic digestion process of sludge due to its simplicity and speed compared to measuring COD and TS/VS. They also suggested the use of studying enzymatic activities compared to physiochemical parameters such as COD since physiochemical parameters have the drawback of not describing the progression of microorganisms throughout the stabilization process (Barragan Sanchez et al., 2006).

Zupancic and Ros (2008) compared the effect of using pure oxygen to air at different temperatures for aeration during the aerobic digestion of sludge. They also compared the effect of aerobic

hydraulic retention time on two-stage anaerobic-aerobic sludge digestion by keeping the anaerobic digestion HRT constant at 5 days while varying the oxygen-aerated aerobic digestion's HRT between 5 and 10 days. They compared these changes in variables by measuring COD removal, among other parameters, throughout the digestion process. They found that there were two ranges, 38°C and 25-30°C, where successful oxygen-aerated aerobic digestion occurred as these were the ranges where the most COD was removed. Air-aerated aerobic digestion also had two ranges where the most COD was removed, 38°C and 50-58°C. For the second part of their experiment, they found that as the aerobic HRT increased so did the COD removal which indicated that sludge stabilization was more effective at an aerobic digestion HRT of 10 days compared to 5 days in the two-stage anaerobic-aerobic sludge digestion process (Zupancic & Ros, 2008).

Joo et al. (2016) used COD removal as one of the parameters to evaluate the effectiveness of using a pressurized ultra filtration membrane with a mesh screen (MS-PUFM) to optimize two-phased anaerobic sludge digestion. MS-PUFM was investigated due to the membrane fouling problems associated with membrane bioreactors. They found the two-phased anaerobic digestion coupled with the MS-PUFM was more effective than the typical two-phased anaerobic digestion without MS-PUFM as the COD removal rates were 97% and 40%, respectively (Joo et al., 2016). Using the results from this stability parameter, they suggested that using MS-PUFM had more practical feasibility for the disposal of sludge with a high solids content than other current commercial system processes such as anaerobic biofilters or up-flow anaerobic sludge beds.

Even though COD can be used as a good monitoring tool to measure the stabilization process of sludge, it still has its drawbacks. One of its limitations is its inability to describe the microorganism activity throughout the process. Secondly, COD measurements involve lab-based methods which does not allow for in-line and real-time characterization and monitoring of sludge.

2.3.3 Pathogen Reduction

There are microorganisms living in the human gastrointestinal system which are excreted and end up at the wastewater treatment plant and then within the sludge produced from wastewater treatment processes. Human beings who are infected with or carrying a disease can have pathogenic organisms such as bacteria, viruses, protozoa and helminths within their gastrointestinal systems meaning these pathogenic organisms will end up in sludge and can pose health risks. The pathogenic organisms can cause diseases such as typhoid fever, diarrhea and cholera and can even result in death in locations lacking sanitation (Tchobanoglous & Burton, 1991). Since sludge treatment methods such as lime stabilization, anaerobic and aerobic digestion reduce the pathogen concentration within sludge, pathogen reduction has been used as a parameter to monitor the treatment of sludge (Sanin et al., 2011). Pathogen reduction is also a parameter which is used in many countries for regulation for final disposal of sludge (USEPA, 1995).

One method used to determine the pathogen reduction in sludge is to monitor the reduction in an indicator organism. A common indicator organism used is *E. coli*, which is an organism that is found in the intestines of all warm-blooded animals (Sanin et al., 2011). Most strains of *E.coli* are not harmful, however some can cause illness in humans such as diarrhea and fever. *E. coli* is used because it is assumed to be more resistant to stabilization processes compared to pathogenic organisms which means a reduction in *E. coli* would mean a reduction in pathogens.

In recent years, different methods have been used to determine the pathogen reduction in sludge. Lloret et al. (2012) analyzed *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens* spores and total coliforms to determine if autothermal thermophilic aerobic digestion could remove the pathogens included in the Proposal for a European Directive on spreading sludge on land and produce a stable sludge. *Salmonella* spp. and *Clostridium perfringens* spores were analysed by

using a lab-based method known as denaturing gradient gel electrophoresis (DGGE). The total coliforms and *E. coli* were quantified by being plated and incubated for 24 hours. They concluded that the autothermal thermophilic aerobic digestion process gave Class A biosolids as the *Salmonella* spp. and *E. coli* were completely eliminated. However, they were unable to obtain advanced sludge status due to the presence of *Clostridium perfringens* spores in the treated sludge (Lloret et al., 2012).

Chen et al. (2012) used pathogen reduction as a parameter to assess the effect of retention time in a mesophilic anaerobic digester on the stabilization of sludge. To determine the pathogen reduction, they measured *E. coli*, *Salmonella* sp. and *Shigella* sp. concentrations in the sludge at different solids retention times from the mesophilic anaerobic digestion process using the most probable number and quantitative polymerase chain reaction (qPCR) methods. *E. coli* and *Salmonella* sp. were measured as they are used in the pathogen requirements for the classification of biosolids while *Shigella* sp. was chosen as there is limited information on the survival of this pathogen during anaerobic digestion. The qPCR method was used in addition to the more common most probable number method to see if it could supplement the culture-based method as the qPCR method can detect viable, but non-culturable bacteria which can reactivate and is a faster method. Their results showed that the mesophilic anaerobic digestion process removed *E. coli* and *Salmonella* sp. and that the removal efficiency of these pathogens increased as the solids retention time increased from 11 days to 16 days to 25 days. However, the *Shigella* sp. was resistant to the anaerobic digestion as there was little removal. Similar results were obtained for pathogen reduction using both methods.

Indicator organisms may be ideal to use for quantifying pathogens in drinking water due to the low concentrations of pathogenic organisms, but it does not provide accurate results for sludge where

pathogen concentrations can be much higher. There are a great number of pathogens in sludge which means using any one or few organisms as indicators can be misrepresentative as seen in the research carried out by Chen et al. (2012). The two pathogens which are used in biosolids regulations in the US were at acceptable levels, however *Shigella*, another pathogen, was resistant to the anaerobic digestion process and was still present in the treated sludge. This shows the issue of using pathogen reduction as a stability parameter for sludge as it is difficult to quantify the pathogen reduction due to the great number of pathogens present. In addition, the methods used to determine the pathogen reduction are time consuming, lab-based methods meaning there is a time gap between when the sludge treatment is complete and when the results are known.

2.3.4 Odour

Odours within sludge mainly come from S-containing or N-containing compounds, including hydrogen sulfide (H_2S), ammonia (NH_3), methyl mercaptan (CH_3SH), dimethyl sulfide ($(CH_3)_2S$) and skatole (C_9H_9N) (Sanin et al., 2011). The S-containing compounds in the sludge are known as volatile organic sulfur compounds and can be produced from a variety of ways, including the degradation of proteins to create H_2S and methanethiol (CH_4S) which produces $(CH_3)_2S$ through methylation.

The reason odour can be used as a measurement for the stabilization of sludge is because odour decreases as sludge is digested in a treatment process with stabilized sludge generally having little to no smell (Kumar, 2006). This is due to the S-containing and N-containing compounds being broken down during the digestion process. The quantitative measurement of odour, however, is not ideal. There are two methods for collection of foul gas samples from sites containing sludge for quantitative measurements. One method to collect odourous gas from the site containing sludge

is to use an empty container to collect the odourous air. The second method uses a vessel containing an absorbent material to capture the pungent gas. These samples can then be taken to the laboratory where analytical methods such as gas chromatography can be applied to the samples (Sanin et al., 2011). From gas chromatography, concentrations of specific compounds within the samples can be determined.

Kumar (2006) used gas chromatography of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide to quantify odour measurements for the investigation into sequential anaerobic-aerobic digestion for odour reduction of digested sludge. By measuring organic sulfur concentrations, Kumar concluded that odour generation decreases with an increase in the anaerobic digestion period and that an even greater decrease was observed for sequential anaerobic-aerobic digestion. The issue with using this quantitative measurement of odour though is that gas chromatography can only show you the concentrations of the compounds within the gas but cannot express how strong of an odour the gas has. This can only be done by the human nose (Sanin et al., 2011).

At the site, an olfactometer can be used to estimate the odour of the sludge using the nose. This piece of equipment includes a small chamber of odourless air where the nose can be placed to smell. Specified volumes of the pungent gas are placed within the chamber until a threshold is detected by the nose (Sanin et al., 2011). The odour number is then determined from the number of dilutions that were required to reach the threshold and can be used as an indication of the stability of the sludge.

The European Environment Agency suggested a similar method to determine the stability of sludge where a panel of people with sensitive olfactory skills were asked to sniff progressively diluted odourous gases until the smell could no longer be detected (Bresters et al., 1997). The issues with

these methods used to estimate the odour of sludge using the nose is that olfactometers in different laboratories may give different results because this is a subjective method where it is dependent on the olfaction of the people carrying out the analysis (Sanin et al., 2011).

Using odour as a stability parameter for regulation is not ideal as it is site specific and difficult to obtain quantitative measurements (Sanin et al., 2011). Disposal of odourous sludge in a rural area could seem reasonable with no humans around but disposing the same sludge in an urban area with a higher population density could be very problematic. This would result in the creation of different thresholds of odour for stabilization dependent on disposal location which should not be the case. Also, measuring compound concentrations do not give a complete indication of how odourous the gas is as this can only be done by the human nose, which then results in subjective analysis. Lastly, any non-subjective odour analysis must involve lab-based measurements which can be time-consuming and limits process control from being achieved.

2.3.5 Specific Oxygen Uptake Rate (SOUR)

The specific oxygen uptake rate (SOUR) is a parameter that can only be used in aerobic digestion as an indication of the stability of the sludge. The SOUR is the milligrams of oxygen used by the microorganisms per gram of volatile suspended solids per hour (Kazimierczak, 2012). It has previously been used as a method to determine the biodegradable matter in a substance in addition to being used to analyze the quality of activated sludge. A high SOUR indicates that the sludge is very active, therefore meaning that biodegradable matter is being consumed as the microorganisms within the sludge use the oxygen to break down the OM (Sanin et al., 2011). As the sludge stabilizes the SOUR decreases and eventually equilibrates as the biological activity declines due to the minimal amount of biodegradable material remaining for the microorganisms to consume.

Barragan Sanchez et al. (2006) also used SOUR as a parameter to indicate sludge stabilization during aerobic digestion. They were measuring variations in microbial activity parameters and comparing them to more traditional parameters throughout the course of the aerobic digestion. They found that at the beginning of the digestion process the SOUR readings were low which could have been attributed to the aerobic microorganisms getting acclimatised to their surroundings. As the digestion continued the SOUR readings increased as microorganisms began consuming the OM. After day 46, the SOUR decreased exponentially as there was little OM left for the microorganisms to consume and they began to die off (Barragan Sanchez et al., 2006). On day 110 they could classify the sludge as "Category A" sludge since it had a SOUR of less than 1.5 mgO_2/g TS h, which is a requirement for aerobically digested sludge by the US EPA. They could also correlate the esterase activity to the SOUR as there was an initial decrease in esterase activity as the microorganism adapted to their surroundings. After that there was an increase in esterase activity until day 46 when the maximum value was recorded. After that the esterase activity values gradually decreased until the end of the experiment (Barragan Sanchez et al., 2006). There are issues associated with using SOUR as an indication of the level of treatment of sludge in the aerobic digestion process. If the sludge becomes poisoned by a toxic chemical it can cause a drop in the SOUR. This would be troublesome because it would seem that the treatment is complete, however this drop would be due to the toxic chemical killing the microorganisms and there would still be OM present, indicating unstable sludge. In addition, different microorganisms will have different SOURs which can cause problems. For example, protozoa in aerobic sludge produce a high SOUR which would be thought to indicate that there is a lot of biological activity occurring within the sludge and that treatment is still ongoing (Sanin et al., 2011). However, protozoa are only present when the sludge is stabilized so excess treatment could occur if SOUR

was used as the only indicator of sludge treatment as the SOUR would still be high. This could result in unnecessary additional operational costs. Like the previous parameters, SOUR is a lab-based method which results in a reliance on manual control for sludge treatment and management.

2.3.6 Gas Production

Similar to SOUR indicating the level of biological activity in aerobic digestion of sludge, gas production can be representative of the level of biological activity in anaerobic digestion and can therefore be used to monitor the level of treatment in the anaerobic digestion of the sludge. The gas produced from anaerobic digestion usually consists of 65-70% CH_4, 25-30% CO_2 and small amounts of other gases (Tchobanoglous & Burton, 1991).

At the beginning, there will be minimal gas production as the anaerobic bacteria adapt to their settings. Once the bacteria are acclimatised, OM is broken down with the end products including CH_4 and CO_2 gases. Gas will continue to be produced throughout the digestion process if there is biological activity and OM present (Sanin et al., 2011). When there is no more CH_4 and CO_2 gas being produced, it can be used as an indication that the sludge is stabilized because there is no more OM remaining to be broken down and converted to gas. Gas produced during anaerobic digestion can then be collected and analyzed using gas chromatography to determine the concentrations of CO_2 and CH_4 present in the gas, giving an indication of the stability of the sludge. Like using SOUR for aerobic digestion, if toxic materials are present within the sludge the anaerobic bacteria may be unable to produce CH_4 and CO_2. If gas production is used as the monitoring tool, it would seem as though the treatment was complete as there would be no gas being produced. However, the sludge would still be unstable and it would be due the toxic materials

inhibiting the bacteria from producing gas. In addition, CO_2 and CH_4 concentrations using gas chromatography must be determined in a lab, which means results are not obtained in real-time.

2.4 Potential real-time tools and methods for sludge monitoring

Even though the previous methods described that are currently used for sludge monitoring can be effective at assessing the quality of sludge, they are all either lab-based measurements or unreliable parameters when solely used. This limits process control and optimization as there is a time lag between when the sludge treatment is complete and when the results are known.

Therefore, there is a need for new approaches to optimize the operation of sludge treatment processes as there are currently very few real-time monitoring tools and technologies for sludge treatment. The development of tools and methods that can be used for in-line and real-time characterization and monitoring of sludge would allow determining, modeling and predicting sludge behaviour and flow characteristics through treatment processes, thereby optimizing process control based on this knowledge.

2.4.1 Ultraviolet/visible (UV-Vis) spectrophotometry

When electromagnetic radiation in the ultraviolet (UV) and visible (Vis) spectrum interacts with matter in a sample, the matter absorbs light (Owen, 1996). The measured absorbance can then be used in quantitative analysis of a sample by correlating the absorbance of the sample to the desired parameter, such as concentration, with statistical methods. Two common statistical methods that are used to do this are multivariate regression (MLR) and principal components regression (PCR). Both methods use the absorbance data for a specified wavelength range and the measured value of the parameter of interest which is calculated using a different method. Equations with regression

coefficients are developed which can then be used to predict the parameter of interest using absorbance data instead of calculating the parameter in a more time-consuming fashion (Mark & Workman, 2007).

In order to compare developed equations for varying wavelength ranges, statistical parameters such as the Coefficient of Determination (R^2) and the Residual Mean Square Error (RMSE) can be used. The R^2 is the regression sum of squares that is expressed as a fraction of the total sum of squares. As the value of R^2 approaches 1, the better fit the model is for the measured data. RMSE is a measure of the differences between measured values and those predicted by a model (Berthouex & Brown, 2002).

There are multiple types of spectrophotometers, including lab spectrophotometers and real-time spectrophotometers. For lab spectrophotometers, a sample is placed within the machine and the absorbance is measured. Real-time spectrophotometers can measure absorbance in real-time in-line at treatment plants, thereby allowing real-time optimization.

In the water and wastewater industry, UV/Vis spectrophotometers have been used to monitor the quality of water in fresh lakes, wastewater treatment plants and sewers. Lepot et al. (2016) used the UV/Vis spectrum to correlate the absorbance of samples from sewers, rivers, and wastewater treatment plants to water quality parameters such as total suspended solids (TSS), COD and sCOD (soluble COD) (Lepot et al., 2016). They did this by applying statistical methods to the absorbance data sets to correlate the data to the TSS and COD concentrations. They then used statistical criteria to determine if the methods were accurate. In their study, they found that linear regression was more effective at estimating TSS and COD concentrations compared to partial least squares using the UV/Vis spectrophotometer.

2.4.2 Rheology

The next method that has the potential to be used as a monitoring technology is rheology. Rheology is a holistic descriptor encompassing the physical, biological and chemical changes that occur in sludge and is superior to single descriptive parameters such as TS, VS or COD. Rheology provides information on the overall sludge matrix and can be used to predict sludge behaviour and achieve real-time optimization. It studies the deformation and flow of matter and includes measuring the flow characteristics (shear rate, shear stress, yield stress, rheogram peaks, etc.) of fluids (Ormeci, 2007). A torque rheometer is a device which can be used to determine the rheological properties of a fluid such as sludge. It consists of a shaft connected to an impeller that rotates at a desired speed within a cup in which the sludge is placed. As the impeller rotates it measures the sludge's torque by measuring the sludge's resistance to shear over a period of time. From this a torque rheogram can be produced. There are also torque rheometers that can be installed in-line at a wastewater treatment plant which allows real-time optimization.

There are two different torque rheometry methods that can be used to determine the rheological properties of sludge. The first method is known as the totalized torque (TTQ) method. TTQ is a term that is used to describe the area under a torque-time curve, representing the total energy dissipation which can be used to estimate the overall network strength of the sludge (Ormeci et al., 2004, Ormeci & Abu-Orf, 2005). This method was used to determine the optimum polymer dose during dewatering in lab-scale and full-scale tests at treatment plants (Abu-Orf & Ormeci, 2005; Ormeci & Abu-Orf, 2006; Ormeci, 2007). A decrease in TTQ values with increasing polymer dose corresponds to the optimum polymer dose. Comparing the TTQ of conditioned sludge samples is advantageous over using other rheological parameters such as the yield stress or yield point as

these parameters are not able to monitor changes that the sludge can undergo during conditioning (Ormeci et al., 2004).

The second method that can be used to establish the rheological properties of sludge is known as the direct injection method. The direct injection method uses unconditioned sludge and only utilizes the peaks observed after direct injection of a chemical, such as a polymer, into the sludge sample to assess the overall network strength of the sludge. This method has also been used to determine the optimum polymer dose during dewatering in lab-scale and full-scale tests at treatment plans (Ormeci, 2007; Murray & Ormeci, 2008). Peak heights from the rheogram increase with increasing polymer dose until the optimum dose is reached at the maximum peak height. After this, peak heights gradually decrease with increasing polymer doses in the overdose region. This method is advantageous compared to rheological methods using concentric cylinder rheometers as it allows direct injection of chemicals during measurements which is not allowed by concentric cylinder rheometers.

2.4.3 Near-Infrared Spectroscopy (NIS)

Another region of the spectrum which has been used as a tool for real-time monitoring is the near-infrared spectrum. Near-infrared spectroscopy (NIS) involves absorbance in the wavelength range of 700 nm to 2,500 nm and is a vibrational type of spectroscopy (Pascoa et al., 2008). Chemical bonds in the sample give off vibrations which causes absorbance in the near infrared wavelength region.

Reed et al. (2011) used NIS combined with principle components analysis to monitor the progress of the anaerobic treatment of sludge. They did this by producing predictive models that used the absorbance values found in the near-infrared spectrum of the samples to predict volatile fatty acids,

bicarbonate alkalinity, and TS and VS values throughout the anaerobic digestion of sludge. The predictive models were able to track the relevant stabilization parameters as coefficients of efficiency for the parameters were 0.75, 0.75, 0.71, and 0.69 for the TS, VS, bicarbonate alkalinity, and volatile fatty acids, respectively. Reed et al. (2011) also found that NIS with principle components analysis was able to differentiate the sludge at the different anaerobic digestion stages as it distinguished WAS, primary, feed (Primary:WAS, 70:30) and digested sludge from each other.

Galvez-Sola et al. (2013) also used NIS to estimate parameters of sewage sludge. In their study, they found that NIS could estimate parameters of sewage sludge such as total organic matter, total organic carbon, total nitrogen, water-soluble carbon, extractable organic carbon, fluvic-acid like carbon, electrical conductivity, Mg, Fe, and Cr, among other parameters using partial least squares regression.

2.4.4 Electrical Impedance Spectroscopy (EIS)

Electrical impedance spectroscopy (EIS) is another application which has the potential to be used as a real-time monitoring tool and for sludge characterization. EIS is a non-destructive tool which characterizes the electrical properties of materials and has been used in a variety of fields (Moreno et al., 2014). EIS is carried out by applying an alternating current (AC) or voltage to a sample at varying frequencies (Scholz & Bond, 2010). The sample's electrical response to the varying frequencies is measured and analyzed.

EIS already has applications in sludge treatment as the electrical properties of sludge have been correlated to the rheological properties of sludge during the anaerobic digestion process, potentially allowing a new method for sludge characterization and treatment monitoring (Dieudé-

Fauvel et al., 2014). It has also been investigated how total solids content affects the correlation between electrical and rheological properties. It was found that the sludge showed dual rheological and electrical behaviour, which were both separated by a critical solid content (Ségalen et al., 2015).

2.4.5 Image Based Analysis

Imaged based analysis (IBA) is a potential sludge treatment on-line monitoring application which has become more common in the wastewater industry as it has been used on-line to characterize the floc distribution and to determine the structure of sludge flocs (Govoreneanu, et al., 2004; Liao, et al., 2006).

Mesquita et al. (2011) recently used IBA with chemometrics to improve the activated sludge system performance. Using IBA, they collected information on the aggregated and filamentous bacteria, the biomass composition, and the viable/damaged bacteria (Mesquita et al., 2011). They then completed analytical measurements used to monitor activated sludge systems such as TSS, sludge volume index, and COD. Principle components analysis was used to correlate IBA data to these analytical measurements, showing that IBA data can identify activated sludge deficiencies.

Based on the studies carried out on the methods listed above and considering the cost of the equipment and their suitability/availably for real-time analysis and data collection, UV-vis spectrophotometry and rheology were chosen in this study to examine their potential use as sludge treatment monitoring technologies. They were also chosen because NIR and EIS have already been used to monitor the anaerobic digestion of sludge and IBA has been used to monitor activated sludge. No research has been found on the use of UV-vis absorbance to monitor the aerobic digestion of sludge as it has only been found to be used to monitor wastewater treatment. Rheology

has previously been used to optimize sludge dewatering, but there is no research on using it to detect changes in multivalent cation and extracellular polymer concentrations in anaerobically digested sludge or to monitor the aerobic treatment of sludge. If these tools are successful, they could be used for in-line and real-time characterization and monitoring of sludge and optimization of treatment performance at treatment plants.

Chapter 3 – Materials and Methods

This research was carried out in two parts. The first part involved investigating the use of UV/Vis spectrophotometry and rheology to determine the treatment performance of aerobic digestion of sludge. The second part of the research explored using rheology to monitor and predict changes in sludge characteristics relevant to treatment. The methods for the two parts are provided below.

3.1 Aerobic Digestion Experiments

The experiments carried out for each sample collected from the aerobic batch reactor in part one of the research are summarized in the flow chart seen in Figure 3 and then are described in further detail in the following sections.

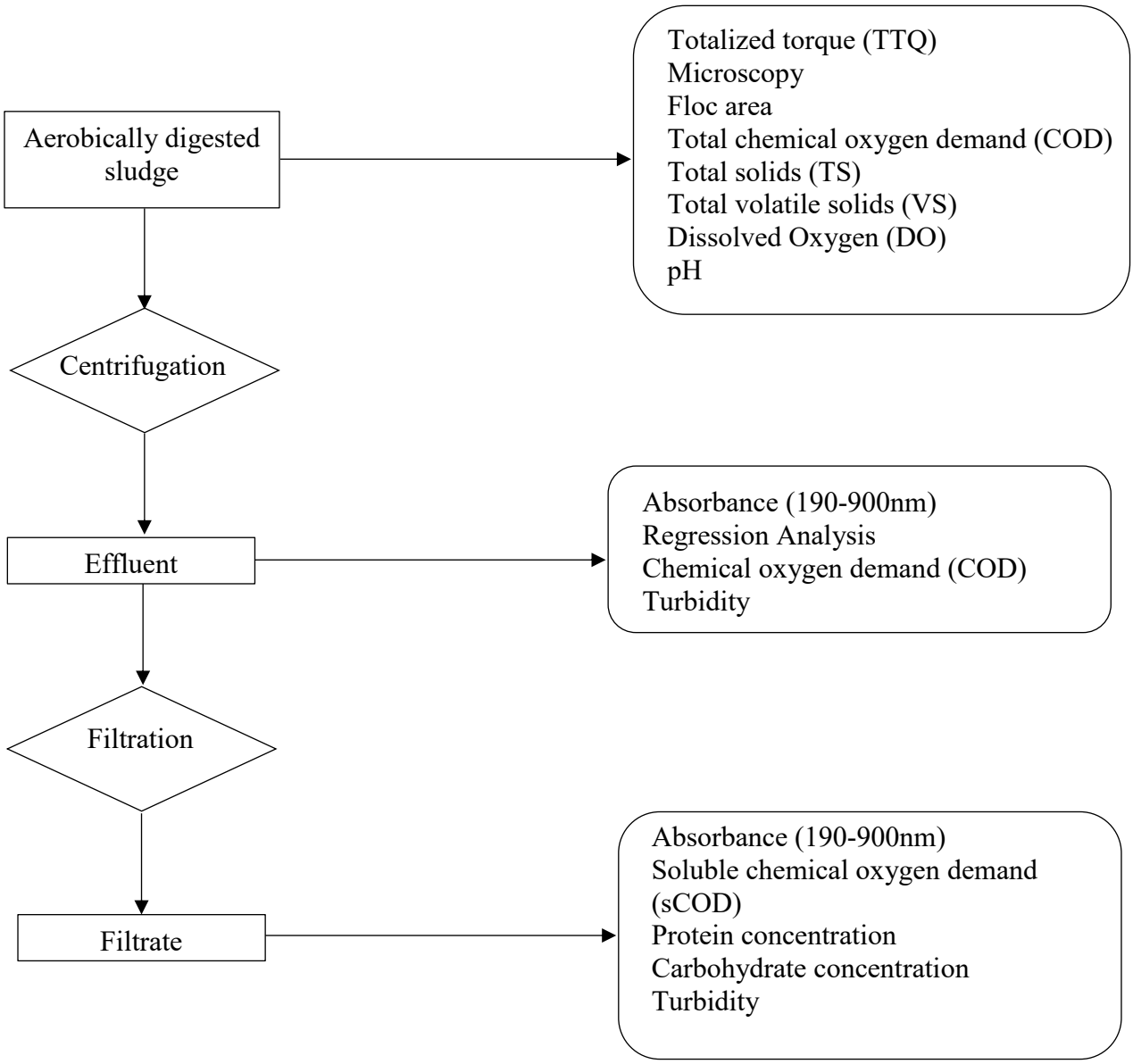

Figure 3. Flow chart of experimental process carried out for part one of research.

3.1.1 Batch aerobic reactor setup

A 20 L jug was used as the lab-scale batch aerobic reactor. Within the jug, 10 L of primary sludge and 10 L of WAS were placed. Eight air diffusers were positioned at the bottom of the jug to provide aeration to the sludge mixture throughout the entire experiment. These eight air diffusers

were connected to a tube which was connected to an air valve that controlled the amount of air that was provided from the air pump. Samples of approximately 700 mL were withdrawn from the reactor each time analysis was completed. A diagram of the lab-scale aerobic reactor can be seen below in Figure 4.

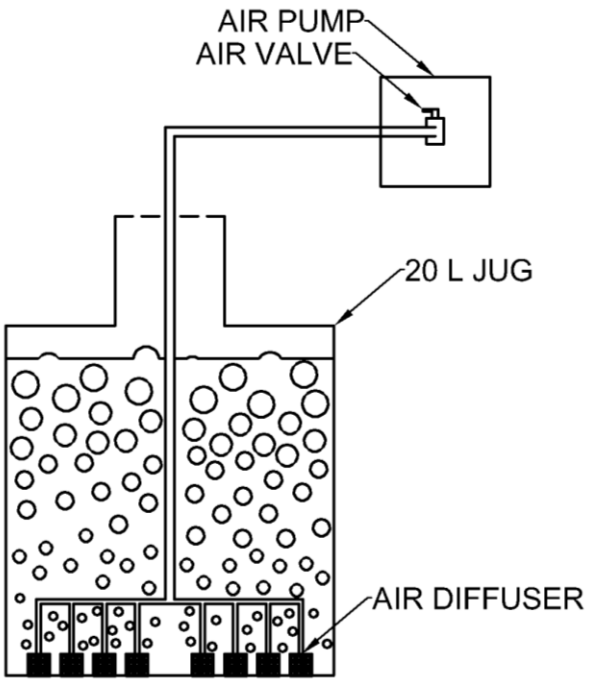

Figure 4. Batch aerobic reactor set-up.

Experiments were carried out using two different batches of mixed 50% primary and 50% waste activated sludge (WAS) samples that were at different strengths. The normal strength sludge (COD = 22,700 mg/L, VS = 13,845 mg/L) represented the strength of mixed primary sludge and WAS under daily operational conditions. The high strength sludge (COD = 37,173 mg/L, VS = 20,657 mg/L) had an initial organic loading rate higher than usual.

COD, TS/VS, absorbance, regression analysis and turbidity measurements were carried out on both batches. Rheological, microscopic, filtrate protein, filtrate carbohydrate, dissolved oxygen (DO), and pH measurements were additionally carried out on the normal strength sludge. All measurements were taken in replicates of 3.

3.1.2 Rheological measurements: TTQ method

The rheological measurements of each sludge sample were measured using the lab-scale Floccky Tester (manufactured by Koei Industries Inc., Japan) torque rheometer. The Floccky Tester can be seen in Figure 5 below. The Floccky tester also has a full-scale model that can installed and used at treatment plants.

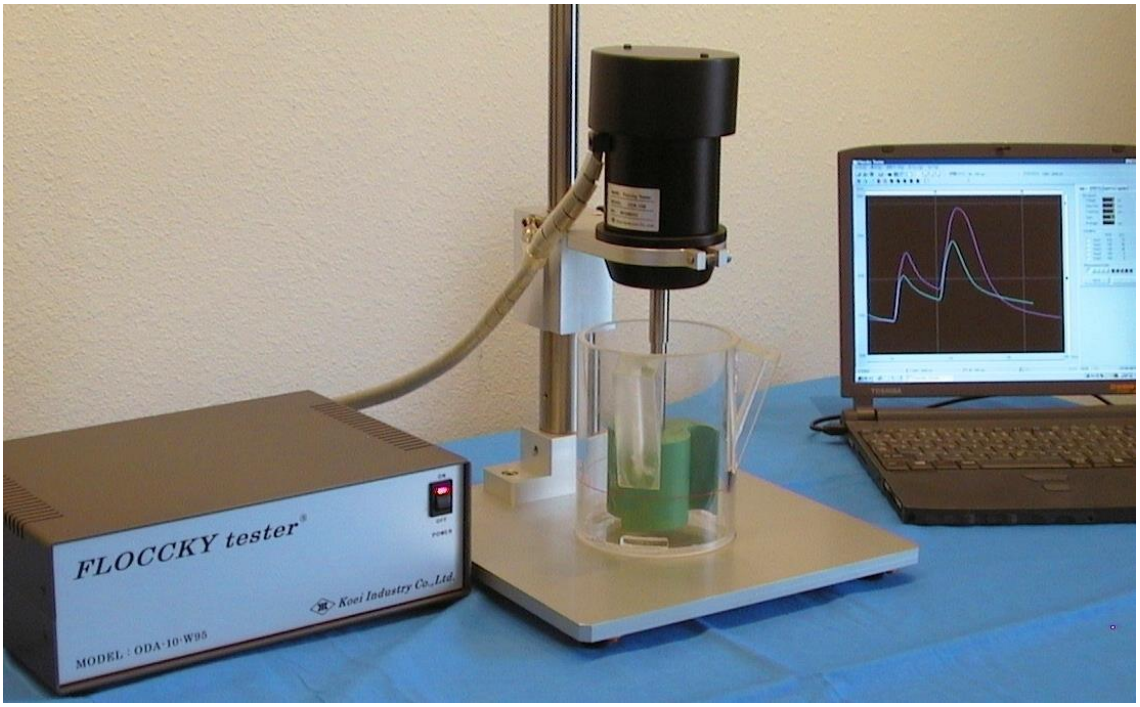

Figure 5. Floccky Tester.

An impeller speed of 300 rpm was chosen for the mixture of primary sludge and WAS due to previous studies (Ormeci et al., 2004). A duration of 120 seconds was selected as this timespan was deemed sufficient to observe the rheological characteristics of the sludge (Murray & Ormeci, 2008).

Three 200 mL subsamples were individually placed in the cylindrical jar of the torque rheometer to obtain three replicates. For each 200 mL sample, the torque rheometer impeller rotated at 300 rpm for 120 seconds and the torque was measured over time. The measurement of torque over time produces a torque rheogram. The subsamples were discarded after each run due to the thixotropic nature of sludge. The Floccky Tester's software reports the area under the torque-time graph, also known as totalized torque (TTQ), and the peak height which can be used to compare the rheological properties of the sludge samples.

3.1.3 Absorbance

The Cary 100 Bio UV-Visible Spectrophotometer was used to measure the absorbance of the effluent and the filtrate in the wavelength range of 190nm – 900nm. A quartz cuvette was used for all measurements and was cleaned thoroughly between measurements. Two samples with dilution factors of 100:1 and 20:1 were used each time for the effluent. Two samples with dilution factors of 50:1 and 5:1 were used each time for the filtrate. Three replicates were measured from each sample to ensure accuracy. A subsample of approximately 5 mL was taken from the sample and placed in the cleaned cuvette. The cuvette was placed in the spectrophotometer and the lid was closed. The spectrophotometer then scanned the sample for the entire wavelength range, 190nm-900nm and recorded absorbance measurements.

