Waste Activated Sludge Pre-Treatment with Chlorine Dioxide: Its Impact on Pre-Existing Sludge Bulking and its Effect on Solubilization and Anaerobic Digester Performance

by

Abisola Olubodun

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WASTE ACTIVATED SLUDGE PRE-TREATMENT WITH CHLORINE DIOXIDE: ITS IMPACT ON PRE-EXISTING SLUDGE BULKING AND ITS EFFECT ON SOLUBILIZATION AND ANAEROBIC DIGESTER PERFORMANCE

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ABSTRACT

A number of advanced pre-treatment techniques and methods have been evaluated for the sole purpose of improving digestibility of waste activated sludge. The pre-treatment of waste activated sludge (WAS) offers the benefit of releasing solubilized substrates, making them readily available to be utilized in the anaerobic digestion process. Other potential benefits include: reducing shock loading to the digester, improving overall digestibility and potentially providing filament / foaming control. Chlorine dioxide, a well-known disinfectant and oxidizing agent has been utilized in many drinking water processes around the world. Its use in wastewater treatment processes however is limited; especially in Canada where legislation has prevented its use for final effluent disinfection. As an oxidizing agent, chlorine dioxide induces cell rupture resulting in the release of soluble material, which when fed into the digester, may serve as readily available substrate for active microorganisms. This mode of action creates the potential for chlorine dioxide to be used as a sludge pre-treatment agent to improve digester performance and in alleviating pre-existing filamentous sludge bulking. This study was conducted using waste activated sludge obtained from the City of Winnipeg's South End Water Pollution Control Centre (SEWPCC), with the following objectives:

1. Determine the efficacy of chlorine dioxide in alleviating pre-existing filamentous sludge bulking;

2. Determine chlorine dioxide ability to increase WAS solubilization; and

3. Define impact of chlorine dioxide on anaerobic digester performance.

WAS pre-treatment using chlorine dioxide was found to be effective in alleviating filamentous bulking. This is significant as filamentous bulking in the activated sludge may lead several problems downstream. Following pre-treatment, sludge bulking was determined to be alleviated as observed by photomicrographic evidence and as measured by a 57% decrease in the stirred sludge volume index (sSVI).

Particulate COD solubilization increased by 60%, 76%, and 74% over the untreated sludge for WAS pre-treated with 25, 50, and 100 mg ClO_2/L (v/v), respectively.

The pre-treatment of sludge using chlorine dioxide did not have any negative impact on digester performance although it also did not lead to improved performance. The volatile solids destruction and COD removal remained unchanged for both untreated and pre-treated sludge. Chlorine dioxide pre-treatment did not affect anaerobic digestion even at the lowest SRT evaluated; it is possible to decrease the digester SRT to as low as 6 days while maintaining the solids destruction and COD removal capability. Biogas production did not improve with increasing chlorine dioxide dosage during pre-treatment but also was not hindered by the pre-treatment agent.

Chlorine dioxide was shown to alleviate filamentous bulking and improve solubility and has the potential to improve digester performance without negative impacts to the digester. However, the full benefit of the pre-treatment method may only be realized for complex “difficult to disintegrate” sludge types.

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Chapter 1. Introduction and General Objectives

Biological wastewater treatment is an effective and relatively economical method for pollutant removal in both domestic and industrial wastewaters. Sludge disposal may represent as much as half of the current operating costs of a wastewater treatment plant (Appels, et al., 2008). Proper treatment and management of the sludge waste stream is essential for volume reduction, pathogen destruction, vector attraction reduction and organic matter stabilization if such waste is to be safely reused or properly disposed of.

Some well-known methods of sludge treatment include alkaline stabilization, aerobic digestion, anaerobic digestion, and composting (Metcalfe & Eddy, Inc. , et al., 2013). Anaerobic digestion is generally preferred over other sludge treatment methods due to benefits listed as follows:

1. High calorific value biogas is produced;
2. Reduction in the volume of final solids and disposal costs;
3. Improved solids dewatering properties; and
4. The production of stabilized Class A or Class B biosolids.

These advantages have led to an ever increasing use of anaerobic digestion for sludge management. It is therefore no surprise why many research efforts continue to be devoted towards seeking methods for enhanced, more economical and efficient digester operations.

Waste activated sludge (WAS), which is typically removed from return activated sludge (RAS), contain mainly biomass. The semi-rigid structure of the microbial cell wall protect the bacteria from degradation (Appels, et al., 2008) and without external influences, will require a relatively long hydraulic retention time to disintegrate. In addition, large particles with lower surface-to-volume ratios are known to be hydrolysed more slowly than smaller particles (Vavilin, et al., 1996). Where the substrate is in particulate or highly complex form such as in sludge containing mainly biomass or more complex cellulosic polymers, the rate of degradation is especially limited by the disintegration of these complex structures (Ladisch, et al., 1983; Grethlein, 1984).

The pre-treatment of sludge prior to anaerobic digestion offers a means by which the overall digestion process can be optimized. Pre-treatment includes the use of applied methods and techniques to enhance the disintegration of biomass cell wall and the breakdown of extracellular particulate and complex organic matter; thereby facilitating the release of soluble and readily biodegradable substrate for input into the main anaerobic digester. Sludge pre-treatment may involve the physical separation of the hydrolysis and methane-forming phases (Cohen, et al., 1979) of anaerobic digestion; thereby providing optimal environmental conditions for the different microbial groups involved. The different pre-treatment methods known today are generally based on enhancing the rate of hydrolysis via mechanical / physical, thermal, chemical, or biological means, and combinations of these. Generally, the results include an increase in substrate turnover, improved process stability (Cohen, et al., 1979), reduced sludge quantities, alleviation of bulking sludge and improved digestibility of sludge.

Sludge bulking is a common occurrence in wastewater treatment processes. This condition is generally defined by poor settling characteristics of sludge resulting in high effluent suspended solids from the clarifying unit and / or foaming in the sludge digester. Bulking due to the presence of filamentous organisms is more common than other types of bulking (Metcalfe & Eddy, Inc. , et al., 2013). The City of Winnipeg's largest wastewater treatment facility, the North End Water Pollution Control Centre (NEWPCC) (which receives sludge from the South End Water Pollution Control Centre (SEWPCC)) has, in the past, faced major challenges with digester mixing and foaming that have been attributed to filamentous bacteria. Filamentous organisms in the activated sludge can quickly proliferate causing excessive foaming in the anaerobic sludge digesters. If not addressed in a timely manner, the foaming can block biogas vent pipes, inhibiting biogas escape and resulting in a buildup of internal pressure within the vessel. Temporary control measures including chlorination have been practiced at the plant as a means of alleviating pre-existing sludge bulking. The use of chlorine however raises concerns about the formation of trihalomethanes and other disinfection by-products. It is therefore necessary to evaluate other means of pre-treatment in attempts to eliminate such concerns.

Chlorine dioxide is a strong oxidizing agent and its use as a disinfectant in drinking water treatment is increasingly being recognized and accepted worldwide. Its use in wastewater treatment processes however is vastly under-utilized. Similar to ozone, chlorine dioxide is a strong oxidant with demonstrated ability to disintegrate sludge solids (Wang , et al., 2011), rupturing the bacterial cell wall to release soluble intercellular components. While ozone-pre-treated sludge has been shown to increase biogas production by as much as 200% (Ak, et al., 2013), to our knowledge, no such studies has been performed with chlorine dioxide. Chlorine dioxide is more cost effective than ozone and unlike chlorine; it does not generate harmful by-products (Wang , et al., 2011). However, it does dissociate to produce the by-products, chlorite and chlorate ions, both of which can be potentially toxic. It is therefore important that the choice of chlorine dioxide as a sludge pre-treatment agent does not negatively impact the anaerobic digester performance.

The objective of this study was therefore to evaluate the impact of chlorine dioxide as a sludge pre-treatment agent. This work was conducted in three major parts:

a) First, the ability of chlorine dioxide to alleviate pre-existing sludge bulking was evaluated using the sludge volume index analysis as an indication of sludge settling characteristic. Comparisons were made with varying concentrations of chlorine dioxide in order to investigate the effect of the different chlorine dioxide dosages in improving sludge settling and consequently to determine the optimal chlorine dioxide dosage for such purpose.
b) Second, the potential for improving the solubilization of sludge was evaluated. The release of solubilized substrate was measured by analyzing the soluble COD increase following pre-treatment.
c) In the third phase, the impact of the pre-treated sludge on the anaerobic digester was determined. Pre-treated sludge was fed into a semi-continuously-operated anaerobic digester operating at the mesophilic temperature of 35 ^{o}C. Its performance was compared to two anaerobic digesters operating simultaneously and with the same operating conditions; the first receiving un-treated sludge and the other receiving a 2-day pre-fermented sludge.

Chapter 2. Literature Review

2.1 OVERVIEW OF ANAEROBIC DIGESTION

It is safe to say that anaerobic digestion is the oldest natural form of waste stabilization. In nature, many microorganisms, in the absence of oxygen are capable of solubilizing organic and inorganic compounds (principally sulfate) into oxidized and reduced forms producing mainly methane, carbon dioxide, and hydrogen sulfide gases (Metcalfe & Eddy, Inc. , et al., 2013);

$$\text{Organic matter} + H_2O \longrightarrow CH_4 + CO_2 + \text{New biomass} + NH_3 + H_2S + \text{heat}$$

As compared to aerobic processes where a large portion of the energy of the substrate is wasted as heat, anaerobic digestion produces little free energy with most of the energy conserved in methane gas as a by-product (Winter, 1984). Anaerobic digestion has the added advantage of energy recovery (as methane gas), lower biomass yields, greater solids destruction, and increased throughput although start-up and system recovery times take significantly longer (Table 2-1). Microorganisms involved in anaerobic digestion prefer to harness their energy into biogas production as opposed to cell synthesis as compared to their aerobic counterpart. This allows for a significant decrease in new biomass production and therefore less reactor volume requirements. Biogas in the form of methane gas may be captured and utilized as an alternative fuel source.

Table 2- 1: A general comparison of the conventional high-rate anaerobic and aerobic digestion processes; source: (Metcalfe & Eddy, Inc. , et al., 2013).

OPERATING CONSIDERATIONS	ANAEROBIC	AEROBIC
Energy requirements	Power requirements for heating and mixing; minimal when biogas is recovered	Energy-intensive due to mixing and aeration requirements
Organic loading rates, kg COD/m^3.d	3.2 – 32	0.5 – 3.2
Typical SRT, days	15 – 20	40 – 60
Degree of Stabilization (SRT dependent), %VSR	56 – 65	35 – 50
Start-up and recovery rates	Slow; requires skilled operation and recovery may take several weeks	Faster; operation is relatively easy, may only require days
Alkalinity requirements	Low to none during stable operation	May be high during nitrification
Sensitivity to external variables	High; methanogens are very sensitive to operational fluctuations	Also susceptible although to a lesser degree
Energy production	Highly valuable methane gas produced	No valuable energy produced
Supernatant quality	Poor; higher content of nitrogen and phosphorous	Lower content of nitrogen and phosphorous possible

2.2 ANAEROBIC DIGESTION PHASES

There are three major biochemical reactions that must take place for the complete anaerobic digestion of organic matter (Figure 2-1); each reaction involving different species of microorganisms. The first reaction namely *hydrolysis* occurs when complex and higher-molecular weight organic compounds such as polysaccharides, lipids and protein molecules are broken down into their respective soluble and simple monomeric forms such as monosaccharides, fatty acids, and amino acids. In this phase, fermentative bacteria produce extracellular enzymes to facilitate the breakdown of these complex compounds from particulate to soluble components. Enzymes involved may include amylases for the breakdown of starches, lipases for the breakdown of lipids, and proteases for the breakdown of proteins.

Simultaneous with hydrolysis, soluble compounds serving as electron donors and acceptors, are degraded further into volatile fatty acids (VFA) such as acetate, propionate, and butyrate. This is accompanied by the production of hydrogen (H_2) and carbon dioxide (CO_2) gases. This step is known as *acidogenesis* (also may be referred to as *fermentation*). For longer-chained fatty acids such as propionate and butyrate, an intermediate pathway termed *acetogenesis* must take place. This will involve 1) further degradation of the higher organic acids into acetate (Metcalfe & Eddy, Inc. , et al., 2013) via β-oxidative cleaving (Vaccari, et al., 2006) and 2) the conversion of CO_2 and H_2 to produce acetate. The final products of fermentation are mostly acetate, hydrogen and CO_2; all serving as organic substrate for methane producing organisms.

The final phase of anaerobic digestion known as *methanogenesis* utilizes the products of fermentation, into gaseous end products i.e. methane and carbon dioxide. This phase is completed by two groups of methanogens (i.e. methane-producing microorganisms). The first group, *aceticlastic methanogens* split acetate into methane and carbon dioxide. The second group, hydrogen-utilizing methanogens reduce hydrogen using carbon dioxide as the electron acceptor to produce methane. With a significant amount of carbon dioxide initially being oxidized to acetate, about 70 percent of the methane produced will be from the reduction of acetate by *aceticlastic methanogens* (Appels, et al., 2008), (Klass, 1984), (Liu & Whitman, 2008), and (Winter, 1984). Zeikus (1977) explains that the preference for one substrate over another is dependent on environmental differences.

Methanogenesis has been observed to occur in many habitats such as the rumen and intestinal tract of animals, in sediments of aquatic habitats, and in thermal springs (Zeikus, 1977).

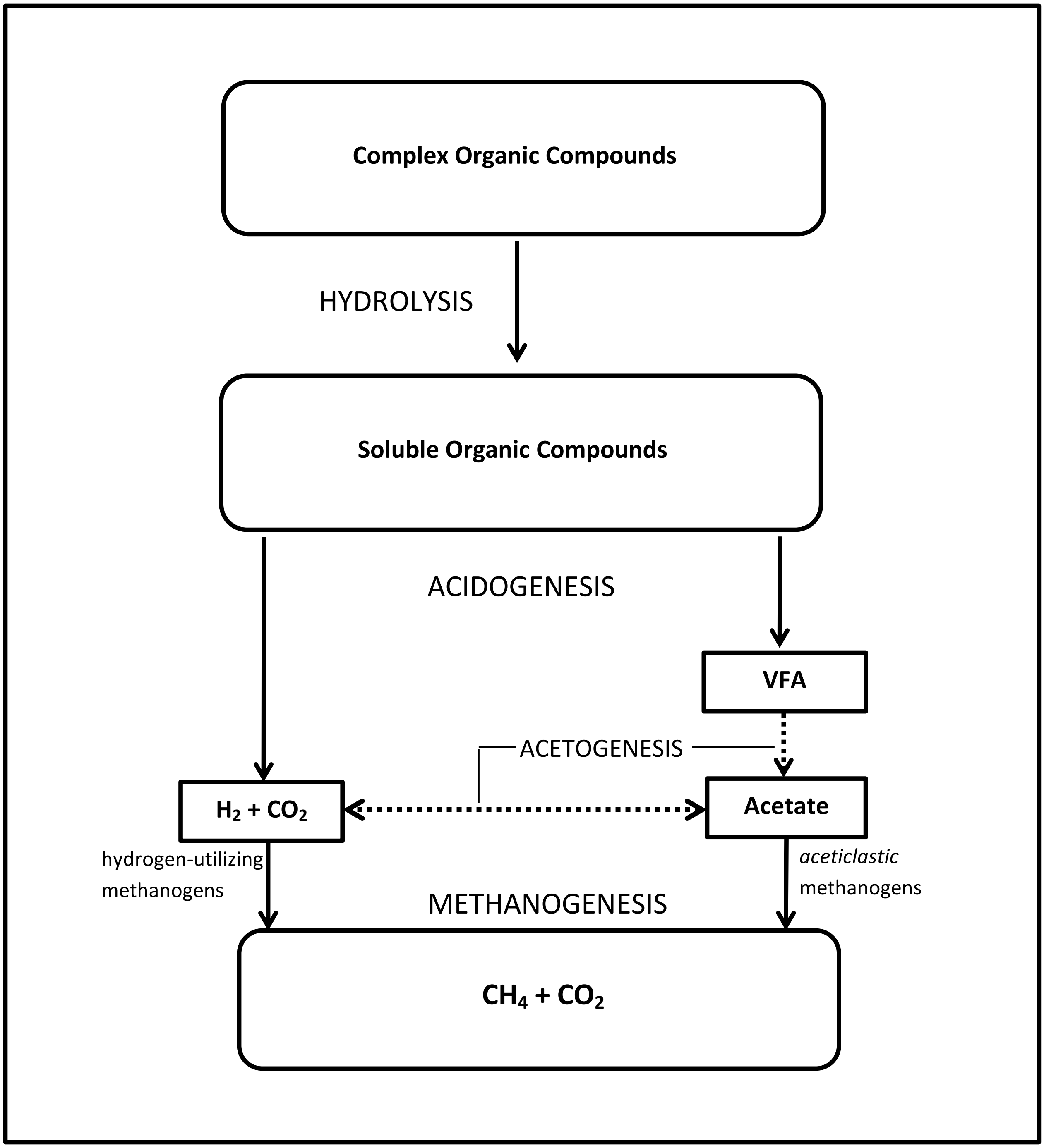

Figure 2- 1: General Schematic of the Anaerobic Digestion Process; adapted from (Metcalfe & Eddy, Inc. , et al., 2013).

2.3 THE PRE-TREATMENT OF SLUDGE

The conventional single-staged anaerobic digestion set-up usually requires a long retention time, larger footprint and is readily susceptible to toxic shock loadings. The pre-treatment of sludge prior to entry into the digester offers the advantage of improving the solubility of the sludge which may result in an enhancement of the rate of digestion, significantly shortening the retention time and in some cases, producing class A biosolids.