3.1.4 Regression Analysis

To determine regression coefficients which could be used with the absorbance data to predict effluent COD, sCOD, and VS using effluent and filtrate absorbance, the MATLAB software was used. Within MATLAB, two functions were created to be used for multivariate regression (MLR) and principle components regression (PCR) (Mark & Workman, 2007). These functions had the absorbance data for the specified wavelength range and the COD measurements as inputs and the regression coefficients as outputs. The script for the MLR function can be seen in Figure 6 below.

```
function [ b, cest ] = MLRfunc( A,c )
%MLRfunc determines regression coefficients using multivariate regression

%Inputs:
%A = absorbance data determined using UV/VIS Spectrophotometer
%c = concentration data determined using HACH method

%Outputs:
%b = regression coefficients for absorbance data to predict concentration

b = inv(A'*A)*A'*c;

cest = A*b;

end
```

Figure 6: Script for MLR function.

The script for the PCR function can be seen in Figure 7 below.

```
function [ b, cest ] = PCRfunc( A, c, n)
%PCRfunc determines regression coefficients using multivariate regression

%Inputs:
%A = absorbance data determined using UV/VIS Spectrophotometer
%c = concentration data determined using HACH method
%n = number of wavelengths absorbance recorded at

%Outputs:
%b = regression coefficients for absorbance data to predict concentration
%cest = predicted concentration using regression coefficients

[U,S,V] = svd(A);

T = A*V;

b = V(:,1:n)*inv(S(1:n,1:n))*U(:,1:n)'*c;

cest = (T*V')*b;
end
```

Figure 7. Script for PCR function.

The measured parameter found using lab-based methods and varying wavelength ranges from the UV/vis spectrum inputted into the MLR and PCR functions seen in Figure 6 and 7, respectively. The standard deviations for the measured parameters and measured wavelength ranges were not considered in the regression analysis. From these functions, regression coefficients were developed which could be used with the measured absorbance in a regression equation to predict a parameter such as VS, effluent COD or sCOD. The regression analysis procedure is summarized in the flowchart below in Figure 8.

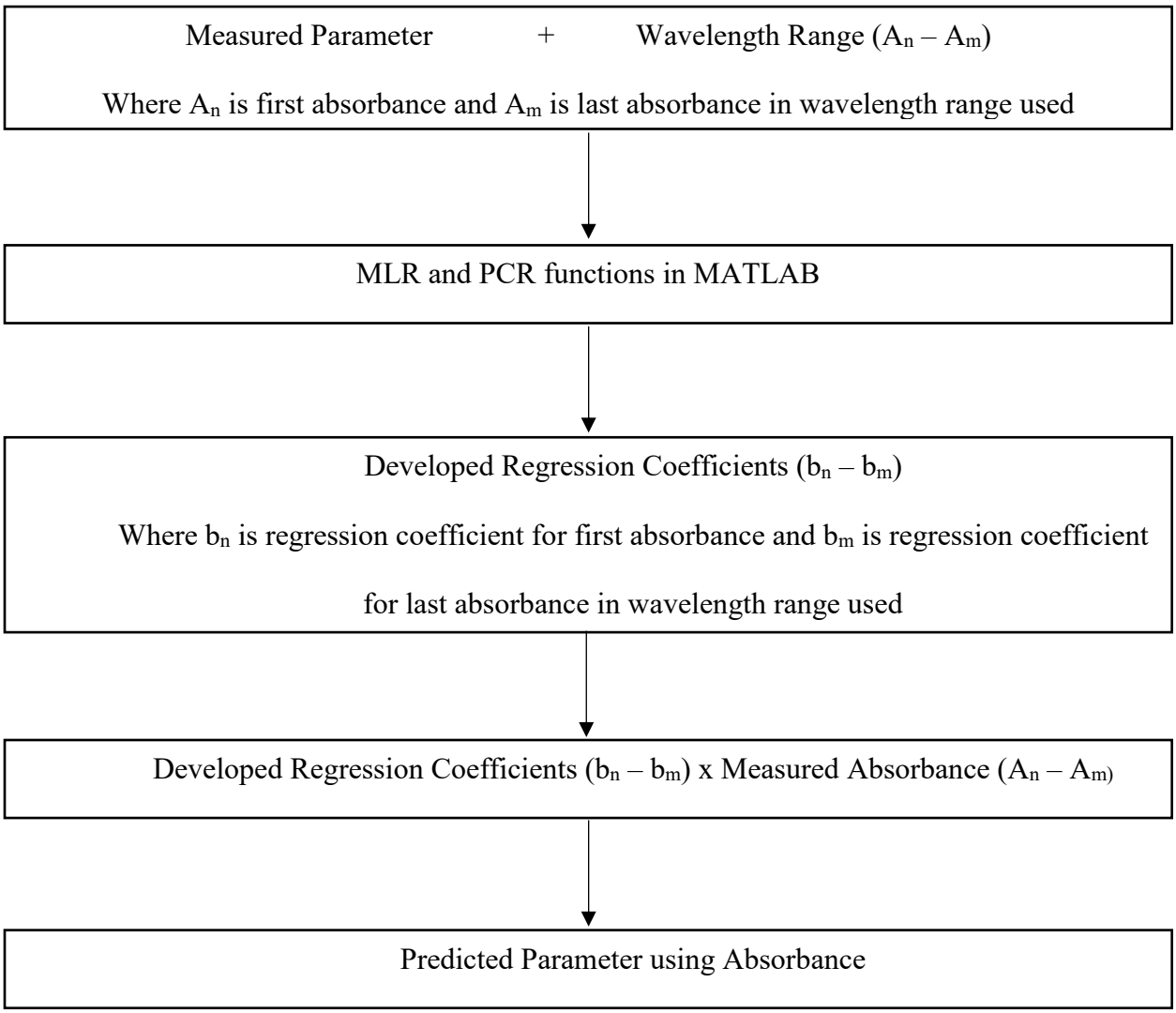

Figure 8. Flowchart for regression analysis process.

Multiple wavelengths were examined and the accuracy of the regression equations used to predict the measured parameter were compared based on Coefficient of Determination (R^2), Residual Mean Square Error (RMSE), and Standard Error.

The Coefficient of Determination (R^2) is the regression sum of squares that is expressed as a fraction of the total sum of squares.

The Residual Mean Square Error (RMSE) is the measure of the differences between the measured and predicted values.

The Standard Error is a measure of the accuracy of predictions made using a regression line.

3.1.5 Microscopy

The Nikon Eclipse Inverted Research Microscope was used to study the microscopic properties of the sludge. A Fisherbrand disposable inoculating loop was used to withdraw 10 microliters of sludge. The sample was placed in a MatTek glass bottom microwell dish and was diluted with 3 mL of D.I. water. The dish was gently shaken to mix the sample. The sample was then placed on the microscope stage and five minutes were given to allow the floc particles to settle to the bottom of the glass bottom microwell dish.

The microscope and the QImaging Retiga EXi Fast 1394 camera were turned on while the floc particles settled. The Nikon NIS-Elements Advanced Research program, version 3.2, was then opened on the computer. This program is connected to the camera and microscope, allowing images viewed through the microscope to be captured as pictures on the computer using the camera. Once the five minutes was complete, the sample was brought into focus. Next, the battlement technique was used to ensure the sample was a homogenous mixture. The battlement technique involves moving the microscope a field along the edge of the sample, a field up, one along and then one down, until the entire sample has been viewed. Five images which best represented the sample were captured as JPG files using the NIS-Elements Advanced Research program and saved.

3.1.6 Floc Area

Area of the flocs was used as a tool to monitor the changes in sludge structure. The areas of the flocs in the five captured images were measured using the Area Tool in the NIS-Elements Advanced Research program as it was calibrated to measure micrometers in images. The software was used to draw the perimeter around each floc and then the area of the floc would be calculated using the Area Tool. The floc area measurements were recorded to a statistics table which could be exported as a Microsoft Excel file.

3.1.7 Chemical Oxygen Demand (COD)

Total COD refers to the COD of the sludge, effluent COD is the COD of the effluent after centrifugation process and sCOD is the COD of the filtrate that is passed through the 0.45 μm pore size filter. The total COD, effluent COD and sCOD were each measured in 3 replicates using HACH Method 8000, High Range, 20-1,500 mg/L COD for each sample (HACH, 2005). A dilution factor of 25:1 was used for total COD, a dilution factor of 5:1 was used for effluent COD and a dilution factor of 2:1 was used for soluble COD (sCOD).

3.1.8 Total solids (TS) and total volatile solids (VS)

To measure the TS and VS of the sludge within the reactor, Standard Methods 2540 B and 2540 E were used (APHA, AWWA, & WEF, 2005). To prepare, 30 (3 replicates per analysis) 50 mL aluminum dishes were labeled and placed in a furnace at 550 °C for one hour. They cooled in a desiccator for 30 minutes and then they were labeled and weighed using an analytical balance. Three 10 mL subsamples were taken from the 700 mL sample using a wide-mouth plastic pipette. Each replicate was then placed in the oven at 105 °C and was left in the oven for at least 24 hours

to ensure the sample dried. Once the sample dried, it was cooled, and then the dish and dry sample were weighed using an analytical balance. After being weighed, the dish and dry sample were placed in a furnace for at least 2 hours at 550 °C. After cooling in a desiccator, the ignited sample and dish were weighed using an analytical balance. The TS and VS were then calculated using the following equations.

$$TS = \frac{B-A}{V} \quad \text{Equation 2}$$

Where: TS = total solids of sample (mg/L)

A = mass of dish (mg)

B = mass of dish and dry sample (mg)

V = volume of sample (L)

$$VS = \frac{B-C}{V} \quad \text{Equation 3}$$

Where: VS = total volatile solids of sample (mg/L)

C = mass of dish and ignited sample (mg)

3.1.9 Protein

The filtrate protein concentration was measured using Coomassie Brilliant Blue G-250 reagent and the Bradford method (Bradford, 1976). A bovine serum albumin (BSA) solution was created by adding 10 mg of BSA powder to 100 mL of deionized (D.I.) water. The solution was continuously stirred until the BSA powder had completely dissolved in the D.I. water. The BSA solution was diluted to concentrations between 0 and 100 mg/L to create a calibration curve which is shown in Figure 7. The calibration curve was linear up to 50 mg/L. Test tubes were filled with 5 mL of the Coomassie Brilliant Blue G-250 reagent and 3 mL of each standard or diluted filtrate sample. A dilution factor of 20:1 was used for the filtrate samples as this dilution factor gave

absorbance measurements within the working range of the calibration curve. The cap was placed on the test tube and inverted several times to mix the solution. Ten minutes were given to allow the reaction in the test tube to proceed at room temperature. During this time, the DR2800 HACH spectrophotometer was turned on and set to a wavelength of 595 nm. It was then zeroed with a test tube containing D.I. water. After the ten-minute reaction period, the absorbance of each standard or diluted filtrate sample was measured after wiping the test tube clean with a Kimwipe. The absorbance of the blank sample, containing 3 mL of D.I. water and 5 mL of Coomassie Brilliant Blue G-250 reagent was subtracted from the absorbance of all standards and samples. The protein concentration measurements of the diluted filtrate samples were then back calculated using the linear regression line generated from the calibration curve seen in Figure 9.

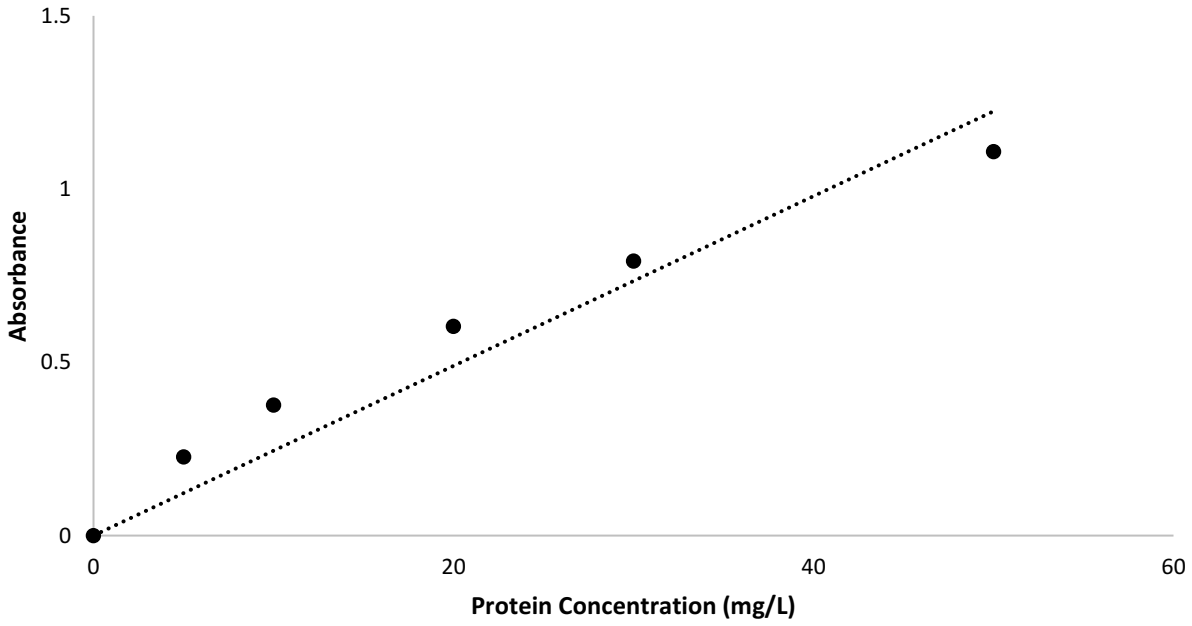

Figure 9. Calibration curve for BSA between 0 and 50 mg/L. It has an equation of line of $y=0.0245x$ and a $R^2=0.93$.

3.1.10 Carbohydrate

The filtrate carbohydrate concentration of the sample was measured using the Anthrone method (Morris, 1948). A 95% sulphuric acid solution with 2 g/L of anthrone reagent was created by adding 1 g of anthrone reagent powder to 500 mL of 95% sulphuric acid. Galactose stock solutions were prepared by adding 10 mg of 98% pure galactose powder to 100 mL of D.I. water. The solution was continuously stirred until the 98% pure galactose powder had completely dissolved in the D.I. water. The galactose solution was diluted to concentrations between 0 and 100 mg/L to create a calibration curve shown in Figure 10. The calibration curve was linear up to 100 mg/L. Test tubes were filled with 6 mL of the 95% sulphuric acid solution with 2 g/L of anthrone reagent and 3 mL of each standard or diluted filtrate sample. A dilution factor of 20:1 was used for the filtrate samples as this dilution factor gave absorbance measurements within the working range of the calibration curve. The cap was placed on the test tube and inverted several times to mix the solution. Ten minutes were given to allow the reaction in the test tube to proceed at room temperature. During this time, the DR2800 HACH spectrophotometer was turned on and set to a wavelength of 620 nm. It was then zeroed with a test tube containing D.I. water. After the ten minute reaction period the absorbance of each standard or diluted filtrate sample was measured after wiping the test tube clean with a Kimwipe. The absorbance of the blank sample, containing 3 mL of D.I. water and 6 mL of 95% sulphuric acid solution with 2 g/L of anthrone reagent was subtracted from the absorbance of all standards and samples. The carbohydrate concentration measurements of the diluted filtrate samples were then back calculated using the linear regression line generated from the calibration curve seen in Figure 10.

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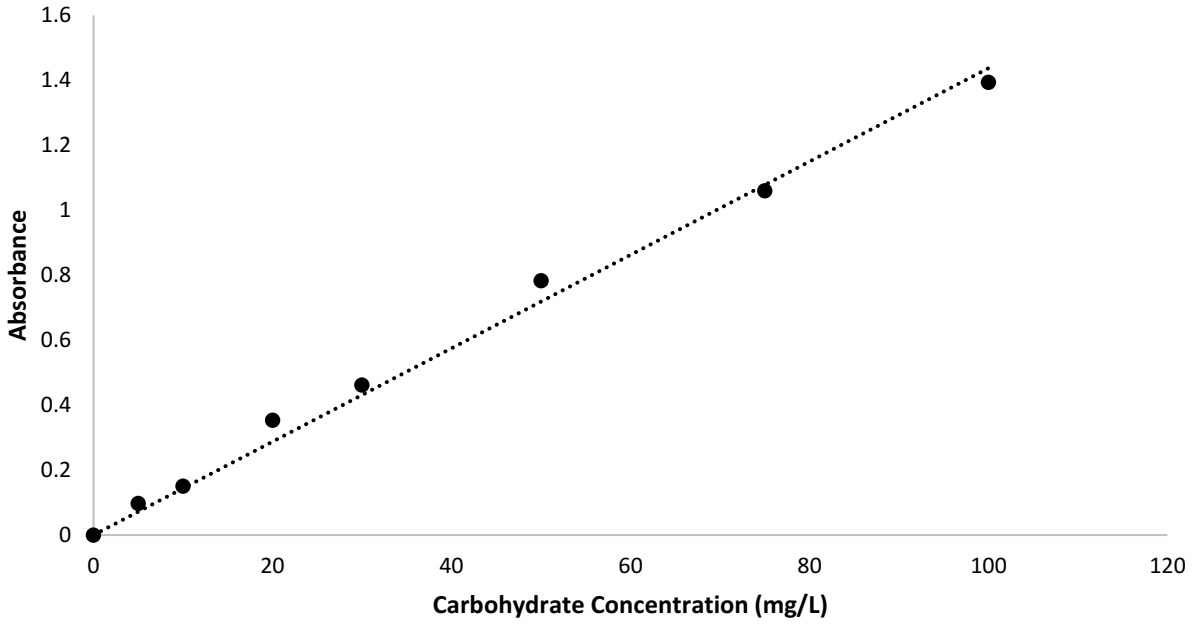


Figure 10. Calibration curve for galactose between 0 and 100 mg/L. It has an equation of line of y=0.0144x and a R^2=0.99.

3.1.11 Turbidity

The turbidity of the effluent and filtrate was measured using the 2100AN Turbidimeter. The empty sample cell was rinsed with D.I. three times and dried prior to each measurement. The sample cell was filled with approximately 30 mL of the sample and the cap was placed on the sample cell. The outside of the sample cell was then cleaned with a soft, lint-free cloth to remove water spots and fingerprints. Next, a small bead of silicone oil was applied to the outside of the sample cell from the top to bottom. An oiling cloth was then used to distribute the oil equally to the outside surface of the sample cell and to remove any excess oil. The sample cell was gently inverted to mix the sample and then was placed in the sample cell holder with the triangle on the sample cell aligning with the reference mark on the sample cell holder (HACH, 2014). The cover was closed and then turbidity measurements were taken at 0 s, 10 s, 20 s and 30 s. An average of these four

measurements was taken as the turbidity measurement for the sample. Three samples each were measured every time for the effluent and the filtrate. A dilution factor of 20:1 was used for the effluent and a dilution factor of 2:1 was used for the filtrate.

3.1.12 Dissolved Oxygen (DO)

The DO of the sludge sample was measured using the Orion RDO Dissolved Oxygen Probe. Prior to each measurement the DO probe had to be calibrated using the Orion calibration sleeve. The cap was removed from the calibration sleeve and the sponge from the cap was taken out. The sponge was saturated with distilled water and squeezed until the excess water came out of the sponge. The calibration sleeve was reassembled. The thread ring was unscrewed and removed from the RDO sensor. The calibration sleeve was placed over the front of the RDO sensor and then the sleeve was screwed onto the sensor. Instructions were followed on the Thermo Scientific Orion Star A Series portable meter to perform a water-saturated air calibration.

When DO measurements were taken, the RDO probe was first rinsed off with distilled water, blotted dry with a lint-free tissue and inserted into the sample. The RDO sensor was immersed in the sludge solution and then a reading was obtained using the Thermo Scientific Orion Star A Series portable meter.

3.1.13 pH

The pH of the sample was measured using the Orion 8107UWMMD Ross Ultra pH/ATC Triode electrode and Thermo Scientific Orion Star A Series portable meter. A 40 mL subsample of sludge was withdrawn from the sample and placed in a 50 mL graduated cylinder. The electrode was withdrawn from the electrode storage bottle and rinsed with D.I. water three times. The portable

meter was turned on. The pH electrode was dried and placed in the subsample and was gently rotated while the portable meter obtained a reading. Once a finalized reading was obtained the pH probe was rinsed three times again with D.I. water and was placed back in the electrode storage bottle.

3.1.14 Sludge Samples

Primary sludge and WAS samples were collected from the Robert O. Pickard Environmental Centre (ROPEC) located in Ottawa (Ontario, Canada).

3.1.15 Centrifugation

To be able to measure the change in the characteristics of sludge supernatant (effluent) during digestion, centrifugation was used to separate the sludge solids and liquids. Approximately 500 mL of sludge was separated into two bottles which were placed in the Sorvall Legend RT+ Centrifuge. The sludge was centrifuged at 7,500 G for ten minutes. The effluent was then carefully removed from each bottle and placed in a beaker for further analysis. The residual solids portion of the sludge was discarded.

3.1.16 Filtration

To be able to measure the change in the characteristics of the filtrate during digestion, a filtration apparatus was used with a 0.45 μm pore size filter and a vacuum-pressure pump. Filtration allowed removal of the organic and inorganic particles compared to the centrifuged samples. Approximately 200 mL of effluent from the centrifugation process was used for the filtration

process with filters being replaced once they became fouled. The filtrate was removed from the bottom of the filtration apparatus and was placed in a beaker for further analysis.

3.2 Rheological Characterization

The TTQ method and direct injection method are summarized in the flow chart seen in Figure 11 and then are described in further detail in the following sections.

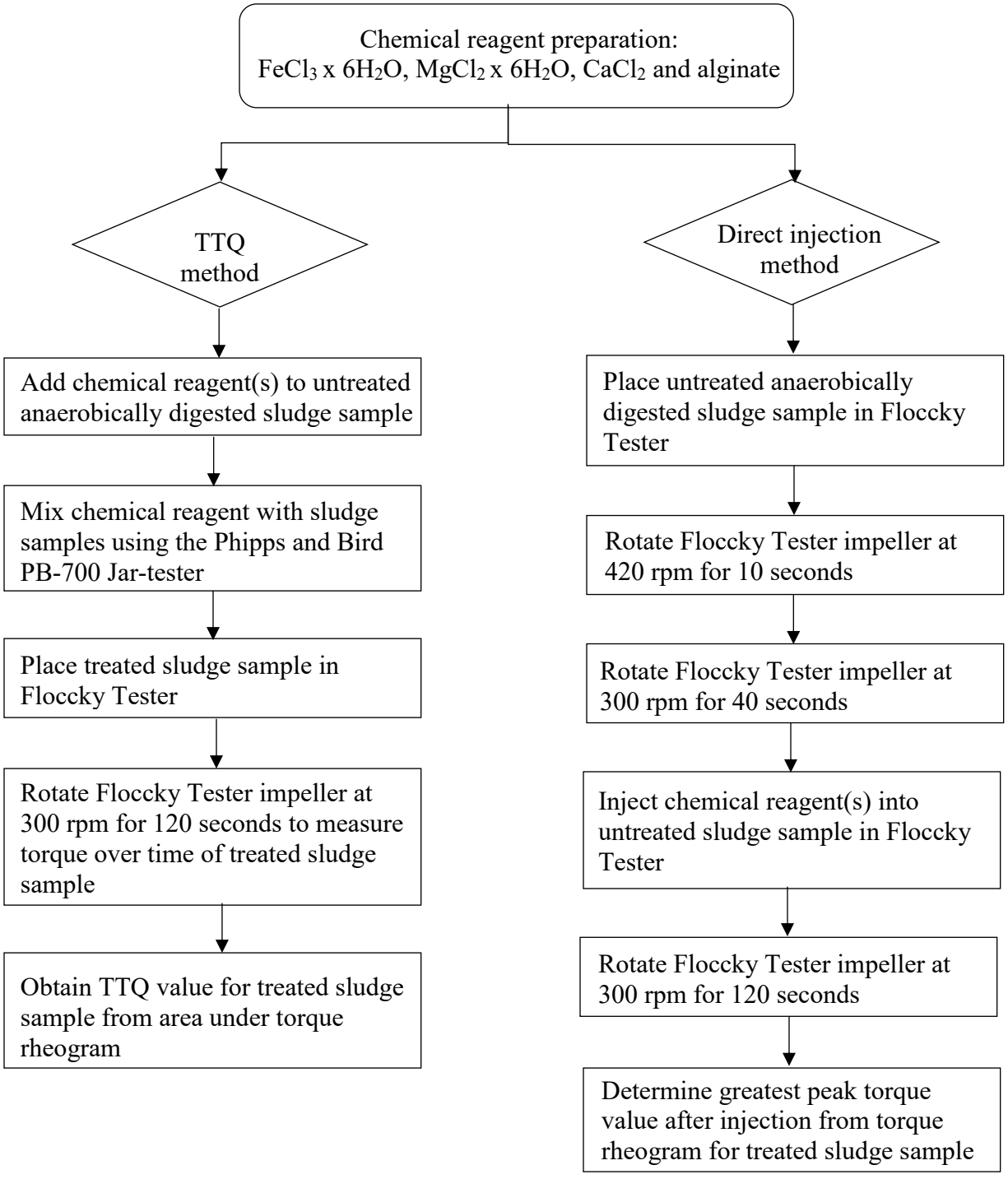


Figure 11. Flow chart of experimental processes carried out for the totalized torque and direct injection methods used in part two of research.

3.2.1 Rheological measurements: Totalized torque (TTQ) method

TTQ method uses pretreated samples for rheological tests and measures the area under the torque-time rheograms. This method was used when the sludge was pretreated with $MgCl_2$ x $6H_2O$, $CaCl_2$, $FeCl_3$ x $6H_2O$, alginate, and $CaCl_2$ and alginate as explained in sections 2.3 and 2.4 below.

Three 200 mL subsamples were individually placed in the cylindrical jar of the torque rheometer to obtain three replicates. For each 200 mL sample, the torque rheometer impeller rotated at 300 rpm for 120 seconds and the torque was measured over time (Ormeci et al., 2004). The subsamples were discarded after each run due to the thixotropic nature of sludge. The Floccky Tester's software reports the area under the torque-time graph, also known as TTQ, and the peak height which were used to compare the rheological properties of the sludge samples.

3.2.2 Rheological measurements: Direct injection method

The direct injection method uses raw (untreated) sludge for rheological tests and directly injects the chemical into the sample during testing and observes the size of the peaks formed in the rheograms after the injection of the chemical. The direct injection method is described below for $FeCl_3$ x $6H_2O$ and it was similarly used for $MgCl_2$ x $6H_2O$, $CaCl_2$ and alginate.

The Floccky Tester was used again for these measurements. Eighteen 190 mL sludge samples (3 per Fe^{+3} concentration, 3 untreated) were individually placed in the cylindrical jar of the torque rheometer. For each sample, the impeller first rotated at a speed of 420 rpm for 10 seconds to suspend the settled solids and homogeneously mix the sludge (Murray & Ormeci, 2008). Next, the sludge was mixed at 300 rpm for 40 seconds to provide a baseline for the 190 mL of untreated sludge. At the beginning of the next step, 10 mL of the varying Fe^{+3} concentration solutions were

injected into the sludge through one of the ports in the cup (see Figure 4) and the sludge was mixed at 300 rpm for 120 seconds. A peak was observed right after the solution injection.

When the direct injection method was used for the combination of $CaCl_2$ and alginate, the same process described above was carried out, however, when the injection occurred, 10 mL of a 5,000 mg/L alginate solution and 10 mL of the varying Ca^{+2} concentration solutions were injected into the sludge through two of the ports in the cup simultaneously.

3.2.3 Sludge pretreatment with $MgCl_2$ x $6H_2O$, $CaCl_2$, $FeCl_3$ x $6H_2O$, and alginate for TTQ method

The process described below for $FeCl_3$ x $6H_2O$ was also used for $MgCl_2$ x $6H_2O$, $CaCl_2$ and alginate.

190 mL sludge samples were dosed with 10 mL of the 5 different $FeCl_3$ x $6H_2O$ solutions, resulting in a total sample volume of 200 mL each run. For each $FeCl_3$ x $6H_2O$ concentration, 3 replicates were made, resulting in 15 samples. These 200 mL samples were then mixed on the Phipps and Bird PB-700 Jar-tester for two minutes at 200 rpm. In addition to these treated sludge samples, three 200 mL untreated sludge samples were also prepared to act as a baseline.

3.2.4 Sludge pretreatment with $CaCl_2$ and alginate for TTQ method

180 mL sludge samples were dosed with 10 mL of alginate solution with a concentration of 5,000 mg/L and 10 mL of the 5 different $CaCl_2$ solutions, resulting in a total sample volume of 200 mL each run. For each varying $CaCl_2$ concentration, 3 replicates were made, resulting in 15 samples. These 200 mL samples were mixed on the Phipps and Bird PB-700 Jar-tester for two minutes at 200 rpm. The alginate solution was added at time zero with the $CaCl_2$ solution being added ten

seconds into the mixing. In addition to these treated sludge samples, three 200 mL untreated sludge samples were prepared to act as a baseline.

3.2.5 Variation of total solids content for TTQ method

The anaerobically digested sludge obtained from ROPEC was found to have a TS of 1.76%. One 800 mL sludge sample was diluted with 200 mL of D.I. water to achieve a TS of 0.91%. The next 800 mL sludge sample was centrifuged at 2,000 G for six minutes in the Sorvall Legend RT+ Centrifuge. Once the effluent was removed, the dewatered sludge sample was found to have a TS of 3.25%. The final 800 mL sludge sample was centrifuged at 6,000 G for 6 minutes. After removing the effluent, the dewatered sludge sample had a TS of 4.23%.

3.2.6 Microscopy

See section 1.5 for the description of the method used for microscopy. Four samples were analyzed using the microscope which were 200 mL of untreated sludge, 190 mL of sludge treated with 10 mL of 138,800 mg/L $CaCl_2$ (50,000 mg/L Ca^{+2}), 190 mL of sludge treated with 10 mL of 242,000 mg/L $FeCl_3$ x $6H_2O$ (50,000 mg/L Fe^{+3}), and 190 mL of sludge treated with 10 mL of 5,000 mg/L alginate and 10 mL of 138,800 mg/L $CaCl_2$ (50,000 mg/L Ca^{+2}).

3.2.7 Floc area

See section 1.6 for the description of the method used to determine the area of the flocs in the captured images of the untreated and treated sludges.

3.2.8 Sludge samples

Anaerobically digested sludge samples were collected from the ROPEC located in Ottawa (Ontario, Canada). This plant employs mesophilic anaerobic digestion.

3.2.9 Preparation of chemical reagents

3.2.9.1 Magnesium chloride hexahydrate ($MgCl_2$ x $6H_2O$)

A 200 mL solution of 209,160 mg/L $MgCl_2$ x $6H_2O$ (25,000 mg/L Mg^{+2}) was initially prepared by adding 41.8 g of $MgCl_2$ x $6H_2O$ to 200 mL of D.I. water. From this, 50 mL solutions with $MgCl_2$ x $6H_2O$ concentrations of 41,830 mg/L (5,000 mg/L Mg^{+2}), 83,660 mg/L (10,000 mg/L Mg^{+2}), 125,490 mg/L (15,000 mg/L Mg^{+2}) and 167,330 mg/L (20,000 mg/L Mg^{+2}) were prepared by adding the required volumes of D.I. water and the 209,160 mg/L $MgCl_2$ x $6H_2O$ solution. These solutions were prepared for both the TTQ method and direct injection method. For the direct injection method, an additional solution with an $MgCl_2$ x $6H_2O$ concentration of 418,300 mg/L (50,000 mg/L Mg^{+2}) was prepared.

3.2.9.2 Calcium chloride ($CaCl_2$)

A 200 mL solution of 69,380 mg/L $CaCl_2$ (25,000 mg/L Ca^{+2}) was initially prepared by adding 13.9 g of $CaCl_2$ to 200 mL of D.I. water. From this, 50 mL solutions with $CaCl_2$ concentrations of 13,880 mg/L (5,000 mg/L Ca^{+2}), 27,750 mg/L (10,000 mg/L Ca^{+2}), 41,630 mg/L (15,000 mg/L Ca^{+2}) and 55,500 mg/L (20,000 mg/L Ca^{+2}) were prepared by adding the required volumes of D.I. water and the 69,380 mg/L $CaCl_2$ solution. An additional solution with a $CaCl_2$ concentration of 138,800 mg/L (50,000 mg/L Ca^{+2}) was prepared. These solutions were prepared for both the TTQ method and direct injection method.

3.2.9.3 Ferric chloride hexahydrate ($FeCl_3$ x $6H_2O$)

A 200 mL solution of 121,000 mg/L $FeCl_3$ x $6H_2O$ (25,000 mg/L Fe^{+3}) was initially prepared by adding 24.2 g of $FeCl_3$ x $6H_2O$ to 200 mL of D.I. water. From this, 50 mL solutions with $FeCl_3$ x $6H_2O$ concentrations of 24,200 mg/L (5,000 mg/L Fe^{+3}), 48,410 mg/L (10,000 mg/L Fe^{+3}), 72,610 mg/L (15,000 mg/L Fe^{+3}) and 96,800 mg/L (20,000 mg/L Fe^{+3}) were prepared by adding the required volumes of D.I. water and the 121,000 mg/L $FeCl_3$ x $6H_2O$ solution. These solutions were prepared for both the TTQ method and direct injection method. For the direction injection method, an additional solution with a $FeCl_3$ x $6H_2O$ concentration of 242,000 mg/L (50,000 mg/L Fe^{+3}) was prepared.

3.2.9.4 Alginate

Three different sets of alginate solutions were prepared for experiments.

For the TTQ method, a 200 mL solution of 25,000 mg/L alginate was initially prepared by adding 5 g of alginate to 200 mL of D.I. water. From this, 50 mL solutions with alginate concentrations of 1,000 mg/L, 2,000 mg/L, 3,000 mg/L, 4,000 mg/L, 5,000 mg/L and 10,000 mg/L were prepared by adding the required volumes of D.I. water and the 25,000 mg/L alginate solution.

For the direct injection method, a 200 mL solution of 50,000 mg/L alginate was initially prepared by adding 10 g of alginate to 200 mL of D.I. water. From this, 50 mL solutions with alginate concentrations of 5,000 mg/L, 10,000 mg/L, 15,000 mg/L, 20,000 mg/L and 25,000 mg/L were prepared by adding the required volumes of D.I. water and the 50,000 mg/L alginate solution.

When the TTQ method and direct injection method were carried out using $CaCl_2$ and alginate together, a 300 mL solution of 5,000 mg/L alginate was prepared by adding 1.5 g of alginate to 300 mL of D.I. water for each method.

3.2.10 Statistical Methods

The Data Analysis Tool ANOVA: Single Factor was used for statistical analysis to determine if there was significant change in the TTQ, peak torque and increase in torque values with increasing chemical concentration. The concentrations were placed in one row in Excel and the rheological parameter (TTQ, peak torque, increase in torque) was placed in the other row. These rows were then selected for ANOVA: Single Factor analysis and the p-value was provided for the data in a summary table.

Chapter 4 – Utilization of ultraviolet/visible spectrophotometry and rheology for monitoring of aerobic digestion of sludge

Abstract

There is currently a lack of in-line and real-time monitoring tools for sludge treatment which limits the sludge treatment process efficiency. Therefore, there is a need for new sludge treatment monitoring tools which have the potential to be used in-line and real-time because this would result in decreased operational costs, the time it takes to obtain results and the environmental impact of sludge treatment process. Two methods which were investigated as sludge monitoring tools were UV-vis spectrophotometry and rheology. To investigate their potential use as sludge monitoring tools, two batches of sludge, one normal strength and one high strength, were treated using aerobic digestion. Absorbance and rheology measurements, in addition to other common sludge stabilization measurements and microscopy, were taken throughout the aerobic digestion process to examine if there were any correlations between the potential real-time monitoring tools and current sludge monitoring parameters. It was found that there were correlations between the current sludge monitoring parameters (e.g. VS reduction, effluent and soluble COD) and the filtrate and effluent absorbance measurements, indicating the potential to use filtrate and effluent measurements for in-line and real-time measurements during aerobic digestion. Filtrate absorbance measurements were found to be a better indicator of sludge treatment due to the effect of particles being removed; however, they are more time-consuming to obtain compared to effluent measurements. It was found that the Floccky Tester was not sensitive enough to monitor rheological changes during aerobic digestion.

Keywords: Ultraviolet-visible spectrophotometry, rheology, real-time monitoring, aerobic digestion, sludge treatment.

4.1 Introduction

Aerobic digestion of sludge involves providing air to a basin containing sludge, causing biodegradable matter within the sludge to break down (Tchobanoglous & Burton, 1991).

The amount of organic matter (OM) present in the sludge can be used to monitor the level of treatment of the sludge within an aerobic reactor. The OM content can be measured by determining the total volatile solids (VS) and the chemical oxygen demand (COD) of the sludge. As the aerobic process progresses, these parameters should decrease as OM is broken down and then stabilize once treatment is complete due to limited biodegradable OM remaining.

Even though these measurements are effective at assessing the treatment progress and the quality of the sludge, the duration for which it takes these tests to be completed is not ideal as they are lab-based measurements. This limits process control and optimization as there is a time lag between when the sludge treatment is complete and when the results are known.

Therefore, there is a need for new approaches to optimize the operation of sludge treatment processes as there are currently very few real-time monitoring tools and technologies for sludge treatment. This research aims to determine tools and methods that can be used for in-line and real-time characterization and monitoring of sludge. This would allow determining, modeling, and predicting sludge behaviour through treatment processes, thereby optimizing process control based on this knowledge. Two methods that have the potential to be used as monitoring technologies are ultraviolet/visible spectrophotometry and rheology.

When electromagnetic radiation in the ultraviolet (UV) and visible (Vis) spectrum interacts with matter in a sample, the matter absorbs light (Owen, 1996). The measured absorbance can then be used in quantitative analysis of a sample by correlating the absorbance of the sample to the desired parameter with statistical methods. Two common statistical methods that are used to do this are

multivariate regression (MLR) and principal components regression (PCR). Both methods use the absorbance data for a specified wavelength range and the measured value of the parameter of interest, which is calculated using a different method. Equations with regression coefficients are developed which can then be used to predict the parameter of interest using absorbance data instead of calculating the parameter in a more time-consuming fashion (Mark & Workman, 2007).

In order to compare developed equations for varying wavelength ranges, statistical parameters such as the Coefficient of Determination (R^2) and the Residual Mean Square Error (RMSE) can be used. The R^2 is the regression sum of squares that is expressed as a fraction of the total sum of squares. As the value of R^2 approaches 1, the better fit the model is for the measured data. RMSE is a measure of the differences between the measured values and those predicted by a model, with a lower RMSE indicating a stronger correlation between the measured and predicted values (Berthouex & Brown, 2002).

There are multiple types of spectrophotometers, including lab spectrophotometers and real-time spectrophotometers. For lab spectrophotometers, a sample is placed within the machine and the absorbance is measured. Real-time spectrophotometers can measure absorbance in-line and in real-time at treatment plants, thereby allowing real-time optimization.

In the water and wastewater industry, UV/Vis spectrophotometers have been used to monitor the quality of water in fresh lakes, wastewater treatment plants (WWTPs) and sewers. Lepot et al. (2016) used the UV/Vis spectrum to correlate the absorbance of samples from sewers, rivers, and WWTPs to water quality parameters such as total suspended solids (TSS), COD and sCOD (soluble COD) (Lepot, et al., 2016). They did this by applying statistical methods to the absorbance data sets to correlate the data to the TSS and COD concentrations. They then used statistical criteria to determine if the methods were accurate. In their study, they found that linear regression (LR)

was more effective at estimating TSS and COD concentrations compared to partial least squares (PLS) using the UV/Vis spectrophotometer.

Another method that has the potential as a monitoring technology is rheology. Rheology is a holistic descriptor encompassing the physical, biological and chemical changes that occur in sludge and is superior to single descriptive parameters such as TS, VS or COD. Rheology provides information on the overall sludge matrix and can be used to predict sludge behaviour and achieve real-time optimization. It studies the deformation and flow of matter and includes measuring the flow characteristics (shear rate, shear stress, yield stress, rheogram peaks, etc.) of fluids (Ormeci, 2007). A torque rheometer is a device which can be used to determine the rheological properties of a fluid such as sludge. It consists of a shaft connected to an impeller that rotates at a desired speed within a cup in which the sludge is placed. As the impeller rotates it measures the sludges torque by measuring the sludges resistance to shear over a period of time. From this a torque rheogram can be produced.

One torque rheometry method that can be used to determine the rheological properties of sludge is the totalized torque (TTQ) method. It uses the area under the torque-time curve (torque rheogram) to represent the overall network strength This method has been used to determine the optimum polymer dose during dewatering in lab-scale and full-scale tests at treatment plants (Abu-Orf & Ormeci, 2005; Ormeci & Abu-Orf, 2006; Ormeci, 2007) but has not been tested for other treatment processes.

The overall goal of this study was to investigate tools and methods that can be used for in-line and real-time characterization and monitoring of sludge, and evaluate whether this information can be used for optimization of treatment processes. The study investigated the use of UV/Vis spectrophotometry and rheology to monitor the treatment progress and performance during aerobic

digestion of sludge. These methods may have the potential to decrease operational costs, the time it takes to obtain results and the environmental impact while increasing the efficiency of sludge treatment processes.

4.2 Materials and Methods

The experiments carried out for each sample collected from the aerobic batch reactor are summarized in the flow chart seen in Figure 12 and then are described in further detail in the following sections.

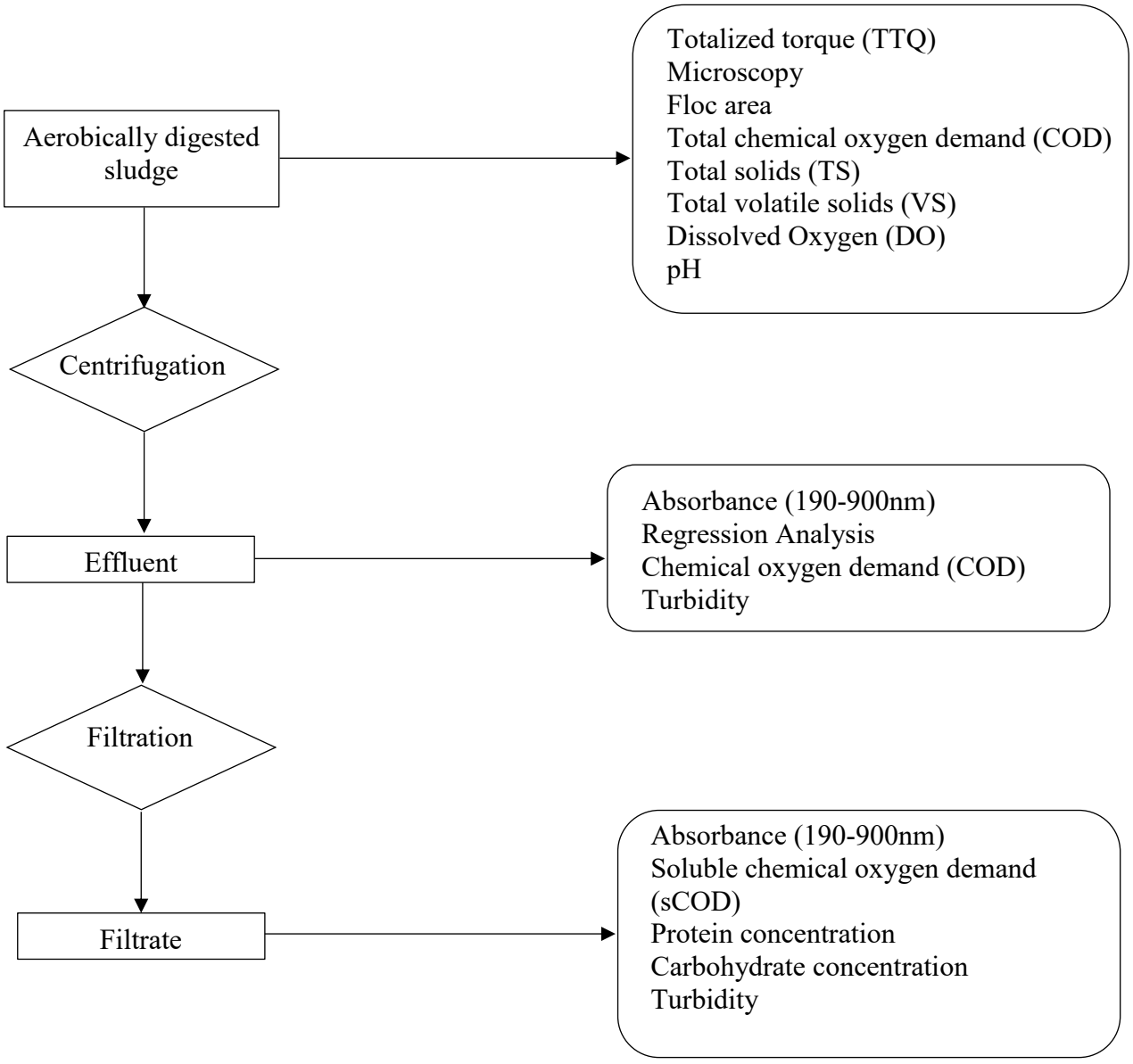


Figure 12. Flow chart of experimental process carried out for part one of research.

4.2.1 Batch aerobic reactor setup

A 20 L jug was used as the lab-scale batch aerobic reactor. Within the jug, 10 L of primary sludge and 10 L of WAS were placed. Eight air diffusers were positioned at the bottom of the jug to

provide aeration to the sludge mixture throughout the entire experiment. These eight air diffusers were connected to a tube which was connected to an air valve that controlled the amount of air that was provided from the air pump. Samples of approximately 700 mL were withdrawn from the reactor each time analysis was completed. A diagram of the lab-scale aerobic reactor can be seen below in Figure 13.

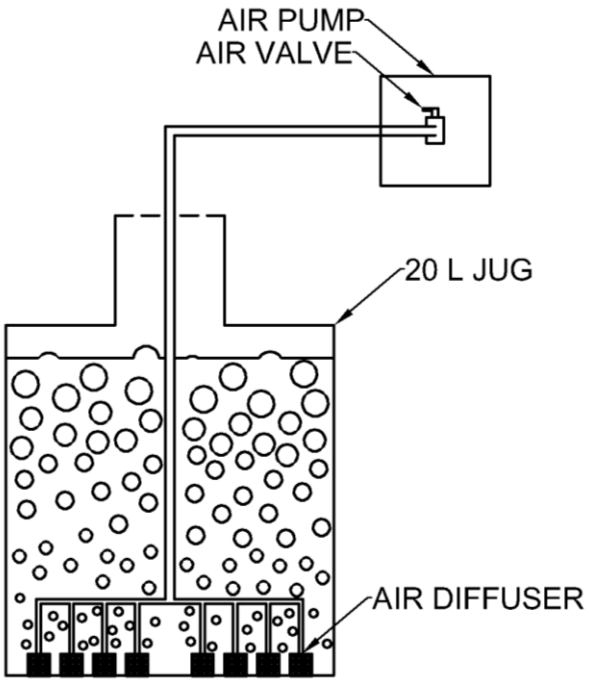


Figure 13. Batch aerobic reactor set-up.

Experiments were carried out using two different batches of mixed 50% primary and 50% waste activated sludge (WAS) samples that were at different strengths. The normal strength sludge (COD = 22,700 mg/L, VS = 13,845 mg/L) represented the strength of mixed primary sludge and WAS under daily operational conditions. The high strength sludge (COD = 37,173 mg/L, VS = 20,657 mg/L) had an initial organic loading rate higher than usual.

Chemical oxygen demand (COD), total solids (TS)/total volatile solids (VS), absorbance scans, regression analysis and turbidity measurements were carried out on both batches. Rheology, microscopy, filtrate protein, filtrate carbohydrate, dissolved oxygen (DO), and pH measurements were additionally carried out on the normal strength sludge. When measurements were carried out, triplicate measurements were taken of each sample,

4.2.2 Rheological measurements: TTQ method

The rheological measurements of each sludge sample were measured using the lab-scale Floccky Tester (manufactured by Koei Industries Inc., Japan) torque rheometer. The Floccky Tester can be seen in Figure 14 below. The Floccky Tester also has a full-scale model that can be installed and used at treatment plants.

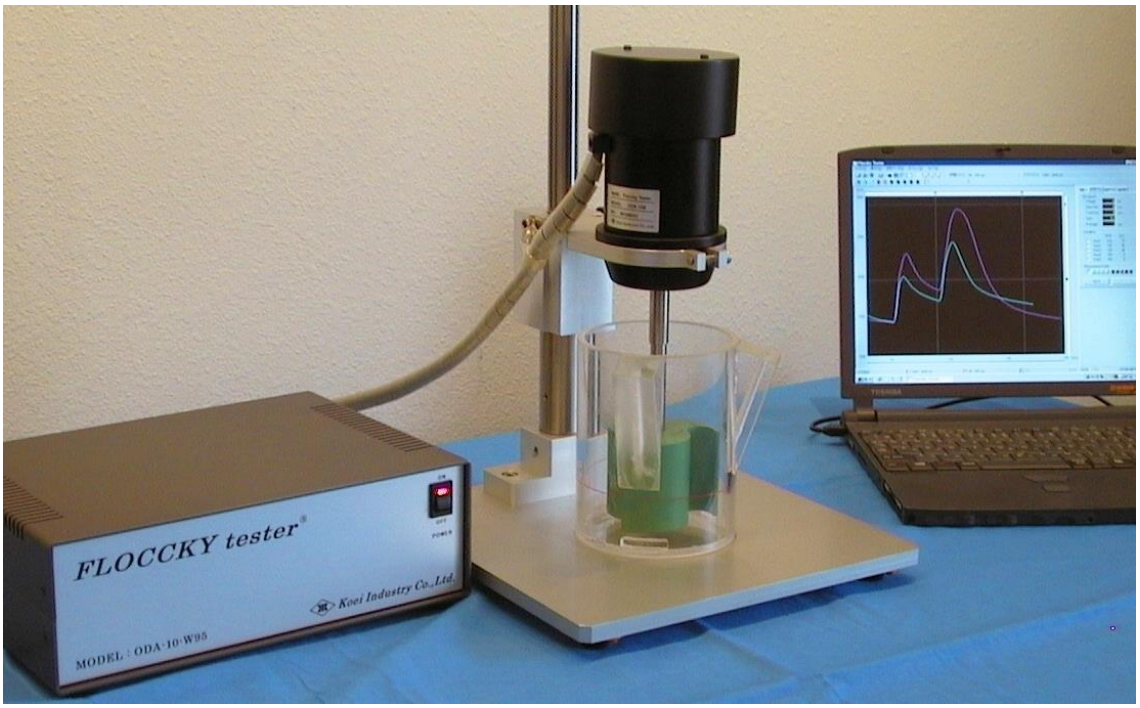


Figure 14. Floccky Tester.

An impeller speed of 300 rpm was chosen for the mixture of primary sludge and WAS due to previous studies (Ormeci et al., 2004). A duration of 120 seconds was selected as this timespan was deemed sufficient to observe the rheological characteristics of the sludge (Murray & Ormeci, 2008).

Three 200 mL subsamples were individually placed in the cylindrical jar of the torque rheometer to obtain three replicates. For each 200 mL sample, the torque rheometer impeller rotated at 300 rpm for 120 seconds and the torque was measured over time. The measurement of torque over time produces a torque rheogram. The subsamples were discarded after each run due to the thixotropic nature of sludge. The Floccky Tester's software reports the area under the torque-time graph, also known as totalized torque (TTQ), and the peak height which can be used to compare the rheological properties of the sludge samples.

4.2.3 Absorbance

The Cary 100 Bio UV-Visible Spectrophotometer was used to measure the absorbance of the effluent and the filtrate in the wavelength range of 190nm – 900nm. A quartz cuvette was used for all measurements and was cleaned thoroughly between measurements. Two samples with dilution factors of 100:1 and 20:1 were used each time for the effluent. Two samples with dilution factors of 50:1 and 5:1 were used each time for the filtrate. Three replicates were measured from each sample to ensure accuracy. A subsample of approximately 5 mL was taken from the sample and placed in the cleaned cuvette. The cuvette was placed in the spectrophotometer and the lid was closed. The spectrophotometer then scanned the sample for the entire wavelength range, 190nm-900nm and recorded absorbance measurements.

4.2.4 Regression Analysis

To determine regression coefficients which could be used with the absorbance data to predict effluent COD, sCOD and VS, the MATLAB software was used. Within MATLAB, two functions were created to be used for multivariate regression (MLR) and principle components regression (PCR) (Mark & Workman, 2007). These functions had the absorbance data for the specified wavelength range and the COD measurements as inputs and the regression coefficients as outputs. The script for the MLR function can be seen in Figure 15 below.

```
function [ b, cest ] = MLRfunc( A,c )
%MLRfunc determines regression coefficients using multivariate regression

%Inputs:
%A = absorbance data determined using UV/VIS Spectrophotometer
%c = concentration data determined using HACH method

%Outputs:
%b = regression coefficients for absorbance data to predict concentration

b = inv(A'*A)*A'*c;

cest = A*b;

end
```

Figure 15: Script for MLR function.

The script for the PCR function can be seen in Figure 16 below.

```
function [ b, cest ] = PCRfunc( A, c, n)
%PCRfunc determines regression coefficients using multivariate regression

%Inputs:
%A = absorbance data determined using UV/VIS Spectrophotometer
%c = concentration data determined using HACH method
%n = number of wavelengths absorbance recorded at

%Outputs:
%b = regression coefficients for absorbance data to predict concentration
%cest = predicted concentration using regression coefficients

[U,S,V] = svd(A);

T = A*V;

b = V(:,1:n)*inv(S(1:n,1:n))*U(:,1:n)'*c;

cest = (T*V')*b;
end
```

Figure 16. Script for PCR function.

The measured parameter found using lab-based methods and varying wavelength ranges from the UV/vis spectrum inputted into the MLR and PCR functions seen in Figure 15 and 16, respectively. The standard deviations for the measured parameters and measured wavelength ranges were not considered in the regression analysis. From these functions, regression coefficients were developed which could be used with the measured absorbance in a regression equation to predict a parameter such as VS, effluent COD or sCOD. The regression analysis procedure is summarized in the flowchart below in Figure 17.

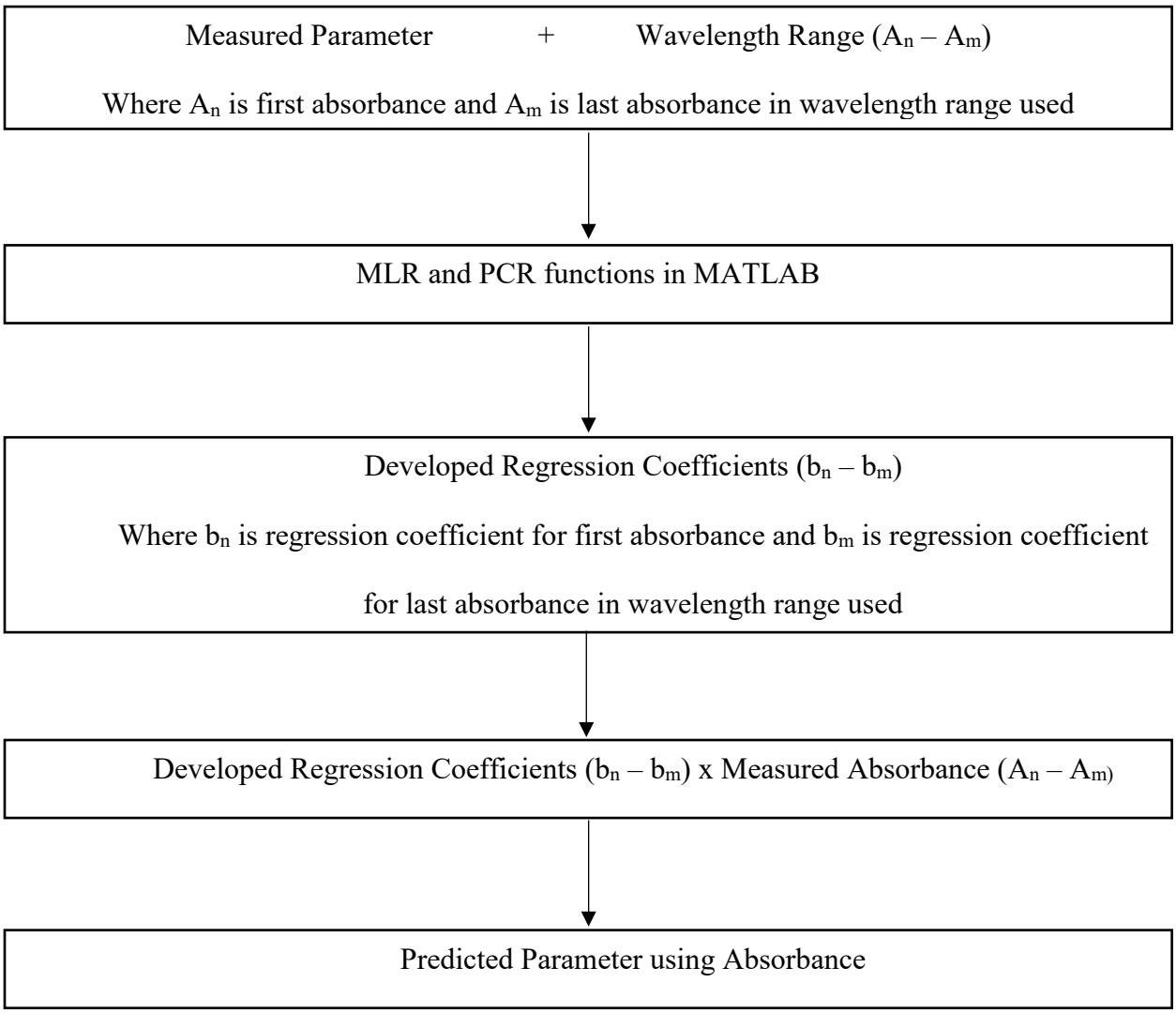


Figure 17. Flowchart for regression analysis process.

Multiple wavelengths were examined and the accuracy of the regression equations used to predict the measured parameter were compared based on Coefficient of Determination (R^2), Residual Mean Square Error (RMSE), and Standard Error.

The Coefficient of Determination (R^2) is the regression sum of squares that is expressed as a fraction of the total sum of squares.

The Residual Mean Square Error (RMSE) is the measure of the differences between the measured and predicted values.

The Standard Error is a measure of the accuracy of predictions made using a regression line.

4.2.5 Microscopy

The Nikon Eclipse Inverted Research Microscope was used to study the microscopic properties of the sludge. A Fisherbrand disposable inoculating loop was used to withdraw 10 microliters of sludge. The sample was placed in a MatTek glass bottom microwell dish and was diluted with 3 mL of D.I. water. The dish was gently shaken to mix the sample. The sample was then placed on the microscope stage and five minutes were given to allow the floc particles to settle to the bottom of the glass bottom microwell dish.

The microscope and the QImaging Retiga EXi Fast 1394 camera were turned on while the floc particles settled. The Nikon NIS-Elements Advanced Research program, version 3.2, was then opened on the computer. This program is connected to the camera and microscope, allowing images viewed through the microscope to be captured as pictures on the computer using the camera. Once the five minutes was complete, the sample was brought into focus. Next, the battlement technique was used to ensure the sample was a homogenous mixture. The battlement technique involves moving the microscope a field along the edge of the sample, a field up, one along and then one down, until the entire sample has been viewed. Five images which best represented the sample were captured as JPG files using the NIS-Elements Advanced Research program and saved.

4.2.6 Floc Area

Area of the flocs was used as a tool to monitor the changes in sludge structure. The areas of the flocs in the five captured images were measured using the Area Tool in the NIS-Elements Advanced Research program as it was calibrated to measure micrometers in images. The software was used to draw the perimeter around each floc and then the area of the floc was calculated using the Area Tool. The floc area measurements were recorded to a statistics table which could be exported as a Microsoft Excel file.

4.2.7 Chemical Oxygen Demand (COD)

Total COD refers to the COD of the sludge, effluent COD is the COD of the effluent after centrifugation and sCOD is the COD of the filtrate that is passed through the 0.45 μm pore size filter. The total COD, effluent COD and sCOD were each measured in 3 replicates using HACH Method 8000, High Range, 20-1,500 mg/L COD for each sample (HACH, 2005). A dilution factor of 25:1 was used for total COD, a dilution factor of 5:1 was used for effluent COD and a dilution factor of 2:1 was used for sCOD.

4.2.8 Total solids and total volatile solids

To measure the TS and VS of the sludge within the reactor, Standard Methods 2540 B and 2540 E were used (APHA, AWWA, & WEF, 2005). To prepare, 30 (3 replicates per analysis) 50 mL aluminum dishes were labeled and placed in a furnace at 550 °C for one hour. They cooled in a desiccator for 30 minutes and then they were labeled and weighed using an analytical balance. Three 10 mL subsamples were taken from the 700 mL sample using a wide-mouth plastic pipette. Each replicate was then placed in the oven at 105 °C and was left in the oven for at least 24 hours

to ensure the sample dried. Once the sample dried, it was cooled, and then the dish and dry sample were weighed using an analytical balance. After being weighed, the dish and dry sample were placed in a furnace for at least 2 hours at 550 °C. After cooling in a desiccator, the ignited sample and dish were weighed using an analytical balance. The TS and VS were then calculated using the following equations.

$$TS = \frac{B-A}{V} \quad \text{Equation 4}$$

Where: TS = total solids of sample (mg/L)

A = mass of dish (mg)

B = mass of dish and dry sample (mg)

V = volume of sample (L)

$$VS = \frac{B-C}{V} \quad \text{Equation 5}$$

Where: VS = total volatile solids of sample (mg/L)

C = mass of dish and ignited sample (mg)

4.2.9 Protein

The filtrate protein concentration was measured using Coomassie Brilliant Blue G-250 reagent and the Bradford method (Bradford, 1976). A bovine serum albumin (BSA) solution was created by adding 10 mg of BSA powder to 100 mL of deionized (D.I.) water. The solution was continuously stirred until the BSA powder had completely dissolved in the D.I. water. The BSA solution was diluted to concentrations between 0 and 100 mg/L to create a calibration curve which is shown in Figure 15. The calibration curve was linear up to 50 mg/L. Test tubes were filled with 5 mL of the Coomassie Brilliant Blue G-250 reagent and 3 mL of each standard or diluted filtrate sample. A dilution factor of 20:1 was used for the filtrate samples as this dilution factor gave

absorbance measurements within the working range of the calibration curve. The cap was placed on the test tube and inverted several times to mix the solution. Ten minutes were given to allow the reaction in the test tube to proceed at room temperature. During this time, the DR2800 HACH spectrophotometer was turned on and set to a wavelength of 595 nm. It was then zeroed with a test tube containing D.I. water. After the ten minute reaction period, the absorbance of each standard or diluted filtrate sample was measured after wiping the test tube clean with a Kimwipe. The absorbance of the blank sample, containing 3 mL of D.I. water and 5 mL of Coomassie Brilliant Blue G-250 reagent was subtracted from the absorbance of all standards and samples. The protein concentration measurements of the diluted filtrate samples were then back calculated using the linear regression line generated from the calibration curve seen in Figure 18.

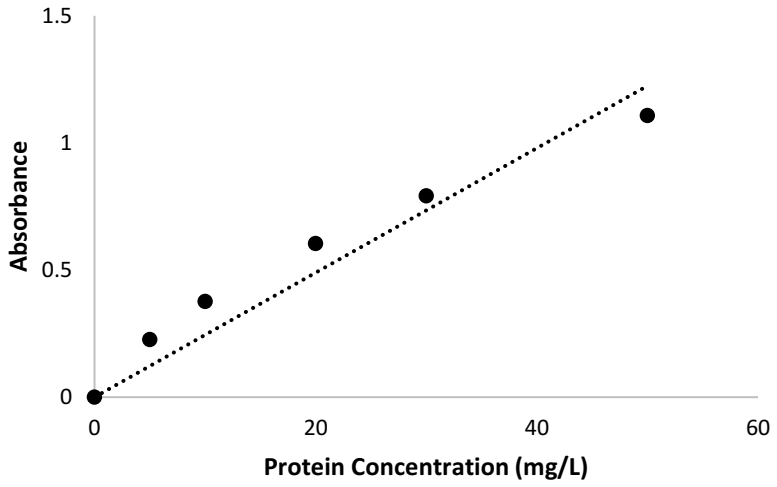


Figure 18. Calibration curve for BSA between 0 and 50 mg/L. It has an equation of line of $y=0.0245x$ and a $R^2=0.93$.

4.2.10 Carbohydrate

The filtrate carbohydrate concentration of the sample was measured using the Anthrone method (Morris, 1948). A 95% sulphuric acid solution with 2 g/L of anthrone reagent was created by adding 1 g of anthrone reagent powder to 500 mL of 95% sulphuric acid. Galactose stock solutions were prepared by adding 10 mg of 98% pure galactose powder to 100 mL of D.I. water. The solution was continuously stirred until the 98% pure galactose powder had completely dissolved in the D.I. water. The galactose solution was diluted to concentrations between 0 and 100 mg/L to create a calibration curve shown in Figure 16. The calibration curve was linear up to 100 mg/L. Test tubes were filled with 6 mL of the 95% sulphuric acid solution with 2 g/L of anthrone reagent and 3 mL of each standard or diluted filtrate sample. A dilution factor of 20:1 was used for the filtrate samples as this dilution factor gave absorbance measurements within the working range of the calibration curve. The cap was placed on the test tube and inverted several times to mix the solution. Ten minutes were given to allow the reaction in the test tube to proceed at room temperature. During this time, the DR2800 HACH spectrophotometer was turned on and set to a wavelength of 620 nm. It was then zeroed with a test tube containing D.I. water. After the ten minute reaction period the absorbance of each standard or diluted filtrate sample was measured after wiping the test tube clean with a Kimwipe. The absorbance of the blank sample, containing 3 mL of D.I. water and 6 mL of 95% sulphuric acid solution with 2 g/L of anthrone reagent was subtracted from the absorbance of all standards and samples. The carbohydrate concentration measurements of the diluted filtrate samples were then back calculated using the linear regression line generated from the calibration curve. The calibration curve is presented in Figure 19.

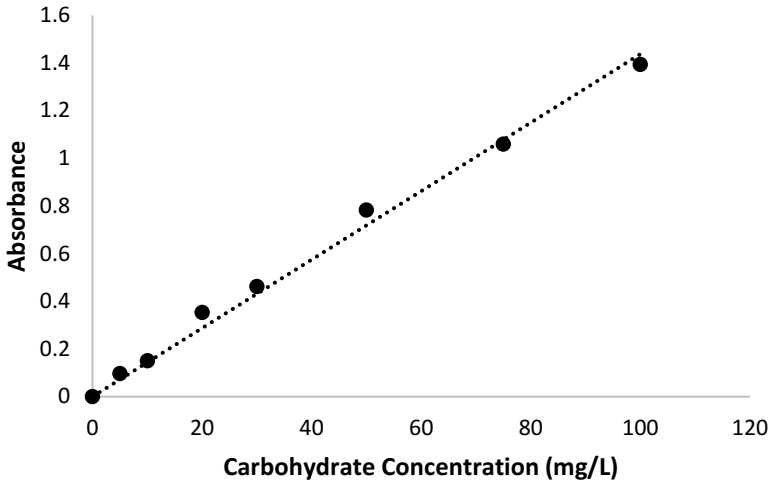


Figure 19. Calibration curve for galactose between 0 and 100 mg/L. It has an equation of line of y=0.0144x and a R^2=0.99.

4.2.11 Turbidity

The turbidity of the effluent and filtrate was measured using the 2100AN Turbidimeter. The empty sample cell was rinsed with D.I. three times and dried prior to each measurement. The sample cell was filled with approximately 30 mL of the sample and the cap was placed on the sample cell. The outside of the sample cell was then cleaned with a soft, lint-free cloth to remove water spots and fingerprints. Next, a small bead of silicone oil was applied to the outside of the sample cell from the top to bottom. An oiling cloth was then used to distribute the oil equally to the outside surface of the sample cell and to remove any excess oil. The sample cell was gently inverted to mix the sample and then was placed in the sample cell holder with the triangle on the sample cell aligning with the reference mark on the sample cell holder (HACH, 2014). The cover was closed and then turbidity measurements were taken at 0 s, 10 s, 20 s and 30 s. An average of these four measurements was taken as the turbidity measurement for the sample. Three samples each were

measured every time for the effluent and the filtrate. A dilution factor of 20:1 was used for the effluent and a dilution factor of 2:1 was used for the filtrate.