Though the anaerobic digestion process has been well delineated over many years, conflicting conclusions remain on which of the aforementioned anaerobic digestion stages is overall rate-limiting. While some believe the *hydrolysis* of particulates to soluble substrates to be rate-limiting (Appels, et al., 2008; Vavilin, et al., 1996; Ladisch, et al., 1983; Grethlein, 1984; Ghosh & Conrad, 1974; Eastman & Ferguson, 1981), others have considered it to be the *methanogenesis* reactions (Pfeffer, et al., 1967; Zeikus, 1977; Rittman & McCarty, 2001), mostly due to the slow growth and poor adaptability of methanogens to varying environmental conditions and sensitivity to inhibition by the products of *acidogenesis* (Cohen, et al., 1979).

With reference to the anaerobic digestion of secondary sludge or sludge containing complex organic matter, *hydrolysis* is ultimately considered the rate-limiting step. This is mostly due to the high biomass content of this sludge stream. Secondary sludge (RAS or WAS) is known to be more difficult to digest than primary sludge (PS). The bacterial cell envelope is a semi-rigid structure which provides sufficient intrinsic strength to protect the cell from osmotic lysis. Microbial cell walls contain glycan strands cross-linked by peptide chains, causing resistance to biodegradation (Appels, et al., 2008). Most of the organics in WAS is contained within the microbial cell; hydrolysis must occur to disintegrate the cell wall and the release of valuable carbon substrates into bulk solution (Hwang, et al., 1997). Typically, PS is mixed with WAS and digested as a mixture in a process known as *cofermentation*, to mitigate the complex nature of WAS. The benefit of *cofermentation* has been reported by (Yuan, et al., 2010).

From another perspective, *methanogenesis* is rate-limiting considering the slow growth of methanogens and their vulnerability to varying environmental conditions. Complete anaerobic digestion involves a consortium of heterotrophic and autotrophic microorganisms that differ widely with respect to their physiology and nutritional requirements (Cohen, et al., 1979). Both groups of organisms must maintain a syntrophic relationship, promoting the direct transfer of hydrogen between the different species in order to maintain minimal hydrogen concentrations and partial pressure in the reactor. Accumulation of intermediate products especially unionized volatile fatty acids (VFAs) is especially toxic to methanogens; direct conversion of VFAs is necessary in facilitating the equilibrium of the fermentation reaction.

A separate pre-treatment process allows for the physical separation of the hydrolysis phase and the methane-forming phase (Cohen, et al., 1979) of anaerobic digestion; thereby providing optimal environmental conditions for both microbial groups to thrive.

Several methods of sludge pre-treatment have been studied and are increasingly being integrated in real-scale. All pre-treatment methods ultimately result in the lysis and disintegration of biomass cells, thus releasing soluble intracellular material into the liquid phase, increasing the solids surface-to-volume ratio, and transforming refractory organic material into biodegradable species (Appels, et al., 2008). Many sludge pre-treatment methods have been proven effective at improving the anaerobic digester performance. The different pre-treatment methods are generally based on enhancing hydrolysis rates via mechanical / physical, thermal, chemical, or biological means, and combinations of these. Although each method operates via different modes and techniques, the ultimate objective is to disintegrate complex matter into solubilized form which can be assimilated readily by microorganisms in the main digestion stage. The product of pre-treatment is essentially sludge with reduced viscosity and increased solubilized material measured as soluble COD (sCOD).

2.3.1 Methods of Sludge Pre-treatment

Mechanical / Physical Sludge Pre-treatment

Mechanical pre-treatment involve subjecting sludge to physical forces to precipitate shear stresses and disintegrate cells to facilitate the release of soluble intercellular fragments. Some examples of mechanical pre-treatment include the use of stationary or moving mills, high pressure homogenization, microwave, and sonication (the use of ultrasound). Increased volatile solids destruction has been observed with increasing pressure amounts (Hwang, et al., 1997). Mechanical pre-treatment may be used in combination with other pre-treatment methods to further enhance solubilization.

Advantages of mechanical pre-treatment include;

1. Reduction in sludge volume,
2. Filamentous control,
3. Improved effluent quality, and
4. Enhanced dewaterability of the digested solids.

While some disadvantages may include;

1. Limited full-scale application, and

2. Dependent on the mechanical disintegration of bacteria.

Ultrasound is a form of physical treatment employing sounds or vibrations at the ultrasonic frequency to induce cavitation in sludge. Cavitational collapse produces intense local heating and high pressure on liquid–gas interface, turbulence and high shearing phenomena in the liquid phase (Bougrier, et al., 2005). Ultrasound pre-treatment may only require short exposure times between 1 and 2 minutes as shown in Tiehm, et al., (1997). Appels, et al., (2008) stated enhanced biogas production by about 50% and improved volatile solids destruction in the range of 40% to 55% have been achieved with this method of pre-treatment. Reports cited however show less achievements; Bougrier, et al., (2005) reported biogas production increases of 25 – 30 %. Zorba, et al., (2010) reported a 15% increase in methane production for sonicated WAS and Tiehm, et al., (1997) only observed a 10% increase in VSR with no consequent improvement in biogas production, over a control digester receiving raw sludge. Tiehm, et al., (1997) however observed stable operations at SRTs as low as 8 days even though VSR at this retention time decreased slightly. Lower SRT operations are advantageous due to smaller footprint requirements. Advantages of ultrasound pre-treatment include;

1. Small footprint requirements,
2. Increased biogas production,
3. May alleviate foaming due to high OLR or filamentous organisms, and
4. Energy consumption relatively small.

While some disadvantages include;

1. High capital cost,
2. Varied results, and

3. No impact on foaming caused by chemical surfactants or polymers.

Thermal Sludge Pre-treatment

Thermal pre-treatment involves the use of heat to induce disintegration of sludge. The sludge is generally subjected to elevated temperatures (in the range of 150 – 200 ^{o}C) and pressure for a short period of time. The high temperature application disrupts the chemical bonds of the cell wall and membrane (Appels, et al., 2008), breaking up its rigid structure and releasing soluble cell material.

An example of a thermal pre-treatment is the commercially available, Cambi process. The Cambi unit involves first dewatering the sludge to 14 – 18 percent dry solids (DS) then preheating close to operating temperature. The sludge is then moved to a reactor vessel, where it is further heated to 160 – 180 °C with direct steam injection and under pressure for approximately 30 minutes (Parker & Beland, 2003). This method is known to produce solids solubilization of approximately 30% and increases to biogas production by 150% (Appels, et al., 2008). The sludge is also sterilized through the process to produce Class A biosolids.

Advantages of the Cambi process include;

1. Class-A biosolids production,
2. Long-term stability of sludge,
3. Increased solids destruction and decreased sludge volume,
4. Enhanced dewaterability of the digested solids,
5. Increased biogas production,
6. No foaming issue, and
7. May be an economical alternative.

While some disadvantages include;

1. Capital and operating costs,
2. Pre-dewatering requirements,
3. High odour potential, and
4. Requires high level of operation and maintenance skill.

Biological Sludge Pre-treatment

This pre-treatment method relies on the addition of specific bacterial strains and/or enzymes to promote the rate of enzymolysis. Appels, et al., (2008) referenced that the addition of the *Geobacillus* so. strain AT1 induced enhanced protease activity, producing a 210% increase in biogas production. Observations from a pilot-scaled study of enzymic hydrolysis show improved pathogen reduction, improved volatile solids destruction and increased biogas production (Mayhew, et al., 2003).

Biological methods may also be used in the control of bulking due to filamentous organisms. As described in the sludge pre-treatment section, biological control involves the addition of specific bacterial strains and/or enzymes to promote the rate of enzymolysis. Recent studies have shown that higher forms of organisms e.g. bacteriophages and rotifers can be used in the control of sludge bulking. These organisms actually consume filamentous organisms and with a faster growth rates can lead to reductions in the population of filamentous organisms. The rotifer, *Lecane inermis*, was shown to improve sludge sedimentation properties and reduce the total length of filaments in comparison with a control unit. The rotifer was also shown to have the ability to consume more than one filamentous organism; *Microthrix parvicella* and *Nostocoida limicola* and Type 021N (Kocerba-Soroka, et al., 2013) and (Kocerba-Soroka, et al., 2013). More importantly the inclusion of rotifers was shown to not adversely impact nutrient removal efficiencies. Reports on the potential of bacteriophages in improving sludge bulking can be reviewed in (Withey, et al., 2005).

Chemical Sludge Pre-treatment

Chemical pre-treatment exposes sludge to chemicals in concentrations that hydrolyze the cell wall and membrane. Three major operating principles of chemical pre-treatment exists;

i. Acid / Alkaline hydrolysis,
ii. Ozonation, and
iii. Advanced oxidation methods.

In acid / alkaline pre-treatment, an acid or a base is added to the sludge exposing the sludge to extreme pH levels. At extreme pH values, cell walls tend to rupture, releasing soluble cellular component. However the addition of mineral acids or bases may increase the salinity of the treated sludge (Parker & Beland, 2003). Alkaline pre-treatment has been shown to be more effective than the acid counterpart (Zorba, et al., 2010) and (Liu, et al., 2012). Prior to entering the digester, the pH must be neutralized to maintain stable digester operations. This increases chemical consumption and associated cost which can be a disadvantage for this method.

Oxidative methods rely on the use of strong oxidants to lyse microbial cells and increase sludge solubilisation. Oxidants have generally been used as tertiary disinfectants in drinking water applications. Most common oxidants are ozone, hydrogen peroxide, chlorine and chlorine dioxide.

Sludge pre-treatment using ozone has been well studied in literature. *Ozone (O_3)* is a strong oxidizing agent consisting of three oxygen atoms. The ozone molecule is very unstable and therefore is not likely to create residuals in the applied medium. Ozone may act specifically or non-specifically to oxidize organic compounds. Its nonspecific reactive nature is claimed to be responsible for the disintegration of sludge (Ak, et al., 2013).

$$3\,O_2 \rightleftharpoons 2O_3$$

Ozone may be introduced to thickened sludge prior to digestion. This method of sludge pre-treatment has been shown to improve gas production and increase VSR. Ak, et al., (2013) reported an impressive increase between 33% and 200% in biogas production with varying dosages of ozone in pretreated sludge feed reactor over the control. This is similar to results of (Weemaes, et al., 2000) who reported increases between 23% and 55% in biogas production, also for varying ozone dosages. Other comparable results have been reported by (Bougrier, et al., 2007), (Park, et al., 2008) and (Silvestre, et al., 2014). The concentration of ozone employed in these studies ranged from 0.05 and 1.5 g O_3/g TSS.

Similar literature on the improvement of digester performance for chlorine however remains non-existent. This is most likely because of the potential of chlorine to bind to organic and inorganic matter to form undesirable halogenated compounds. However, oxidizing agents' ozone, chlorine and hydrogen peroxide are readily used in the control of bulking (Saayman, et al., 1998) and (Sezgin, et al., 1978). Just as in low substrate conditions where the diffusion gradient increases from the floc surface to the core allowing filaments more exposure to food in the bulk solution (Heukelekian, 1941), it is postulated here that extended filaments are also more susceptible to toxic chemicals or disinfectants that may be present in the liquid medium. The application principle of these methods is then simple: since filamentous bacteria causing bulking sludge are positioned mostly outside of the floc, they are more susceptible to oxidants than the floc forming bacteria (Martins, et al., 2004).

When introduced in appropriate proportions, chlorine may attack the protruding filaments without destroying the good floc structure (Smith & Purdy, 1936). The City of Winnipeg wastewater treatment process includes pre-chlorination (with the use of hypochlorite) of the return activated sludge (RAS) presumably, to prevent the proliferation of filamentous microorganisms in the activated sludge bioreactor. It is unknown however whether chlorine or other chemicals are added to the waste activated sludge (WAS) for the said purpose of sludge bulking in the anaerobic digester. Chlorine is also typically sprayed over the surface of reactors or clarifiers when excessive foaming is observed. Literature in support of chlorine as a sludge pre-treatment agent for the primary purpose of reducing filamentous organisms however remains non-existent.

Ozone however has been used for filamentous control and in improving sludge properties. (Nilsson, et al., 2014) achieved a dSVI reduction from 170 to 100 mL/g following ozone treatment at a concentration of 2.8–5.0 g O3 /kg SS. This was achieved without any negative impacts on downstream removal efficiencies. Other reports in support of ozonation to reduce filamentous propagation include (Saayman, et al., 1998).

Like other chemical control agents, it is postulated here that the effectiveness of chlorine dioxide in alleviating filamentous organisms is as a function of exposure. Due to the cost of ozonation and the potential for chloramine formation and known health concerns associated with chlorination, chlorine dioxide may be a viable alternative. Literature on this topic are however non-existent. Further studies are needed to support its use in the pre-treatment of sludge. This report will aim to show that like other pre-treatment methods, chlorine dioxide pre-treatment may results in improved sludge characteristics without negatively impacting digester performance.

With the use of chemical agents being non-specific, their application may have a negative impact on floc formers and slow growing bacteria which take a longer time to recover hence applications must be carried out with utmost caution. For chemical pre-treatment, considerations for chemical cost and safe use must also be considered.

2.3.2 Sludge Bulking and Pre-treatment for Filament Control

The overall success of any water or wastewater treatment is determined by the efficiency of the liquid/solids separation unit. Gravitational settling is the most cost effective methods used for solids separation but its efficacy is largely dependent on the formation of large, dense and stable floc structures (Seka & Verstraete, 2003) as defined by Newton and Stokes' *Law of Sedimentation*:

$$v_{p(t)} = \sqrt{\frac{4g}{3C_d}\left(sg_p - 1\right)d_p}$$

where $v_{p(t)}$ = particle terminal velocity, LT^{-1} (m/s), g = acceleration due to gravity, LT^{-2} (9.81 m/s^2),

C_d = drag coefficient (unitless),

sg_p = specific gravity of the particle, and

d_p = diameter of particle, L (m).

As particles coalesce to form floc structures, the diameter (d_p) and density (sg_p) increases, allowing for settling at a faster velocity ($v_{p(t)}$). In many cases however, floc structures may not compact well or even compact at all; the result of which is poor settling and escape of particles (as suspended solids) in the effluent, a condition referred to as *sludge bulking* (Sezgin, et al., 1978). Sludge bulking is most commonly attributed to the presence of excess filamentous organisms although other forms of bulking are possible such as an excessive presence of extracellular polymeric substances (EPS) production (Metcalfe & Eddy, Inc. , et al., 2013) or from the formation of diffuse pin floc.

Filamentous bulking i.e. bulking due to filamentous microorganisms occur when filaments extend from the floc unit, forming inter- and intra- bridging interactions between floc structures hence disrupting its physical compactness. These filaments are single-celled microorganisms attached to one another end- to-end (Metcalfe & Eddy, Inc. , et al., 2013). In reasonable numbers, filamentous microorganisms contribute to the structural integrity of floc structure. Sezgin, et al., (1978) stated that when filaments are insufficient, floc will be weak with tendencies to break apart into pin-floc when exposed to even minimal turbulence. But in excess, the filaments protrude out of the floc, forming bridging interactions between floc structures, thus increasing the floc surface area to mass ratio and resulting in poor settling (Metcalfe & Eddy, Inc. , et al., 2013) . Common filamentous microorganisms are *Microthrix parvicella, Nocardia spp., Thiotrix spp*., *Sphaerotilus natans* etc. These organisms are known to proliferate under adverse conditions of low substrate environments including low dissolved oxygen, low nutrient and low food to microorganism (F/M) ratios [(Sezgin, et al., 1978) and (Palm, et al., 1980)].

Early investigations of (Heukelekian, 1941) explained that the obligatory nature of floc-formers for dissolved oxygen hinders their biochemical activity in low dissolved oxygen conditions. With the aid of protruding filaments, filamentous microorganisms tend to thrive in these (low oxygen) conditions; consequently creating a bulky sludge situation. In addition, *Microthrix parvicella* and *Nocardia spp.,* possess hydrophobic cell surfaces that attach to air bubbles, increasing the floc buoyancy and causing excessive foaming (Metcalfe & Eddy, Inc. , et al., 2013). Palm, et al. (1980) and Sezgin, et al. (1978) presented a correlation between the settling properties of activated sludge as assessed by methods such as the zone settling velocity (ZSV) and sludge volume index (SVI) and the quantitative amount of filaments extending from the floc units. The SVI indicates the volume occupied by 1 g of sludge after 30 minutes of settling (Mohlman, 1934). A value of 100mL/g is considered good settling sludge while above 150mL/g is typically associated with filamentous growth (Palm, et al., 1980).

During wastewater treatment, filamentous organisms quickly proliferate when subjected to adverse conditions. When filamentous sludge is introduced into the digester, excessive foaming may occur due to the formation of gaseous products and the attachment of the filaments to these products thus increasing floc buoyancy. If not addressed in a timely manner, excessive foaming may block biogas vent pipes, inhibiting biogas escape and resulting in a buildup of internal pressure within the digester unit. The City of Winnipeg's largest wastewater treatment facility, the North End Water Pollution Control Centre (NEWPCC) (which receives sludge from the South End- and West End- Water Pollution Control Centre (SEWPCC and WEWPCC)) faced major challenges with digester mixing and foaming that were attributed to filamentous bacteria. In one major occasion, excessive foaming in the digesters plugged the gas release line and pressure relief valves causing extensive structural damages to the units (Oleszkiewicz, et al., 2010). Other potential issues of foaming are; health and safety related-issues (e.g. slip hazards), disruption of digester heating system, non-uniform SRTs, odour releases, and an overall disruption of digester performance (Shimp, et al., 2010).