4.2.12 Dissolved Oxygen

The dissolved oxygen (DO) of the sludge sample was measured using the Orion RDO Dissolved Oxygen Probe. Prior to each measurement the DO probe had to be calibrated using the Orion calibration sleeve. The cap was removed from the calibration sleeve and the sponge from the cap was taken out. The sponge was saturated with distilled water and squeezed until the excess water came out of the sponge. The calibration sleeve was reassembled. The thread ring was unscrewed and removed from the RDO sensor. The calibration sleeve was placed over the front of the RDO sensor and then the sleeve was screwed onto the sensor. Instructions were followed on the Thermo Scientific Orion Star A Series portable meter to perform a water-saturated air calibration.

When DO measurements were taken, the RDO probe was first rinsed off with distilled water, blotted dry with a lint-free tissue and inserted into the sample. The RDO sensor was immersed in the sludge solution and then a reading was obtained using the Thermo Scientific Orion Star A Series portable meter.

4.2.13 pH

The pH of the sample was measured using the Orion 8107UWMMD Ross Ultra pH/ATC Triode electrode and Thermo Scientific Orion Star A Series portable meter. A 40 mL subsample of sludge was withdrawn from the sample and placed in a 50 mL graduated cylinder. The electrode was withdrawn from the electrode storage bottle and rinsed with D.I. water three times. The portable meter was turned on. The pH electrode was dried and placed in the subsample and was gently

rotated while the portable meter obtained a reading. Once a finalized reading was obtained the pH probe was rinsed three times again with D.I. water and was placed back in the electrode storage bottle.

4.2.14 Sludge Samples

Primary sludge and waste activated sludge (WAS) samples were collected from the Robert O. Pickard Environmental Centre (ROPEC) located in Ottawa (Ontario, Canada).

4.2.15 Centrifugation

To be able to measure the change in the characteristics of sludge supernatant (effluent) during digestion, centrifugation was used to separate the sludge solids and liquids. Approximately 500 mL of sludge was separated into two bottles which were placed in the Sorvall Legend RT+ Centrifuge. The sludge was centrifuged at 7,500 G for ten minutes. The effluent was then carefully removed from each bottle and placed in a beaker for further analysis. The residual solids portion of the sludge was discarded.

4.2.16 Filtration

To be able to measure the change in the characteristics of the filtrate during digestion, a filtration apparatus was used with a 0.45 μm pore size filter and a vacuum-pressure pump. Filtration allowed removal of the organic and inorganic particles compared to the centrifuged samples. Approximately 200 mL of effluent from the centrifugation process was used for the filtration process with filters being replaced once they became fouled. The filtrate was removed from the bottom of the filtration apparatus and was placed in a beaker for further analysis.

Data points for “Day 1” are for the day when the primary and WAS were obtained from the wastewater treatment plant and were placed in the aerobic reactor. Two hours of aeration were provided before “Day 1” measurements were taken to ensure a homogenous mixture.

4.3 Results and Discussion

Experiments were carried out in two parts. Part 1 used regular strength mixed primary sludge and waste activated sludge (WAS) to represent sludge from daily operations while Part 2 used high strength mixed primary sludge and WAS to represent sludge with a high organic loading rate and to evaluate if differences in trends would be observed due to the strength of the sludge. In Part 1, TS, VS, total COD, effluent COD, soluble COD (sCOD), effluent absorbance, filtrate absorbance, filtrate protein concentration, filtrate carbohydrate concentration, effluent turbidity, filtrate turbidity, dissolved oxygen (DO), pH, totalized torque (TTQ), rheogram peak height, microscopy images of sludge flocs, and average floc area were monitored during the aerobic digestion of regular strength sludge. In Part 2, TS, VS, total COD, effluent COD, sCOD, effluent absorbance, filtrate absorbance, effluent turbidity, and filtrate turbidity were monitored throughout the aerobic digestion of the high strength mixed primary sludge and WAS. For both parts, regression analysis was carried out on the effluent and filtrate absorbance measurements with effluent COD, sCOD and VS to determine if a correlation could be made between the absorbance measurements and one of the sludge stabilization indicators. Trends from both experiments were also compared to assess the effect that sludge strength has on patterns observed for various measurements during aerobic digestion. Triplicate measurements were carried out on samples used, three different samples were not used for measurements. Doing triplicate measurements on the same sample

caused some data points to have minimal standard deviation in the figures below in this section. However, each figure plotted below does have error bars associated with each data point, they just may not be visible.

4.3.1 Part 1. Regular strength sludge measurements

4.3.1.1 Total solids and total volatile solids

The measured total solids (TS) concentration and total volatile solids (VS) concentration of the sludge mixture over the course of the aerobic treatment are shown in Figure 20 below.

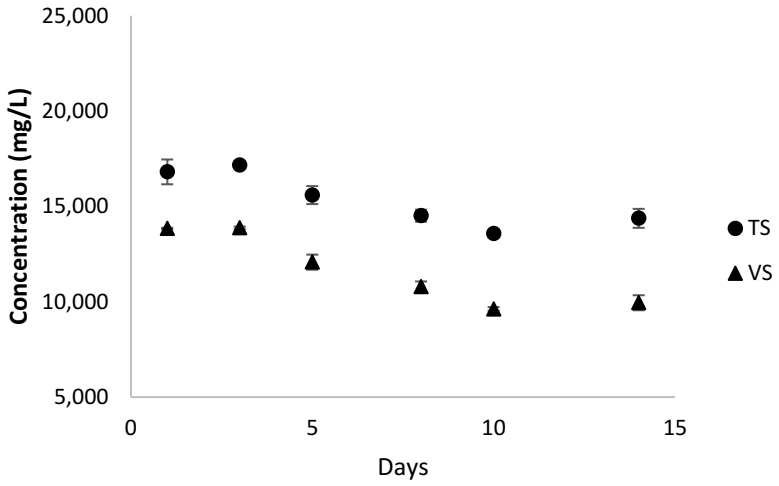


Figure 20. TS and VS concentrations over course of aerobic treatment in reactor.

It can be observed from Figure 20 that both the TS and VS concentrations of the sludge mixture gradually decrease over the course of the experiment. A total volatile solids reduction (VSR) of 43% was observed over the course of the experiment. The presence of oxygen within the air assisted microorganisms in breaking down the biodegradable organic matter (volatile solids)

within the sludge into CO_2 and H_2O, decreasing the TS and VS concentrations (Tchobanoglous & Burton, 1991).

It can also be seen from Figure 20 that there is very minimal change in TS and VS values from day 10 onward. This stabilization of VS values indicates the limited biodegradable OM remaining in sludge and near completion of the aerobic digestion.

4.3.1.2 Chemical Oxygen Demand (COD)

4.3.1.2.1 Total COD

The total COD, obtained using the HACH method, for the sludge over the aerobic treatment period can be seen in Figure 21 below.

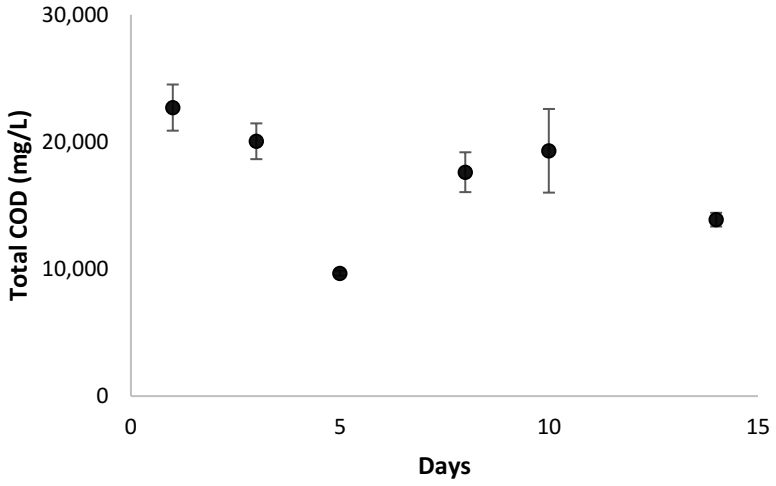


Figure 21. Total COD over course of aerobic treatment in reactor.

In Figure 21 there is a total COD removal of 39% over the course of the aerobic treatment. Like VS, total COD is a measure of the amount of organic matter (OM) in the sludge (Tchobanoglous

& Burton, 1991). As the aerobic treatment process progresses the biodegradable OM will get broken down by aerobic microorganisms, decreasing the total COD of the sludge (Kazimierczak, 2012).

The total COD value for day 5 in Figure 21 is much lower than the other values and does not follow the trend. This can be associated with error in obtaining a sludge subsample for the total COD measurement as it is possible the sample was not stirred enough to ensure a homogeneous sample to withdraw the subsample from. For this reason, day 5 can be considered an outlier.

From Figure 21 it is also observed that, when taking the error bars on day 10 into consideration, COD values begin to approach stabilization around day 10. This stabilization in COD values around day 10 is similar to the stabilization of VS values seen in Figure 20. Since both parameters are a measurement of the OM present, the results from Figures 20 and 21 indicate that the aerobic treatment of the sludge mixture was largely achieved after ten days.

4.3.1.2.2 Effluent COD

The effluent COD was obtained by centrifuging the sludge sample and performing the HACH method on the liquid portion after centrifugation. Effluent samples were not filtered. In Figure 22 shown below, the effluent COD over the course of the experiment is presented.

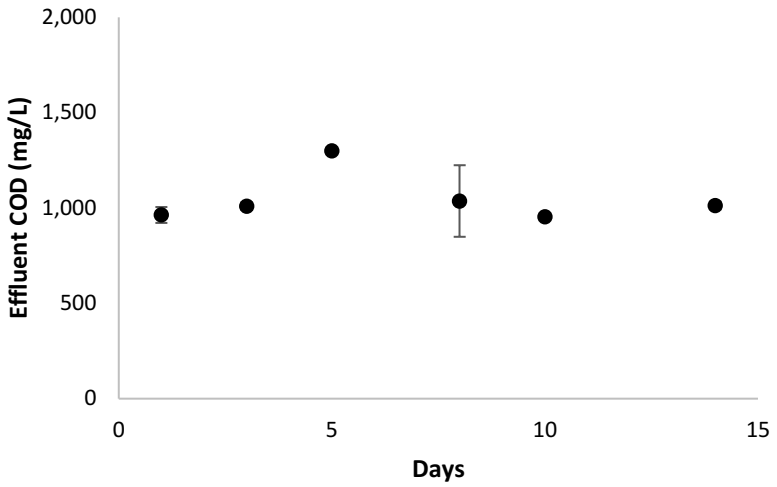


Figure 22. Effluent COD over course of aerobic treatment in reactor.

Figure 22 shows a peak in effluent COD on day 5. The initial increase in effluent COD can be explained by the solubilisation of the OM in sludge and its release to the supernatant. After day 5, a decrease in effluent COD is observed as the biodegradable OM is consumed by the aerobic bacteria. On day 10 the lowest effluent COD is observed and then it levels off.

The advantage of using effluent directly without filtration is that this would be easier to employ at treatment plants during operation. However, one drawback of using effluent measurements to monitor the aerobic digestion process is the presence of suspended organic matter in the effluent which may impact the COD measurements. During the centrifugation process, liquids and solids in the sludge are separated by the applied centrifugal force, however, it is difficult to ensure complete separation of the liquids and solids. This causes smaller particles to remain in the effluent which may also increase the turbidity and impact absorbance based COD measurements using the HACH method. Therefore, using effluent COD can make it more difficult for patterns in the data to be realized.

4.3.1.2.3 Soluble COD (sCOD)

The effect of suspended matter on COD measurements can be removed if the effluent is passed through a 0.45 μm filter and the COD of the filtrate (sCOD) is measured. The solubilization and consumption of biodegradable OM concentration was monitored by measuring the sCOD of the filtrate during the aerobic digestion and the results are presented in Figure 23 below.

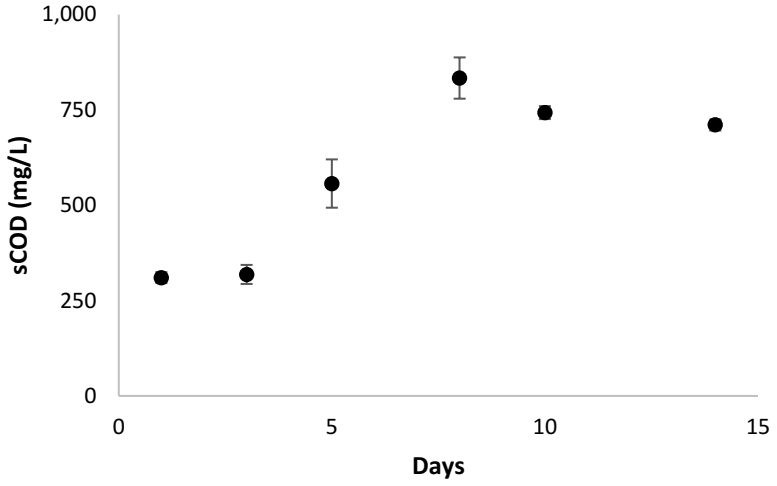


Figure 23. Soluble COD over course of aerobic treatment in reactor.

Figure 23 shows an increase in sCOD up to day 8. After day 8 it begins to level off. This initial increase in sCOD can be attributed to the breakdown of the OM. Sludge flocs consist of biopolymer matrices which contain extracellular polymers (ESP) such as proteins and lipids bridged together by divalent cations (Higgins & Novak, 1997B). During aerobic digestion, these matrices are broken down and the ESPs and other organic matter are released into solution, causing an increase in sCOD. Figure 23 shows that the sCOD values stabilize near the same time that

common sludge stabilization indicators such as VS and total COD stabilize, indicating a correlation between sCOD and the level of sludge treatment.

The effect of particles becomes evident when comparing the effluent COD in Figure 22 and the sCOD in Figure 23 as there is a more significant increase in sCOD (p-value = 1.46×10^{-9}) compared to the increase in effluent COD (p-value = 4.30×10^{-3}). This shows that filtrate measurements can be a more accurate representation of the level of treatment of sludge as the effect of particles is negated.

4.3.1.3 Absorbance

Each time the effluent COD and sCOD were measured, absorbance measurements from 190-900 nm were taken of the effluent and filtrate at dilutions of 20:1 and 5:1, respectively. Different wavelengths were looked at to determine if there could be any correlation found between the absorbance at a specified wavelength and one of the other measured parameters using regression analysis. In particular, wavelengths 190 nm and 254 nm were investigated. The 190 nm wavelength was looked at because it was at this wavelength that the greatest amount of light was absorbed. Some of the biopolymers were reported to absorb light at 190 nm (Ormeci, 2015), and organic compounds without double bonds tend to absorb light at lower wavelengths. The 254 nm wavelength was investigated because dissolved OM can be detected at this wavelength (Rogas et al., 2016).

4.3.1.3.1 Effluent absorbance

The effluent absorbance values were first analyzed to determine if correlations between absorbance values and stabilization parameters could be found. Absorbance values at 190 nm (20X) for the effluent are shown over the aerobic treatment process in Figure 24 below.

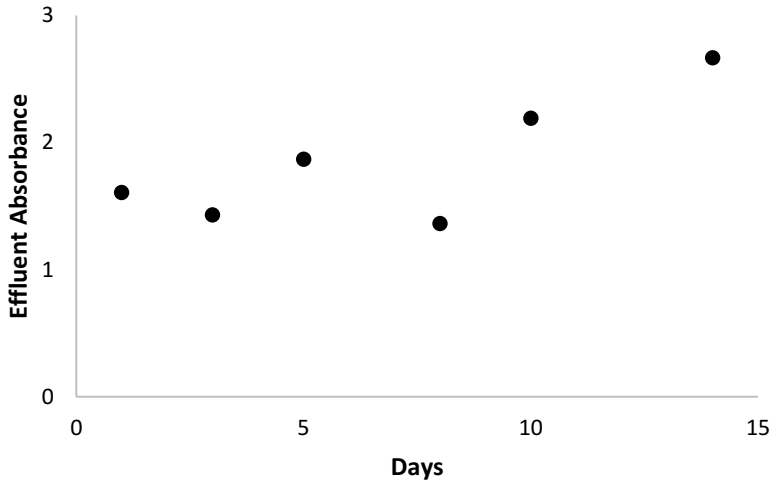


Figure 24. Effluent absorbance at 190 nm (20X) over course of aerobic treatment in reactor.

Limited change in effluent absorbance at 190 nm is observed up to day 8 in Figure 24. After this, there is an increase in effluent absorbance at 190 nm until day 15. The fluctuation in effluent absorbance values is likely related to the presence of particles in the effluent samples as the centrifugation process does not ensure complete separation of the liquid (effluent) and the solids.

Changes in absorbance values throughout the aerobic digestion were mainly due to the OM breaking down and releasing organic compounds into the effluent and also biodegradable organic matter consumed by aerobic bacteria simultaneously. Many organic compounds, such as sugars, have been strongly absorbed in the wavelength range around 190 nm (Paredes et al., 2006). The

results showed that 190 nm absorbance values continued to increase past day 10 when common sludge stabilization indicators such as VS and COD began to stabilize.

The next wavelength that was examined was 254 nm. The effluent absorbance at 254 nm (20X) over the course of the experiment is shown in Figure 25 below.

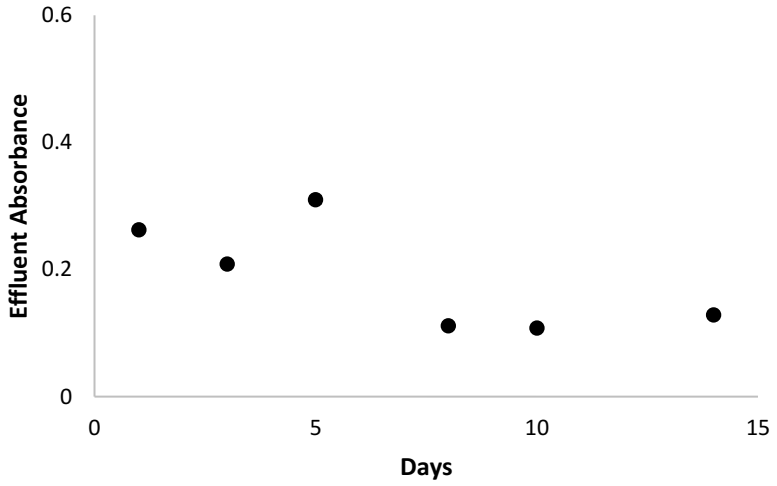


Figure 25. Effluent absorbance at 254 nm (20X) over course of aerobic treatment in reactor.

In Figure 25 a peak in effluent absorbance was observed at 254 nm on day 5. A small change in effluent absorbance at 254 nm was observed after the peak on day 5, and it appears that the absorbance values stabilized after this day. Dissolved OM is both released (Hansen et al., 2016) and consumed (the biodegradable portion) during aerobic digestion which could explain the fluctuation observed on day 5.

It can be seen from Figure 25 that the effluent absorbance at 254 nm follows a somewhat similar trend to the effluent COD in Figure 22. Regression analysis may be able to generate an equation that uses absorbance values centering around 254 nm to predict the effluent COD of the sludge.

Using effluent absorbance to determine COD would be advantageous over using the HACH method which takes over 2 hours.

4.3.1.3.2 Effluent absorbance regression analysis

To further investigate the correlation between the effluent COD and absorbance at 254 nm, they were both plotted on the same graph as seen in Figure 26.

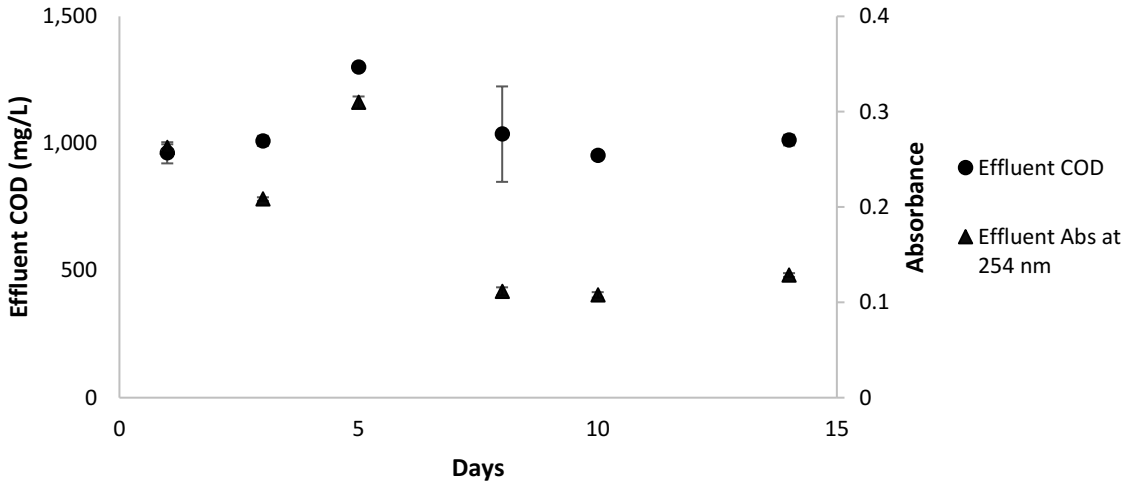


Figure 26. Comparison of effluent COD and effluent absorbance at 254 nm over course of aerobic treatment in reactor.

From Figure 26 similar trends of the COD and absorbance data are clear. Regression equations that could be used to predict the effluent COD were developed by applying MLR and PCR, two common regression methods, to wavelength ranges centering around 254 nm. These predictive models were then compared using statistical parameters such as the Coefficient of Determination (R^2) and the Residual Mean Square Error (RMSE) as these parameters provided an indication of how well the regression equation predicted the COD values. Upon comparing multiple wavelength ranges centering around 254 nm, it was determined that the most accurate equation to predict the

effluent COD was found for the wavelength range of 247-261 nm using PCR. It resulted in a R^2 of 1.000, Standard Error of 0.016 and a RMSE of 0.085. The equation developed using PCR can be seen below in Equation 6.

$$COD_{effluent} = -113{,}450xA_{247} + 420{,}370xA_{248} - 465{,}130xA_{249} - 179{,}050xA_{250} + 415{,}860xA_{251} + 76{,}730xA_{252} + 118{,}060xA_{253} + 491{,}880xA_{254} - 405{,}340xA_{255} - 560{,}560xA_{256} + 78{,}950xA_{257} - 19{,}280xA_{258} - 546{,}640xA_{259} + 81{,}900A_{260} + 608{,}980A_{261}$$

Equation 6

Where:

$COD_{effluent}$ = COD of effluent (mg/L)

$A_{\#}$ = absorbance of effluent diluted 20:1 measured at wavelength

Effluent COD measurements found using the HACH method were compared to the COD values predicted using Equation 6 and are presented below in Figure 27.

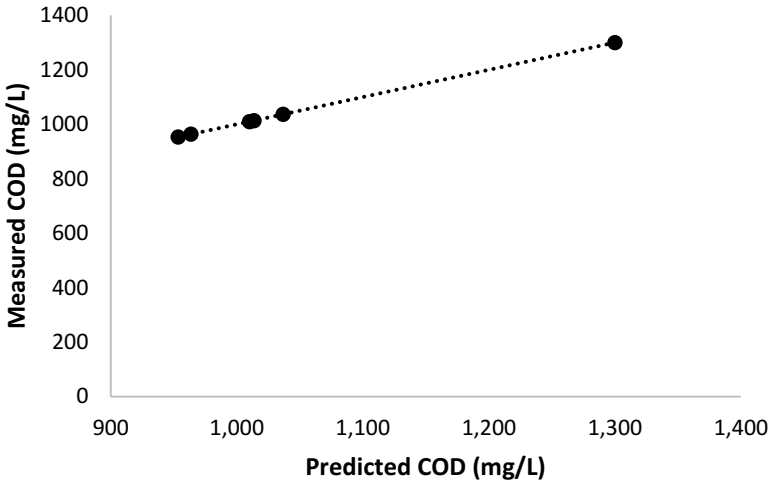


Figure 27. Comparison of normal strength effluent COD measured using HACH method to normal strength effluent COD predicted using Equation 6 (R^2 = 1.000).

From Figure 27 it can be observed that Equation 6 accurately predicts the effluent COD using the absorbance data. This shows that effluent COD measurements can be predicted in real-time using UV/Vis absorbance measurements, which would allow real-time process monitoring and control. The regression analysis process described above was also carried out for effluent absorbance at 254 nm with VS and effluent absorbance at 190 nm with effluent COD and VS. The results from the regression analysis for the four different sets of data are presented in Table 1.

Table 1. Summary of regression analysis results for regression equation created using normal strength effluent absorbance and sludge parameters.

Wavelength of focus (nm)	Regression Method	Parameter	Wavelength Range of Regression Equation	R^2	Standard Error	RMSE
190	PCR	Effluent COD	190-220	1.000	0.003	0.003
	PCR	VS	190-204	1.000	0.531	1.775
254	PCR	Effluent COD	247-261	1.000	0.016	0.085
	PCR	VS	248-260	1.000	0.209	1.279

It can be observed from Table 1 that the effluent COD had a stronger correlation with effluent absorbance compared to the VS. Similar trends were found between the effluent absorbance at 190 nm and 254 nm with effluent COD which could have resulted in a more accurate equation being developed using effluent absorbance to predict effluent COD compared to VS. Effluent COD measurements are also done on the effluent sample while VS measurements are done on the sludge sample which also could have attributed to effluent COD having a stronger correlation with effluent absorbance compared to VS. The PCR regression method was more effective at generating accurate regression equations compared to the MLR regression method.

4.3.1.3.3 Filtrate absorbance

The filtrate samples were obtained after filtering the effluent samples through a 0.45 µm filter in order to remove the particles. The absorbance values at 190 nm (5X) during the aerobic treatment can be seen in Figure 28 below.

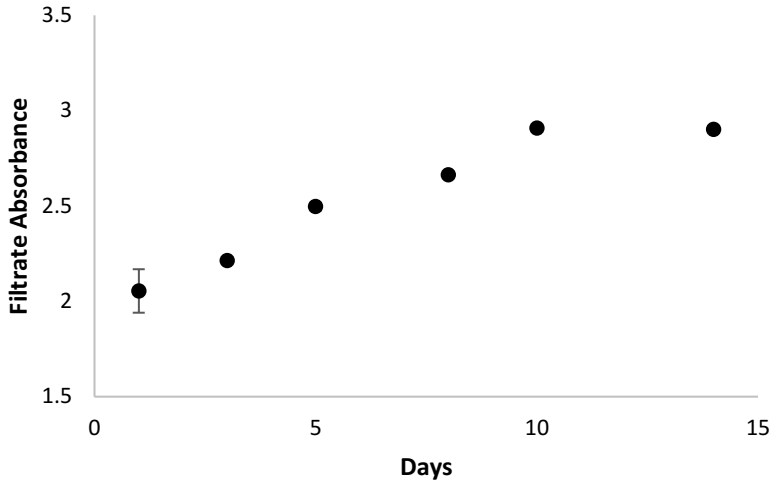


Figure 28. Filtrate absorbance at 190 nm (5X) over course of aerobic treatment in reactor.

In Figure 28 there is a gradual increase in filtrate absorbance values at 190 nm until day 10. The absorbance values level off after day 10 and no significant change is observed. The stabilization of filtrate absorbance values from day 10 on is similar to the trend observed in Figure 20 as there was a lack of change observed in VS after day 10. As the aerobic digestion proceeds, sludge flocs consisting of biopolymer matrices get broken down (Higgins & Novak, 1997B). As these biopolymer matrices break down the ESPs get released into solution, which could cause the filtrate absorbance at 190 nm to increase as more biopolymers get added to the solution (Paredes et al., 2006). Polysaccharides for example, one of the main components of ESPs, do not absorb light >200 nm (Lindon et al., 2016; Schever, 2012; Schnabel, 2007). For proteins, another main component of ESPs, 190 nm has been found to be the best wavelength for quantification (Baret et al., 1997). After day 10, the biodegradable OM is largely consumed causing the absorbance values at 190 nm to level off. The correlation between the absorbance of the filtrate at 190 nm and common sludge monitoring parameters such as total COD and VS indicate that there is also

potential to use the filtrate absorbance at 190 nm to monitor aerobic sludge treatment and regression analysis for the filtrate should also be investigated.

Since dissolved OM is typically measured at 254 nm, the filtrate absorbance at 254 nm was measured and the results are presented below in Figure 29.

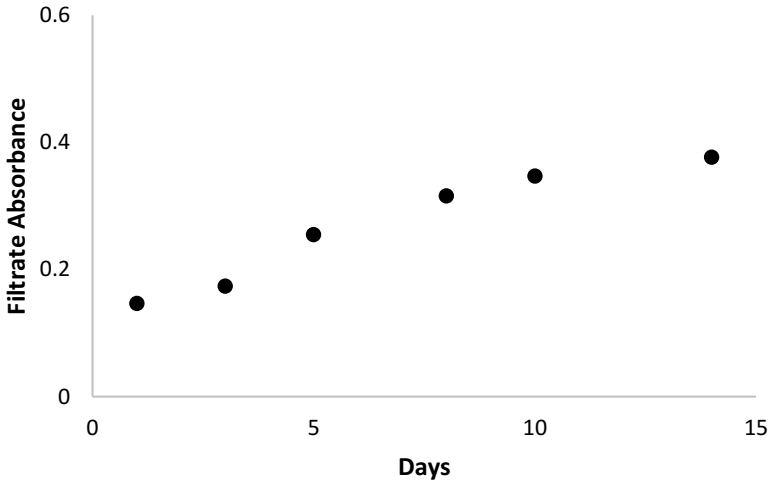


Figure 29. Filtrate absorbance at 254 nm (5X) over course of aerobic treatment in reactor.

The filtrate absorbance at 254 nm in Figure 29 follows a somewhat similar trend to Figure 28, and the absorbance values appear to level off from day 10 onwards. As previously mentioned for the effluent, dissolved OM has been measured at 254 nm which could cause filtrate absorbance values at 254 nm to increase throughout the aerobic digestion process as OM is broken down and released to the supernatant (Hansen et al., 2016). It would be expected that the filtrate absorbance values at 254 nm would stabilize after day 10, similar to the filtrate absorbance values at 190 nm, as a limited amount of OM remains after day 10. Organic compounds have been measured at both 190 nm and 254 nm, however a greater amount of light is absorbed at 190 nm compared to 254 nm which could

cause measurements at 190 nm to be a better indicator of the amount of OM present in the filtrate (Rogas et al., 2016; Hansen et al., 2016; Paredes et al., 2006).

4.3.1.3.4 Filtrate absorbance regression analysis

A similar regression analysis process which was carried out for the effluent was carried out for the filtrate. Regression equations to predict sCOD and VS were created using filtrate absorbance wavelength ranges focusing around 190 nm and 254 nm. The results for the regression analysis for the four sets of data are presented below in Table 2.

Table 2. Summary of regression analysis results for regression equation created using normal strength filtrate absorbance and sludge parameters.

Wavelength of focus (nm)	**Regression Method**	**Parameter**	**Wavelength Range of Regression Equation**	**R^2**	**Standard Error**	**RMSE**
190	PCR	sCOD	190-198	1.000	0.274	0.217
	PCR	VS	190-214	1.000	0.357	0.594
254	PCR	sCOD	242-266	1.000	0.270	0.232
	PCR	VS	238-270	1.000	0.452	2.710

From Table 2 it can be observed that sCOD has a stronger correlation with filtrate absorbance compared to VS as both filtrate sCOD regression equations have lower standard error and RMSE values compared to the filtrate VS regression equations. The stronger correlation between sCOD and filtrate absorbance measurements could be associated with sCOD and filtrate absorbance measurements being conducted on the filtrate sample while VS measurements are conducted on the sludge sample. The similarity in sCOD regression analysis parameters between the wavelength

ranges focusing on 190 nm and 254 nm could be associated with similar trends being observed for the absorbance at 190 nm and 254 nm, as shown in Figures 28 and 29, respectively.

When comparing the statistical analysis carried out for the effluent and the filtrate in Tables 1 and 2, respectively, it can be observed that the regression equations used to predict the effluent COD had the lowest Standard Error and RMSE, with the wavelength range from 190-220 nm providing a regression equation with the lowest Standard Error and RMSE. However, regression equations using filtrate absorbance were more accurate at predicting VS compared to regression equations using effluent absorbance.

4.3.1.4 Filtrate Protein

Proteins are one of the main components of biopolymers. For this reason, the filtrate protein concentration was measured over the aerobic digestion process using the Bradford Method and the results can be seen in Figure 30 below.

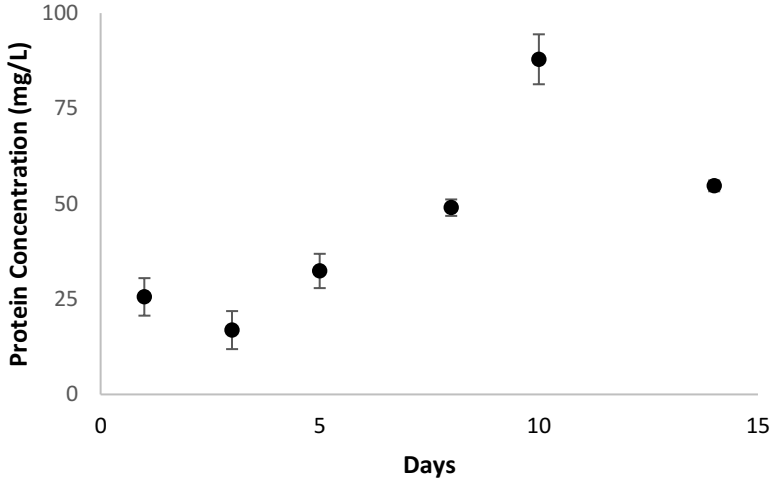


Figure 30. Filtrate protein concentration measured using the Bradford Method over course of aerobic treatment in reactor.

It is observed from Figure 30 that the filtrate protein concentration increases up to day 10. After this there is a decrease in filtrate protein concentration. Since proteins are a main component of biopolymers, the filtrate protein concentration will continue to increase as the biopolymers are broken down and the constituents, including proteins, become solubilized. This occurs up to day 10 as after that VS and COD values stabilize which indicates limited biodegradable OM remains to be consumed. The decrease on day 14 is likely due to the utilization of protein as substrate by sludge bacteria or it can be an experimental error.

In addition to the Bradford Method, protein concentration can also be measured at 280 nm as proteins strongly absorb light at 280 nm and they have been measured at this wavelength in other studies (Cutler, 2004). The filtrate absorbance values at 280 nm are presented below in Figure 31.

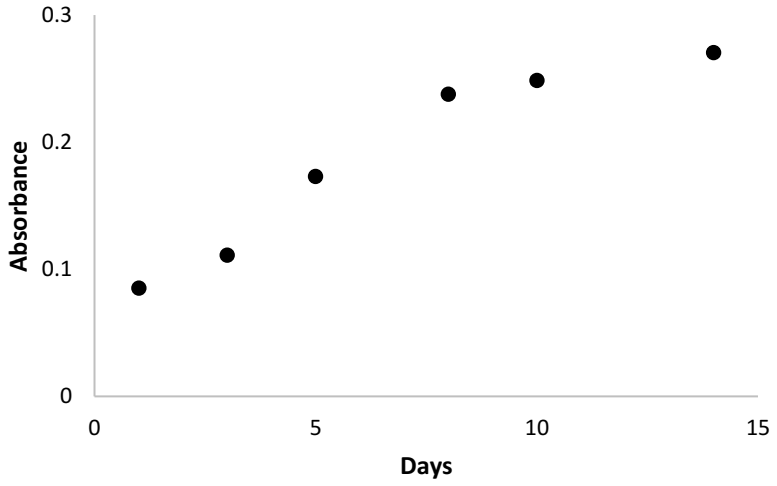


Figure 31. Filtrate absorbance at 280 nm over course of aerobic treatment in reactor.

It can be seen by from Figure 31 that when the protein was quantified using the absorbance at 280 nm there is no decrease from day 10 to day 14 as there was when measuring the protein content using the Bradford method. Figure 28 indicates that protein measurements begin to level off around day 10, similar to the VS, total COD and absorbance measurements at 190 nm because when there is limited biodegradable OM remaining in the treated sludge, protein concentrations in the filtrate will begin to stabilize. This shows that there is a potential correlation between filtrate protein measurements and the treatment level of the sludge.

The wavelength of 280 nm is also used in a ratio with the absorbance at 260 nm as a typical method to compare the nucleic acid content in proportion to the amount of protein in a solution (Kim et al., 2008). The ratios of filtrate absorbance values at 280 nm to 260 nm were determined over the course of the aerobic treatment and are shown in Figure 32 below.

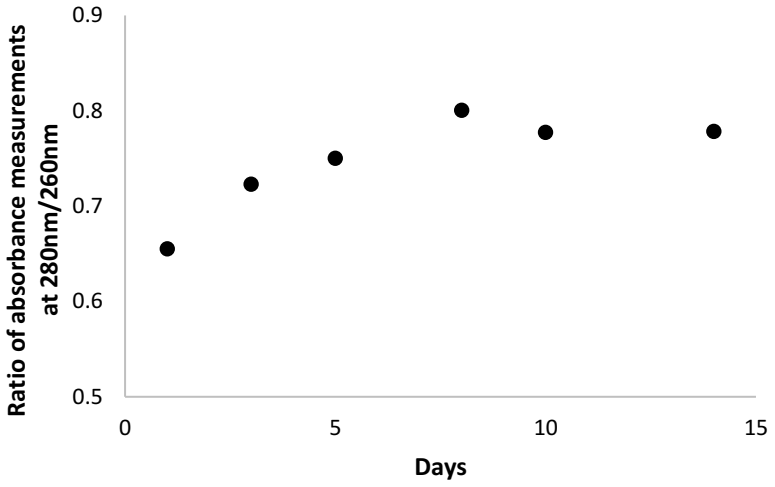


Figure 32: Ratio of filtrate absorbance at 280 nm to 260 nm over course of aerobic treatment in reactor.

In Figure 32 there is a gradual increase in the ratio of 280 to 260 absorbances and then it levels off at day 10. The similar trends observed in Figure 31 and 32 indicate that there is no significant change in the quantity of DNA in the filtrate suggesting a stable microbial population.

4.3.1.5 Filtrate Carbohydrate

The filtrate carbohydrate concentration is another important component of biopolymers and was measured using the Anthrone Method over the course of the aerobic treatment. The results are presented in Figure 33 below.

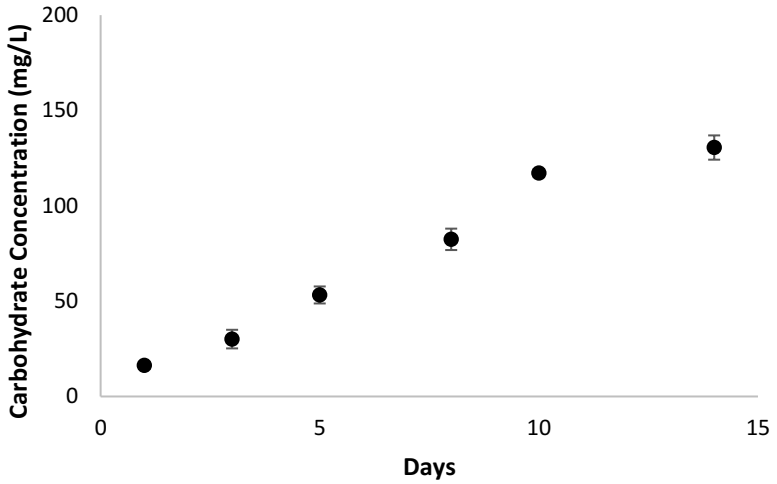


Figure 33. Filtrate carbohydrate concentration measured using the Anthrone Method over course of aerobic treatment in reactor.

A steady increase in filtrate carbohydrate concentration can be observed throughout the course of the aerobic digestion process in Figure 33. There is a gradual increase from day 1 to day 10. This stabilization of carbohydrate concentration values from day 10 onwards was expected due to the

aerobic treatment of the sludge approximately taking 10 days. Carbohydrates are biopolymers like proteins and therefore get released into solution during the aerobic digestion. When aerobic digestion is close to completion limited carbohydrates are released into solution, causing carbohydrate concentration measurements to stabilize.

The carbohydrate concentration measurements using the Anthrone method also follow a similar trend to the filtrate absorbance values at 190 nm shown in Figure 28. This could have occurred because carbohydrates have been measured at 190 nm (El-Rassi, 2002). This means that filtrate carbohydrate content has the potential to be quantified in real-time and in-line during sludge treatment using UV-vis spectrophotometry instead of using the Anthrone method which can be time consuming.

4.3.1.6 Turbidity

4.3.1.6.1 Effluent

The turbidity was measured to determine the approximate quantity of suspended solids in the solutions. Below in Figure 34 the effluent turbidity over the course of the aerobic reactor experiment is presented.

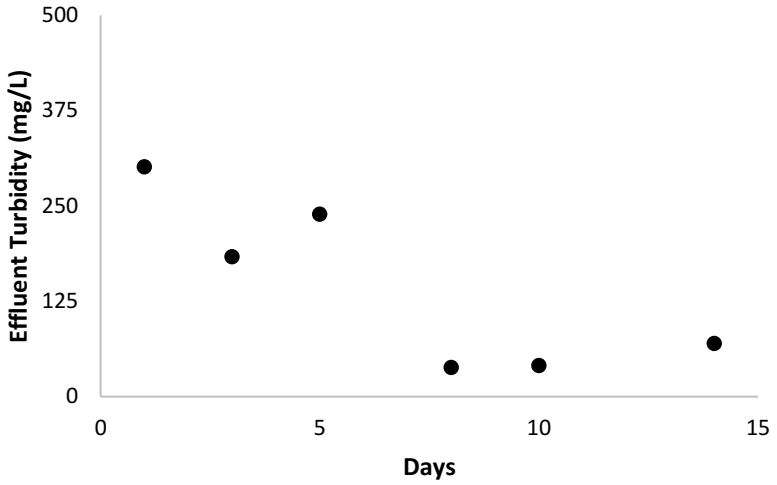


Figure 34. Effluent turbidity over course of aerobic treatment in reactor.

It can be seen from Figure 34 that there is an overall decrease in effluent turbidity and it has a similar trend with the effluent absorbance at 254 nm (Figure 25). This may point to the influence of particles and turbidity in unfiltered effluent samples on absorbance measurements. Therefore, even though filtration requires an additional step, measurements from filtrate would be more reliable than the measurements from effluent.

4.3.1.7 Dissolved Oxygen (DO)

The oxygen provided to the reactor throughout the experiment was monitored by measuring the dissolved oxygen (DO) content of the sludge. During the initial period of treatment, low DO concentrations in the sludge were observed as the oxygen provided was being consumed by the OM (Kazimierczak, 2012). However, as treatment neared completion DO values in the sludge started to increase as there was limited OM remaining to consume the provided oxygen.

4.3.1.8 pH

The pH of the sludge was monitored throughout the experiment and a small decrease was observed. This decrease could be associated with the nitrification process occurring in the sludge after the easily biodegradable OM in the sludge has been oxidized (Barragan Sanchez et. al., 2006).

4.3.1.9 Totalized Torque (TTQ)

The rheological properties of the sludge were studied to examine how they were affected by aerobic digestion and to determine if rheological methods had potential to be used as a monitoring tool. The totalized torque (TTQ) values produced from the rheograms for the sludge over the course of the aerobic digestion are presented in Figure 35 below.

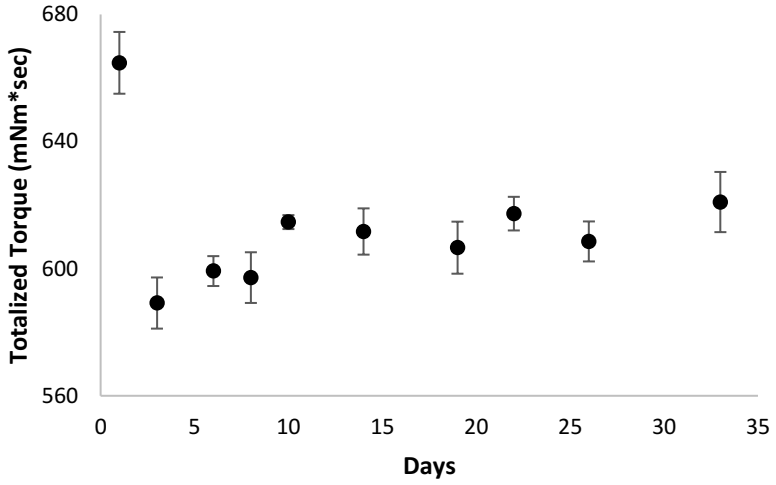


Figure 35. TTQ values over course of aerobic treatment in reactor.

A steep decrease in TTQ is observed from day 1 to day 3 in Figure 35. There is a slight increase, but limited change in TTQ values throughout the rest of the aerobic treatment. Greater changes in

TTQ were expected during the aerobic digestion process due to the TS and VS decreasing throughout the treatment process and previous correlations found between the rheological properties of sludge and the solids content (Eshtiaghi et al., 2013). As aerobic digestion proceeds particles get broken down, resulting in less resistance to the applied shear from the rheometer and therefore lower expected TTQ values throughout the progression of the aerobic treatment. It is possible that this method is not sensitive enough to recognize slight changes in the VS of the sludge during the aerobic digestion. Since VS is a parameter that can be used to monitor the treatment level of sludge and the TTQ values cannot distinguish slight changes in VS, TTQ does not appear to be an ideal tool to monitor aerobic digestion.

Another method for analyzing the rheological properties of the sludge is investigating the peak heights from the torque rheograms for the sludge samples. The peak heights from the torque rheograms for the sludge samples over the course of the aerobic digestion were also measured and are presented in Figure 36.

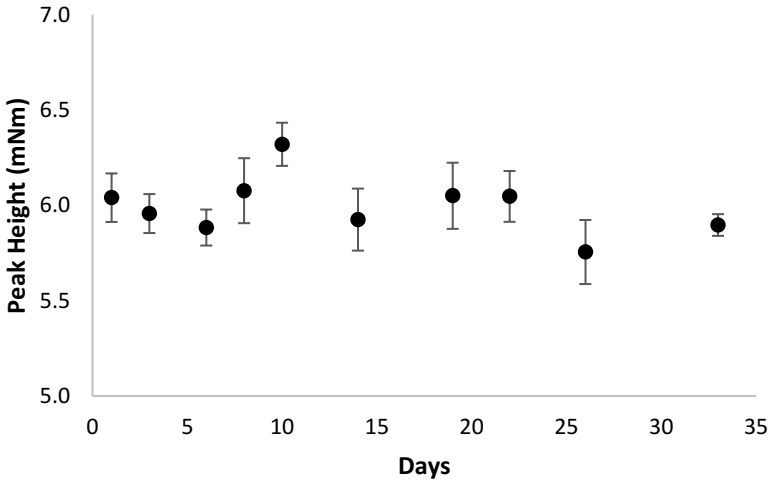


Figure 36: Rheogram peak height values over course of aerobic treatment in reactor.

It can be seen from Figure 36 that minimal change in peak height values occurs over the course of the treatment. Peak height values were expected to decrease as the aerobic digestion proceeded due to the number of particles present in the sludge gradually decreasing. The trend observed in Figure 36 is similar to the trend in Figure 35 as limited change in values is detected over the course of the aerobic treatment. This was expected as they are both measurements of the rheological properties and indicators of network strength. Figure 36 confirms that rheological measurements (TTQ and peak height) from the Floccky Tester are not sensitive enough to be used as a sludge monitoring method for aerobic digestion.

4.3.1.10 Microscopy

Microscopy images were taken of sludge flocs throughout the course of the experiment to assess the impact that aerobic digestion has on the sludge network and floc structure. Microscopy images of the sludge taken on day 1 are shown in Figure 37 below.

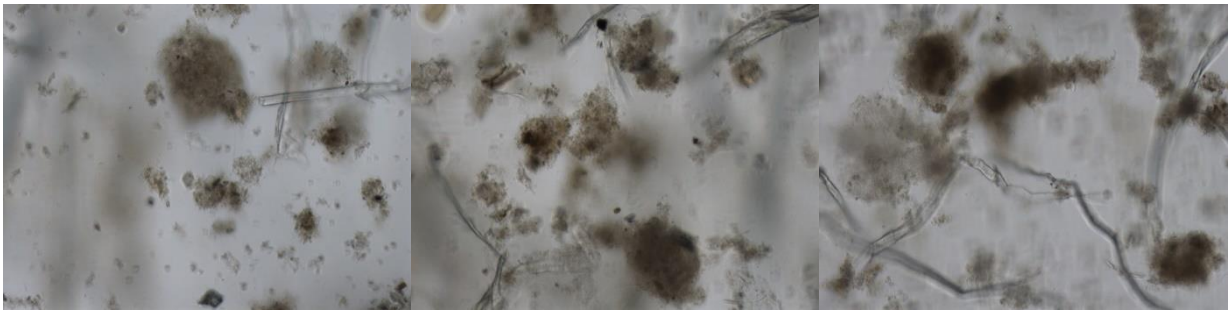


Figure 37: Microscopy images of the sludge mixture taken on day 1 (20X).

Microscopy images of the sludge were taken on day 8 and are presented in Figure 38.

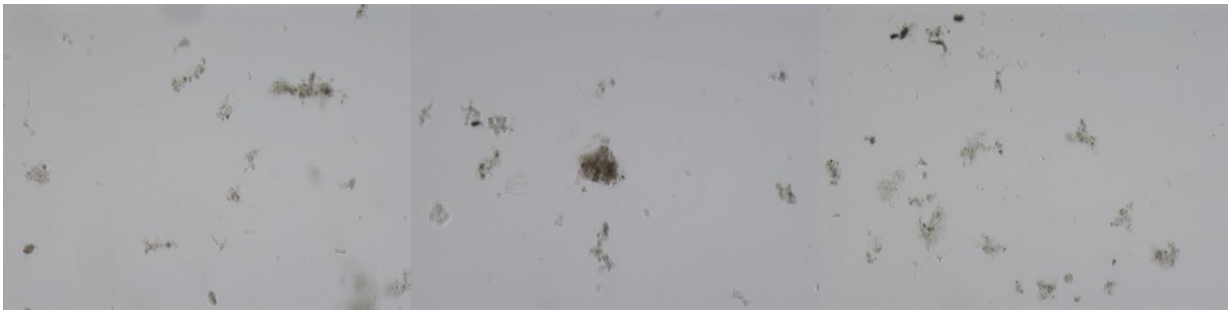


Figure 38. Microscopy images of the sludge mixture taken on day 8 (20X).

Microscopy images of the sludge were taken on day 14 and are presented in Figure 39.

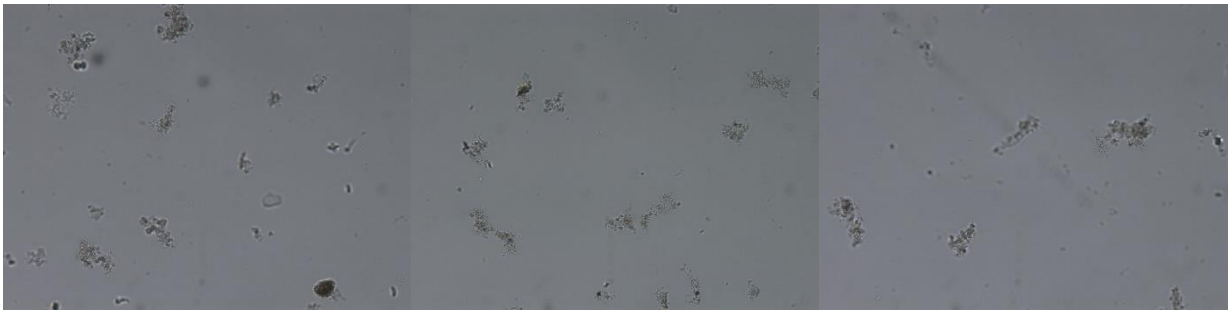


Figure 39: Microscopy images of the sludge mixture taken on day 14 (20X).

The microscopy images of the sludge taken on day 33 are shown below in Figure 40.

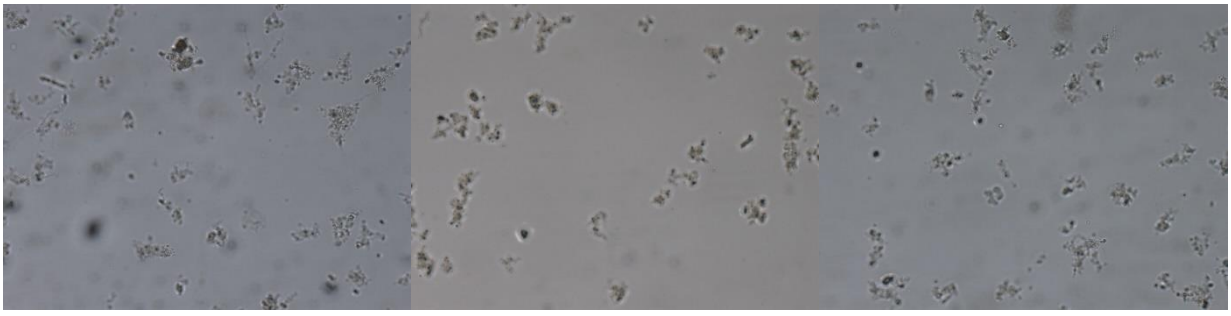


Figure 40: Microscopy images of the sludge mixture taken on day 33 (20X).

It can be visually observed from Figures 37 and 38 that there is a substantial decrease in floc size from day 1 to day 8. When comparing Figures 38, 39, and 40, there seems to be minimal change in floc size from day 8 to day 33.

To be able to quantify the average floc size for each microscopy image of the sludge, the Area Tool in the NIS-Elements Advanced Research program was used to measure the area of each individual floc present in the microscopy image and then the average floc size was calculated. The average sludge floc area over the course of the aerobic treatment is presented in Figure 41 below.

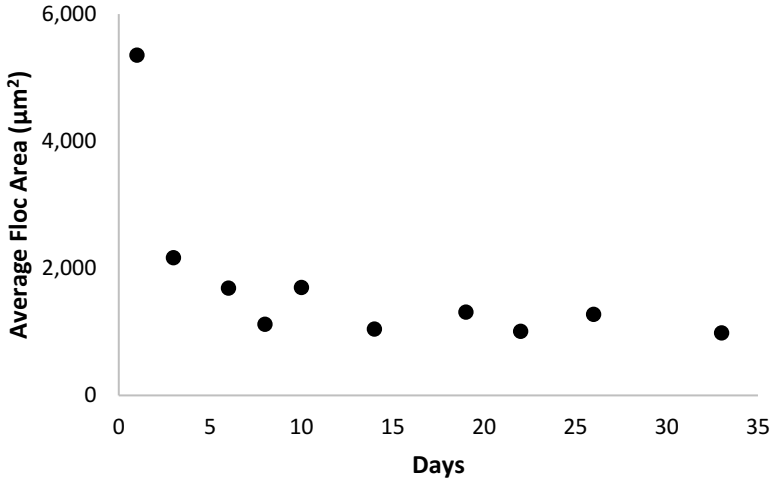


Figure 41. Average floc area over course of aerobic treatment in reactor.

A rapid decrease in average floc area occurs from day 1 to day 3 in Figure 41 and then continues until day 8. Beyond day 10, there is not a significant change in floc size which confirms the findings from the COD, TS, VS, and absorbance measurements. By day 10, the disintegration of the floc structure and decomposition of biodegradable organic matter is largely achieved. The microscopy data also confirm the gradual disruption of sludge network and the inability of the

totalized torque measurements to pick up these changes using the Floccky tester. Other rheometers with more sensitive impellers could be able to detect these changes.

4.3.2 Part 2. High Strength Sludge Measurements

The impact of there being a higher than normal initial loading rate was examined and the results are presented and discussed in the following section. Since the initial strength of this sludge was higher, longer aerobic digestion times were tested.

4.3.2.1 Total solids and total volatile solids

The TS and VS concentrations over the course of the aerobic reactor experiment can be seen in Figure 42.

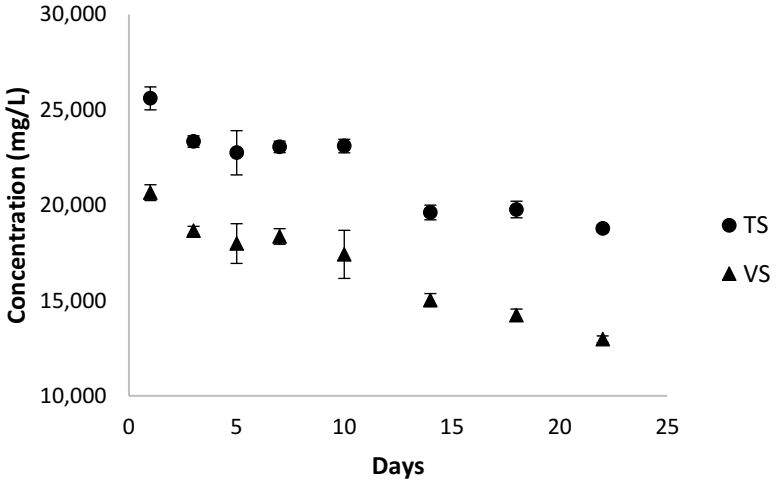


Figure 42. TS and VS concentrations over course of aerobic treatment in reactor.

It can be observed from Figure 42 that there is a decrease in both TS and VS concentrations of the sludge mixture during the aerobic treatment with TS/VS values beginning to level off at day 14. A volatile solids reduction (VSR) of 37% was observed over the course of the aerobic digestion process due to OM breaking down and the biodegradable fraction being consumed by the aerobic bacteria. A VSR of 37% also indicates that the sludge treatment is close to completion as VSRs close to 40% are assumed to be the stabilization limit (Kazimierczak, 2012).

4.3.2.2 Chemical Oxygen Demand (COD)

4.3.2.2.1 Total COD

Total COD is another common parameter used to monitor sludge treatment and the total COD of the sludge over the course of the aerobic treatment can be seen below in Figure 43.

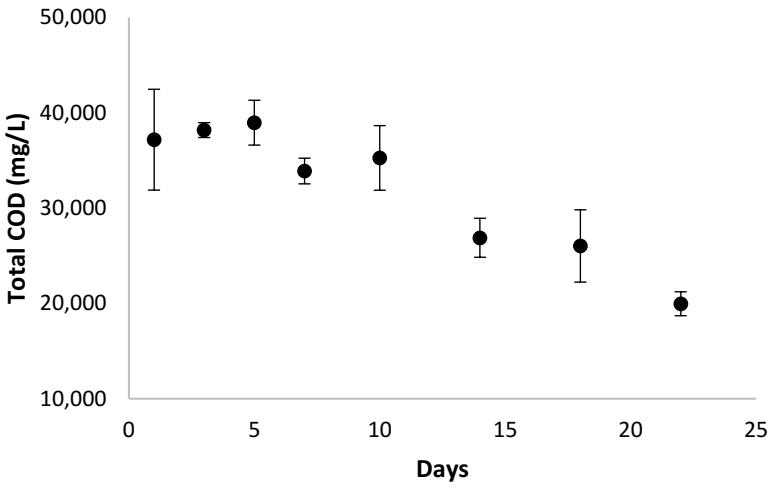


Figure 43. Total COD over course of aerobic treatment in reactor.

From Figure 43 a gradual decrease in total COD can be observed throughout the aerobic digestion process with values beginning to level off around day 14, resulting in a total COD removal of 46%. As previously mentioned for the normal strength sludge, the total COD decreases as organic particles get broken down and their biodegradable constituents get consumed by aerobic bacteria. Since the total COD begins to level off at day 14, like the VS concentration in Figure 42, the aerobic treatment of the high strength sludge is approaching completion by day 14.

4.3.2.2.2 Effluent COD

Similar to the process for the normal strength sludge, the high strength sludge sample was placed in a centrifuge and the effluent was extracted from the centrifugation process. The effluent COD over the course of the aerobic treatment can be seen below in Figure 44.

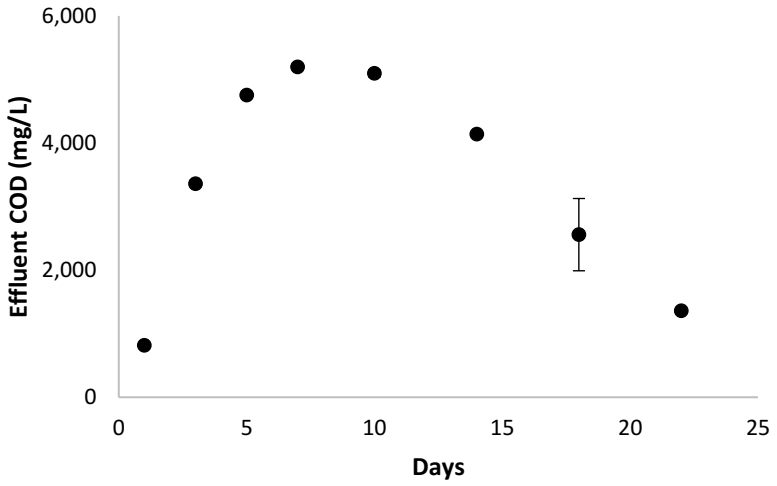


Figure 44. Effluent COD over course of aerobic treatment in reactor.

Figure 44 shows that the effluent COD gradually increases until day 7. This initial increase in effluent COD can be explained by the solubilisation of the OM in the sludge and its release to the supernatant. After day 7, the effluent COD continues to decrease until the end of the treatment as aerobic microorganisms consume the solubilized biodegradable OM. Allowing the reactor to run for a longer duration allowed to see the complete trend of initial COD release and consumption later by aerobic bacteria.

4.3.2.2.3 Soluble COD (sCOD)

The high strength effluent was passed through a 0.45 μm filter so the filtrate could be analyzed. The sCOD throughout the aerobic treatment is presented below in Figure 45.

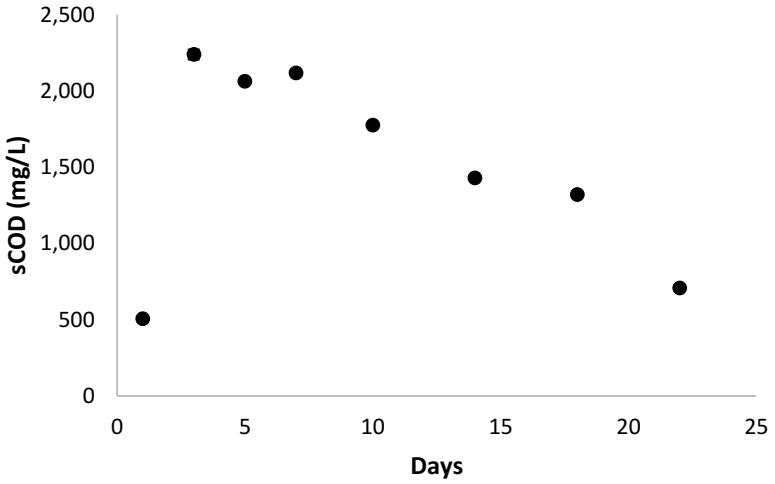


Figure 45. Soluble COD over course of aerobic treatment in reactor.

From Figure 45 there is a rapid increase in sCOD from day 1 to day 3. There is a gradual decrease in sCOD throughout the rest of the experiment. This steep initial increase in sCOD is caused by

many sludge flocs breaking down during the initial stages of the aerobic digestion process. The sludge flocs, consisting of biopolymer matrices of ESPs bridged together by cations, release the ESPs into solution. Since these extracellular particles can dissolve into solution, they will pass through the 0.45 μm filter and cause sCOD values to increase during the initial stages of the sludge treatment. As treatment progresses and less OM is released to solution a decline in sCOD is observed as aerobic microorganisms consume the dissolved biodegradable ESPs.

4.3.2.3 Absorbance

Like the normal strength sludge, absorbance measurements from 190-900 nm were taken of the effluent and filtrate at dilutions of 20:1 and 5:1, respectively. Different wavelengths were looked at to determine if there could be any correlation found between the absorbance at a specified wavelength and one of the other measured parameters using regression analysis. After examining multiple wavelengths, the wavelengths 190 nm and 254 nm were chosen again. The wavelengths of 190 nm and 254 nm were of special interest due to the correlations previously found at these wavelengths for the normal strength filtrate and effluent.

4.3.2.3.1 Effluent absorbance

The first wavelength that was examined for the effluent was 190 nm and the absorbance measurements at 190 nm throughout the aerobic digestion can be seen in Figure 46.

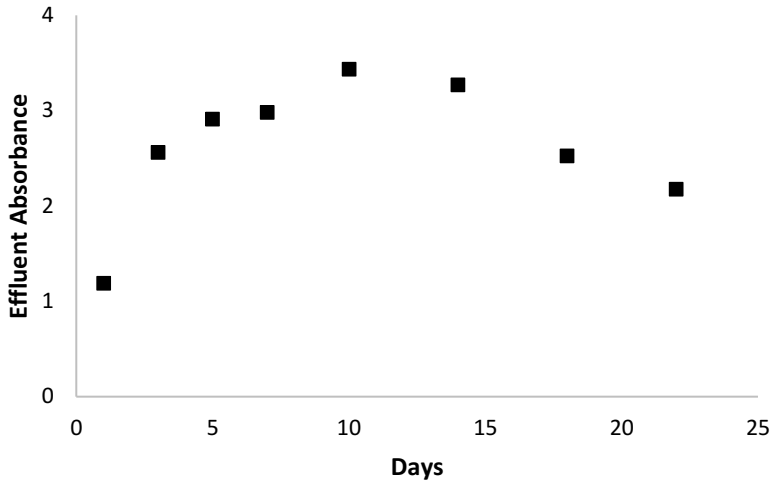


Figure 46. Effluent absorbance at 190 nm (20X) over course of aerobic treatment in reactor.

It can be seen from Figure 46 that there is a gradual increase in effluent absorbance at 190 nm from day 1 to day 10. The absorbance at 190 nm then decreases until the end of the aerobic digestion process. Absorbance values increasing up to day 10 can be associated with multiple organic compounds absorbing light at 190 nm (Paredes et al., 2006). Aerobic treatment causes the decomposition of OM and the release of organic compounds into solution. As the number of organic compounds in the solution increases so will the absorbance. The absorbance values stabilize from day 10 to day 14 and then a gradual decrease in values is seen throughout the rest of the treatment which could be caused by dissolved biodegradable OM being consumed during the aerobic digestion.

The wavelength of 254 nm was examined next and the effluent absorbance values at 254 nm are shown in Figure 47 below.

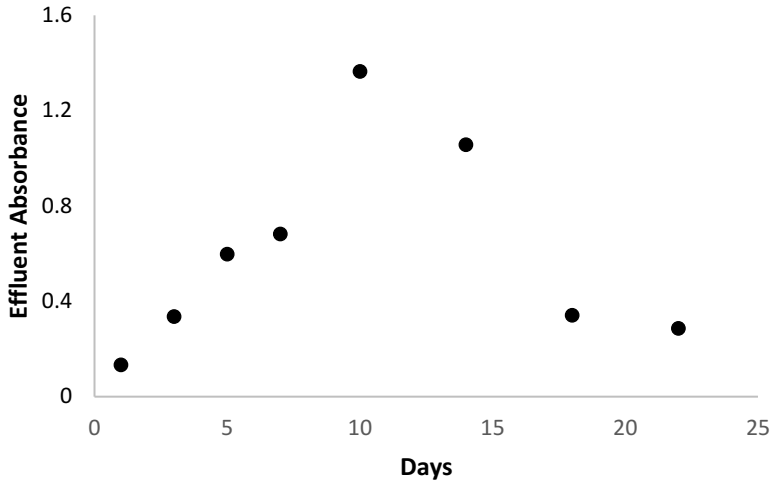


Figure 47. Effluent absorbance at 254 nm (20X) over course of aerobic treatment in reactor.

An increase in effluent absorbance at 254 nm can be seen up to day 10 in Figure 47. After day 10 the effluent absorbance decreases and then levels off. The trend being observed can be explained by dissolved OM being measured at 254 nm, and the role aerobic bacteria and dissolved OM have in the aerobic digestion of sludge (Hansen et al., 2016). It can be observed that absorbance values at 254 nm begin to decline on day 14. The absorbance values beginning to decrease after day 14 could be explained by the consumption of biodegradable OM by aerobic bacteria.