Sludge bulking may be controlled using two strategies; specific and non-specific methods. The non-specific methods comprise the use of non-selective chemicals such as chlorine, ozone and hydrogen peroxide while specific methods are preventive methods that have the goal to favour the growth of floc-forming bacterial structures at the expense of filamentous bacterial structures (Martins, et al., 2004). In the case of the latter, a selector is provided with environmental conditions (high dissolved oxygen, nutrient and food to microorganism (F/M) ratios) which may inhibit or suppress the growth of filamentous organisms. The use of selectors to promote these conditions have been shown to be effective at controlling the presence of filamentous organisms (Al-Mutairi, 2009), (Azimi & Zamanzadeh, 2006) and (Fainsod, et al., 1999). The selector promotes the growth of floc formers which can outgrow the filamentous organisms as a result of a higher substrate uptake and storage rates. A successful application of selectors relies on detailed knowledge of: (i) physiology and substrate requirement of the filamentous microorganisms, (ii) wastewater composition and (iii) substrate removal kinetic in the selector system (Al-Mutairi, 2009). The selector may be operated under aerobic, anaerobic or anoxic conditions.

Non-selective methods may involve the introduction of non-selective chemicals such as chlorine and ozone. Chlorine is typically sprayed over the surface of activated sludge reactors or clarifiers when excessive foaming is observed. Ozone has been used for filamentous control (Nilsson, et al., 2014) and (Saayman, et al., 1998).

Due to the cost of ozonation and the potential for chloramine and THM formation associated with chlorination, it is important to investigate other alternatives for sludge pre-treatment and filamentous bulking mitigation.

2.4 THE USE OF CHLORINE DIOXIDE AS A SLUDGE PRE-TREATMENT AGENT

Chlorine dioxide (ClO_2) is a strong oxidizing agent with high oxidation potential and strong antimicrobial properties. It is effective over a wide range of pH (3 to 8 pH units) and has strong biocidal activity against a broad spectrum of microorganisms including bacteria, fungi, yeast, and mold (Ray, et al., 2013). Chlorine dioxide is commonly used in drinking water treatment for pathogen reduction, iron and manganese oxidation, taste and odour reduction, and sometimes for sulfide oxidation (Aieta & Berg, 1986). As a disinfectant, chlorine dioxide has been shown to be more effective than chlorine (Benarde, et al., 1967), with 2.63 times the oxidizing power of chlorine (Metcalfe & Eddy, Inc. , et al., 2013). In addition, chlorine dioxide produces very few disinfection by-products (DBPs); it does not react with ammonia or humic substances to form carcinogenic compounds like chloramines and trihalomethanes (THMs) (US EPA, OW, Office of Ground Water and Drinking Water, April 1999).

The specific mode by which chlorine dioxide interacts with microorganisms for pathogen kill remains inconclusive. Its bactericidal action may involve direct oxidation of proteineous substances including certain amino acids, enzymes and outer membrane proteins. The study of (Benarde, et al., 1967) showed that chlorine dioxide inhibits protein synthesis immediately upon contact with microorganisms. Protein inhibition was reported as the primary cause of lethality. However, (Berg, et al., 1986) identified the primary cause of cell death as nonspecific oxidative damage to the inner membrane leading to significant efflux of potassium (K^+) and the consequent loss of permeability control. Chlorine dioxide has also been reported to react with free fatty acids (Ghanbari, et al., 1982) and irreversibly with the sulfhydryl group, which is essential for the activity of many enzymes (Roller, et al., 1980). Seemingly, its effectiveness as a bactericide may involve several non-specific interactions which include the inactivation of critical enzyme systems or disruption of protein synthesis and alteration of inner membrane proteins and lipids (Metcalfe & Eddy, Inc. , et al., 2013; US EPA, OW, Office of Ground Water and Drinking Water, April 1999; Roller, et al., 1980).

Nonetheless, these interactions ultimately result in biomass cell disintegration and release of intracellular material both of which are proposed to be beneficial during sludge pre-treatment. The reduction of pathogens or non-beneficial microorganisms reduces substrate competition in the anaerobic digester while the release of intracellular material as a result of pre-treatment increases solubilized substrate availability.

Chlorine dioxide has been researched and applied as a disinfectant of pathogenic microorganisms in drinking water treatment applications, as a bleaching agent in pulp and paper milling, and even on crops, pre- and post- harvest, to reduce spoilage. For the reasons above, chlorine dioxide has the potential to be used as an effective agent in sludge pre-treatment; unfortunately, literature on this topic is very limited. A sludge disintegration study by Wang, et al., (2011) showed a 58% reduction in excess sludge production from a sequencing batch reactor receiving chlorine dioxide. This was comparably better than when chlorine was used. Its application also did not adversely affect the effluent quality thus making its use in the treatment train more desirable. Chlorine dioxide pre-treatment also has the added benefit of reducing odours; mainly those generated by decaying organic matter, hydrogen sulfide and phenolic compounds (US EPA, OW, Office of Ground Water and Drinking Water, April 1999).

Chlorine dioxide is highly reactive and unstable, and cannot be compressed or stored commercially in gaseous form as it is highly explosive under pressure (US EPA, OW, Office of Ground Water and Drinking Water, April 1999). It is therefore necessary to synthesize onsite prior to application. Chlorine dioxide can be synthesized relatively easily from the oxidation of sodium chlorite with (i) gaseous chlorine (Cl_2), (ii) aqueous chlorine (e.g. hypochlorous acid), or (iii) a strong acid (e.g. Hydrochloric acid);

$$2\ NaClO_2 + Cl_{2(g)} \rightarrow 2\ ClO_{2(g)} + 2\ NaCl \ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{(i)}$$

$$2\ NaClO_2 + HOCl \rightarrow 2\ ClO_{2(g)} + 2\ NaCl + NaOH \ldots\ldots\ldots\ldots\text{(ii)}$$

$$5\ NaClO_2 + 4HCl \rightarrow 4\ ClO_{2(g)} + 5\ NaCl + 2H_2O \ldots\ldots\ldots\ldots\text{(iii)}$$

In contrast with chlorine, chlorine dioxide does not hydrolyze extensively in water but remains in solution as a dissolved gas in the pH range of 2-10. It does however disproportionate in alkaline solution to give a 1:1 molar ratio of chlorite (ClO_2^-) and chlorate (ClO_3^-) ion (Aieta & Berg, 1986);

$$2ClO_2 + 2OH^- \longrightarrow ClO_2^- + ClO_3^- + H_2O$$

Aqueous solutions will remain stable when kept in cool temperatures and away from light sources.

Chapter 3. Detailed Research Objectives

Although chlorine dioxide popularity is making strides as a disinfectant, its potential use in sludge treatment is only slowly becoming realized. Based on similarities in the mode of disinfection between ozone and chlorine dioxide, it is postulated here that the utilization of the latter is likely to result in an increased solubilization of particulate matter and as a consequence, a potential to improve digester performance by way of increased biogas production, improved volatile solids destruction and improved COD removal. It is also postulated that pre-treatment using chlorine dioxide will be effective at mitigating pre-existing filamentous sludge bulking. Chlorine dioxide however is a strong oxidant with potential to disintegrate into the toxic by-products, chlorite and chlorate which may trickle into the digestion process negatively impacting beneficial microorganisms. Studies on chlorine dioxide as a sludge pre-treatment agent remain limited. It is therefore necessary to evaluate this topic extensively on a small scale prior to any pilot- or full-scale implementation. This will also help to assess the cost of implementation against savings from realized benefits.

The objectives of this research were:

- To evaluate the effect of chlorine dioxide in improving sludge settling characteristics by partial degradation of pre-existing filaments in bulking sludge,
- To assess the potential to improve solubilization of particulate matter following pre-treatment, and
- To evaluate the subsequent effect of the pre-treatment method on the anaerobic digestion process.

Chapter 4. Methodology

4.1 CHLORINE DIOXIDE PREPARATION

A chlorine dioxide stock was prepared according to method 4500-ClO_2B of the Standards Methods for the Examination of Water and Wastewater (APHA, 1998). The set-up is illustrated in Figure 4-1 and pictured in Figure 4-2 below. The concentration of ClO_2 stock solution was measured and verified before each application using the HACH DR/2500 spectrophotometer and method: 8138-Direct Reading Method HR of the HACH DR/2500 Handbook (2003). This method of measurement was determined to be comparative to the DPD method (method 10126-DPD) of the HACH DR/2500 Handbook (2003)).

During sludge pre-treatment, the concentrated stock solution was diluted with the raw sludge to obtain a final concentration of 25, 50, and 100 mg ClO_2/L (v/v) which were the dosages used throughout this thesis. These dosages were chosen based on similar literature by (Wang, et al., 2013) where chlorine dioxide was assessed for activated sludge disintegration in a sequencing batch reactor.

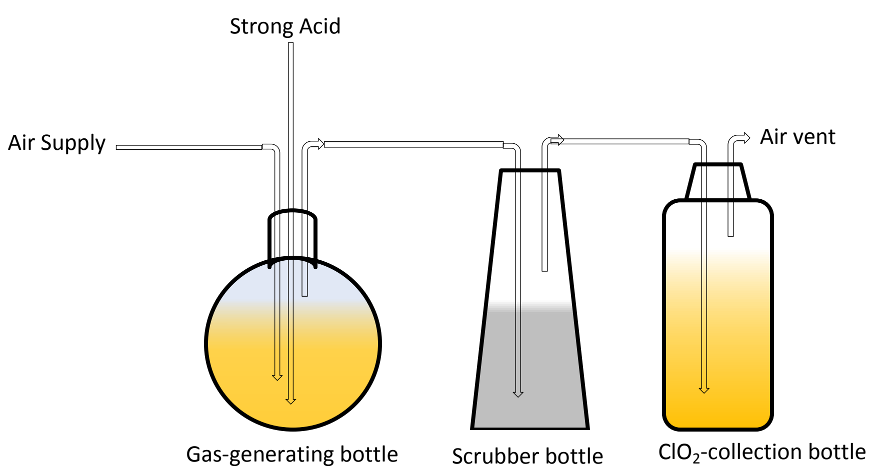

Figure 4- 1: Configuration of chlorine dioxide generator used in this study (Adapted from (APHA, 1998)).

Figure 4- 2: Photograph of Chlorine Dioxide setup during preparation.

4.2 EXPERIMENTAL SETUP

4.2.1 Sludge Settling Evaluation

A series of batch tests was conducted to evaluate the sludge volume index of waste activated sludge (WAS) following pre-treatment with chlorine dioxide. The WAS used in this study was obtained from the City of Winnipeg South End Water Pollution Control Center (SEWPCC) (Manitoba, Canada) and refrigerated at 4^{o}C. The refrigerated sludge was used within 48 hours of collection.

The setup utilized four 4 L wide-mouthed glass jars placed on magnetic stirrer units. Each reactor received a pre-determined amount of ClO_2 prepared stock to achieve a final concentration of 0, 25, 50, and 100 mg ClO_2/L in a 3 L volume of sludge. The reactors received continuous slow mixing throughout the study period. 1 L samples were extracted (and subsequently returned) from each reactor every two hours over a 12-hour study period for the determination of COD (total and soluble), suspended solids, stirred sludge volume index (sSVI) and microscopic observation. Sample withdrawal was completed using the Masterflex L/S model 77200-60 peristaltic pump. The collected sample was returned to the main bulk solution immediately following analysis.

4.2.2 Sludge Solubilization Evaluation

For each pre-treatment performed throughout this study, the soluble- and total- COD analysis was completed for both pre-treated WAS and untreated WAS. For this thesis, we only report the solubilization results determined during the sludge settling evaluation described above.

4.2.3 Semi – Continuous Reactor Set-up

For this study, three 4 L wide-mouthed glass jars (manually graduated) were used as anaerobic digesters. Each reactor was tightly fitted with a rubber stopper with appropriately-sized holes created to allow for feeding/sampling and biogas escape. The biogas produced from the reactors was transported via tubing into polypropylene bottles where gas production was measured by the volume displacement method. The collection bottles were air-tight, manually graduated vessels each containing 6 L of deionized water saturated with 2.1 kg of NaCl, 300 mL of H_2SO_4 and 0.18 g of methyl orange to prevent gas from dissolving.

The reactors were placed on a magnetic stirrer to provide continuous mixing throughout the experimental period. All of the digesters were operated with an active volume of 3 L in a temperature controlled walk-in chamber maintained at 35 ^{o}C. All connections or potential sources of gas escape was reinforced with a silicone sealant and tested occasionally for leaks. The experimental setup of the digesters is illustrated in Figure 4-3 and actual setup is pictured in Figure 4-4.

To start up, the bench-scale mesophillic anaerobic digesters were equally seeded with a mixture of digestate from a previous experiment (which utilized co-thickened sludge obtained from the City of Winnipeg's North End Water Pollution Control Center (NEWPCC) (Manitoba, Canada) and operated at an SRT of 15 days). The reactor volumes were topped up to 3 L with fresh untreated WAS obtained from the City of Winnipeg's South End Water Pollution Control Center (SEWPCC) (Manitoba, Canada). All three reactors were then operated semi-continuously (with WAS only) and maintained at an SRT of 10 days.

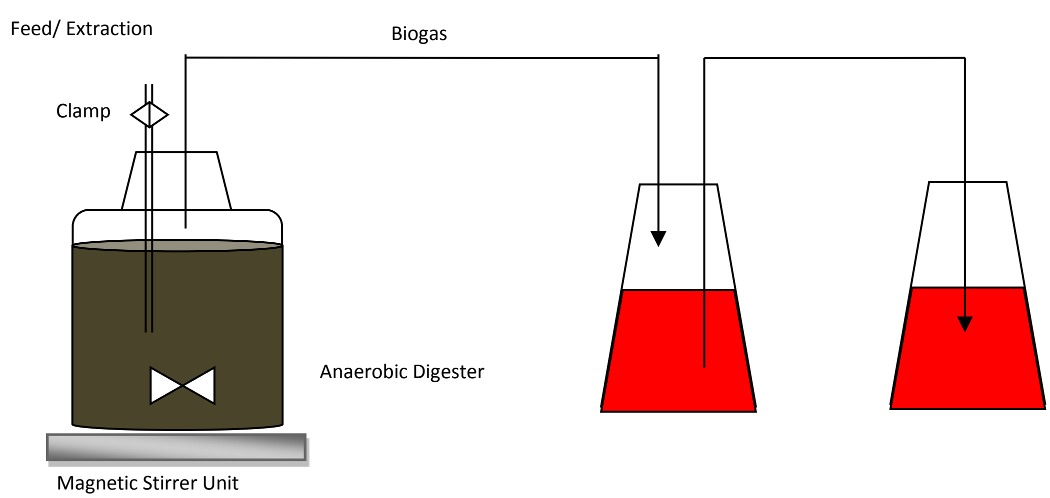

Figure 4- 3: Schematic representation of the semi-continuous anaerobic digester set-up.

Figure 4- 4: Photograph of the semi-continuous anaerobic digester set-up.

This process continued until steady state was reached (indicated by a stable pH). After which, the feeding continued with varying sludge types as indicated below. Once daily, the biogas volume was recorded; the appropriate volume of reactor content was then removed and replaced equal volumes of new sludge content.

This study utilized three anaerobic digester setup operated simultaneously with feed variations as follows:

- Reactor 1: Control; fed with untreated WAS
- Reactor 2: Fed with 1.5-day fermented WAS
- Reactor 3: Fed with chlorine dioxide-pretreated WAS.

Fresh WAS was collected once a week and stored in a 4^{o}C refrigerator until ready for use. The feed sludge fermentation and chlorine dioxide pre-treatment were performed at room temperature (21^{o}C), each utilizing a 1 L glass beaker and mixer apparatus.

For sludge pre-fermentation without ClO_2, the WAS is allowed to mix in the beaker (covered with parafilm) for 2 days before feeding to the digester (Reactor 2). For chlorine dioxide pre-treatment, a pre-determined amount of chlorine dioxide was introduced into the sludge to achieve a final concentration of 50 mg ClO_2/L in the beaker. The chlorine dioxide treated sludge was allowed to mix slowly in the beaker for 4 hours before feeding to the digester (Reactor 3).

4.2.4 Respirometer (Batch Assay) Set-up

To further evaluate the effect of chlorine dioxide sludge pre-treatment on anaerobic digestion, several batch experiments were completed using a respirometer unit. The respirometer used was the AER-800 respirometer unit supplied by Challenge Technology, Springdale, AR.

The seed used is digested sludge obtained from the City of Winnipeg North End Water Pollution Control Center (NEWPCC) (Manitoba, Canada). The WAS used was obtained from either the City of Winnipeg South End- or North End- Water Pollution Control Center (SEWPCC or NEWPCC) (Manitoba, Canada).

For the first assay, three independent trials were conducted with each experimental condition completed in duplicate. Each setup (trial) was conducted at the same time and allowed to operate for 7 days before decommissioning and proceeding with the next trial. Here, varying concentrations of chlorine dioxide on different types of sludge was evaluated as the substrate input. Initially, the amount of substrate and seed used in the respirometer bottles was determined using the methodology of (Moody, et al., 2011) using a substrate to seed ratio (VS_{sub}:VS_{inoc}) of 1:1. The substrate being WAS pre-treated with a pre-calculated amount of chlorine dioxide to achieve final concentrations of 0, 25, 50, and 100 mgClO_2/l. 600 ml graduated polypropylene bottles with screw caps fitted with septa were used as digester bottles. The substrate and seed volume for each reactor was calculated using a substrate to seed ratio (VS_{sub}:VS_{inoc}) as above to make up 67% of the reactor capacity. The reactor was then topped up to 80% capacity with a prepared solution of nutrient media according to (Moody, et al., 2011).

A second assay was completed to evaluate the effect of applying a dechlorinating agent to the pre-treated WAS prior to anaerobic digestion. The dechlorinating agent used was sodium sulfite. One trial was completed with five experimental conditions, each condition setup in duplicate. The experimental conditions evaluated are as follows:

i. Untreated raw WAS as substrate (control),
ii. Chlorine dioxide pre-treated WAS as substrate,
iii. Chlorine dioxide pre-treated WAS + 25 mg/l of sodium sulfite,
iv. Chlorine dioxide pre-treated WAS + 50 mg/l of sodium sulfite, and
v. Chlorine dioxide pre-treated WAS + 100 mg/l of sodium sulfite.