Like the normal strength sludge, similar trends were found between the effluent absorbance at 254 nm and the effluent COD in Figure 41 as a peak is reached close to day 10 and then there is a decline in measurements.

4.3.2.3.2 Effluent absorbance regression analysis

The same regression analysis method used for the normal strength sludge was used for the high strength sludge. Wavelength ranges of the effluent absorbance focusing around 190 nm and 254

nm were used in regression analysis with effluent COD and VS data. The results from the regression analysis for the high strength effluent are presented in Table 3.

Table 3. Summary of regression analysis results for regression equation created using high strength effluent absorbance and sludge parameters.

Wavelength of focus (nm)	Regression Method	Parameter	Wavelength Range of Regression Equation	R^2	Standard Error	RMSE
190	PCR	Effluent COD	190-220	1.000	0.747	3.808
	PCR	VS	190-220	1.000	18.875	32.376
254	PCR	Effluent COD	240-268	1.000	7.875	19.449
	PCR	VS	242-266	1.000	6.773	10.330

Table 3 shows a stronger correlation between effluent COD and absorbance at 190 nm compared to any of the other data sets.

4.3.2.3.3 Filtrate absorbance

To evaluate if the higher organic load had an effect on the filtrate absorbance at 190 nm, the high strength filtrate absorbance at 190 nm was measured and the results are presented in Figure 48 below.

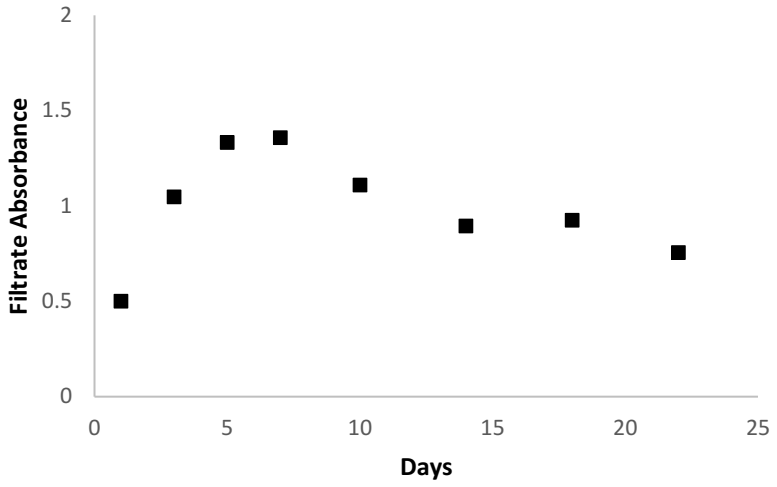


Figure 48: Filtrate absorbance at 190 nm (50X) over course of aerobic treatment in reactor.

For Figure 48, a defined peak is observed close to day 7 and then there is a gradual decrease until day 14 where values begin to level off. Biopolymers, such as carbohydrates and proteins, have been found to absorb light at 190 nm and get released into solution during aerobic treatment (Lindon et al., 2016; Baret et al., 1997). As the treatment progresses, aerobic bacteria begin to consume the biodegradable biopolymers which causes absorbance values to decline. Near the completion of the treatment, absorbance values begin to stabilize as limited available biopolymers remain.

The impact of wavelength on the high strength filtrate absorbance was examined by investigating 254 nm, the results are below in Figure 49.

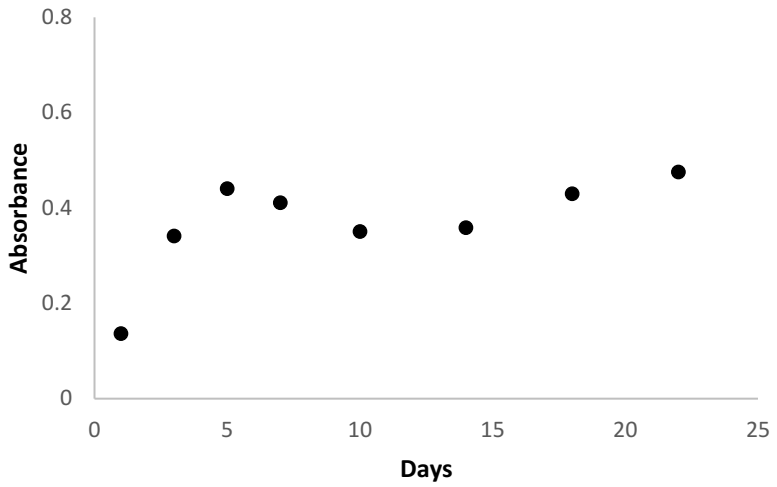


Figure 49. Filtrate absorbance at 254 nm (5X) over course of aerobic treatment in reactor.

A peak in filtrate absorbance at 254 nm is observed on day 5 followed by a localized drop around day 10 and then a steady increase in Figure 49.

4.3.2.3.4 Filtrate absorbance regression analysis

Regression analysis was also carried out for the filtrate. Regression equations to predict sCOD and VS values were generated using wavelength ranges focusing on 190 nm and 254 nm. The results are presented below in Table 4.

Table 4. Summary of regression analysis results for regression equation created using high strength filtrate absorbance and sludge parameters.

Wavelength of focus (nm)	Regression Method	Parameter	Wavelength Range of Regression Equation	R^2	Standard Error	RMSE
190	PCR	sCOD	190-200	1.000	0.232	0.951
	PCR	VS	190-214	1.000	0.264	0.399
254	PCR	sCOD	238-270	1.000	0.229	0.305
	PCR	VS	241-267	1.000	2.669	12.326

The regression equations developed for sCOD were generally more accurate than the regression equations developed for VS. PCR was found to be a more effective regression method for generating accurate regression equations compared to MLR.

When compared to the regression equations developed using the effluent absorbance in Table 3, the filtrate regression equations in Table 4 were more accurate at predicting the sludge stabilization parameters as for each parameter the filtrate regression equation had a lower Standard Error and RMSE.

4.3.2.4 Turbidity

4.3.2.4.1 Effluent

Turbidity, which has previously used for monitoring water quality, was first measured for the effluent and the results throughout the aerobic digestion are shown below in Figure 50.

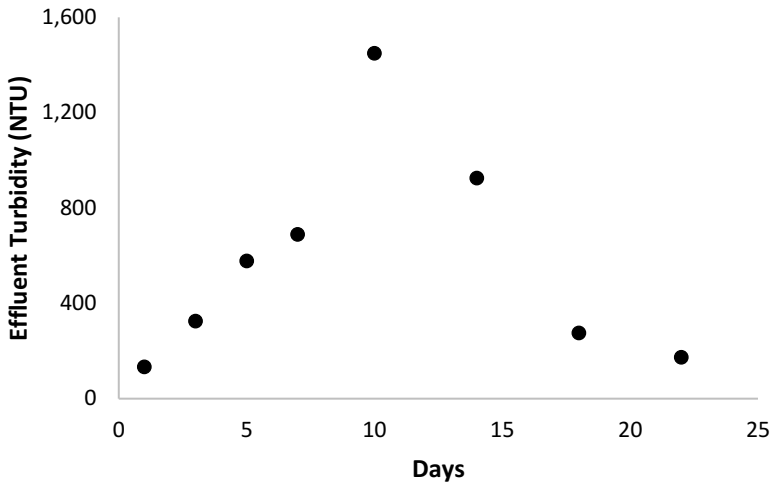


Figure 50. Effluent turbidity over course of aerobic treatment in reactor.

An increase in effluent turbidity can be seen up to day 10 in Figure 50. After day 10 the effluent turbidity decreases and then begins to level off around day 18 until the end of the treatment. The turbidity was expected to initially increase as the number of particles increases due to organic matter breaking down and decrease with the consumption of the biodegradable OM by aerobic bacteria.

4.3.2.5 Comparison to Normal Strength Measurements

Trends in the data found for the high strength sludge were similar to the trends found for the normal strength sludge. In addition to this, similar VSRs and total COD removals were found for the normal and high strength sludge as there were VSRs of 43% and 37%, respectively, and total COD removals of 39% and 46%, respectively. However, there were differences in the data such as peak values and the time at which the parameters stabilized. In general, the high strength sludge parameters had higher and more defined peaks and the time at which parameters stabilized was

later in the aerobic digestion process. This can be attributed to the higher organic load and the longer retention time for the high strength sludge.

4.4 Conclusion

The objective of these aerobic reactor experiments was to determine if UV/Vis spectrophotometry and rheology could be used to monitor the level of treatment and performance during the aerobic digestion of sludge.

Two batches of sludge were used to evaluate the potential of these two methods to be used as monitoring tools, with one batch representing daily operational sludge characteristics while the second batch represented sludge with a higher initial organic loading rate. The normal strength sludge seemed to be approaching completion near day 10 as VS and total COD, two common parameters used to monitor sludge treatment, began to stabilize near this day due to limited biodegradable OM remaining in the sludge. A VSR and total COD removal of 43% and 39% were observed respectively. Due to the greater initial organic load of the high strength sludge, the longer retention time and a similar amount of oxygen supplied, treatment of the high strength sludge appeared to approach completion around 14 days as VS and total COD measurements began to stabilize. A VSR of 37% and a total COD removal of 46% were observed throughout the aerobic treatment.

Filtrate absorbance measurements at 190 nm were found to stabilize around the time that aerobic treatment neared completion. This can be associated with biopolymers, such as carbohydrates and proteins, absorbing light at 190 nm and their role in the aerobic digestion process of sludge. As the aerobic digestion proceeds, OM is broken down and biopolymers within the OM get released into solution causing filtrate absorbance values at 190 nm to increase. A decrease in absorbance values

can be attributed to a greater amount of biodegradable biopolymers being consumed by aerobic bacteria compared to being released to solution. A stabilization of filtrate absorbance values at 190 nm is then observed as limited available biodegradable biopolymers remain, indicating treatment is close to completion. This shows that filtrate absorbance measurements at 190 nm have the potential to be used as an in-line and real-time sludge treatment monitoring tool.

To further examine if biopolymers were released to solution, the quantity of two main biopolymers, carbohydrates and proteins, in the filtrate were measured throughout the process. Filtrate protein and carbohydrate measurements, measured using the Bradford and Anthrone methods, respectively, followed similar trends to the absorbance values at 190 nm, with an outlier being found on day 10 of the protein measurements due to experimental error. An additional wavelength, 280 nm, was looked at as protein has been measured at this wavelength before. Filtrate absorbance measurements at 280 nm confirmed day 10 was an outlier. The ratio of filtrate absorbance at 280 nm to 260 nm was also looked at to determine the ratio of nucleic acid to protein content in the filtrate. It could be concluded from this ratio that there was no significant change in the quantity of DNA in the filtrate suggesting a stable microbial population.

Similar trends were found between the effluent absorbance at 254 nm, effluent COD, and effluent turbidity. Since centrifugation is not a completely efficient process, suspended particles can remain in the effluent solution. The suspended particles remaining in the effluent solution cause the turbidity to impact the absorbance based COD measurements, resulting in an inaccurate representation of the level of treatment of the sludge. This particle effect could also be the reason why filtrate measurements were found to have a stronger correlation to the level of treatment of the sludge. Effluent absorbance measurements, although not the most ideal monitoring tool, can still give an approximate indication of the level of treatment because once a peak is observed in

effluent measurements, it can be acknowledged that a significant amount of OM has been broken down by this point and the treatment is nearing completion. Therefore, effluent measurements can still be used as an in-line and real-time monitoring tool during aerobic digestion, even if they are not as accurate as filtrate measurements because they are quicker and more rapid to obtain.

Regression analysis was carried out for the normal and high strength effluent with the effluent COD and VS and the filtrate with sCOD and VS using the MLR and PCR regression methods. Accurate regression equations were developed to predict effluent COD, sCOD and VS using effluent and filtrate absorbance wavelength ranges focusing around 190 nm and 254 nm. In general, regression equations developed using filtrate data were more accurate at predicting sludge stabilization parameters which could be associated with the particle effect. Stronger correlations were also found at 190 nm compared to 254 nm which could be attributed to a greater amount of light being absorbed at 190 nm and biopolymers being measured there. This shows that the sludge parameters VS, effluent COD, and sCOD have the potential to be predicted during aerobic digestion of sludge in real-time and in-line using effluent and filtrate absorbance focusing around 190 nm and 254 nm using the PCR regression method, with filtrate absorbance focusing around 190 nm providing the strongest indication of the level of treatment.

Similar trends were found between the normal and high strength sludge parameters, with high strength sludge parameters having higher and more defined peaks and stabilization occurring later on in the digestion process. This is due to the higher organic load and longer retention time for the high strength sludge.

Rheological measurements were also taken to examine their potential use as a monitoring tool for sludge treatment. It was concluded that the Floccky Tester was not sensitive enough to measure the rheological changes that occur during aerobic treatment of sludge as there were no significant

changes in TTQ or peak height values after day 3 throughout the sludge treatment. TTQ and peak height values were expected to decrease throughout the aerobic digestion process due to the decrease in TS and VS and previous correlations observed between rheological properties and total solids content. This shows that the Floccky Tester is not an ideal monitoring tool for aerobic sludge treatment. Microscopy images were also taken to assess the impact that aerobic digestion has on the floc structure. The microscopy data confirmed the gradual disruption of the sludge network and the inability of the totalized torque measurements to pick up these changes during aerobic digestion using the Floccky Tester.

Chapter 5 – Assessment of torque rheology as a monitoring tool for changes in sludge characteristics

Abstract

Multivalent cations and extracellular polymers are essential components of sludge flocs and their concentrations in sludge have a direct impact on the network strength of the sludge and therein the treatment performance. The network strength of the sludge has the potential to be monitored using rheology, which has the possibility to predict sludge behaviour and achieve real-time optimization. To investigate the potential use of rheology for sludge characterization and treatment monitoring, changes in anaerobically digested sludge characteristics were made to examine if they could be detected by rheological measurements using the totalized torque (TTQ) and direct injection methods. It was found that varying the total solids concentration could be detected using the TTQ method as there was a linear increase in TTQ values with increasing total solids concentration. Changes in the trivalent ion, ferric iron, could be detected by rheology as both TTQ and peak torque values increased with increasing ferric iron ion concentration. No significant changes in rheological properties were observed for either divalent cation, Ca^{+2} and Mg^{+2}. There was an increase in TTQ values and peak torque values when the concentration of the extracellular polymer, alginate, was increased, however, when alginate and Ca^{+2} ions were added together to the sludge no significant changes in rheological properties were observed for either method. It was also found that the direct injection method is more sensitive compared to the TTQ method due to the greater percent increase in peak torque values compared to TTQ values for all chemicals.

Keywords: Rheology, microscopy, floc strength, cations, extracellular polymers, solids content

5.1 Introduction

Multivalent cations and extracellular polymers (ESP) produced by sludge bacteria are known to play a major role in the formation of flocs, network strength of sludge and microbial activity. ESP provide a matrix in which microbes can be aggregated to form flocs (Higgins & Novak, 1997A). ESP are negatively charged and act as branches which are connected to particles and smaller flocs by the multivalent cations, therein forming larger flocs which will result in the sludge having a greater network strength. Addition of biopolymers and cations has been shown to increase floc and network strength in sludge and improve dewaterability (Higgins & Novak, 1997C; Sanin & Vesilind, 1996; Ormeci & Vesilind, 2000). Treatment performance (e.g. activated sludge, anaerobic digestion, thickening, dewatering, odour control, *E. coli* regrowth) has also been shown to improve in lab-scale studies and at treatment plants by adjusting the ratio of monovalent cations to divalent cations (Yagci et al., 2015; Park et al., 2010; Nguyen et al., 2008; Park et al., 2006).

One method which has potential to be used to monitor the network strength of sludge and therein treatment performance is rheology. Rheology provides information on the overall sludge matrix and can be used to predict sludge behaviour and achieve real-time optimization. It is a holistic descriptor that encompasses the physical, biological and chemical changes that occur in sludge. Sludge rheological properties are determined using a torque rheometer, which is a device consisting of a shaft connected to an impeller. As the impeller rotates, it measures the sludge's torque by measuring the sludge's resistance to shear over a period of time. From this a torque rheogram is produced.

There are two different torque rheometry methods that are used to investigate the rheological properties of sludge. The first method is known as the totalized torque (TTQ) method and uses the area under a torque-time curve to represent the total energy dissipation which is used to estimate

the overall network strength of the sludge (Ormeci et al., 2004, Ormeci & Abu-Orf, 2005). This method has already been used to determine the optimum polymer dose for dewatering at a full-scale dewatering operation at a treatment plant (Abu-Orf & Ormeci, 2005; Ormeci & Abu-Orf, 2006; Ormeci, 2007).

The direct injection method is the second method which is used to analyze the rheological properties of sludge. The direct injection method uses unconditioned sludge and only utilizes the peaks observed after direct injection of a chemical into the sludge sample to evaluate the overall network strength of the sludge. The optimum dose has also been identified using this method during dewatering in lab-scale and full-scale tests at treatment plants (Ormeci, 2007; Murray & Ormeci, 2008).

The objective of this study was to investigate whether changes in sludge characteristics such as cation and biopolymer concentrations and solids content of sludge can be detected by rheological measurements, and whether rheology can be used as a tool to monitor the changes in sludge characteristics during treatment. Multivalent cations and extracellular polymers were measured because they affect floc formation. As previously mentioned, extracellular polymers act as branches that connect smaller flocs to the multivalent cations, therein forming large flocs which increases the network strength of the sludge and can improve its dewaterability. Microscopy was additionally carried out on samples containing different cation and extracellular polymer concentrations to asses the effect dosed chemicals had on the size and structure of the flocs.

5.2 Materials and Methods

The effect of total solids concentration was studied by varying the total solids concentration of the anaerobically digested sludge by adding and withdrawing water. Ferric ions were added to the

sludge to examine the effect of trivalent ions on the sludges rheology. The effect of divalent ions on the rheological properties of the sludge was examined by first dosing the sludge with Mg^{+2} ions and then dosing it with Ca^{+2} ions. Alginate was used to observe the effect of ESP on the rheological properties of sludge. Alginate was then used in combination with Ca^{+2} ions to study the combinative effect of divalent ions and ESP on the rheology of the sludge. Lastly, microscopy images were taken of sludge samples dosed with cations and ESP, and the average floc size within the samples was measured to assess the changes in sludge structure.

The TTQ method and direct injection method are summarized in the flow chart seen in Figure 51 and then are described in further detail in the following sections.

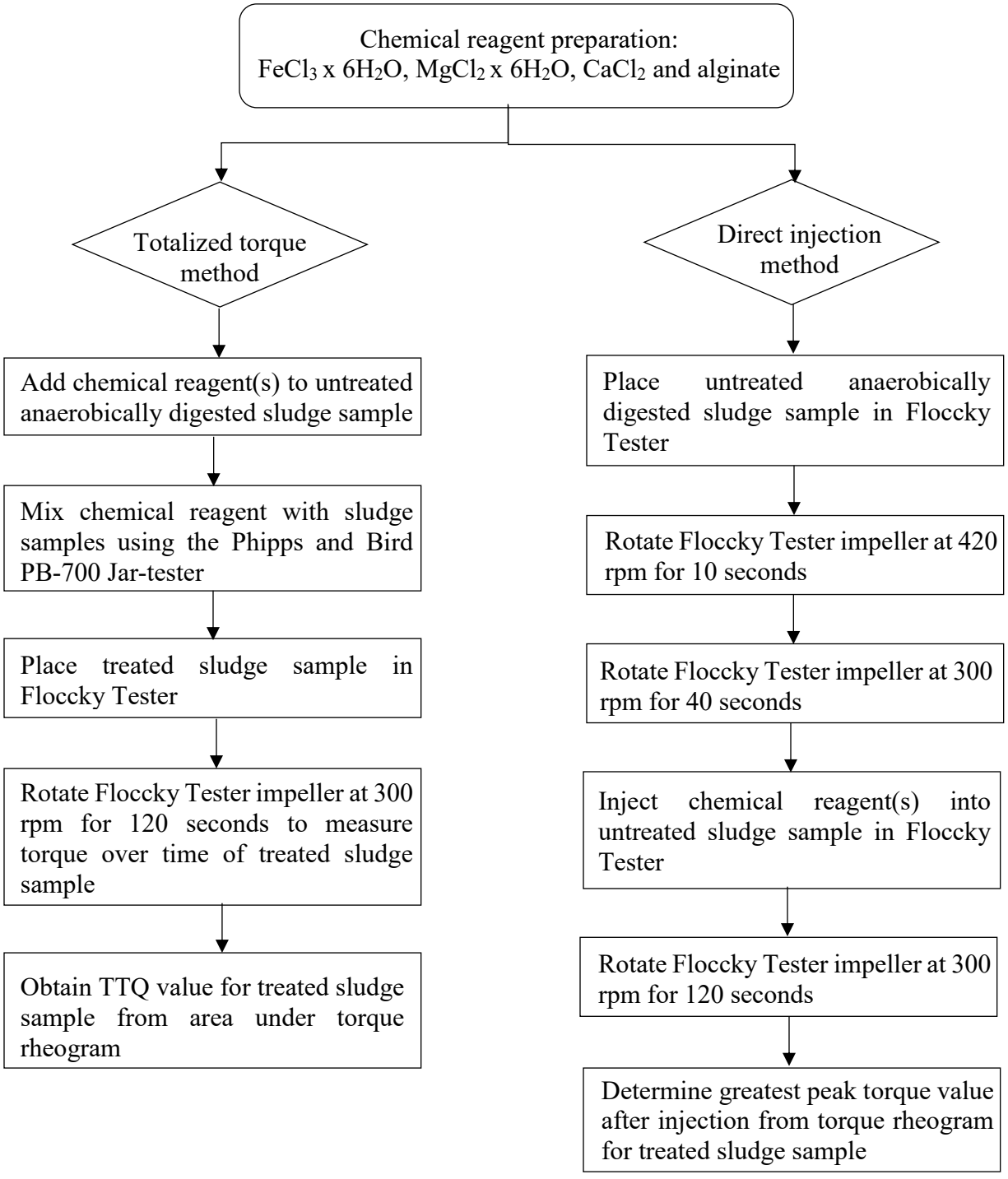


Figure 51. Flow chart of experimental processes carried out for the totalized torque and direct injection methods used in part two of research.

5.2.1 Rheological measurements: Totalized torque (TTQ) method

Totalized torque method uses pretreated samples for rheological tests and measures the area under the torque-time rheograms. This method was used when the sludge was pretreated with $MgCl_2$ x $6H_2O$, $CaCl_2$, $FeCl_3$ x $6H_2O$, alginate, and $CaCl_2$ and alginate as explained in sections 5.2.3 and 5.2.4 below.

Three 200 mL subsamples were individually placed in the cylindrical jar of the torque rheometer to obtain three replicates. For each 200 mL sample, the torque rheometer impeller rotated at 300 rpm for 120 seconds and the torque was measured over time (Ormeci et al., 2004). The subsamples were discarded after each run due to the thixotropic nature of sludge. The Floccky Tester's software reports the area under the torque-time graph, also known as totalized torque (TTQ), and the peak height which were used to compare the rheological properties of the sludge samples.

5.2.2 Rheological measurements: Direct injection method

The direct injection method uses raw (untreated) sludge for rheological tests and directly injects the chemical into the sample during testing and observes the size of the peaks formed in the rheograms after the injection of the chemical. The direct injection method is described below for $FeCl_3$ x $6H_2O$ and it was similarly used for $MgCl_2$ x $6H_2O$, $CaCl_2$ and alginate.

The Floccky Tester was used again for these measurements. Eighteen 190 mL sludge samples (3 per Fe^{+3} concentration, 3 untreated) were individually placed in the cylindrical jar of the torque rheometer. For each sample, the impeller first rotated at a speed of 420 rpm for 10 seconds to suspend the settled solids and homogeneously mix the sludge (Murray & Ormeci, 2008). Next, the sludge was mixed at 300 rpm for 40 seconds to provide a baseline for the 190 mL of untreated sludge. At the beginning of the next step, 10 mL of the varying Fe^{+3} concentration solutions were

injected into the sludge through one of the ports in the cup (see Figure 4) and the sludge was mixed at 300 rpm for 120 seconds. A peak was observed right after the solution injection.

When the direct injection method was used for the combination of $CaCl_2$ and alginate, the same process described above was carried out, however, when the injection occurred, 10 mL of a 5,000 mg/L alginate solution and 10 mL of the varying Ca^{+2} concentration solutions were injected into the sludge through two of the ports in the cup simultaneously.

5.2.3 Sludge pretreatment with $MgCl_2$ x $6H_2O$, $CaCl_2$, $FeCl_3$ x $6H_2O$, and alginate for TTQ method

The process described below for $FeCl_3$ x $6H_2O$ was also used for $MgCl_2$ x $6H_2O$, $CaCl_2$ and alginate.

190 mL sludge samples were dosed with 10 mL of the 5 different $FeCl_3$ x $6H_2O$ solutions, resulting in a total sample volume of 200 mL each run. For each $FeCl_3$ x $6H_2O$ concentration, 3 replicates were made, resulting in 15 samples. These 200 mL samples were then mixed on the Phipps and Bird PB-700 Jar-tester for two minutes at 200 rpm. In addition to these treated sludge samples, three 200 mL untreated sludge samples were also prepared to act as a baseline.

5.2.4 Sludge pretreatment with $CaCl_2$ and alginate for TTQ method

180 mL sludge samples were dosed with 10 mL of alginate solution with a concentration of 5,000 mg/L and 10 mL of the 5 different $CaCl_2$ solutions, resulting in a total sample volume of 200 mL each run. For each varying $CaCl_2$ concentration, 3 replicates were made, resulting in 15 samples. These 200 mL samples were mixed on the Phipps and Bird PB-700 Jar-tester for two minutes at 200 rpm. The alginate solution was added at time zero with the $CaCl_2$ solution being added ten

seconds into the mixing. In addition to these treated sludge samples, three 200 mL untreated sludge samples were prepared to act as a baseline.

5.2.5 Variation of total solids content for TTQ method

The anaerobically digested sludge obtained from ROPEC was found to have a TS of 1.76%. One 800 mL sludge sample was diluted with 200 mL of D.I. water to achieve a TS of 0.91%. The next 800 mL sludge sample was centrifuged at 2,000 G for six minutes in the Sorvall Legend RT+ Centrifuge. Once the effluent was removed, the dewatered sludge sample was found to have a TS of 3.25%. The final 800 mL sludge sample was centrifuged at 6,000 G for 6 minutes. After removing the effluent, the dewatered sludge sample had a TS of 4.23%.

5.2.6 Microscopy

Four samples were analyzed using the microscope which were 200 mL of untreated sludge, 190 mL of sludge treated with 10 mL of 138,800 mg/L $CaCl_2$ (50,000 mg/L Ca^{+2}), 190 mL of sludge treated with 10 mL of 242,000 mg/L $FeCl_3$ x $6H_2O$ (50,000 mg/L Fe^{+3}), and 190 mL of sludge treated with 10 mL of 5,000 mg/L alginate and 10 mL of 138,800 mg/L $CaCl_2$ (50,000 mg/L Ca^{+2}). The Nikon Eclipse Inverted Research Microscope was used to study the microscopic properties of the sludge. A Fisherbrand disposable inoculating loop was used to withdraw 10 microliters of sludge. The sample was placed in a MatTek glass bottom microwell dish and was diluted with 3 mL of D.I. water. The dish was gently shaken to mix the sample. The sample was then placed on the microscope stage and five minutes were given to allow the floc particles to settle to the bottom of the glass bottom microwell dish.

The microscope and the QImaging Retiga EXi Fast 1394 camera were turned on while the floc particles settled. The Nikon NIS-Elements Advanced Research program, version 3.2, was then opened on the computer. This program is connected to the camera and microscope, allowing images viewed through the microscope to be captured as pictures on the computer using the camera. Once the five minutes was complete, the sample was brought into focus. Next, the battlement technique was used to ensure the sample was a homogenous mixture. The battlement technique involves moving the microscope a field along the edge of the sample, a field up, one along and then one down, until the entire sample has been viewed. Five images which best represented the sample were captured as JPG files using the NIS-Elements Advanced Research program and saved.

5.2.7 Floc area

Area of the flocs was used as a tool to monitor the changes in sludge structure. The areas of the flocs in the five captured images were measured using the Area Tool in the NIS-Elements Advanced Research program as it was calibrated to be able to measure micrometers in images. The mouse was used to draw the perimeter around each floc and then the area of the floc was calculated using the Area Tool. The floc area measurements were recorded to a statistics table which could be exported as a Microsoft Excel file.

5.2.8 Sludge samples

Anaerobically digested sludge samples were collected from the Robert O. Pickard Environmental Centre (ROPEC) located in Ottawa (Ontario, Canada). This plant employs mesophilic anaerobic digestion.

5.2.9 Preparation of chemical reagents

5.2.9.1 Magnesium chloride hexahydrate ($MgCl_2$ x $6H_2O$)

A 200 mL solution of 209,160 mg/L $MgCl_2$ x $6H_2O$ (25,000 mg/L Mg^{+2}) was initially prepared by adding 41.8 g of $MgCl_2$ x $6H_2O$ to 200 mL of D.I. water. From this, 50 mL solutions with $MgCl_2$ x $6H_2O$ concentrations of 41,830 mg/L (5,000 mg/L Mg^{+2}), 83,660 mg/L (10,000 mg/L Mg^{+2}), 125,490 mg/L (15,000 mg/L Mg^{+2}) and 167,330 mg/L (20,000 mg/L Mg^{+2}) were prepared by adding the required volumes of D.I. water and the 209,160 mg/L $MgCl_2$ x $6H_2O$ solution. These solutions were prepared for both the TTQ method and direct injection method. For the direct injection method, an additional solution with an $MgCl_2$ x $6H_2O$ concentration of 418,300 mg/L (50,000 mg/L Mg^{+2}) was prepared.

5.2.9.2 Calcium chloride ($CaCl_2$)

A 200 mL solution of 69,380 mg/L $CaCl_2$ (25,000 mg/L Ca^{+2}) was initially prepared by adding 13.9 g of $CaCl_2$ to 200 mL of D.I. water. From this, 50 mL solutions with $CaCl_2$ concentrations of 13,880 mg/L (5,000 mg/L Ca^{+2}), 27,750 mg/L (10,000 mg/L Ca^{+2}), 41,630 mg/L (15,000 mg/L Ca^{+2}) and 55,500 mg/L (20,000 mg/L Ca^{+2}) were prepared by adding the required volumes of D.I. water and the 69,380 mg/L $CaCl_2$ solution. An additional solution with a $CaCl_2$ concentration of 138,800 mg/L (50,000 mg/L Ca^{+2}) was prepared. These solutions were prepared for both the TTQ method and direct injection method.

5.2.9.3 Ferric chloride hexahydrate ($FeCl_3$ x $6H_2O$)

A 200 mL solution of 121,000 mg/L $FeCl_3$ x $6H_2O$ (25,000 mg/L Fe^{+3}) was initially prepared by adding 24.2 g of $FeCl_3$ x $6H_2O$ to 200 mL of D.I. water. From this, 50 mL solutions with $FeCl_3$ x

$6H_2O$ concentrations of 24,200 mg/L (5,000 mg/L Fe^{+3}), 48,410 mg/L (10,000 mg/L Fe^{+3}), 72,610 mg/L (15,000 mg/L Fe^{+3}) and 96,800 mg/L (20,000 mg/L Fe^{+3}) were prepared by adding the required volumes of D.I. water and the 121,000 mg/L $FeCl_3$ x $6H_2O$ solution. These solutions were prepared for both the TTQ method and direct injection method. For the direction injection method, an additional solution with a $FeCl_3$ x $6H_2O$ concentration of 242,000 mg/L (50,000 mg/L Fe^{+3}) was prepared.

5.2.9.4 Alginate

Three different sets of alginate solutions were prepared for experiments.

For the TTQ method, a 200 mL solution of 25,000 mg/L alginate was initially prepared by adding 5 g of alginate to 200 mL of D.I. water. From this, 50 mL solutions with alginate concentrations of 1,000 mg/L, 2,000 mg/L, 3,000 mg/L, 4,000 mg/L, 5,000 mg/L and 10,000 mg/L were prepared by adding the required volumes of D.I. water and the 25,000 mg/L alginate solution.

For the direct injection method, a 200 mL solution of 50,000 mg/L alginate was initially prepared by adding 10 g of alginate to 200 mL of D.I. water. From this, 50 mL solutions with alginate concentrations of 5,000 mg/L, 10,000 mg/L, 15,000 mg/L, 20,000 mg/L and 25,000 mg/L were prepared by adding the required volumes of D.I. water and the 50,000 mg/L alginate solution.

When the TTQ method and direct injection method were carried out using $CaCl_2$ and alginate together, a 300 mL solution of 5,000 mg/L alginate was prepared by adding 1.5 g of alginate to 300 mL of D.I. water for each method.

5.2.10 Statistical Methods

The Data Analysis Tool ANOVA: Single Factor was used for statistical analysis to determine if there was significant change in the TTQ, peak torque and increase in torque values with increasing chemical concentration. The concentrations were placed in one row in Excel and the rheological parameter values (TTQ, peak torque, increase in torque) were placed in the other row. These rows were then selected for ANOVA: Single Factor analysis and the p-value was provided for the data in a summary table.

5.3 Results and Discussion

5.3.1 The effect of total solids (TS)

The TTQ method was used to examine the effect of total solids (TS) on the rheological properties of sludge. The rheograms from anaerobically digested sludges with different TS concentrations are shown in Figure 52A while the TTQ values from those rheograms are shown in Figure 52B, as seen below.

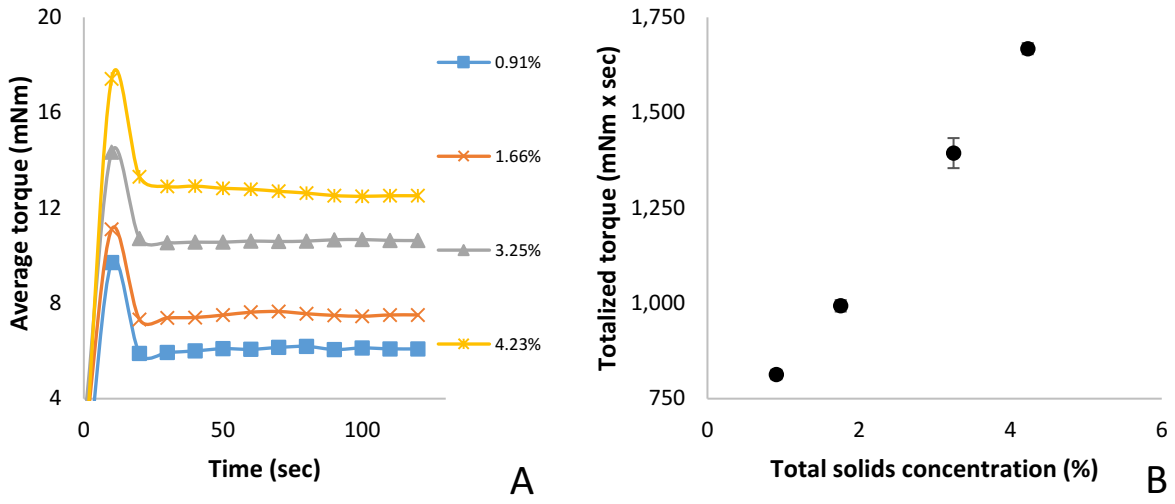


Figure 52. TTQ Method – (A) Rheograms obtained at increasing total solids concentrations. (B) TTQ values for 120 seconds obtained at increasing total solids concentrations.

It is observed from Figure 49 that the TTQ values linearly increase (R^2=1.00) as the TS increases. A greater TS indicates that more solid particles are present in the sludge. As the number of solid particles increases, the torque values measured by the torque rheometer should increase. This is because solutions with higher TS will have greater resistance to the applied shear as they will be more viscous (Eshtiaghi et al., 2013). In addition, a greater number of interparticle interactions occur as the TS increases which can cause larger particles to form (Yeneneh et al., 2016).

5.3.2 The effect of iron

The effect of ferric iron cations on the rheological properties of anaerobically digested sludge was investigated by dosing the sludge with solutions resulting in ferric iron ion concentrations in the sludge ranging from 250 mg/L to 1,250 mg/L, in 250 mg/L increments. These concentrations are higher than ferric ion concentrations typically seen in sludge, which have been measured in the

range of 40 – 340 mg/L, but were chosen because ferric ions are usually added in large quantities during chemical treatment (Park & Novak, 2013).

The TTQ method was used and rheograms for the dosed sludges are seen below in Figure 53A with the TTQ values for 120 seconds from the rheograms shown in Figure 53B.

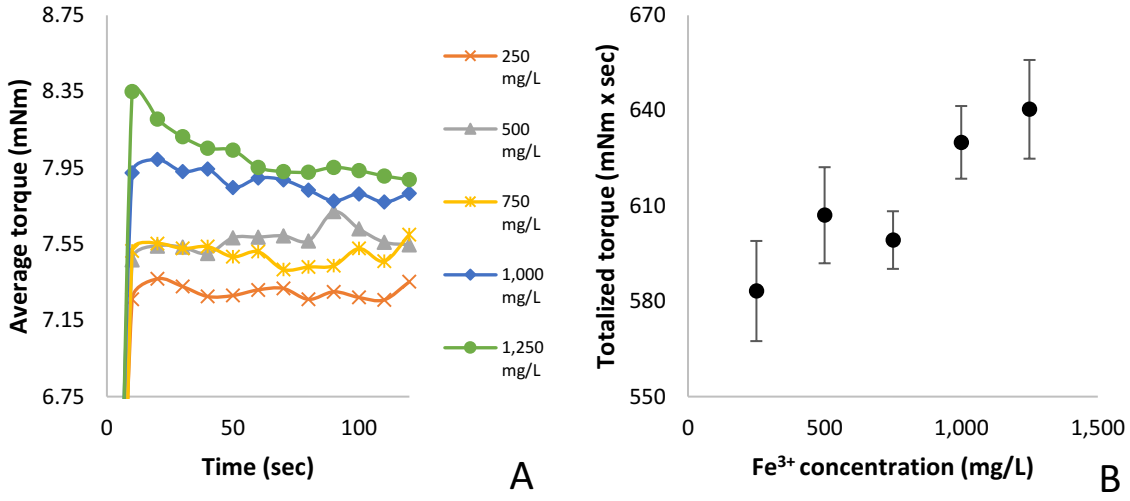


Figure 53. TTQ Method – (A) Rheograms obtained at increasing ferric iron ion concentrations. (B) TTQ values for 120 seconds obtained at increasing iron ion concentrations.

It is observed from both figures that there is a 9.8% increase in TTQ values as the Fe^{+3} ion concentration increases from 250 mg/L to 1,250 mg/L.

The direct injection method was the second method used to study the effects of ferric iron ion concentration on the network strength of the sludge. The rheograms from sludge dosed with varying concentrations of ferric iron ions are shown in Figure 54A. The peak torque values and the maximum increase in torque observed after injection of the ferric iron solution are shown in Figure 54B and Figure 54C, respectively.

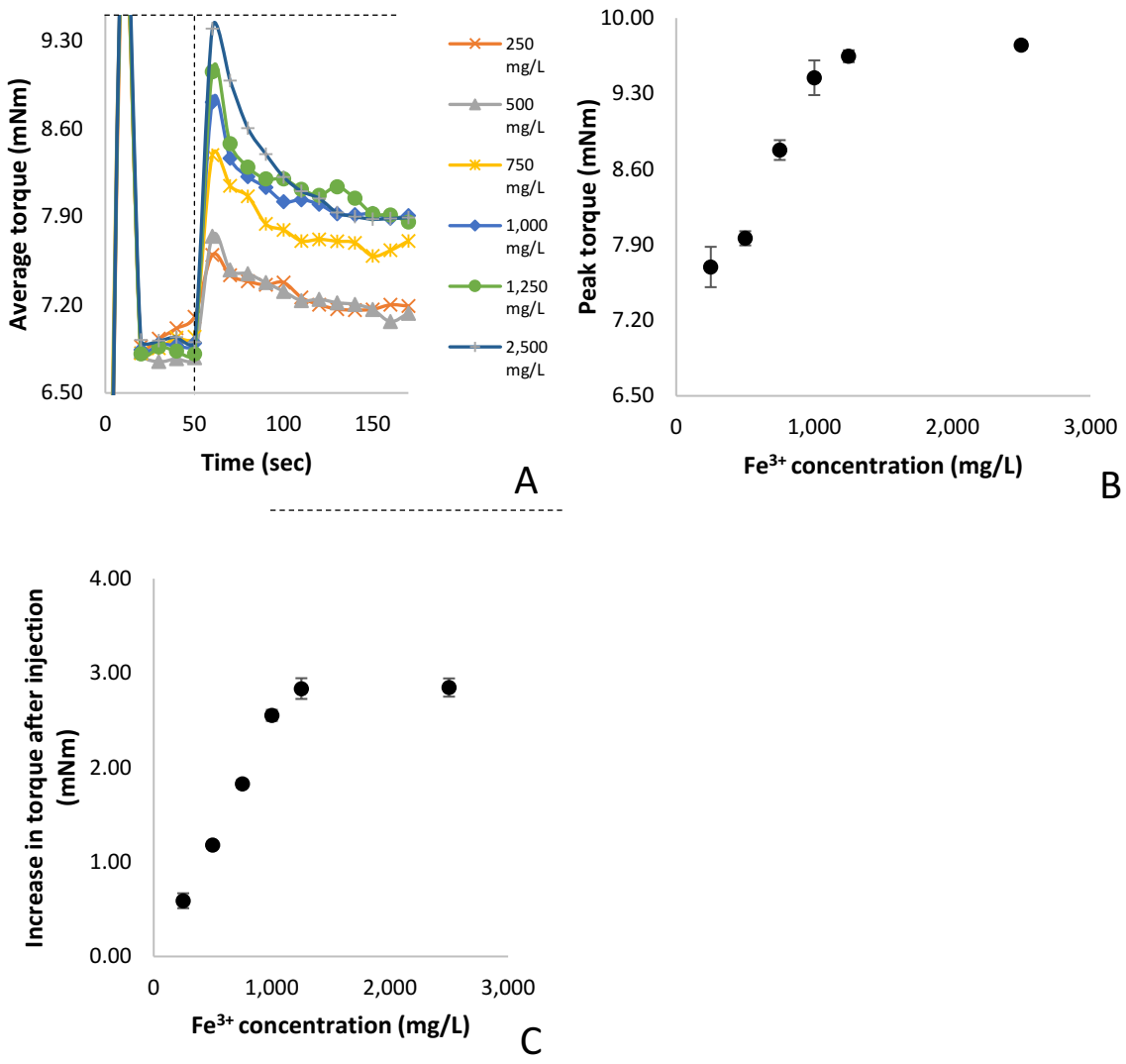


Figure 54. Direct Injection Method – (A) Rheograms obtained at increasing ferric iron ion concentrations. (B) Peak torque values at increasing ferric iron ion concentrations. (C) Increase in torque observed after injection at increasing ferric iron ion concentrations.

It is seen in Figure 54 that there is a similar trend among Figure 54A, B, and C as the peak torque and maximum increase in torque observed after injection become greater with increasing ferric iron ion concentration. As greater amounts of ferric iron ions were injected into the anaerobically digested sludge, larger flocs and a greater sludge network strength was expected as more bonds

between the ESP in the biopolymer matrices could be formed (Park et al., 2010).

The overall sludge floc structure is negatively charged, meaning there are negative adsorption sites for multivalent cations to attach to and form bonds between ESP and negatively charged surfaces (Ormeci & Vesilind, Development of an improved synthetic sludge: A possible surrogate for studying activated sludge dewatering chacteristics, 2000). Since Fe^{+3} is a multivalent cation, as the concentration increases more multivalent cations are present and therefore more bonds between flocs can be formed (Park et al., 2006). This results in larger and stronger flocs being created which will cause a greater resistance to the motion of the impeller in the torque rheometer; therein causing greater TTQ and peak torque values. The influence of ferric iron ions on floc strength and ESP has previously been studied. Park et al. (2006) showed the effect ferric ions had on ESP as they found that WAS with insufficient aluminum and ferric ions had poor binding of biopolymer, which supports the results in Figures 53 and 54.

A similar trend is observed when looking at Figures 53 and 54, which indicates that the results from two methods support each other. This suggests that both the TTQ method and the direct injection method can be used to detect changes in trivalent cations in anaerobically digested sludge.

5.3.3 The effect of magnesium

The anaerobically digested sludge was dosed with Mg^{+2} solutions resulting in Mg^{+2} ion concentrations in the sludge samples ranging from 250 to 1,250 mg/L, in increments of 250 mg/L. These concentrations were chosen as these were similar to the Ca^{+2} ion concentrations used in synthetic sludge (Sanin & Vesilind, 1996).

The TTQ method was first used to determine the effect of the divalent cation concentration on the rheological properties of sludge. The rheograms and the TTQ values generated from these rheograms for the varying Mg^{+2} ion concentrations are seen in Figures 55A and B, respectively.

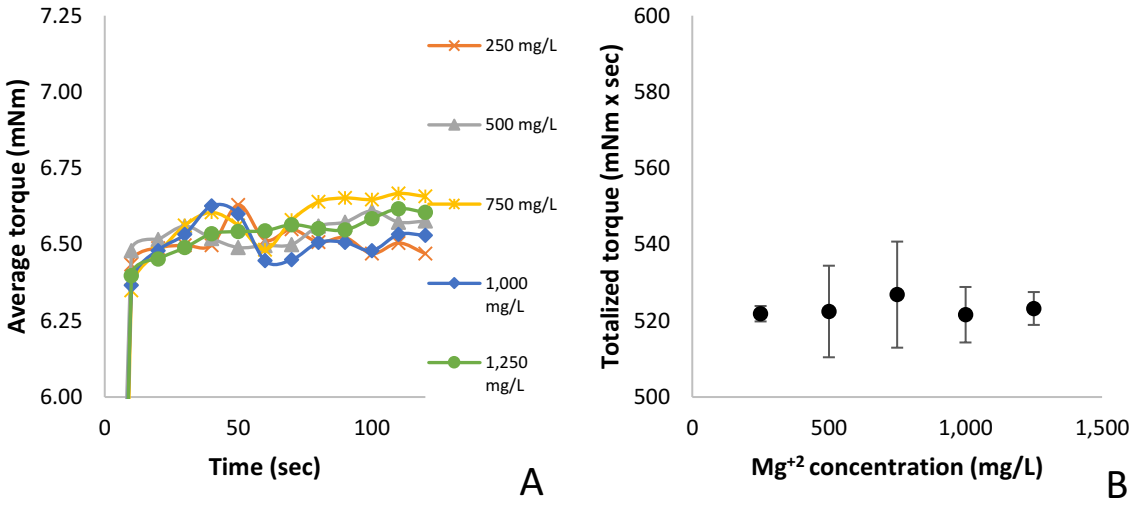


Figure 55. TTQ Method – (A) Rheograms obtained at increasing Mg^{+2} ion concentrations. (B) TTQ values for 120 seconds obtained at increasing Mg^{+2} ion concentrations.

It is observed that the TTQ increases with increasing concentration until 750 mg/L. A 1.0% decrease in TTQ is then observed when the Mg^{+2} concentration increases to 1,000 mg/L, followed by a 0.3% increase as the concentration increases to 1,250 mg/L Mg^{+2}. When error bars were taken into consideration it was apparent that there was no significant variation in the TTQ values (p-value = 0.920) with increasing Mg^{+2} ion concentration.

The direct injection method was then used to examine the effect of Mg^{+2} ion concentration on the rheological properties of the sludge. Based on the inconclusive results from Figure 55, a higher dose of Mg^{+2}, 2,500 mg/L, was added. Rheograms were produced using this method and they are

shown in Figure 56A below, with the peak torque values and the increase in torque observed after injection shown in Figure 53B and C, respectively.

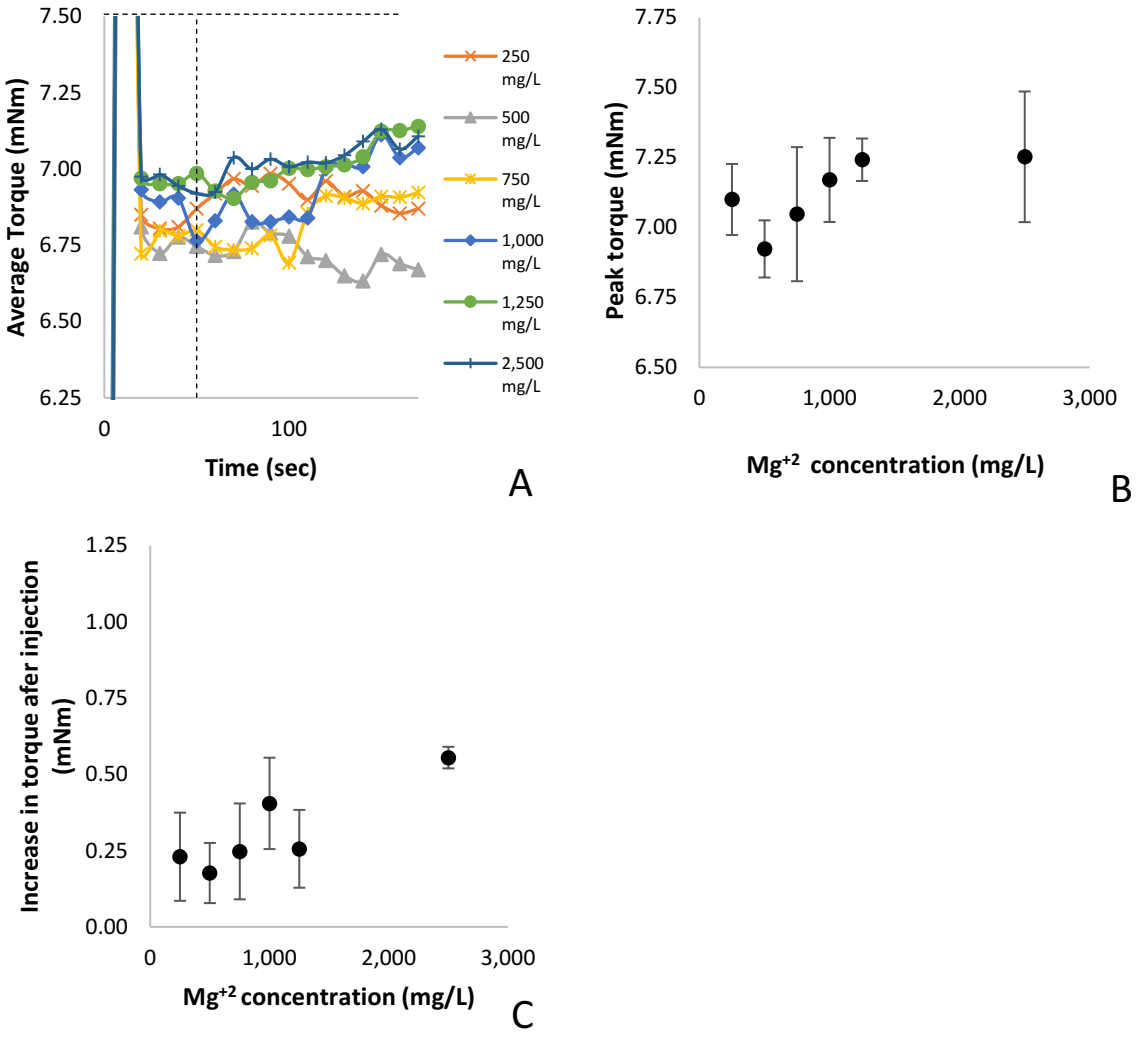


Figure 56. Direct Injection Method – (A) Rheograms obtained at increasing Mg+2 ion concentrations. (B) Peak torque values obtained at increasing Mg+2 ion concentrations. (C) Increase in torque observed after injection at increasing Mg+2 ion concentrations.

In Figure 56B there is a 2.5% decrease in peak torque as the concentration increases from 250 to 500 mg/L, followed by a 4.8% increase in peak torque with increasing Mg^{+2} ion concentration. Figure 56C follows a similar trend, but the drop occurs at 1,250 mg/L, not 500 mg/L. However,

overall there is no significant change in either Figure 56B (p-value = 0.114) or Figure 53C (p-value = 0.501) even when a higher dose of Mg^{+2}, 2.500 mg/L, is used.

This slight difference between the trends of Figures 56B and C is associated with the variation in baseline values prior to injection for the different samples. It is difficult to obtain consistent baseline values prior to injection since each sample of anaerobically digested sludge withdrawn is going to have slightly different characteristics and solids concentration and therefore different rheological properties. To compensate for this variation in baseline values Figure 56C was produced. It looks at the maximum increase in torque observed after injection which is a better representation of the rheological properties of sludge compared to peak torque value as it eliminates the effect of the variability in baseline torque values.

In the results for the concentrations between 250 and 1,250 mg/L for the TTQ method and direct injection method little change is observed in the values. It was expected that as the Mg^{+2} ion concentration increased the network strength would increase as well as TTQ and peak torque values. As the Mg^{+2} ion concentration increases more divalent cations are present within the sludge solution which should enable larger and stronger flocs to be formed. This is because the divalent cations bridge negatively charged functional groups within the biopolymer matrix such as hydroxyl and carboxyl groups (Higgins et al., 2004; Sobeck & Higgins, 2002; Higgins & Novak, 1997A). This would then increase the network strength of the sludge, resulting in increasing TTQ and peak torque values, which was not observed (Ormeci & Abu-Orf, 2005).

The concentrations of Mg^{+2} ions in the anaerobically digested sludge in these experiments ranged from 250-2,500 mg/L. These concentrations are greater than the typical range of concentrations of Mg^{+2} ions found in anaerobically digested sludge, which have been measured in the range of 180 mg/L to 310 mg/L (Antony & Murugavelh, 2018; Fyfe et al., 2016; El-Nahhal et al., 2014;

Gebreeyessus & Jenicek, 2016). Since these concentrations are higher than typical values, flocs within the sludge could already be saturated with divalent cations. This would mean that the presence of new Mg^{+2} ions would not affect the floc structure without the presence of additional ESP.

5.3.4 The effect of calcium

Calcium was the second divalent cation which was used to examine the effect of cations on the rheological properties of sludge. The dosed anaerobic sludge had Ca^{+2} ion concentrations ranging from 250 mg/L to 1,250 mg/L, in increments of 250 mg/L. This concentration range was based on literature as synthetic sludge had Ca^{+2} ion concentrations in this range (Sanin & Vesilind, 1996). The TTQ method was the first method used to study the impact of Ca^{+2} ions on the rheology of sludge. In Figures 57A and B shown below, the rheograms from the TTQ method for sludge dosed with varying concentrations of Ca^{+2} ions and the TTQ values from those rheograms are presented.

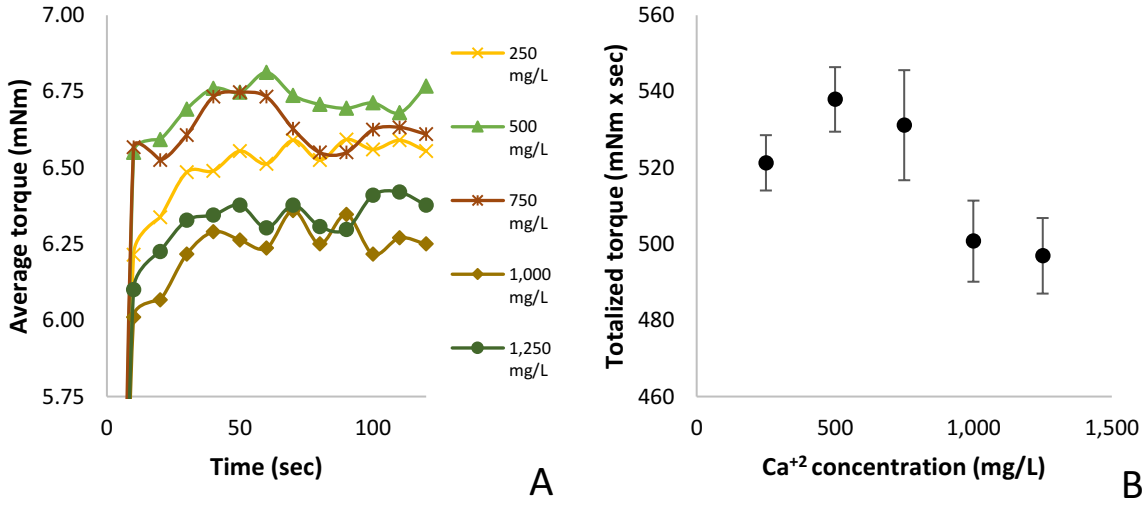
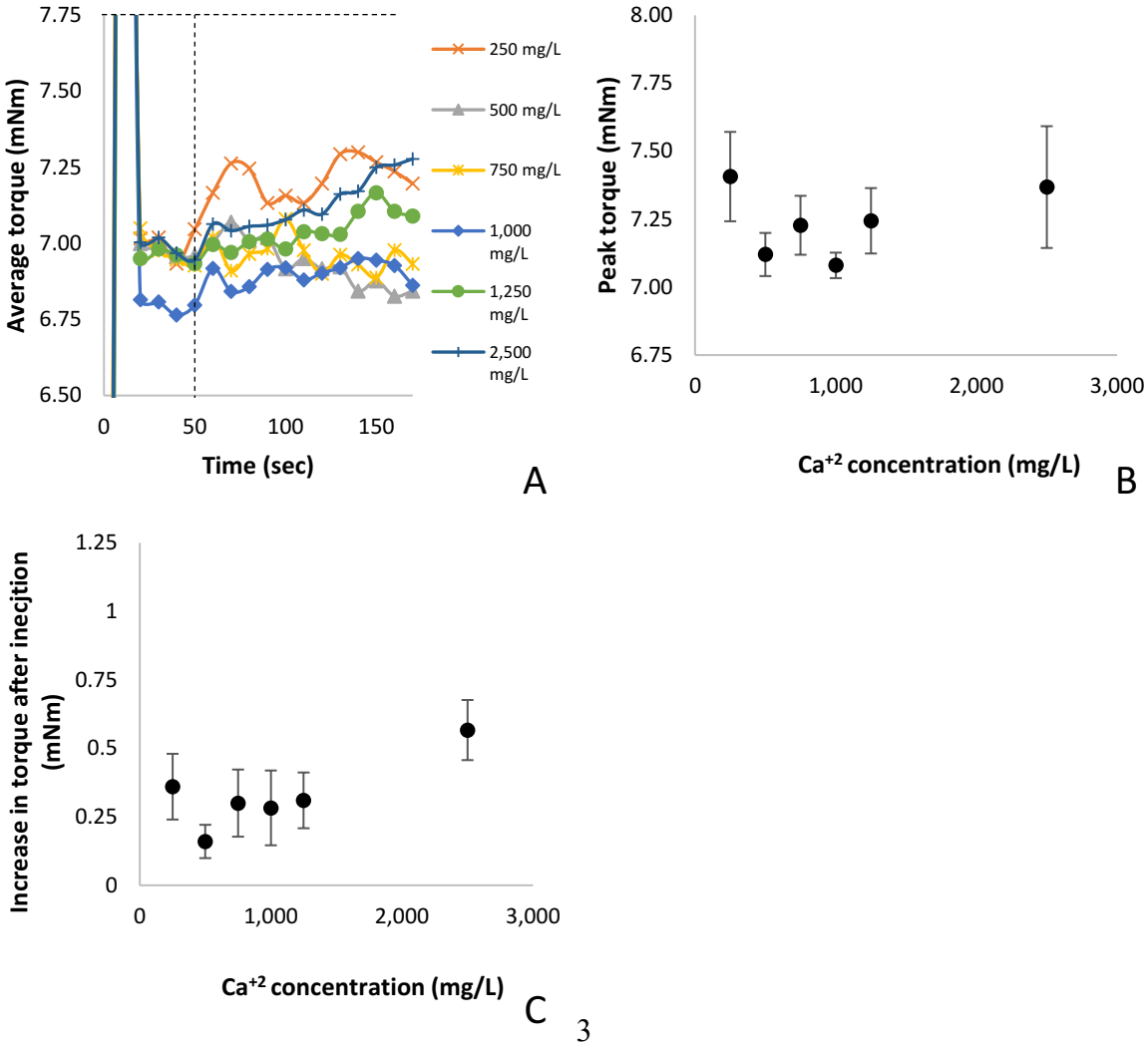


Figure 57. TTQ Method - (A) Rheograms obtained at increasing Ca+2 ion concentrations. (B) TTQ values for 120 seconds obtained at increasing Ca+2 ion concentrations.

It is observed from Figures 57A and B that there is a 3.2% increase in TTQ as the Ca^{+2} ion concentration increases from 250 to 500 mg/L followed by a 7.6% decrease in TTQ as the Ca^{+2} ion concentration increases. Overall there is significant variation (p-value = 0.000555) in TTQ values with increasing Ca^{+2} ion concentration.

Secondly, the direct injection method was used to study how the rheology of sludge was affected by dosing with varying concentrations of Ca^{+2} ions. The rheograms produced for the direct injection method are shown below in Figure 58A. From these rheograms peak torque values and the increase in torque observed after injection were determined and are presented below in Figures 58B and C.



3

Figure 58. Direct Injection Method - (A) Rheograms obtained at increasing Ca+2 ion concentrations. (B) Peak torque values obtained at increasing Ca+2 ion concentrations. (C) Increase in torque observed after injection at increasing Ca+2 ion concentrations.

Similar to Figure 56B and C, there are differences between Figure 58B and C due to varying baseline torque values prior to injection for the samples. The peak torque values in Figure 58B gradually decrease and then increase with increasing Ca^{+2} ion concentration; however, there is an overall 0.5% decrease in peak torque values with increasing Ca^{+2} ion concentration (p-value = 0.0363). In Figure 55C there is a 13.4% decrease in torque after injection up to a Ca^{+2} ion

concentration of 1,250 mg/L. A 83.9% increase in torque after injection is then observed for the sample having a concentration of 2.500 mg/L. However, overall there is no significant variation (p-value = 0.268) in TTQ values with increasing Ca^{+2} ion concentration

The results for Ca^{+2} ions using the both methods are similar to those found for Mg^{+2} ions as no significant changes in the rheological properties of the sludge were observed with increasing Ca^{+2} ion concentration. It appears the TTQ values decrease with increasing Ca^{+2} ion concentration in Figure 57B, but there are large error bars associated with the data points in Figure 57B which makes it difficult to draw conclusions from the figure. Ca^{+2} ion concentrations in digested sludge have been found in the broad range of approximately 270 to 3,000 mg/L and were expected to produce similar results to Mg^{+2} ions as previous research has found that calcium and magnesium ions have the same effect on the activated sludge floc strength when added separately (Fyfe et al, 2016; Gebreeyessus & Jenicek, 2016; El-Nahhal et al., 2014; Higgins et al., 2004; Sobeck & Higgins, 2002).

Also, similar to Mg^{+2} ions, as the Ca^{+2} ion concentration increased the flocs within the sludge were expected to become larger as the divalent cations formed bridges between ESP present in the sludge (Nguyen et al., 2008; Ormeci & Vesilind, 2000; Murthy et al., 1998). This would result in greater network strength and larger values for the TTQ and peak torque caused by the greater resistance to the applied shear (Ormeci & Abu-Orf, 2005).

Sanin and Vesilind (1996) created synthetic sludge using polystyrene latex particles of similar size to bacteria, alginate to represent microbial ESP and Ca^{+2} ions to aid the bridging of the ESP. When they formed synthetic sludge, they found that as they increased their Ca^{+2} ion concentration from 200 to 1,000 mg/L the dewatering properties improved, which is indicative of an increase in floc strength (Sanin & Vesilind, 1996). Even though the floc strength was improved in their

experiments, the dosed anaerobically digested sludge samples used in these experiments had similar concentrations (250-2,500 mg/L) and little changes in the rheological properties were observed. The lack of changes in the rheological properties of the anaerobically digested sludge when dosed with similar concentrations to synthetic sludge could be associated with the anaerobically digested sludge having many constituents in it compared to the synthetic sludge. With many other constituents, the cations are tied in competing reactions which causes the addition of the cations to have little effect on the rheological properties of the sludge.

Both divalent cations by themselves had little effect on the rheological properties of the sludge at the concentrations they were dosed. Not sufficient ESP for the divalent cations to form bonds with is another possibility as to why little change in the rheological properties was observed.

5.3.5 The effect of alginate

Alginate was used to examine the effect of ESP on the rheological properties of anaerobically digested sludge. For the TTQ method, the sludge samples had alginate concentrations ranging from 50 mg to 1,250 mg/L, in increments of 50 mg/L from 50 mg/L to 250 mg/L, then 500 mg/L and 1,250 mg/L. Concentrations from 50 mg/L to 250 mg/L were initially used as this alginate concentration range has been found in synthetic sludge (Sanin & Vesilind, 1996). Sanin & Vesilind (1996) chose these alginate concentrations to simulate a sludge with excess extracellular polymers. After similar results for the increasing alginate concentrations up to 250 mg/L, concentrations of 500 mg/L and 1,250 mg/L were used. Given the lack of variation in results at the lower concentrations (<250 mg/L), for the direct injection method, alginate concentrations ranged from 250 mg/L to 2,500 mg/L, increasing in increments of 250 mg/L up to 1,250 mg/L.

First, the TTQ method was used to study the impact varying doses of alginate had on the rheological properties of anaerobically digested sludge. Rheograms were generated and the TTQ values for 120 seconds from these rheograms are presented below in Figures 59A and B.

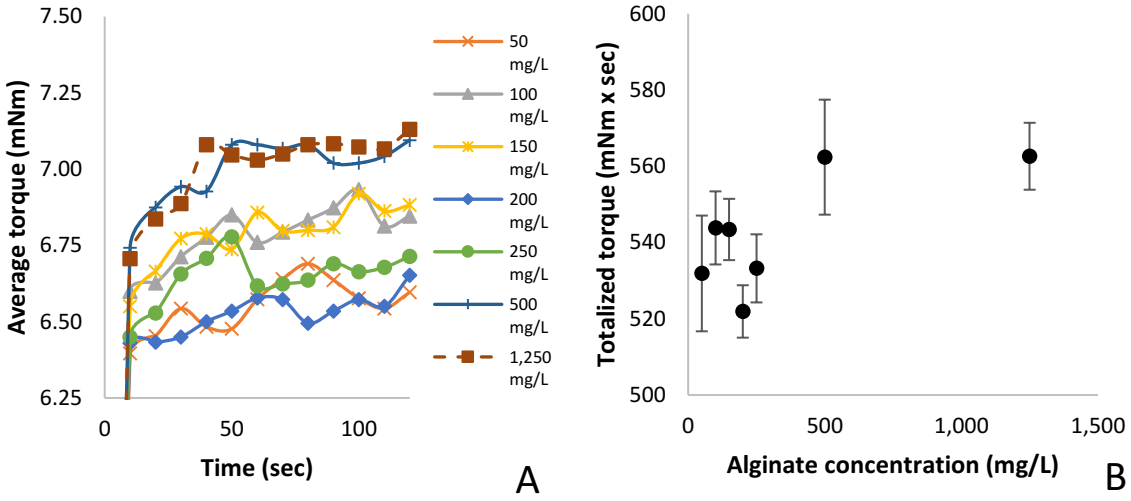


Figure 59. TTQ Method – (A) Rheograms obtained at increasing alginate concentrations. (B) TTQ values for 120 seconds obtained at increasing alginate concentrations.

From Figure 59A and B there is an overall 5.8% increase in TTQ with increasing alginate concentration. There is some fluctuation in the TTQ values between 50 and 250 mg/L, but this is likely due to the concentrations being similar and in a close range.

The direct injection method was used next to see how alginate affected the peak torque values and the increase in torque after injection. In Figure 60A shown below the rheograms for sludge dosed with increasing concentrations are presented. From Figure 60A, Figures 60B and C were produced which show the peak torque and increase in torque observed after injection, respectively.