WAS pre-treatment was completed with a 4 hour reaction time using a pre-calculated amount of chlorine dioxide to achieve a final concentration of 50 mgClO_2/l. The batch assay was allowed to operate for an experimental period of 21 days. Here the amount of substrate and seed used was kept at 15% pre-treated sludge (substrate) to 85% digested sludge (seed).

4.2.5 Bioavailability Determination

The final phase of this thesis involved an evaluation of the bioavailability of the pre-treated sludge using a BOD_5 test.

4.3 ANALYTICAL METHODS

4.3.1 Sludge Settling Evaluation

Total suspended solids (TSS) and volatile suspended solids (VSS) were conducted according to methods 2540D and 2540E, respectively, of the Standards Methods for the Examination of Water and Wastewater (APHA, 1998).

Sludge settling was assessed using the sludge volume index (SVI) analysis which represents the volume occupied by 1 g of sludge after 30 minutes of settling (Mohlman, 1934). The sludge volume index was measured with stirring (sSVI) using the Triton Electrics ‘Type 305’ settleometer.

After obtaining samples for solids analysis and microscopic evaluation, the remainder extracted sample was transferred to a settleometer for sSVI determination. After 30 minutes of settling, the height of the settled sludge and height of the liquid were recorded. Following the settling test, the collected samples were returned to the jar reactor. This study included three trials of the above using fresh sludge obtained from the SEWPCC for each trial.

At a later time, a capillary suction time (CST) test was used to determine sludge dewaterability according to method 2710G in the Standards Methods for the Examination of Water and Wastewater (APHA, 1998). The CST apparatus used was the Type 319 Multi-CST obtained from Triton Electronics.

4.3.2 Microscopic Observation

Sub-samples collected during the sludge settling evaluation above were diluted to obtain a final MLSS of 1500 mg VSS/L. Microscopic observation of gram stains and wet mounts were made under phase contrast at 100x magnification using a binocular microscope (Nikon Eclipse E400). For each prepared slide, three images were captured at random locations using the image capturing attachment and Image-Pro Plus 5.0 software. The filamentous organisms were identified according to the characterization table used by (Strom & Jenkins, 1984).

4.3.3 Sludge Solubilization Evaluation

Hach COD digestion vials were used to determine the COD contents; soluble chemical oxygen demand (sCOD) was determined the same way but for filtered samples, filtered through a 0.45 µm nylon membrane filter. Samples for COD measurement were diluted 10-fold before analysis. The sCOD content was evaluated as a function of total COD (tCOD) in the reactor i.e.

$$sCOD = sCOD \; / \; tCOD$$

4.3.4 Semi-continuous Digester Evaluation

The following parameters were analyzed and monitored in order to evaluate the efficiency of the digester setup and for result compilation.

- pH
- COD (Total and Soluble) concentrations

- Solids concentrations
- Biogas production and composition

The pH in the three digesters was measured daily for the extracted digestate during the feeding/extraction program. The COD and Solids were also analyzed daily but for both feed and extracted reactors samples. The total and soluble COD were measured as described previously using HACH digestion apparatus. Total solids (TS) and volatile solids (VS) were conducted according to method 2540G in the Standards Methods for the Examination of Water and Wastewater (APHA, 1998).

Soluble COD (sCOD) production following WAS pre-treatment was also recorded and the concurrent sCOD utilization and volatile solids destruction was calculated.
Biogas production from each reactor was determined by volume displacement (Figures 4-3 and 4-4). Once daily, before feeding, the volume displaced is recorded and measured against the previous day's recorded volume as in;

$$Total\ biogas\ produced_{day\ n} =$$
$$volume\ in\ collection\ bottle_{day\ n} - volume\ in\ collection\ bottle_{day\ n-1}\ (ml/d)$$

For gas composition determination, biogas samples were collected from the headspace of the collection bottle using a gastight syringe. The syringe was washed with the gaseous content of the headspace three times before the sample is collected, the valve on the syringe is set to the closed position, the syringe is then transported immediately for gas composition analysis. The gas samples were analyzed using a Varian CP 3800 gas chromatography (GC) instrument equipped with a thermal conductivity detector. The optimized GC operating conditions were 250˚C in the injector and 180˚C in the detector. Temperature in the oven was initially set at 40˚C for 1 minute and then ramped up to 100°C at the rate of 20˚C/min for a total running time of 17 minutes. The flow rate of carrier gas helium in the column was constant at 3 mL/min.

4.3.5 Respirometer (Batch Assay) Evaluation

The batch assays were evaluated similar to the semi-continuous set-up above except this was conducted in batch conditions. Initial and final samples were collected and analyzed for:

- COD (Total and Soluble) concentrations
- TS and VS concentrations
- Biogas production and composition

The methodology used is same as previously described for the semi-continuous set-up above with the exception of biogas production information which was obtained automatically using the AER-200 respirometer unit.

4.3.6 Sludge Bioavailability Evaluation

The bioavailability of pre-treated sludge was determined using the 5-day BOD analysis according to method 5210 of the Standards Methods for the Examination of Water and Wastewater (APHA, 1998).

Chapter 5. Results and Discussion

5.1 RESEARCH APPROACH

The purpose of this research was to evaluate the feasibility of using chlorine dioxide as a pre-treatment agent on waste activated sludge (WAS). This was completed in three major parts, namely: 1) an investigation into the alleviation of sludge bulking due to the existence of filamentous organisms; 2) a determination of increased solubility as a result of pre-treatment; and 3) an evaluation of the pre-treatment impact on a semi-continuously operated anaerobic digester and further investigation on the impact when the chlorine dioxide concentration was varied, was applied to different sludge types and was de-chlorinated prior to digestion.

The sludge volume index (SVI) determination was used as a means of measuring the effect of pre-treatment on alleviating sludge bulking. Simultaneously, the disintegration of COD into soluble form was also followed closely. The process was carried out at varying concentrations of chlorine-dioxide in the pre-treated sludge; in part, to obtain an indication of the optimum chlorine dioxide dosage as it relates to the sludge characteristics.

In evaluating the effect of chlorine dioxide pre-treatment on anaerobic digester performance, this research was first completed using semi-continuous bench-scale digesters initially operating at the SRT of 10 days. All relevant digester performance indicators (e.g. pH, TS, VS, COD etc.) were regularly measured and analyzed. As experimentation progressed, the operating SRT was reduced to 8 days and subsequently to 6 days to measure the effect of this change on digester performance.

Several batch experiments were also completed to further investigate the impact of chlorine dioxide sludge pre-treatment on the anaerobic digester. First pre-treatment of different sludge types was evaluated using varying concentrations of chlorine dioxide. Then de-chlorination of the pre-treated sludge prior to introduction into the digester was completed and its effect on digester performance evaluated. Measured performance indicators include solids destruction, COD removal, as well as biogas production and composition. Lastly, bioavailability evaluation was performed on untreated, pre-treated and de-chlorinated pre-treated sludge to evaluate the level of readily biodegradable substrate following pre-treatment.

5.2 EXPERIMENTAL RESULTS

The results of each experiment carried out are presented and discussed in this section.

5.2.1. Sludge Bulking Evaluation

Microscopic Observation

Bulking sludge is most commonly due to the presence of filamentous organisms although less commonly, may also be due to the presence of extracellular biopolymer or diffuse pin-floc. To confirm that the sludge used for this research is indeed bulking with filamentous organisms, a photomicrograph was obtained and is shown in Figure 5-1. It is apparent here that filaments are present in abundant quantities in the sludge used here. These hair-like strands can be seen protruding out from the floc and loosely bridging with adjacent floc. Following chlorine dioxide pre-treatment, the floc structures appear to agglomerate, becoming more compact (Figures 5-2 and 5-3). Protruding filament strands are less visible although can still be seen inter-bridging each floc structure or as shorter, fragmented strands in the bulk space. Wet mount observation (not shown here) presented the filaments to be non-motile while gram stains showed the filaments to be gram positive. According to the filament characterization of (Strom & Jenkins, 1984), the filamentous organism present in the WAS used for this experiment is likely to be *Microthrix parvicella*. This is in conjunction with the City of Winnipeg November 2009 digester foaming incidence report (Oleszkiewicz, et al., 2010).

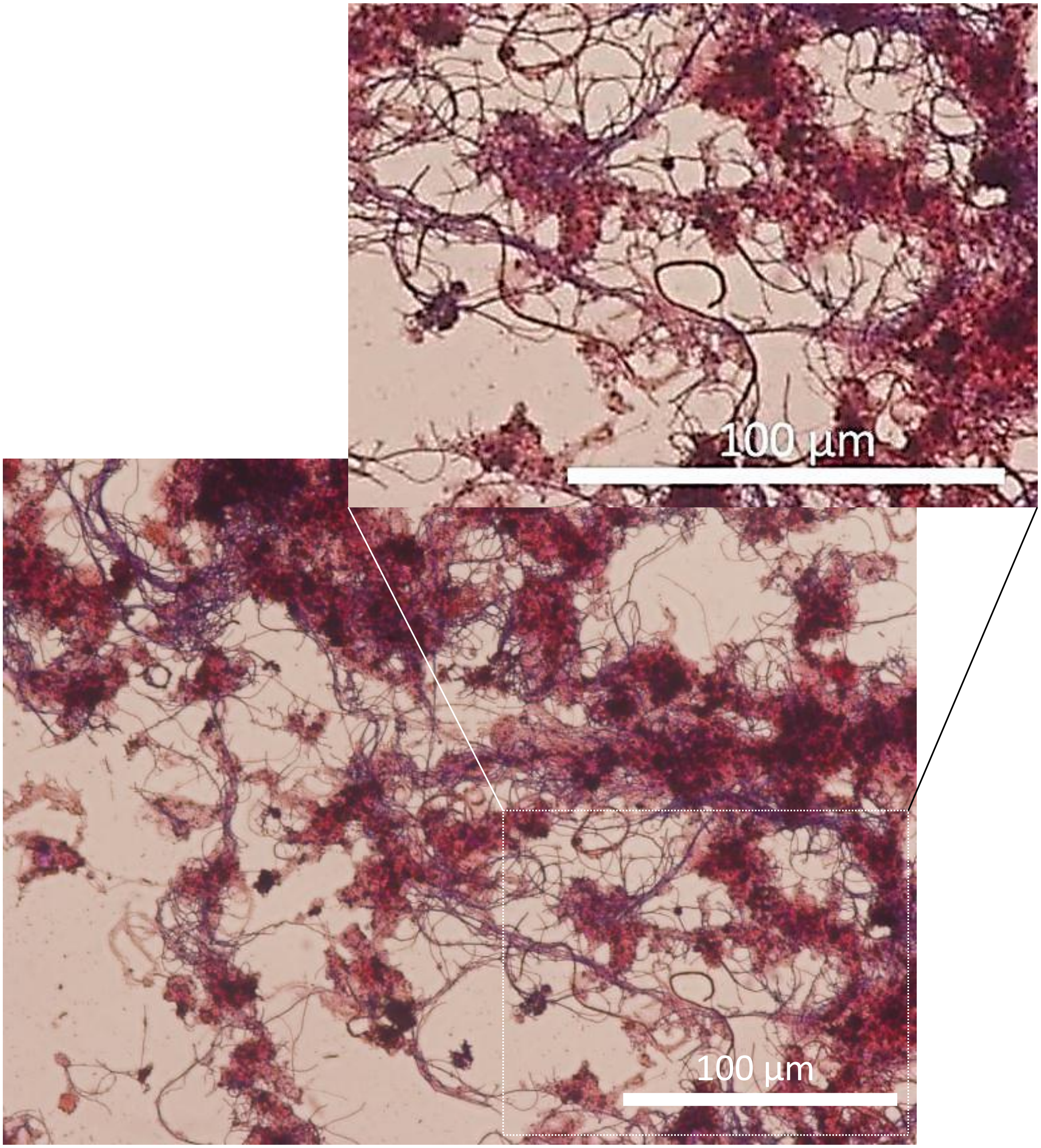

Figure 5- 1: A gram-stained photomicrograph of raw untreated WAS obtained at 100x objective with a Nikon Eclipse E400 microscope (Scale bar = 100 µm).

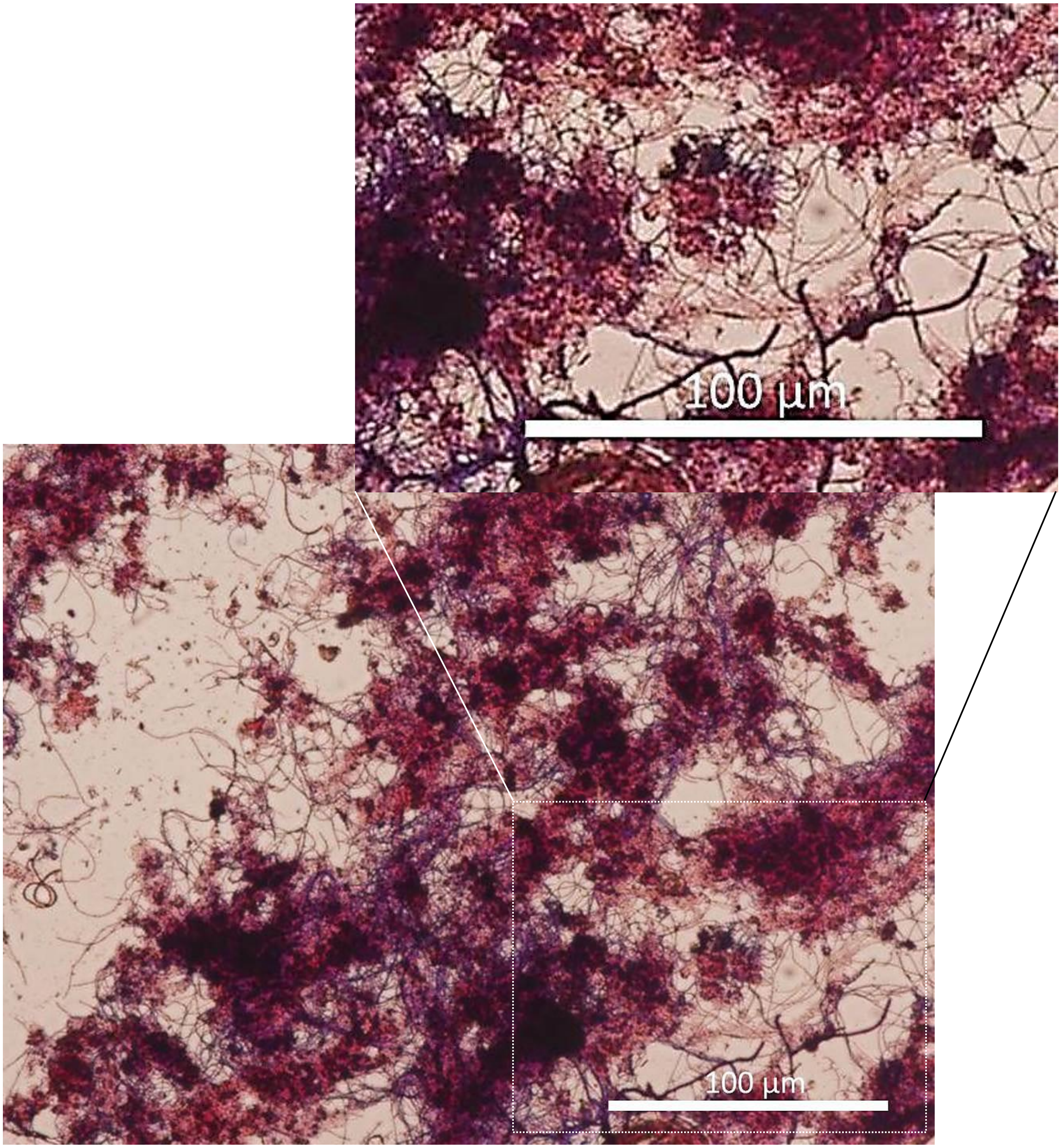

Figure 5- 2: A gram-stained photomicrograph of chlorine dioxide pre-treated WAS (at 50 mgClO2/L v/v) obtained at 100x objective with a Nikon Eclipse E400 microscope (Scale bar = 100 µm).

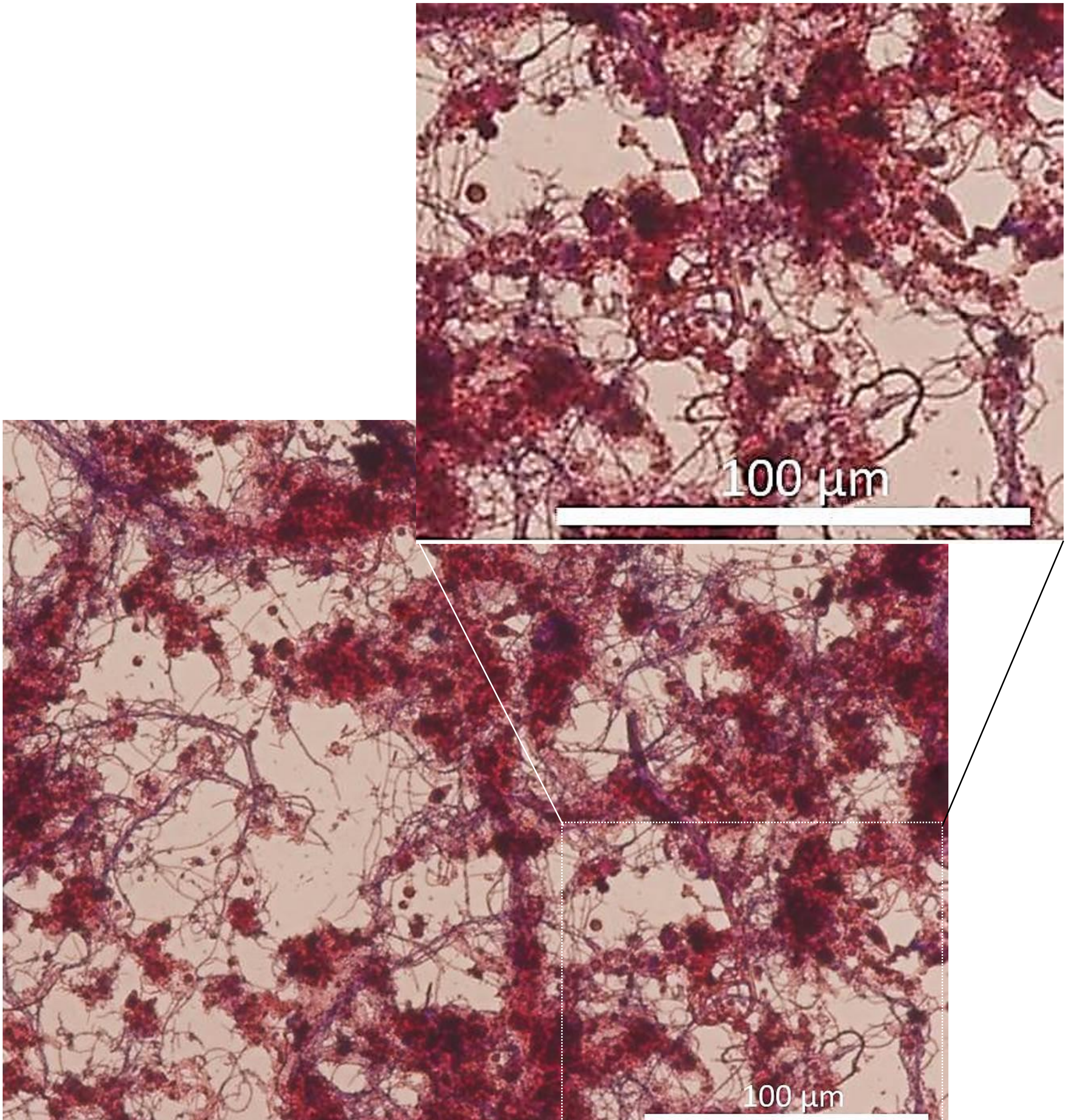

Figure 5- 3: A gram-stained photomicrograph of chlorine dioxide pre-treated WAS (at 100mgClO2/L v/v) obtained at 100x objective with a Nikon Eclipse E400 microscope (Scale bar = 100 µm).

Sludge Volume Index Evaluation

The sludge volume index was measured with stirring using the settling apparatus described in the methodology section. Three trials were performed for the stirred SVI (sSVI) evaluation. Because each trial was performed independently, the sludge used is expected to be at varying stages of bulking. Therefore the results of pre-treatment are shown independently for each trial as in Figure 5-4 below. We assessed the sSVI of the bulking sludge following chlorine dioxide pre-treatment at 0, 25, 50 and 100 mg ClO_2/L (v/v); there was a significant decrease in sSVI when the sludge was pretreated with 50 and 100 mg ClO_2/L (v/v) as compared with untreated sludge (P value < 0.05).

The decrease in sSVI ranged between 28 to 49% and 42 to 57% for the sludge pre-treated at 50 and 100 mg ClO_2/L, respectively. A 25 mg ClO_2/L pre-treatment resulted in no change to the sSVI; in some cases even, the sSVI was observed to increase over time (displayed as a negative % decrease in sSVI in Figure 5-4). This is similar to observations for the control (untreated sludge).

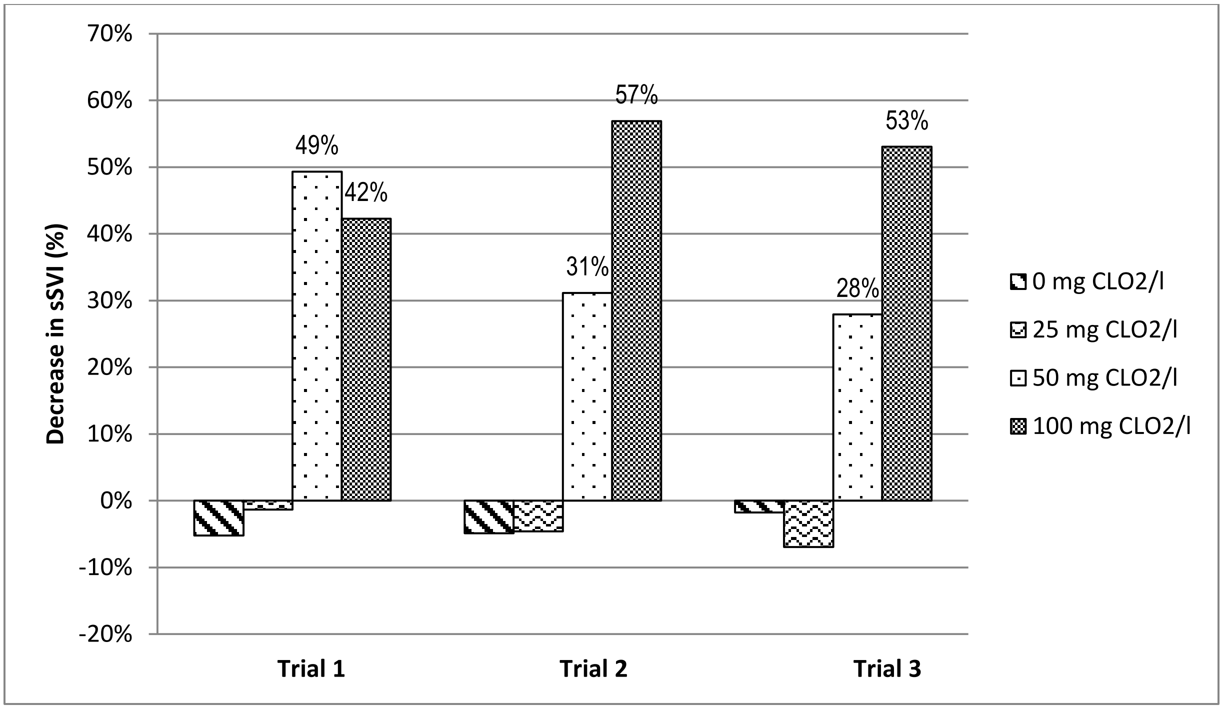

Figure 5- 4: Decrease in the sludge volume index between three independent batch trials following a bulking-WAS pre-treatment with chlorine dioxide at varying concentrations in the sludge; the sludge volume index significantly decreases following pre-treatment with 50 mg/L and 100 mg/L chlorine dioxide (*P* value < 0.05).

The degree by which the sSVI decreases is influenced by the specific chlorine dioxide dosage in the sludge (mg ClO_2 / g VSS). As the specific chlorine dioxide dosage increases, the sSVI can be expected to decrease (Figure 5-5). The specific chlorine dioxide dosage required to produce a significant decrease in sSVI in the bulking sludge was determined to be between 5 and 10 mg ClO_2 / g VSS. Below this threshold, it is possible that: 1) hydrous bulking due to denitrification and the production of nitrogen gas is taking place and / or 2) the presence of chlorine dioxide stimulates certain biochemical reactions including the release of extracellular polymeric substances (EPS) as the bacteria's initial defence mechanism. Therefore the specific chlorine dioxide dosage must be surpassed to counteract either effect in order to product a significant microorganism kill and consequently produce a significant reduction in the sludge bulking.

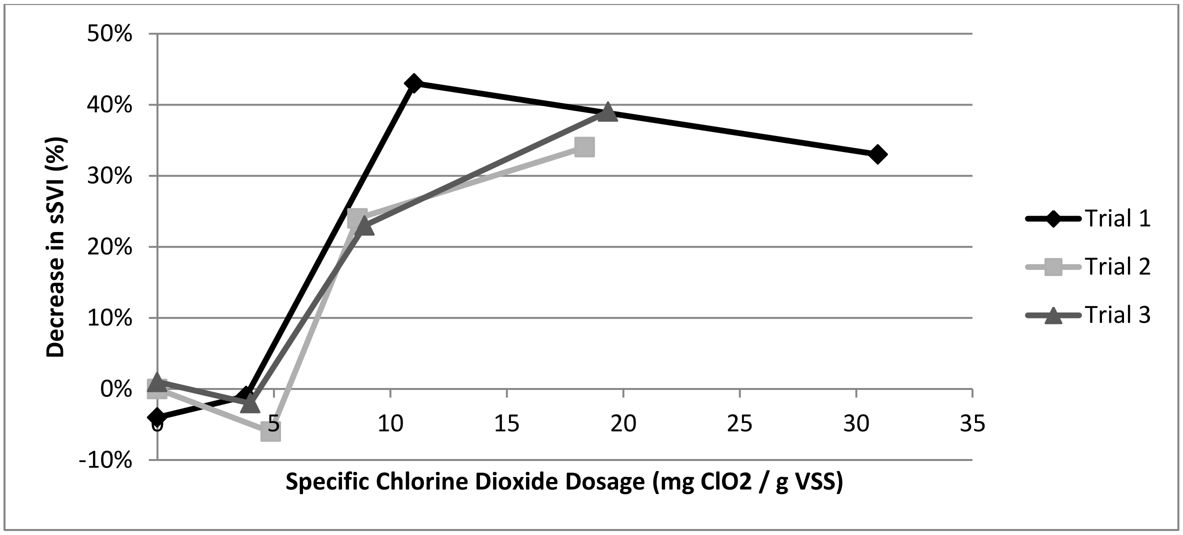

Figure 5- 5: The effect of varying dosages of ClO_2 on the stirred sludge volume index of bulking WAS in a batch study; the specific chlorine dioxide dosage required to produce a significant decrease in sSVI in the bulking WAS was determined to be between 4.6 and 10 mg ClO_2 / g VSS.

<u>Sludge Dewaterability Evaluation</u>

The sludge dewatering characteristics was measured using the capillary suction time (CST) apparatus described in the methodology section. Five iterations were performed for both untreated and pre-treated WAS. The pre-treated WAS received a 50 mg/L concentration of chlorine dioxide. Comparison of pre-treated sludge (M = 40 s, SD = 6.80) and untreated sludge (M = 37.3 s, SD = 3.40) revealed no significant differences in sludge dewaterability between the two; t (6) = -0.75, *ns*, therefore indicating that chlorine dioxide pre-treatment of sludge does not improve dewaterability characteristics at the specific dosage evaluated and for the sludge used. Similar to the findings from the sludge bulking evaluation, it is assumed that a specific chlorine dioxide dosage as a function of solids concentration (mg ClO_2 / g VSS), must be reached in order to produce a positively significant effect on sludge dewaterability. It is believed that in this case, the specific dosage was not reached. The solids content however was not evaluated here and therefore the specific dosage was not determined.

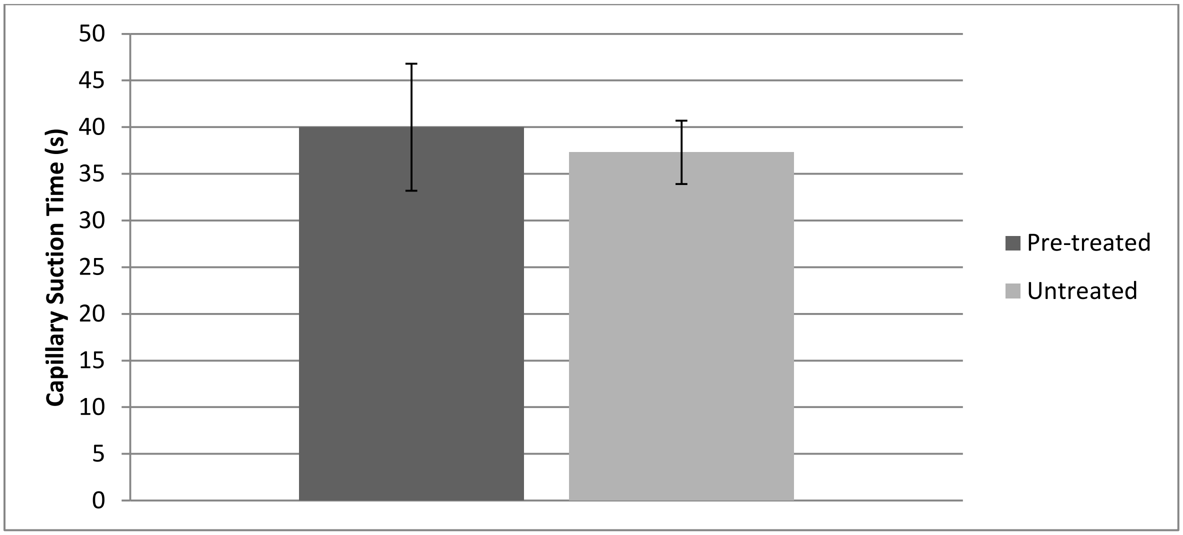

Figure 5- 6: A sludge dewaterability comparison, using the capillary suction time method, between untreated WAS and a pre-treated WAS which received 50 mg/L chlorine dioxide; the pre-treatment method does not significantly impact the dewaterability of the sludge (t (6) = -0.75, *ns*).

5.2.2. Sludge Solubilization Determination

Although both total COD and soluble COD were determined for each operation throughout this thesis, in this section, we report one set of data obtained with varied concentrations of chlorine dioxide.

Here, the COD was measured temporally between three trials. Table 5-1 shows the average total COD (tCOD) as a function of volatile suspended solids (VSS) for each variable completed. The values obtained appear to be in conjunction with stoichiometric determination i.e. 1.42 COD/VSS as reported in (Metcalfe & Eddy, Inc. , et al., 2013). Figure 5-7 shows that the COD/VSS were relatively consistent in each set of tests and also remained constant throughout the study period (P = <0.05). This is of significant importance not only because it assures the sludge integrity is maintained throughout the experimental period but also it provides assurance that complete oxidation to inorganic forms of low calorific carbonaceous compounds is avoided. Chlorine dioxide as a sludge pre-treatment agent facilitates the conversion of complex organic matter to readily-degradable soluble forms which become valuable substrates in the digester. It is therefore imperative that the pre-treatment method utilized is such that complete chemical oxidation to the low calorific carbon dioxide ($CO_{2(g)}$) compound is avoided.

Table 5- 1: COD/VSS of WAS with and without chlorine dioxide at various concentrations remained unchanged in a sludge pre-treatment batch study (*P* = <0.05).

		COD/VSS		
Time (hr)	0 mg CLO_2/L	25 mg CLO_2/L	50 mg CLO_2/L	100 mg CLO_2/L
0	1.45	1.41	1.43	1.42
2	1.48	1.47	1.49	1.40
4	1.49	1.50	1.50	1.40
6	1.49	1.47	1.49	1.43
8	1.50	1.47	1.54	1.50
10	1.49	1.46	1.58	1.46
12	1.46	1.50	1.53	1.41

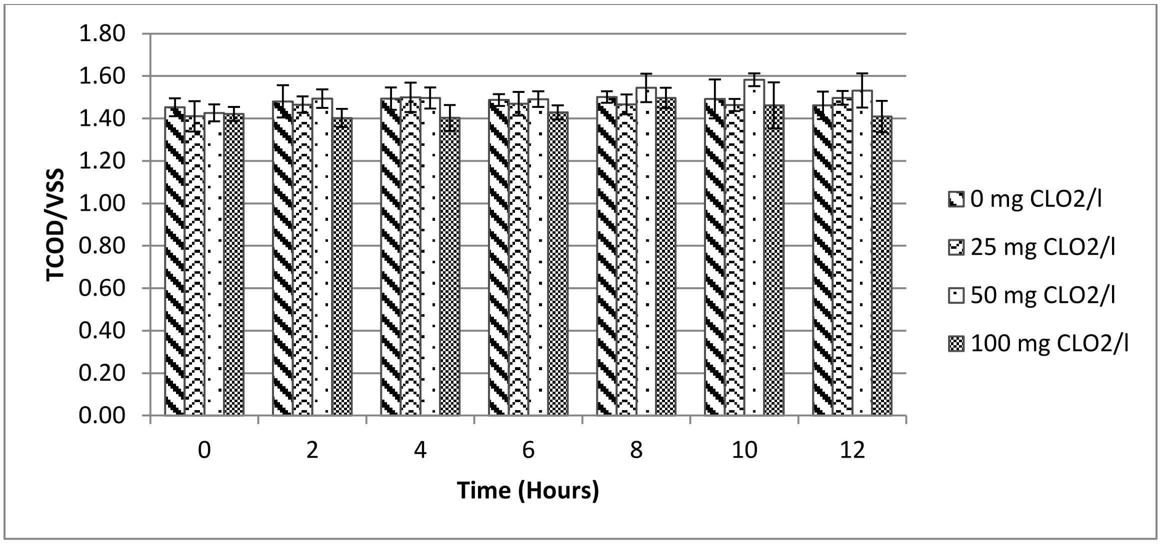

Figure 5- 7: COD/VSS evaluation following WAS pre-treatment with chlorine dioxide in a batch study; the COD as it relates to VSS remained unchanged for both untreated and pre-treated WAS as well as throughout the study period indicating that carbonaceous compounds remained in solution (*P* = <0.05).

The soluble COD release was evaluated as a function of total COD present in the WAS. As shown in figure 5-8, chlorine dioxide pre-treatment of WAS resulted in a significant increase in solubilized COD over the untreated sludge (*P* = <0.05). COD solubilization increased by 60%, 76%, and 74% over the untreated WAS for WAS pre-treated with 25, 50, and 100 mg ClO_2/L (v/v), respectively. Maintaining a 50 mg ClO_2/L (v/v) concentration in the reactor showed the most consistent and highest cumulative increase in solubilized COD as compared with the other input variables. It is important to note that no significant increase of soluble COD was observed over time with each test completed. This suggests that chlorine dioxide oxidative reaction occurs instantly (and not continuing).