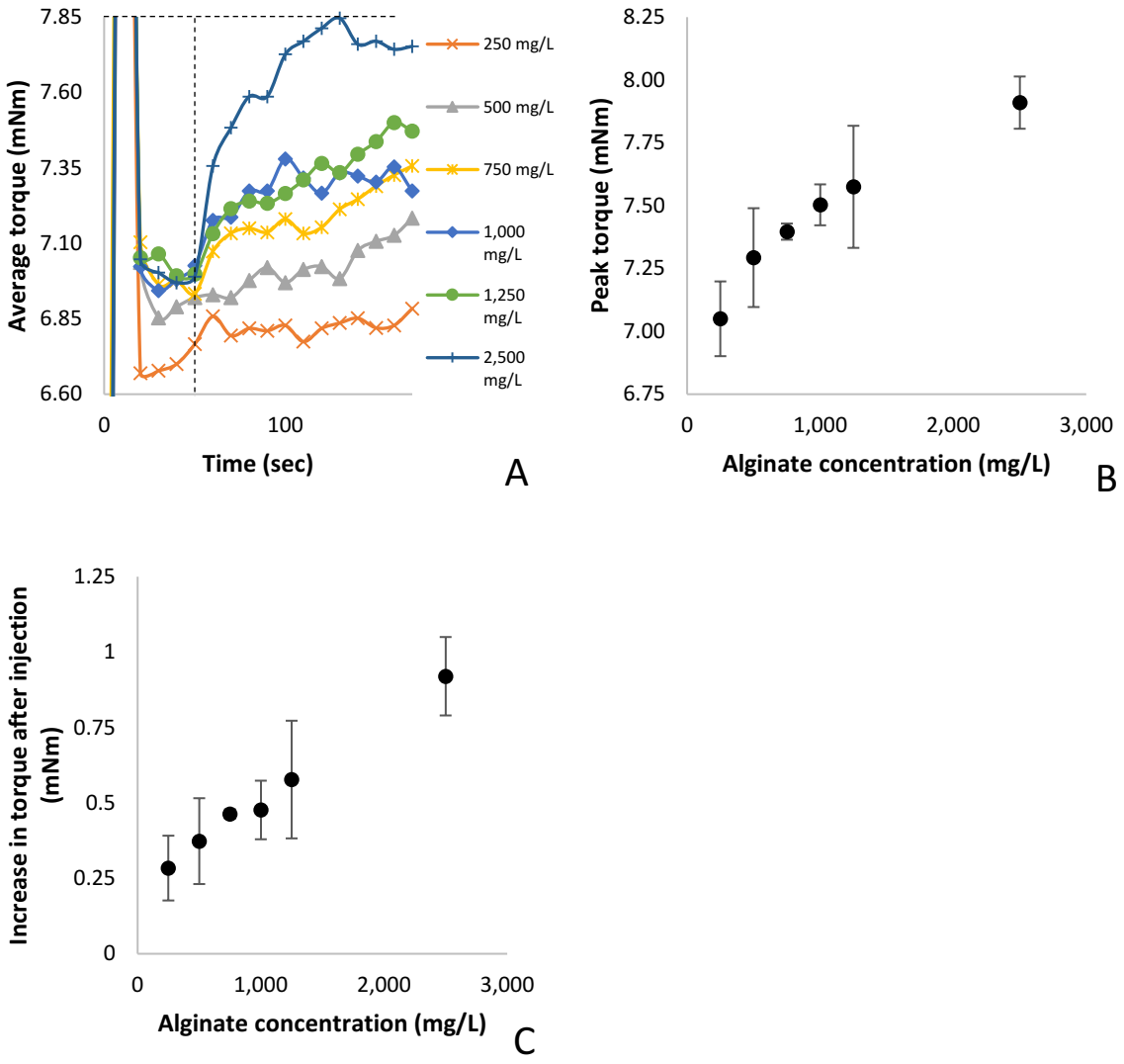


Figure 60. Direct Injection Method – (A) Rheograms obtained at increasing alginate concentrations. (B) Peak torque values obtained at increasing alginate concentrations. (C) Increase in torque observed after injection at increasing alginate concentrations.

From Figures 60A, B and C it is noted that there is a linear increase ($R^2 = 0.93$ for Figure 60B and $R^2 = 0.99$ for Figure 60C) in torque values as the alginate concentration increases when using the direct injection method. Even though the baseline torque values are not consistent in Figure 60A, Figures 60B and C show a very similar pattern.

Results from Figures 59 and 60 show similar trends as there is an increase in all the rheological parameters (TTQ, peak torque and increase in torque after injection) as the alginate concentration increases. This was anticipated to occur because alginate acts as an ESP, which has negatively charged functional groups that are bridged with divalent cations (Nguyen et al., 2008). Having a greater amount of ESP present allows for more flocs to be formed as it provides additional ESP to create matrices with the microbes present in the sludge (Sobeck & Higgins, 2002; Higgins & Novak, 1997C). With available divalent cations in the sludge, more bridges between negative sites on the ESP can be formed which stabilizes the matrix of biopolymers and bacteria and increases the strength of the sludge flocs, therein increasing the TTQ and peak torque (Sobeck & Higgins, 2002).

ESP is composed of carbohydrates, proteins, and lipids and the biodegradable portion is largely consumed during anaerobic digestion of the sludge. There seemed to be no significant effect on the TTQ or peak torque values when the divalent cations, calcium and magnesium, were added. However, when ESP were added, there was an increase in the TTQ and peak torque values since there was not likely sufficient ESP in digested sludge. By increasing the amount of biopolymers, new matrices of biopolymers with microorganisms are formed, which can then be bridged together with available cations, therein increasing the strength of the sludge as seen in Figures 56 and 57 (Nguyen et al., 2008).

5.3.6 The effect of calcium and alginate

Lastly, a combination of Ca^{+2} ions and alginate were added to observe the effect divalent cations and ESP have on the rheological properties of sludge when they are added together. Sludge samples were predosed at a fixed alginate concentration of 250 mg/L and calcium concentrations

ranging from 250 mg/L to 1,250 mg/L, in increments of 250 mg/L. These concentrations were chosen based off literature for synthetic sludge (Sanin & Vesilind, 1996). The TTQ method was first used and the rheograms are shown below in Figure 58A with the TTQ values for 120 seconds from those rheograms shown in Figure 61B, below.

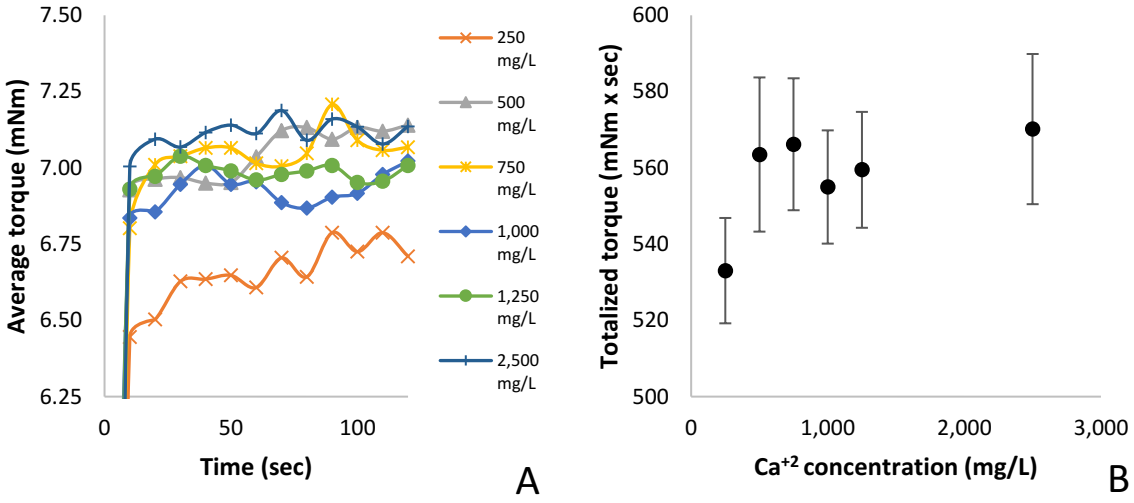


Figure 61. TTQ Method – (A) Rheograms obtained at a constant alginate concentration of 250 mg/L and increasing Ca^{+2} ion concentrations. (B) TTQ values for 120 seconds obtained at a constant alginate concentration of 250 mg/L and increasing Ca^{+2} ion concentrations.

From Figure 61A and B it is observed that there is a 6.2% increase in TTQ from 250 mg/L to 750 mg/L of Ca^{+2} ions, but after that there is only a 0.7% increase observed in the TTQ values as the Ca^{+2} ion concentration increased. When all Ca^{+2} ion concentration data points are compared, there does not appear to be a significant variation in TTQ values with increasing Ca^{+2} ion concentration (p-value = 0.060); however, it should be noted that there is a significant increase (p-value = 0.037) in TTQ when the Ca^{+2} ion concentration is increased from 250 mg/L to 500 mg/L. The jump in TTQ from 250 mg/L to 500 mg/L of Ca^{+2} ions may indicate that when there is free biopolymer

available in sludge, addition of Ca^{+2} ions increases sludge strength until all available sites are saturated with Ca^{+2} ions. Beyond this point, adding more Ca^{+2} ions does not result in further improvements in sludge strength.

The direct injection method was used next. Rheograms were produced for sludge which was simultaneously dosed with a fixed concentration (250 mg/L) of alginate and varying concentrations of Ca^{+2} ions in Figure 62A. Figures 62B and C show the peak torque values and increase observed in torque after injection from the rheograms.

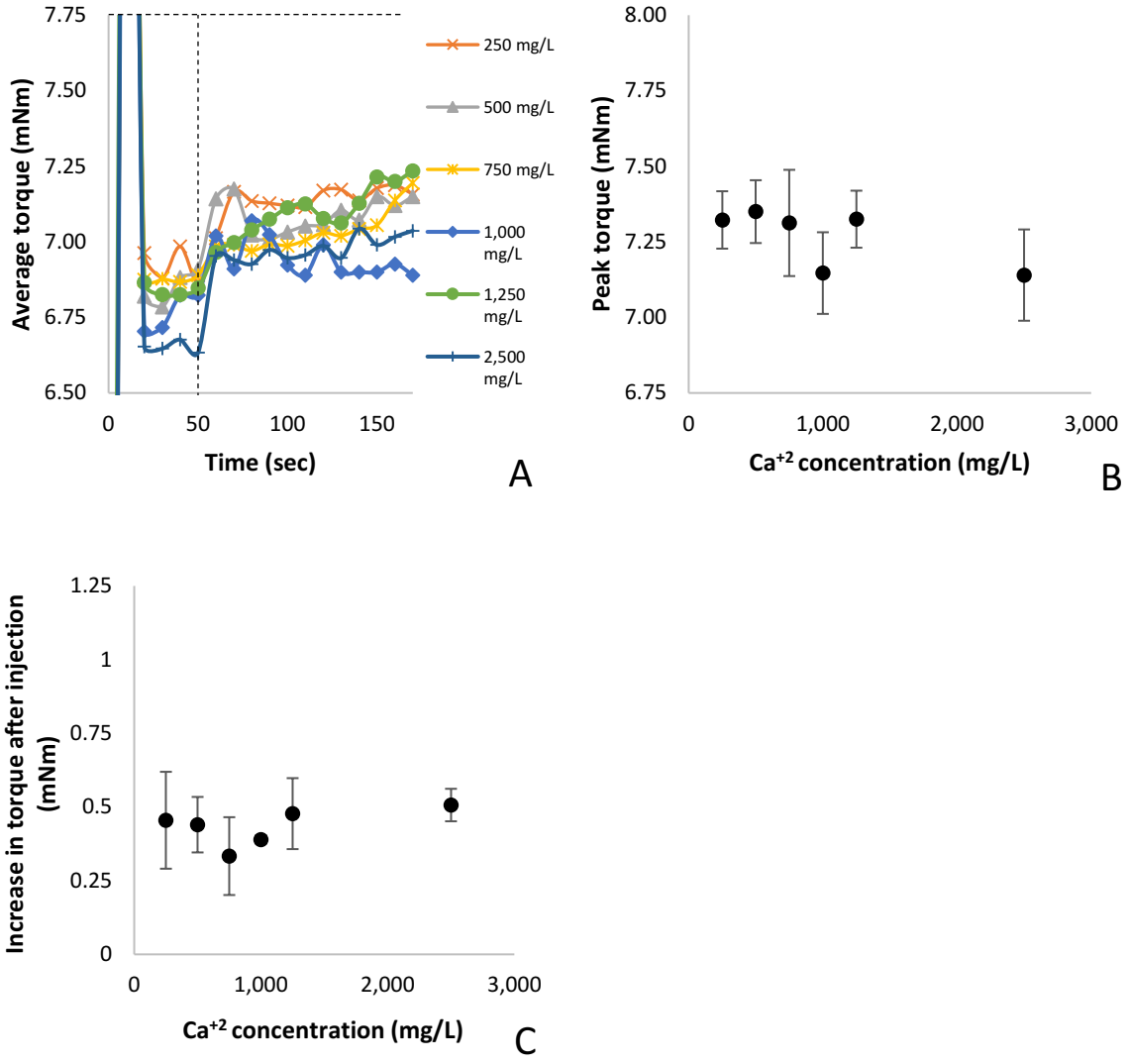


Figure 62. Direct Injection Method – (A) Rheograms obtained at a constant alginate concentration of 250 mg/L and increasing Ca^{+2} ion concentrations. (B) Peak torque values obtained at a constant alginate concentration of 250 mg/L and increasing Ca^{+2} ion concentrations.

The only significant difference between Figures 62B and C is the 2,500 mg/L data point, but this is attributed to its lower baseline torque value as seen in Figure 62A. Figure 62B shows no significant variation (p-value = 0.159) in peak torque values with increasing Ca^{+2} ion concentration

and Figure 62C also shows no significant variation (p-value = 0.532) in increase in torque observed after injection values with increasing Ca^{+2} ion concentration.

The TTQ method provided a better response compared to the direct injection method when adding the two chemicals. This could be attributed to the simultaneous direct injection of Ca^{+2} ions and alginate for the direct injection method. Sequential injection of the chemicals could result in a better response in TTQ and peak torque values with increasing Ca^{+2} ion concentration because if the alginate was added first, as it was for the TTQ method, it would form networks of biopolymers and bacteria and then adding the Ca^{+2} ions second would allow these networks to be bridged together, increasing the overall strength of the sludge.

Based on the experimental results obtained above, the impact of each chemical on sludge network strength was evaluated by comparing the change in TTQ per dose of chemical added.

Table 5. TTQ values in mNm x sec of cations and biopolymers into anaerobically digested sludge for concentrations from 250 to 1,250 mg/L.

Cation/Biopolymer	**Concentration (mg/L)**					**% Increase from 250 mg/L to 1,250 mg/L (%)**
	250	**500**	**750**	**1,000**	**1,250**	
Fe^{+3}	583.23	607.08	599.27	630.01	640.41	9.80
Mg^{+2}	521.85	522.43	526.85	521.58	523.22	0.26
Ca^{+2}	521.30	537.92	531.17	500.75	496.94	-4.67
Alginate	533.32	562.38			562.62	5.49
Ca^{+2} and alginate	533.04	563.49	566.18	554.97	559.47	4.96

It is seen in Table 5 above that Fe^{+3} had the greatest percent increase in TTQ from 250 to 1,250 mg/L. Dosing sludge with trivalent cations causes greater floc stability compared to divalent cations due to the presence of the extra cations (Park et al., 2006). The extra cation of the ferric iron allows more bonds to be formed between the ESP in the biopolymer matrices in the sludge when injected with ferric iron compared to divalent cations such as calcium and magnesium. More bonds being formed results in the sludge having a greater network strength, resulting in greater TTQ values with increasing concentration.. Alginate had the second greatest percent increase followed by calcium and alginate and then the individual divalent cations in Table 5.

In Table 6, the results from the direct injection method are summarized as the increase in torque observed after injection for the various samples are shown.

Table 6. Increase in torque in mNm after direct injection of cations and biopolymers into anaerobically digested sludge.

Cation/Biopolymer	**Concentration (mg/L)**						**% Increase from 250 mg/L to 2,500 mg/L (%)**
	250	**500**	**750**	**1,000**	**1,250**	**2,500**	
Fe^{+3}	0.59	1.18	1.83	2.55	2.84	2.85	383.05
Mg^{+2}	0.23	0.18	0.25	0.41	0.26	0.56	143.48
Ca^{+2}	0.36	0.16	0.30	0.28	0.31	0.57	58.33
Alginate	0.28	0.37	0.46	0.48	0.58	0.92	228.57
Ca^{+2}/alginate	0.46	0.44	0.33	0.39	0.48	0.51	10.87

Similar to Table 5, Fe^{+3} has the greatest percent increase from 250 to 2,500 mg/L with alginate being second. However, in Table 6, Ca^{+2} ions and alginate has the lowest percent increase compared to the individual divalent cations, which was likely because they were dosed simultaneously and not sequentially.

For both methods, dosing the sludge with Mg^{+2} and Ca^{+2} ions resulted in insignificant variation in rheological parameters (TTQ, peak torque, increase in torque observed after injection) as the concentration increased.

5.3.7 Microscopy

Microscopy analysis of sludge structure was carried out in addition to the rheological tests. This was done to assess the effect of dosed chemicals on the size and structure of the flocs. The same concentrations of divalent and trivalent cations were used during microscopy analysis to examine the effect that the additional cation ion had on the average floc area when similar concentrations were used. The microscope images for 3 different views of the same sludge sample with no treatment are shown below in Figure 63.

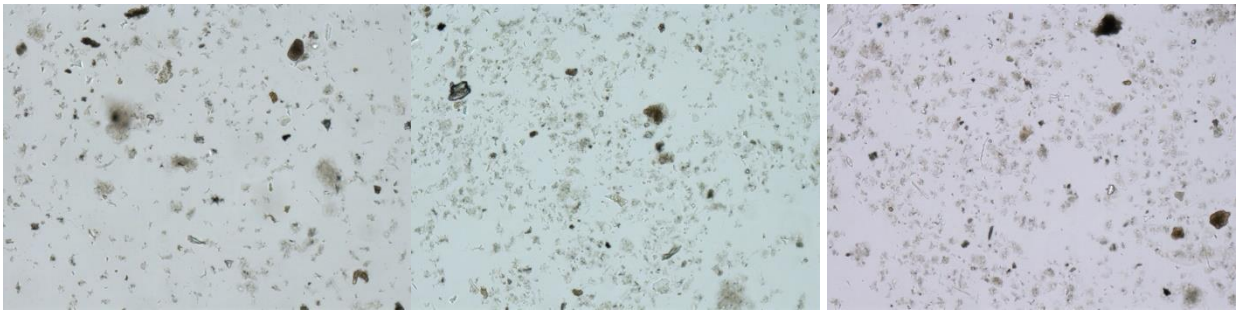


Figure 63. Microscope images for sludge with no treatment.

The microscope images for 3 different views of the same sludge sample with a ferric iron ion concentration of 2,500 mg/L are shown in Figure 64 below.

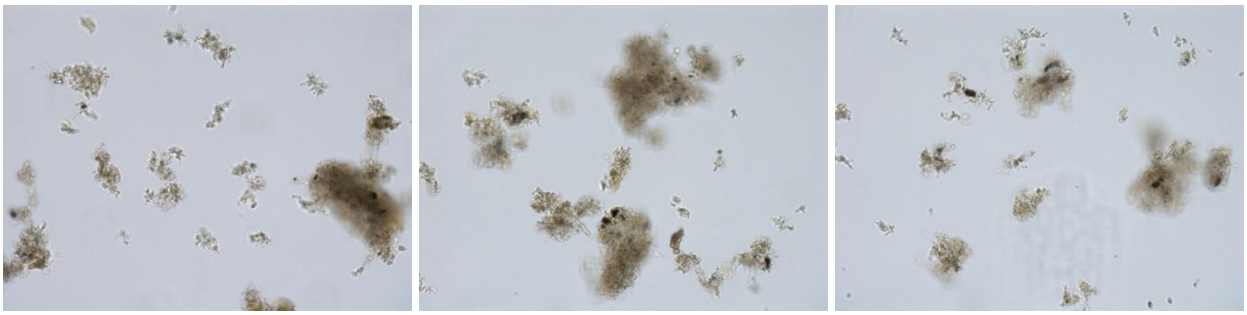


Figure 64. Microscope images for sludge with ferric iron ion concentration of 2,500 mg/L.

The microscope images for 3 different views of the same sludge sample with a Ca^{+2} ion concentration of 2,500 mg/L are shown in Figure 65.

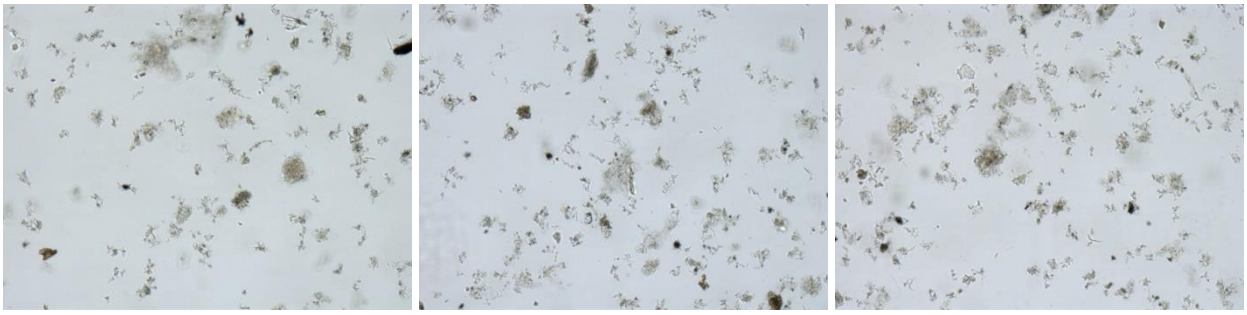


Figure 65. Microscope images for sludge with a Ca+2 ion concentration of 2,500 mg/L.

The microscope images for 3 different views of the same sludge sample with a Ca^{+2} ion concentration of 2,500 mg/L and an alginate concentration of 250 mg/L are shown in Figure 66. The calcium and alginate were sequentially added, with ther alginate being added first.

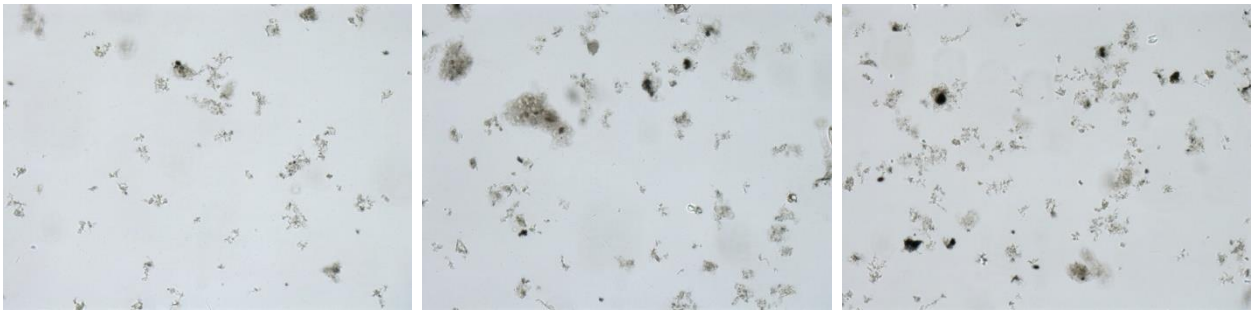


Figure 66. Microscope images for sludge with a Ca^{+2} ion concentration of 2,500 mg/L and an alginate concentration of 250 mg/L.

Floc areas from these microscope images were then calculated and an average floc area was determined. Below in Table 7 the average area calculated for flocs from anaerobically digested sludge dosed with chemical solutions are shown.

Table 7. Effect of cations and biopolymers on average area of anaerobically digested sludge flocs.

Cation/Biopolymers	**Concentration (mg/L)**	**Average Floc Area (μm^2)**
No treatment	N/A	116.31
Fe^{+3}	2,500	2,499.24
Ca^{+2}	2,500	210.65
Ca^{+2}/alginate	2,500/250	264.80

It is observed from Table 7 that the anaerobically sludge that had no chemical added to it had flocs with the smallest average area. The sludge dosed only with divalent cations has the second smallest area followed by the sludge sample dosed with alginate and Ca^{+2} ions. Dosing the sludge with

ferric iron ions resulted in the greatest average floc area. This is attributed to the higher charge valence which results in greater floc stability (Park et al., 2006).

Below in Table 8 a comparison between the average floc area and the corresponding increase in torque after injection of chemicals using the direct injection method is shown.

Table 8. Comparison between floc area and increase in torque after injection of chemicals.

Cation/Biopolymer	**Concentration (mg/L)**	**Average Floc Area (μm^2)**	**Increase in torque after injection (mNm)**
Fe^{+3}	2,500	2,499.24	2.85
Ca^{+2}	2,500	210.65	0.57
Ca^{+2}/alginate	2,500/250	264.80	0.51

It is seen from Table 8 that similar trends for results were found for the average floc area and the rheological properties of the sludge. Ferric iron ions had a much greater floc area and increase in torque after injection compared to the sludge dosed with Ca^{+2} ions and the sludge dosed with Ca^{+2} ions and alginate.

5.4 Conclusion

In this study, the TTQ method and the direct injection method were used to see if changes in sludge characteristics, such as cation and biopolymer concentrations and solids content could be detected by rheological measurements.

Variations in total solids concentration was easily detected using the TTQ method and there was a linear increase (R^2=1.00) in TTQ values with increasing total solids concentration. This is because solutions with higher total solids concentration have a greater number of particles and a higher resistance to the applied shear from the torque rheometer, resulting in higher torque values.

It was found that changes in ferric iron ion concentration could be detected by rheology as both TTQ and peak torque values increased with increasing ferric iron ion concentration. Ferric iron is a trivalent cation and strongly aids coagulation and flocculation processes through charge neutralization of particles.

Significant changes in rheological properties were not observed when the divalent cations (Ca^{+2} and Mg^{+2}) were added to the sludge at the concentrations used in this study as TTQ and peak torque values did not significantly increase with increasing divalent cation concentration. ESP is organic matter and is consumed during anaerobic digestion of sludge. There may not be sufficient amount of ESP available in anaerobically digested sludge for the dosed divalent cations to form bridges between, resulting in no significant change in sludge network strength. In addition, divalent cations added to the sludge may also be consumed in competing reactions.

Alginate was added to the sludge to examine whether changes in biopolymer concentration can be detected with rheological measurements. There was an increase in TTQ values and peak torque values as the alginate concentration increased. Alginate, which is an ESP produced by sludge bacteria, has negatively charged functional groups that are bridged with divalent cations. As the ESP concentration increases, stronger and higher number of flocs are formed.

The addition of cations and biopolymers together was investigated by adding Ca^{+2} ions and alginate to sludge. It was found that for both the TTQ and direct injection methods, no significant changes in rheological properties were observed. The TTQ method provided a better response

compared to the direct injection method because in the direct injection method Ca^{+2} ions and alginate were injected simultaneously while for the TTQ method the chemicals were added sequentially. Sequential injection provides a better response because when the alginate is added first it forms the networks of biopolymers which can then be bridged together by the addition of divalent cations.

When comparing the TTQ and direct injection methods, it was concluded that the direct injection method is more sensitive due to the greater percent increase in peak torque values compared to TTQ values for all chemicals.

Sludge dosed with ferric iron ions had the greatest average floc area while the anaerobically digested sludge that was not dosed had the smallest average floc area. Ferric iron was expected to have the greatest average floc area as it is a trivalent cation which results in greater floc stability and strength.

Addition of ferric iron ions also resulted in a greater increase in torque and totalized torque compared to other divalent cations and alginate.

Chapter 6 – Conclusions

Sludge treatment and management is a growing issue for cities around the world that must be dealt with. There is a reliance on manual control for the operation of sludge treatment processes which is far from ideal. Therefore, there is a need for new approaches to optimize the operation of sludge treatment processes as there are currently very few real-time monitoring tools and technologies for sludge treatment. This research aimed at determining a common practice that can be used for in-line and real-time characterization and monitoring by using ultraviolet/visible (UV/Vis) spectrophotometry and rheology.

The first part of the research investigated the use of UV/Vis spectrophotometry and rheology to monitor the level of treatment and performance during aerobic digestion of normal strength sludge, representing sludge from daily operations, and high strength sludge, representing sludge with a high organic loading rate. The normal strength sludge seemed to approach completion near day 10 as VS and total COD, two common parameters used to monitor sludge treatment, began to stabilize near this day due to limited biodegradable OM remaining in the sludge. A VSR and total COD removal of 43% an 39% were observed respectively. Treatment of the high strength sludge appeared to approach completion around 14 days as VS and total COD measurements began to stabilize. A VSR of 37% and a total COD removal of 46% were observed throughout the aerobic treatment. Filtrate absorbance measurements at 190 nm were found to stabilize around the time that aerobic treatment neared completion. This shows that filtrate absorbance measurements at 190 nm have the potential to be used as an in-line and real-time sludge treatment monitoring tool.

To confirm that biopolymers were released into solution, the quantity of two main biopolymers, carbohydrates and proteins, in the filtrate were measured throughout the process. Filtrate protein

and carbohydrate measurements followed similar trends to the filtrate absorbance values at 190 nm.

Similar trends were found between the effluent absorbance at 254 nm, effluent COD, and effluent turbidity. Since centrifugation is not a completely efficient process, suspended particles can remain in the effluent solution, causing the turbidity to impact the absorbance based COD measurements, resulting in an inaccurate representation of the level of treatment of sludge. Even though effluent absorbance measurements are not the most ideal monitoring tool, it is concluded they can still give an approximate indication of the level of treatment.

Regression analysis was carried out for the normal and high strength effluent with effluent COD and VS and the filtrate with sCOD and VS using the MLR and PCR regression methods. Accurate regression equations were developed to predict effluent COD, sCOD and VS using effluent and filtrate absorbance wavelength ranges focusing around 190 nm and 254 nm. In general, regression equations using filtrate data were more accurate at predicting sludge stabilization parameters which could be attributed to the particle effect. This shows that the sludge parameters VS, effluent COD, and sCOD have the potential to be predicted during aerobic digestion of sludge in real-time and in-line using effluent and filtrate absorbance focusing around 190 nm and 254 nm using the PCR regression method.

Rheological measurements were taken to examine their potential use as a monitoring tool for sludge treatment. It was concluded that the Floccky Tester was not sensitive enough to measure the rheological changes that occur during aerobic treatment of sludge as there were no significant changes in TTQ or peak height values after day 3 throughout the sludge treatment. Microscopy images were also taken to assess the impact that aerobic digestion has on the floc structure. The microscopy data confirmed the gradual disruption of the sludge network and the inability of the

totalized torque measurements to pick up these changes during aerobic digestion using the Floccky Tester.

The second part of the research aimed to explore the use of rheology to optimize process control of sludge treatment. The TTQ method and the direct injection method were used to see if changes in sludge characteristics, such as cation and biopolymer concentrations and solids content could be detected by rheological measurements.

Variations in total solids concentration was easily detected using the TTQ method and there was a linear increase (R^2=1.00) in TTQ values with increasing total solids concentration.

It was found that changes in ferric iron ion concentration could be detected by rheology as both TTQ and peak torque values increased with increasing ferric iron ion concentration.

Significant changes in rheological properties were not observed when the divalent cations (Ca^{+2} and Mg^{+2}) were added to the sludge at the concentrations used in this study as TTQ and peak torque values did not significantly increase with increasing divalent cation concentration.

Alginate was added to the sludge to examine whether changes in biopolymer concentration can be detected with rheological measurements. There was an increase in TTQ values and peak torque values as the alginate concentration increased.

The addition of cations and biopolymers together was investigated by adding Ca^{+2} ions and alginate to sludge. It was found that for both the TTQ and direct injection methods, no significant changes in rheological properties were observed.

When comparing the TTQ and direct injection methods, it was concluded that the direct injection method is more sensitive due to the greater percent increase in peak torque values compared to TTQ values for all chemicals.

Sludge dosed with ferric iron ions had the greatest average floc area while the anaerobically digested sludge that was not dosed had the smallest average floc area.

Addition of ferric iron ions also resulted in a greater increase in torque and totalized torque compared to other divalent cations and alginate.

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Appendix I: Supporting Information for Chapter 5

0 mg/L Explanation

200 mL samples of anaerobically digested sludge (i.e. 0 mg/L) were used as a baseline in the rheological experiments for each concentration used. It was observed that the sample containing 200 mL of sludge had much greater rheological values compared to the samples containing concentrations greater than 0 mg/L, which skewed the results.

The total solids (TS) of the anaerobically digested sludge had been calculated to be 1.66%. Using this information and assuming a 0% TS for the cationic solution, the 190 mL sludge and 10 mL cationic solution was found to have a TS of 1.58%. An equation of a line was then found for Figure 49 (R^2=0.99) and the drop in TTQ from 1.66 to 1.58% was calculated to be 22 mNm x sec. Applying this decrease to all 0 mg/L data points showed that the 0 mg/L data points were lower than the other data points at higher concentrations.

0 mg/L Calculation

Anaerobically digested sludge TS = 16,591 mg/L

$$200\ mL\ of\ dosed\ sludge\ (190\ mL\ sludge\ and\ 10\ mL\ chemical\ solution$$

$$= \frac{\left(0\frac{mg}{L} * 10\ mL\right) + \left(16{,}591\frac{mg}{L} * 190\ mL\right)}{200\ mL} = 15{,}762\frac{mg}{L}$$

Equation of line for Figure 49 = 259.60x + 558.41

$$Predicted\ TTQ\ of\ 200\ mL\ sludge\ using\ equation\ of\ line$$

$$= (259.60 * 16{,}591\frac{m}{L}) + 558.41\ \ = \ 989.11\ mNm * sec$$

$$Predicted\ TTQ\ of\ 190\ mL\ sludge\ and\ 10\ mL\ of\ 0\%\ TS\ solution\ using\ equation\ of\ line$$

$$= (259.60 * \ 15{,}762\frac{m}{L}) + 558.41\ \ = \ 967.58\ mNm * sec$$

$$DIfference\ between\ predicted\ TTQ\ values$$

$$= 989.11\ mNm * \text{sec} \quad - 967.58\ mNm * \text{sec} = 21.53\ mNm * sec$$