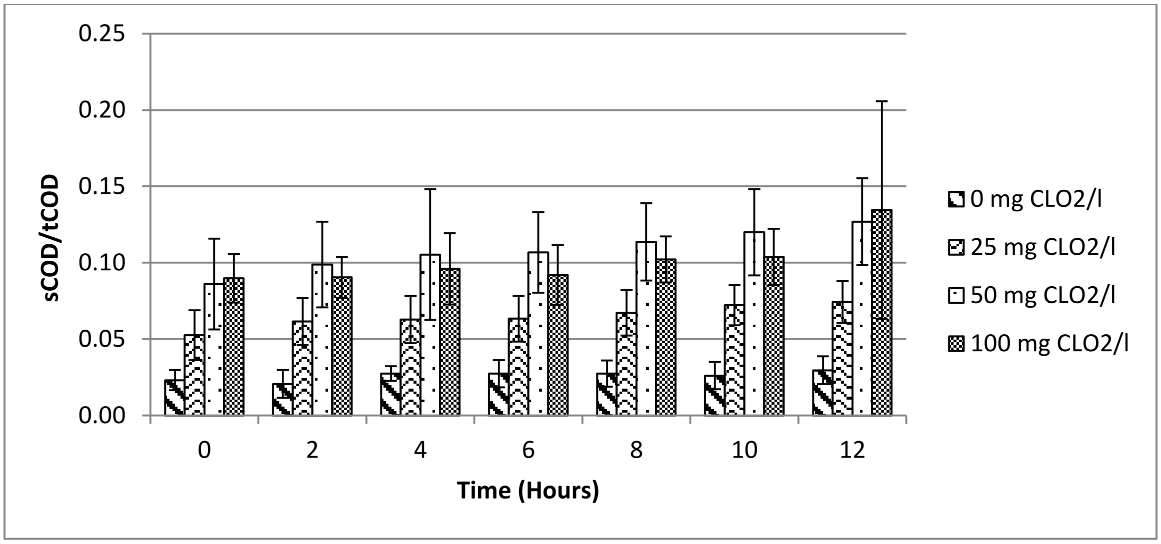

Figure 5- 8: Soluble COD release following WAS pre-treatment with various concentrations of chlorine dioxide in a batch study; COD solubilization significantly increases with increasing chlorine dioxide dosages (*P* = <0.05).

The specific chlorine dioxide dosage in the reactor was determined as a function of VSS for each reactor. Similar to figure 5-5, the soluble COD release can be seen to increase as the specific chlorine dioxide dosage is increased (Figure 5-9). Seemingly, a threshold is reached at about 10 mg ClO_2/g VSS; above this threshold, solubilized COD no longer increases; likely indicating oxidation of carbonaceous compounds at a higher chlorine dioxide dosages. An increase in solubilized carbonaceous compound is expected to result in an improved digestion process unless where the digester organisms are negatively impacted by residual chlorine dioxide or its by-products.

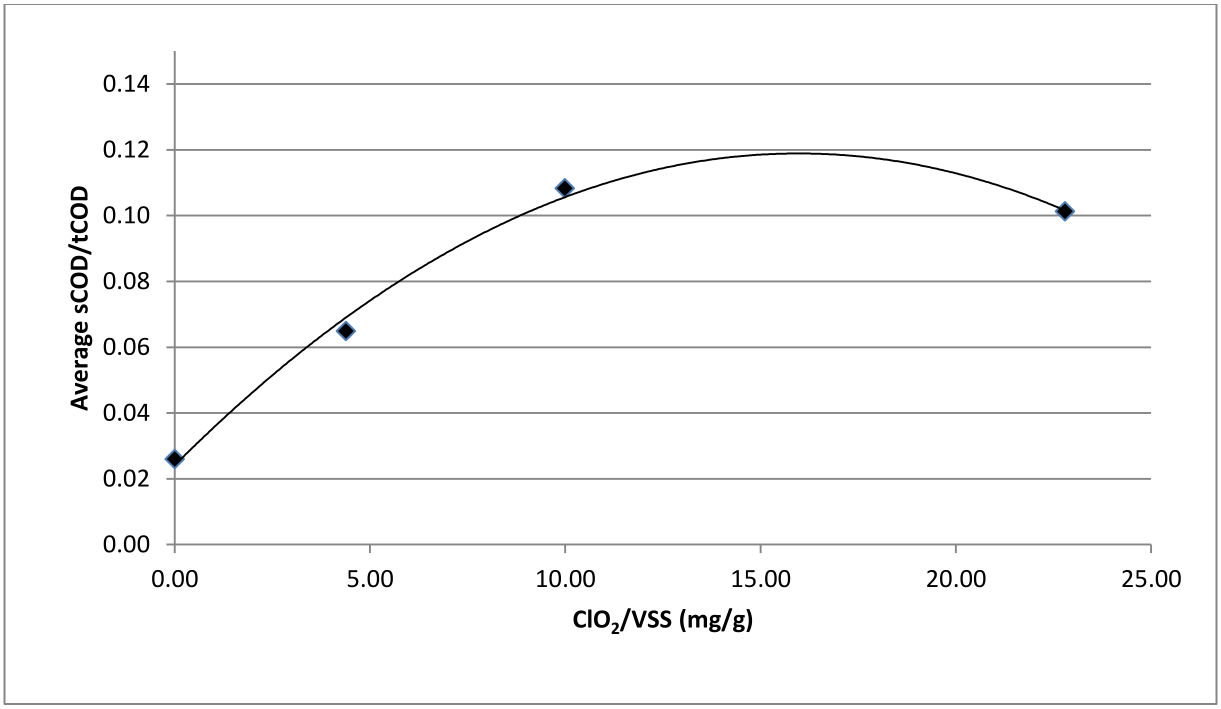

Figure 5- 9: Relationship between the specific chlorine dioxide dosage [ClO_2/VSS (mg/g)] and COD solubilization in pre-treated of WAS during a batch study.

5.2.3 Semi-continuous Digester Operation

pH

The pH is usually the first measure of digester stability. Effective anaerobic digestion occurs near neutral pH with each group of micro-organisms having a different optimum pH range. Methanogenic activities are especially sensitive to unionized forms of VFA which occurs at lower pH values. The optimum operating range for the anaerobic digester is between 6.5 and 7.5 pH units (Appels, et al., 2008); below 6.8 pH units, methanogens become severely inhibited

(Metcalfe & Eddy, Inc. , et al., 2013). A syntrophic relationship has to be maintained whereby methanogens convert fermentation products to methane and carbon dioxide.

The pH in the semi-continuous reactors remained relatively stable between 6.85 – 7.46 pH units throughout the experimental period (Figure 5-10). Steady state was reached early on (Days 0-5) probably because the reactors were seeded with digestate from a previous experiment. No significant variation in pH was observed within each digester throughout the experimental period as well as between the three digesters (*P* = <0.05) as shown in Figure 5-10.

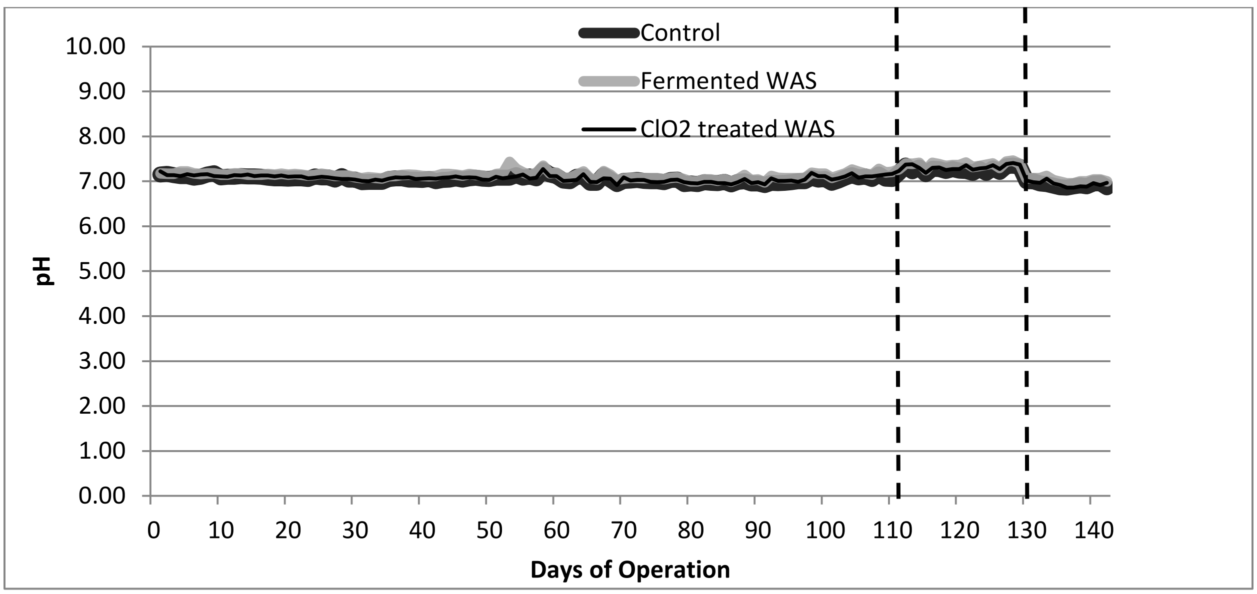

Figure 5- 10: The pH of three semi-continuously-operated anaerobic digesters remain stable within the methanogenic range (Vertical dashed lines indicate changes to SRT – from left to right: 10, 8 and 6 days) (*P* = <0.05).

A slight but not significant increase in pH was observed when the SRT was initially reduced from 10 days to 8 days. This may be attributed to a) washout of organic acids in the reactor and/or b) an initial increase in feed input prompting a temporary spike in ammonia. However the pH remained in the acceptable range for normal digester operations. A further decrease of the

SRT, now to 6 days, resulted in a slight pH depression. This is most likely due to increased solids turnover prompting further methanogens washout and a temporary spike in CO_2 accumulation (carbonic acid), and consequently a slight accumulation of unionized VFA. Throughout the experimental period however, there appeared no reason to adjust the pH therefore supplemental alkalinity was not added to the reactors.

Solids destruction

Solids evaluation specifically volatile solids destruction is an important measure of the degree of stabilization achieved in the anaerobic digestion process. The volatile solids information is also often used to normalize sludge characterization data from sludge of different batches or source. Theoretically, volatile solids destruction of a high-rate complete-mix digester is expected to be;

$$V_d = 13.7 \ln (SRT_{des}) + 18.9$$ (Metcalfe & Eddy, Inc. , et al., 2013)

Where V_d = volatile solids destruction, % and

SRT = time of digestion, d

Total and volatile solids content (TS and VS, respectively) were measured for each input and output in both pre-treatment and digestion phases. The overall TS and VS destruction was determined and the average plotted as shown in Figures 5-11 and 5-12, respectively, for each SRT operation.

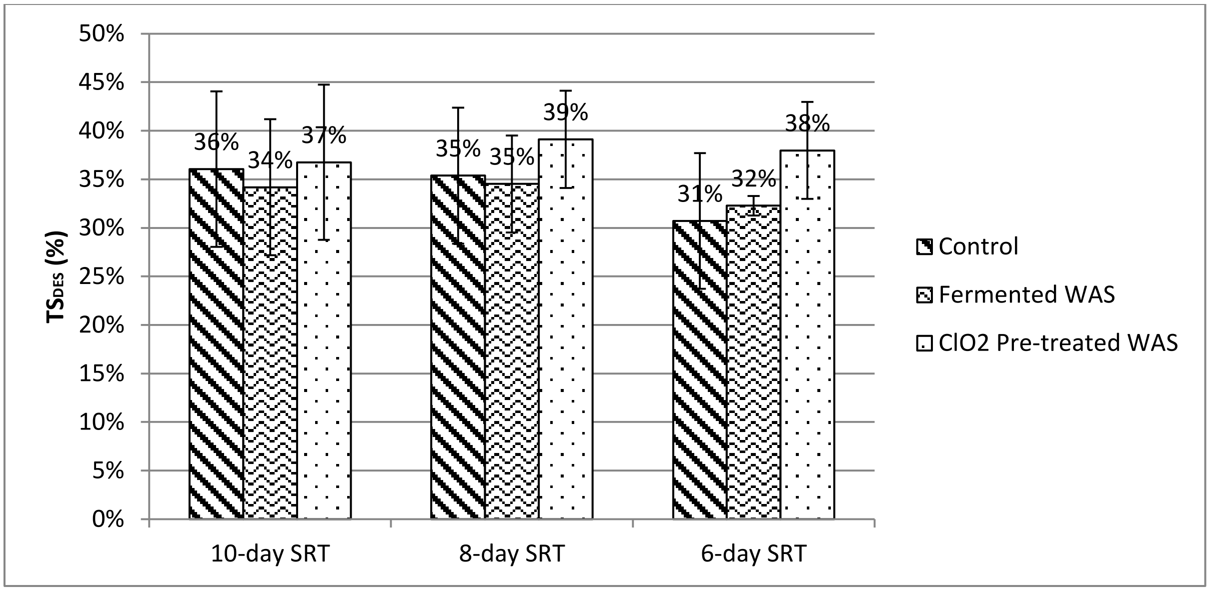

Figure 5- 11: The overall total solids destruction following chlorine dioxide pre-treatment and subsequent anaerobic digestion in semi-continuous reactors operated at varying SRTs; comparison between each condition tested and between each SRT investigated revealed no significant differences (*P* = >0.05).

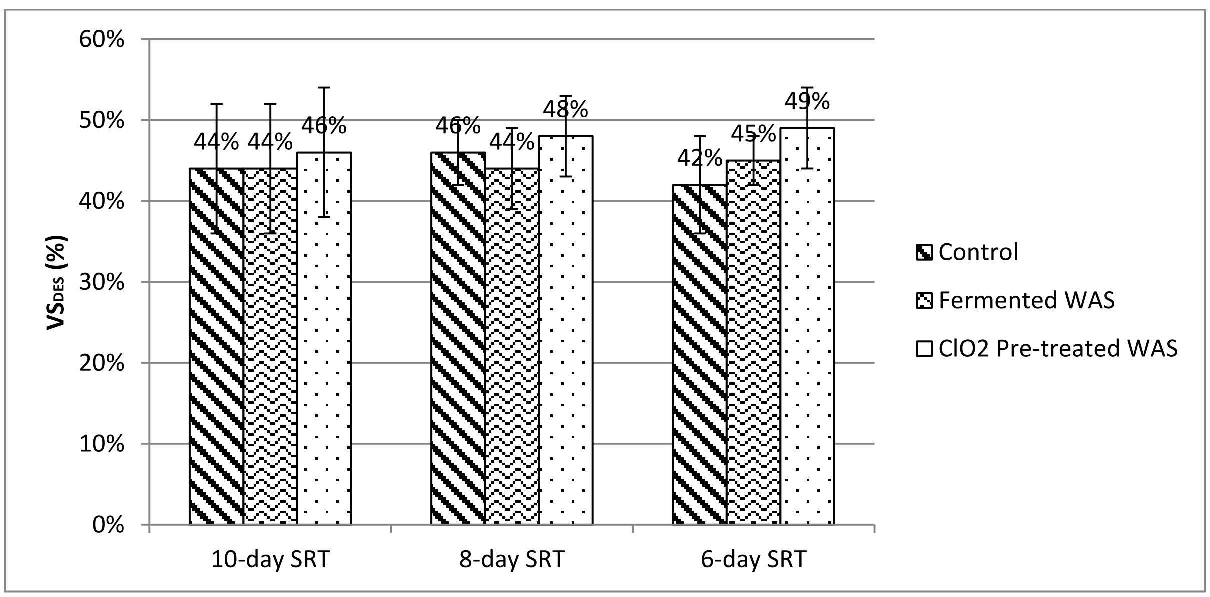

Figure 5- 12: The overall volatile solids destruction following chlorine dioxide pre-treatment and subsequent anaerobic digestion in semi-continuous reactors operated at varying SRTs; comparison between each condition tested and between each SRT investigated revealed no significant differences (*P* = >0.05).

A comparison between the untreated, fermented and pre-treated WAS revealed that there were no significant differences in both TS_{DES} and VS_{DES} (P = >0.05) for each SRT investigated. This shows that the use of chlorine dioxide as a pre-treatment agent does not negatively impact solids destruction in the anaerobic digester. In addition, reducing the SRT did not result in either negative or positive impacts to solids destruction within the digester units. However, the improved solubilization of sludge realized previously did not directly translate into improved solids destruction. It is likely that the improved solubility realized before is as a result of chlorine dioxide's acting on particulates that are already readily degradable and would be effectively degraded in the digester even at the low SRT attempted. Perhaps a less suitable sludge type, such as one experiencing severe bulking or one that has been characterized as being highly complex and difficult to hydrolyze, would benefit from the supplemental pre-treatment stage.

A closer look at the digester units alone showed similar observations. At first glance it appears that the digester receiving chlorine dioxide pre-treated WAS yielded the least solids destruction during the 10 and 8 day operations (Figures 5-13 and 5-14). It was initially thought that the implementation of chlorine dioxide as a pre-treatment agent might induce some inhibition within the digester unit, mainly due to residual chlorine dioxide or the by-product of the oxidative reaction entering into the digester. When chlorine dioxide reacts with organic compounds, the by-product chlorite is formed (Stevens, 1982). Chlorite is an oxidizing agent itself and may negatively affect the digester microbial community. However statistical comparison revealed that the differences between the digester receiving untreated sludge and that receiving pre-treated sludge were insignificant (P = >0.05). Therefore supporting the conclusion that chlorine dioxide may be used as a sludge pre-treatment agent without adverse effects to the anaerobic digester performance.

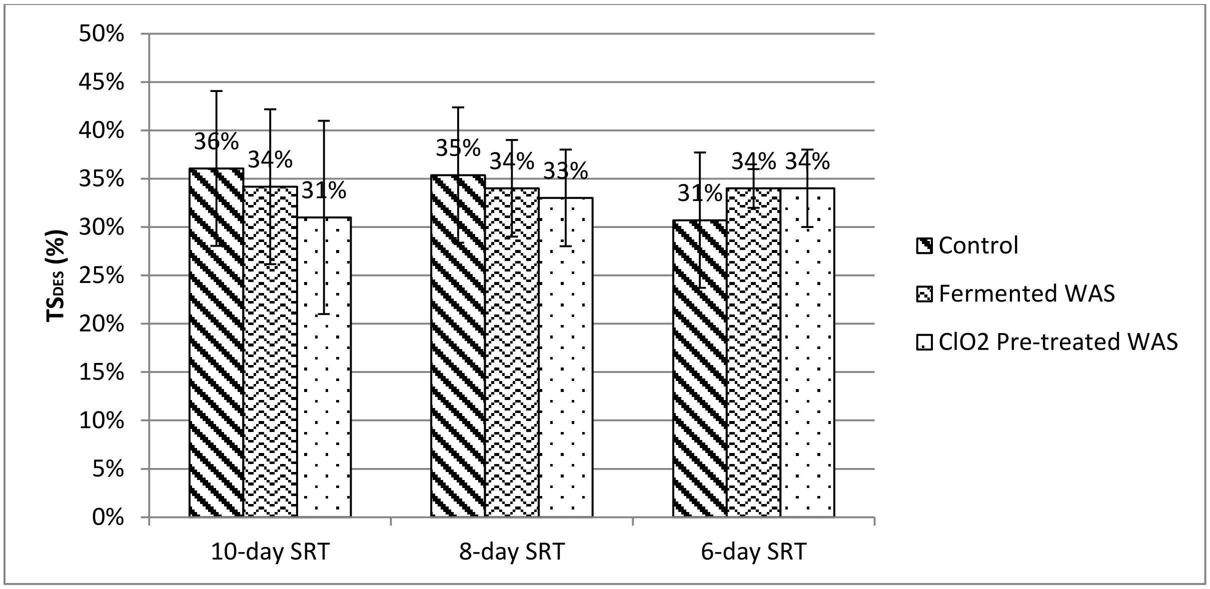

Figure 5- 13: The total solids destruction observed within the anaerobic digester alone, with operations at varying SRTs; comparison between each condition tested and between each SRT investigated revealed no significant differences (*P* = >0.05).

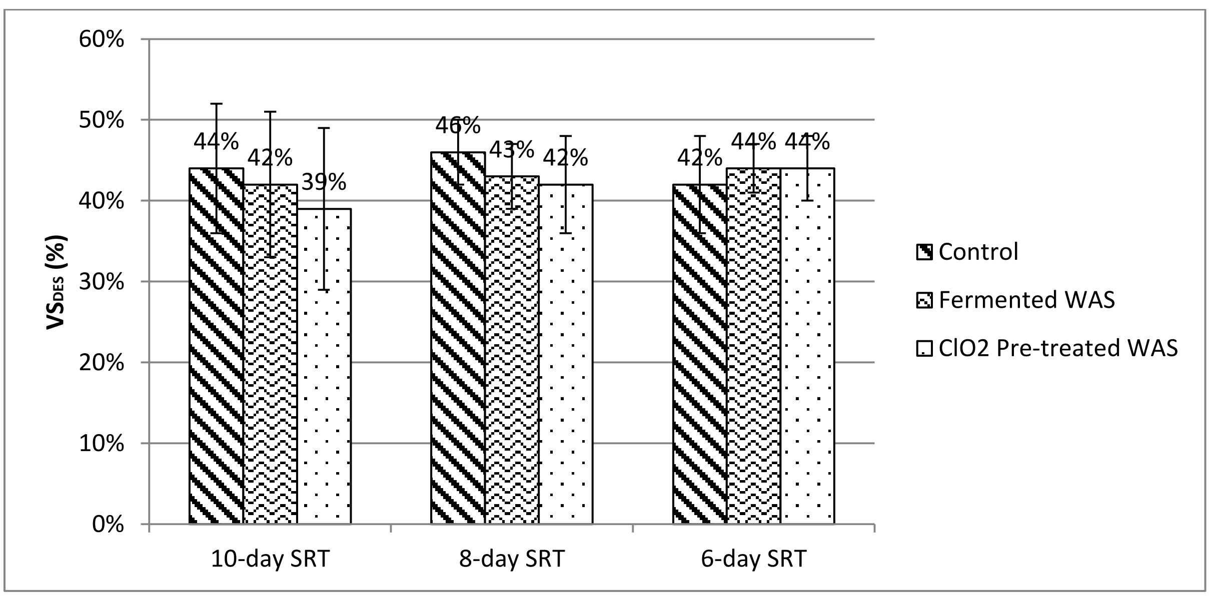

Figure 5- 14: The volatile solids destruction observed within the anaerobic digester alone, with operations at varying SRTs; comparison between each condition tested and between each SRT investigated revealed no significant differences (*P* = >0.05).

COD removal

Similar to the volatile solids destruction, the COD removal is another measure of anaerobic digester performance. As microorganisms degrade organic substrates into stabilized forms COD is expected to decrease. COD removal is concurrent and can be paralleled with volatile solids destruction. Theoretically, the COD/VS has been determined as 1.42 (Metcalfe & Eddy, Inc. , et al., 2013). COD was measured for each digester input and output during the feeding schedule. The COD content was determined and the average overall COD removal is plotted as shown in Figures 5-15 for each SRT operation.

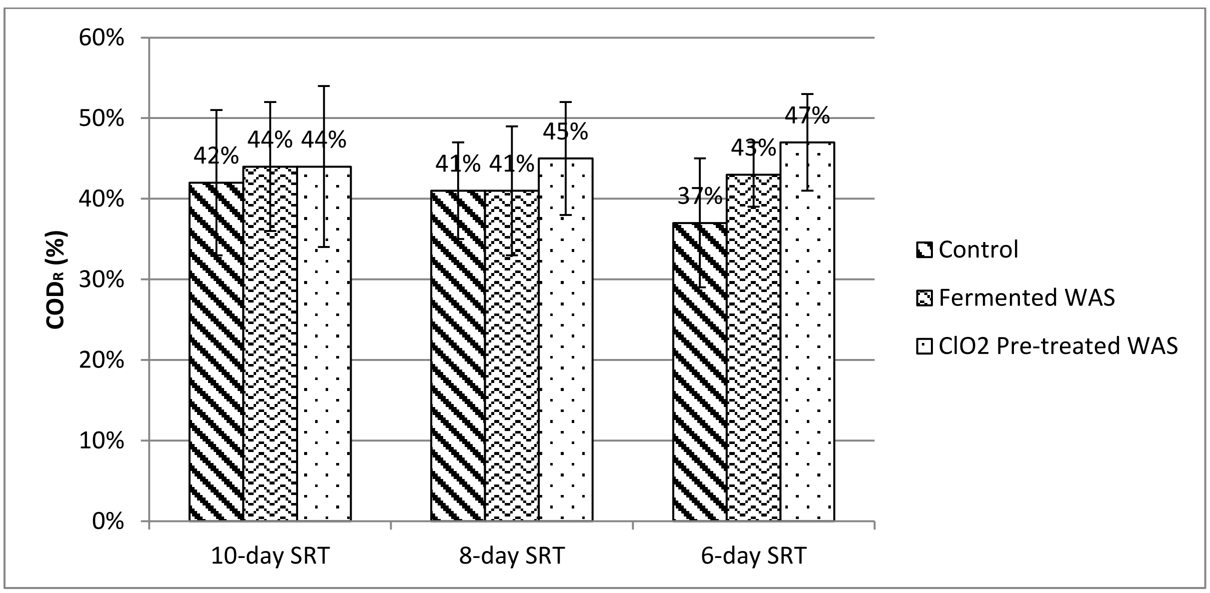

Figure 5- 15: The overall COD removals following chlorine dioxide pre-treatment and subsequent anaerobic digestion in semi-continuous reactors operated at varying SRTs; comparison between each condition tested and between each SRT investigated revealed no significant differences (*P* = >0.05).

A comparison between the untreated, fermented and pre-treated WAS revealed that there were no significant differences in CODr (*P* = >0.05) for each SRT investigated. This correlates with previous observation for solids destruction. In addition, reducing the SRT also did not result in either negative or positive impacts to solids destruction within the digester units. This observation is not surprising since the fermented sludge also does not impact the digester performance thus confirming the explanation that the pre-treatment method utilized here likely acted on particulates that are already readily degradable and would be effectively degraded in the digester nonetheless.

A closer look at the digester units alone showed little significance between the conditions tested with the exception of the 6-day SRT where the results of chlorine dioxide pre-treatment was significantly different than that observed during the 10-day operation (Figures 5-16).

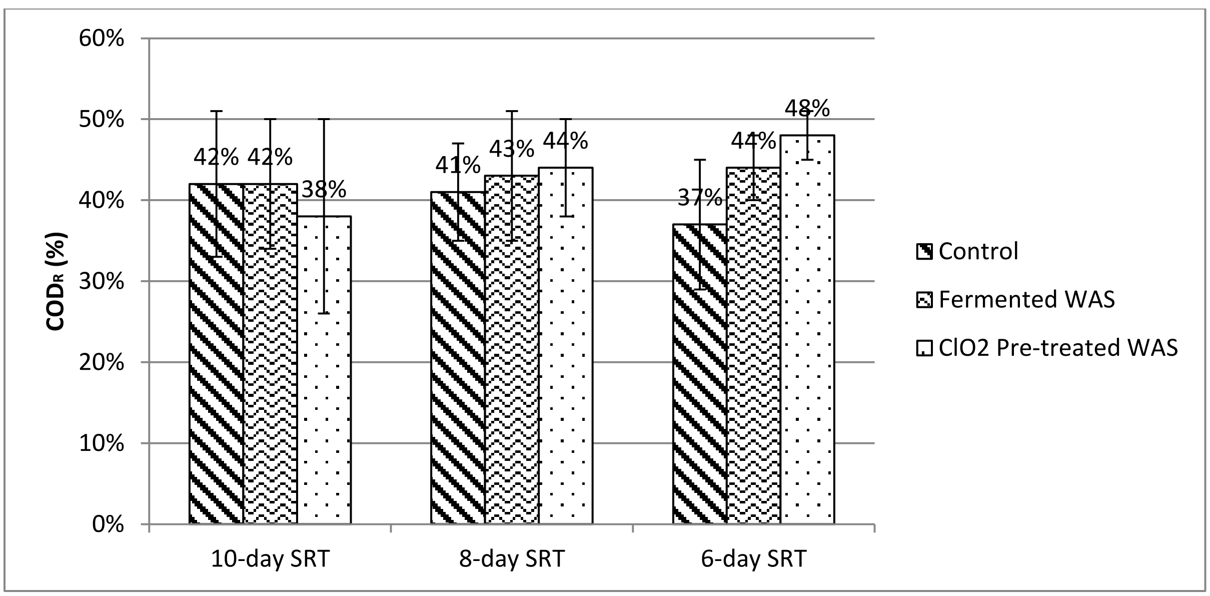

Figure 5- 16: COD removal observed within the anaerobic digester alone, with operations at varying SRTs; comparison between each condition tested and between each SRT investigated revealed no significant differences (*P* = >0.05) except for a significant increase in CODr when the SRT was reduced to 6-day.

It has been shown above, that decreasing the operating SRT does not negatively impact anaerobic digester performance. From the observation here, there is a potential to improve the digester performance although this is only realized at a decreased operating SRT which is mostly affected by the control reactors as the hydrolysis rate within the digester unit become limited.

Biogas and methane production

One of the most important advantages of anaerobic digestion over other methods of solids stabilization is its potential for energy recovery. Biogas in the form of methane (CH_4) and carbon dioxide (CO_2) are generated from the anaerobic degradation of organic matter. In addition to the net production of energy, methane gas is the most reduced state of carbon (Rittman & McCarty, 2001) and therefore represents the complete stabilization of carbonaceous compounds following anaerobic treatment.

The specific gas production is commonly reported as the percentage of volatile solids destroyed with typical values ranging from 0.75 to1.12 m^3/kg (or 12 to 18 ft^3/lb) of volatile solids destroyed. From stoichiometry, the amount of methane produced per unit of COD converted during anaerobic digestion is equal to 0.35 L CH_4/g COD under standard conditions (0^oC and 1 atm) or 0.40 L CH_4/g COD at 35^oC (Metcalfe & Eddy, Inc. , et al., 2013).
The volume and composition of biogas produced was recorded throughout the experimental period and the total volume of gas per operating period was used to calculate the specific gas production, as it relates to volatile solids destroyed. This is presented in Figure 5-17, for each SRT operation. Taking the gas composition into account (Table 5-2), the amount of methane produced per COD removed is presented in Figure 5-18.

The resulting specific gas production values were closest to typical values for the 10-day SRT operation only; these were 0.71, 0.66, and 0.60 m^3/kg VS_{des} for the control, fermented WAS, and ClO_2 pre-treated WAS, respectively. As the SRT decreased, the specific gas production for all conditions decreased (Figure 5-17). This is probably due to methanogens wash out at lower SRT. Both fermented and pre-treated WAS-receiving digesters resulted in less specific gas production than the control. Statistical correlation could not be made as these represent single values (not a series of data).

Gas composition data (Table 5-2) show that all three digesters performed ideally, achieving methane proportions close to typical values of 65 to 75% (Metcalfe & Eddy, Inc. , et al., 2013). Similar to the specific gas production observation, the amount of methane produced per unit of COD converted were closest to typical values (0.40 L CH_4/g COD at 35^oC) for the 10-day SRT operation only; these were 0.37, 0.36, and 0.40 L CH_4/g COD for the control, fermented WAS, and ClO_2 pre-treated WAS, respectively (Figure 5-18). As the SRT decreased, the amount of methane produced decreased (Figure 5-17); also likely due to methanogen wash out. The digesters were monitored and sealed regularly to prevent potential biogas escape.

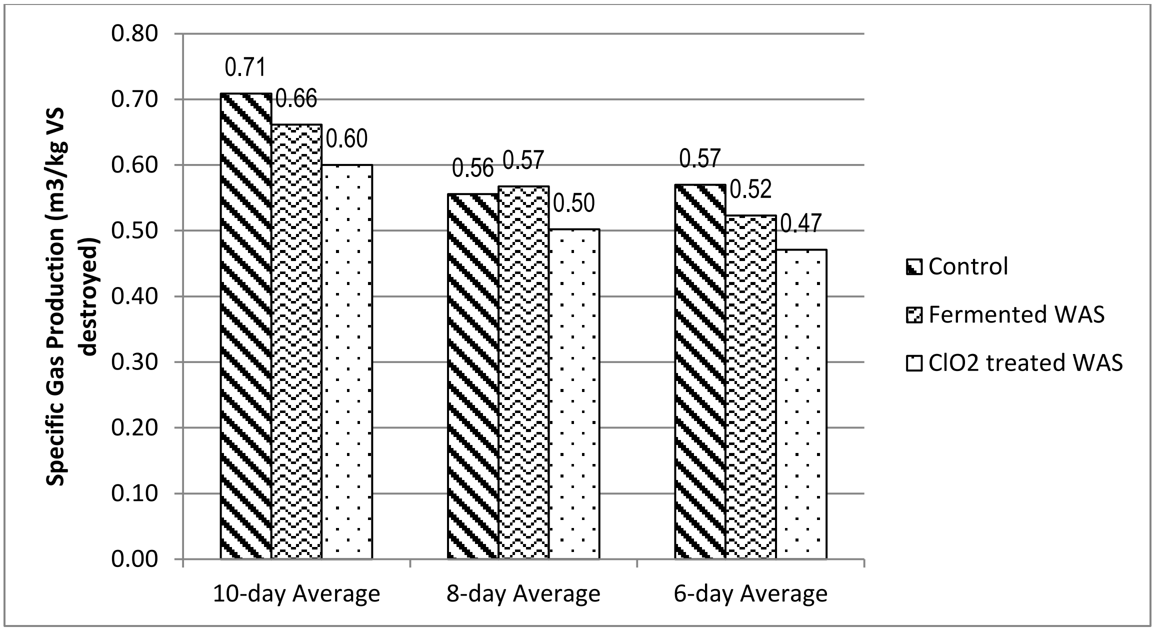

Figure 5- 17: Specific gas production calculated for a semi-continuous digester operation, operating at a 10, 8 and 6-day SRT. The set up included a control reactor (untreated WAS), a 1.5 day fermented WAS and a 50 mg/L ClO_2 pre-treated WAS.

Table 5- 2: Average gas composition in a semi-continuous operated digester (analysis performed at the end of the study period at 6-day SRT).

Digester (feed input)	% Methane	% Carbon dioxide	% Nitrogen
Untreated WAS	73.14 ± 0.06	25.15 ± 0.07	1.71 ± 0.02
Fermented WAS	77.64 ± 0.28	21.30 ± 0.25	1.05 ± 0.03
Chlorine dioxide pre-treated WAS	75.38 ± 0.84	23.49 ± 0.89	1.12 ± 0.06

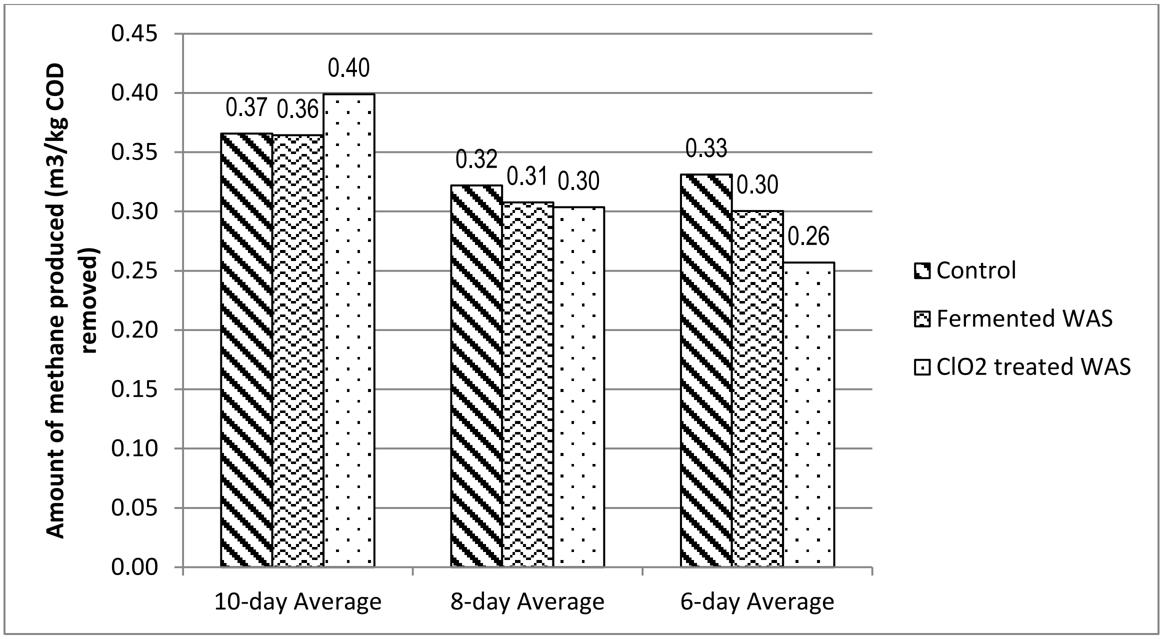

Figure 5- 18: Methane production calculated for a semi-continuous digester operation, operating at a 10, 8 and 6-day SRT. The set up included a control reactor (untreated WAS), a 1.5 day fermented WAS and a 50 mg/L ClO_2 pre-treated WAS.

From the semi-continuous digester operation, we were able to show that pre-treatment of the sludge to increase its solubility does not negatively impact anaerobic digester performance; however it also does not improve same performance significantly. The solids destruction, COD removal and biogas production remained the same for digesters receiving untreated WAS and digesters receiving either fermented or chlorine dioxide pre-treated WAS. In addition, the use of chlorine dioxide as a pre-treatment agent does not inhibit the digester even at the lowest SRT attempted. It is therefore possible to reduce the digester operating SRT to as low as 6-days without significant negative impacts to the digester performance.

5.2.4 Effect of Chlorine Dioxide Concentration and Sludge Type on Anaerobic Digester Operation

Following the semi-continuous operation, batch studies were completed in order to further investigate the effects of chlorine dioxide pre-treatment in the digester units alone (the pre-treated sludge was not analyzed). Here, varied concentrations of the pre-treatment agent were reacted with two sludge types; a secondary (WAS-only) feed sludge and a *co-fermented* (PS/WAS-mixture) feed sludge. This allowed for comparison between different chlorine dioxide dosages and between pre-treatment of two different types of sludge, on the digestion process.

Solids Destruction

The initial (pre-digestion) and final (post-digestion) total and volatile solids (TS and VS, respectively) were measured for each digester unit. The solids content was determined and the average TS and VS destructions were plotted as shown in Figures 5-19 and 5-20, respectively. A comparison between the untreated and pre-treated sludge revealed that there were no significant differences in both TS_{DES} and VS_{DES} (P = >0.05) regardless of the chlorine dioxide concentration for each sludge type used. This is similar to observations from the semi-continuous operation and confirms that the use of chlorine dioxide as a pre-treatment agent does not negatively impact solids destruction in the anaerobic digester.

However, significant differences were observed in some cases when the comparison was made between the WAS-only and the PS/WAS mixture. The PS/WAS mixture experienced a greater TS_{DES} than the WAS-only sludge feed for both untreated and pre-treated feed inputs(P = <0.05). However, VS_{DES} in the PS/WAS mixture was only significantly higher where the pre-treatment utilized 100 mg ClO_2/l. This is expected given the greater hydrolysis rate in PS hence why *cofermentation* is widely practiced. The benefit of *cofermentation* has been reported by (Yuan, et al., 2010).

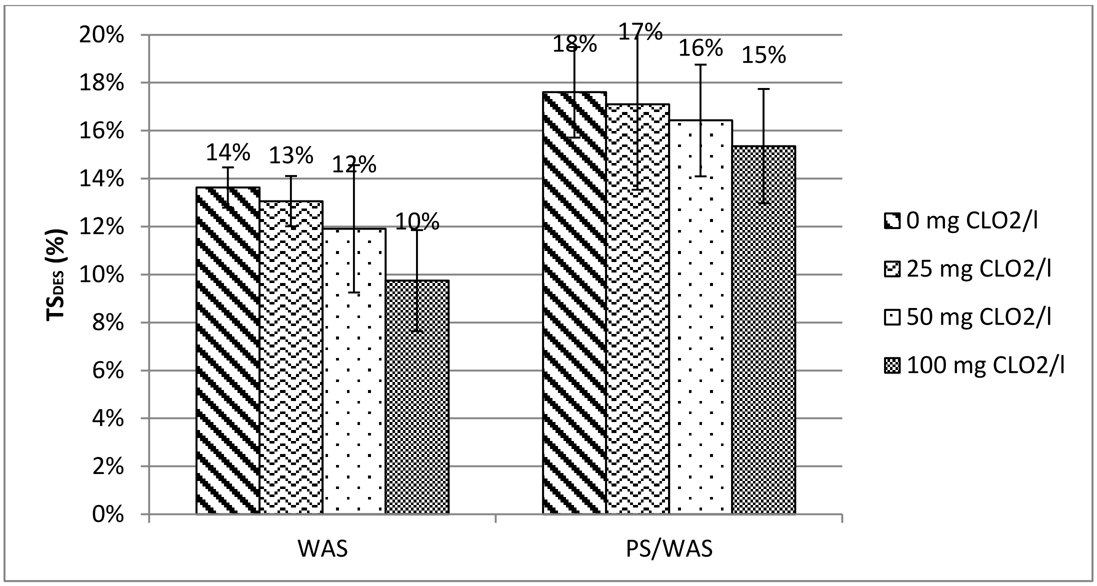

Figure 5- 19: Total solids destruction obtained in batch operated anaerobic reactors receiving varying concentrations of chlorine dioxide in the sludge feed. Comparison revealed that chlorine dioxide pre-treatment had no significant impact on either WAS or PS/WAS mixture (P = >0.05). However, the PS/WAS mixture experienced greater solids destruction than WAS-only sludge feed (P = <0.05).

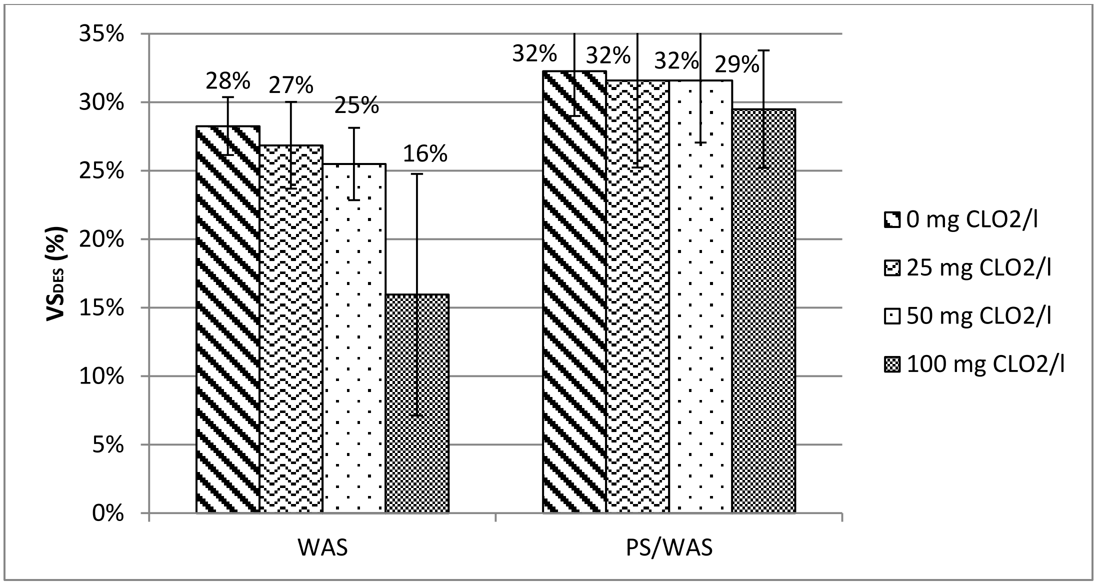

Figure 5- 20: Volatile solids destruction obtained in batch operated anaerobic reactors receiving varying concentrations of chlorine dioxide in the sludge feed. Comparison revealed that chlorine dioxide pre-treatment had no significant impact on either WAS or PS/WAS mixture (*P* = >0.05). However, the PS/WAS mixture experienced greater solids destruction than WAS-only sludge feed (*P* = <0.05).

COD Removal

The initial (pre-digestion) and final (post-digestion) COD were measured for each reactor. Average COD removals (COD_R) were analyzed as shown in Figure 5-21. A comparison between the untreated and pre-treated sludge revealed that there were no significant differences in COD_R regardless of the chlorine dioxide concentration for each sludge type used (P = >0.05). This is also similar to observations from the semi-continuous operation and confirms that the use of chlorine dioxide as a pre-treatment agent does not negatively impact COD removal in the anaerobic digester.

Also here, some significant differences were observed when the comparison was made between the WAS-only and the PS/WAS mixture. The *co-fermented* mixture experienced a greater COD_R

than the WAS-only sludge feed for both untreated and 25 mg ClO_2/l pre-treated feed inputs(P = <0.05). This is expected given the greater hydrolysis rate in PS hence why *cofermentation* is widely practiced.

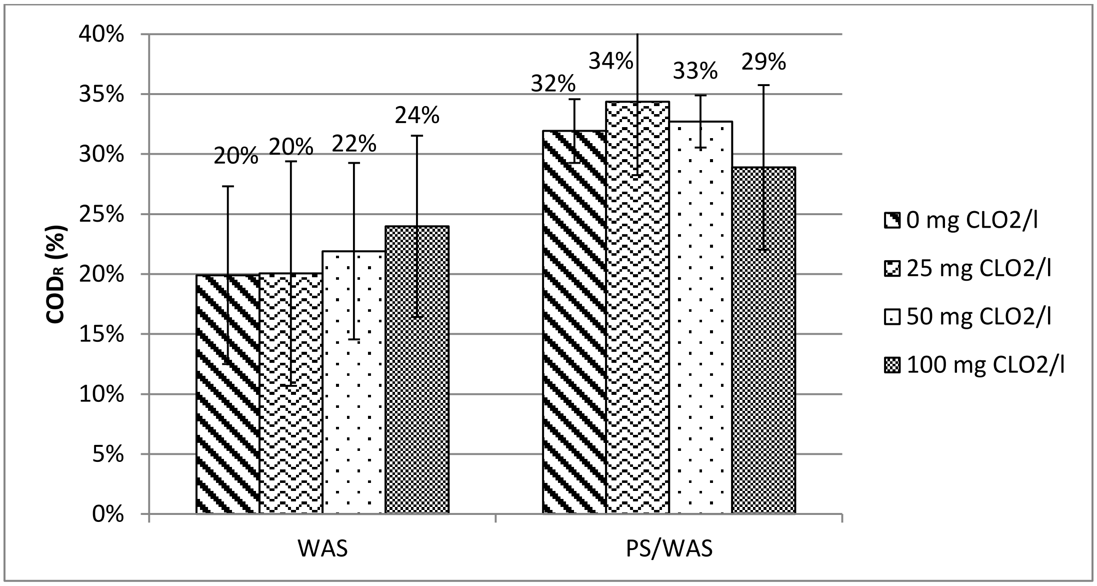

Figure 5- 21: Total COD removal obtained in batch operated anaerobic reactors receiving varying concentrations of chlorine dioxide in the sludge feed. Comparison revealed that chlorine dioxide pre-treatment had no significant impact on either WAS or PS/WAS mixture (*P* = >0.05). However, the PS/WAS mixture experienced greater COD removal than WAS-only sludge feed (*P* = <0.05).

<u>*Biogas and methane production*</u>

The total biogas production (by volume) was measured throughout the experimental period and the average values between three replicates are reported in Figures 5-22 and 5-23 for both reactors receiving WAS-only and a PS/WAS mixture, respectively. Similar to previous observation for the semi-continuous operation, there were no significant differences in biogas production between the untreated and pre-treated sludge feeds regardless of the chlorine dioxide concentration (P = >0.05).

Not surprisingly, reactors receiving pretreated *co-fermented* mixture experienced nearly twice as much biogas production than the reactors receiving only pretreated WAS. The PS/WAS mixture experienced a greater volume of biogas production than the WAS-only sludge feed for both 50 and 100 mg ClO_2/l pre-treated feed inputs (P = <0.05). This may be attributed to a higher hydrolysis rate and therefore higher food to microorganism ratio typical of primary sludge.

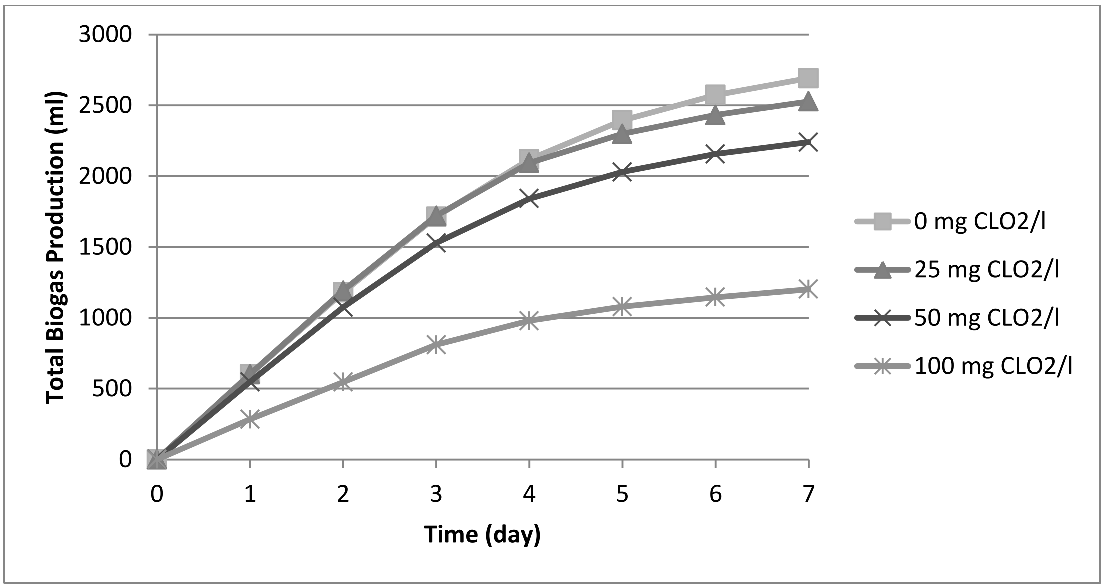

Figure 5- 22: Total biogas produced in batch operated anaerobic reactors receiving varying concentrations of chlorine dioxide in the WAS-only sludge feed. Comparison revealed that chlorine dioxide pre-treatment had no significant impact on biogas production in the digester (*P* = >0.05).

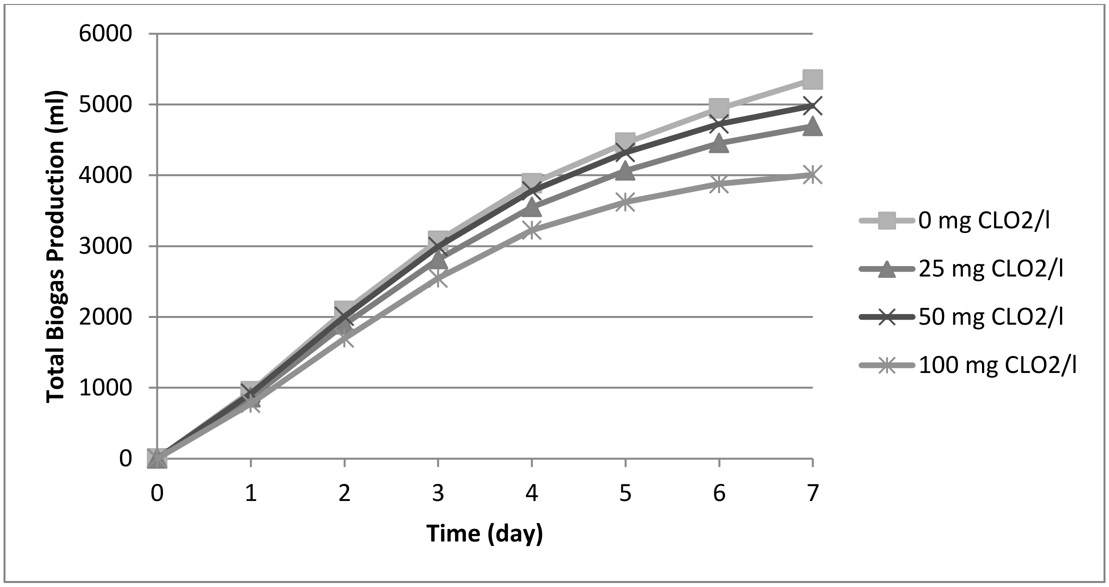

Figure 5- 23: Total biogas produced in batch operated anaerobic reactors receiving varying concentrations of chlorine dioxide in the PS/WAS sludge feed. Comparison revealed that chlorine dioxide pre-treatment had no significant impact on biogas production in the digester (*P* = >0.05).

From this batch study, we were able to show that pre-treatment of the sludge to increase its solubility does not negatively impact anaerobic digester performance; however it also does not improve same performance significantly. The solids destruction and COD removal remained the same for digesters receiving untreated sludge and digesters receiving chlorine dioxide pre-treated sludge, regardless of the sludge type. Also increasing the chlorine dioxide concentration in the pre-treated sludge does not have any significant effect on these parameters. As expected, *cofermentation* showed improved biogas production than a WAS-only feed. This is due to a higher food to microorganism ratio in the mixed sludge.

5.2.5 De-chlorination of Pre-treated Sludge and its Effect on Anaerobic Digester Operation

It was initially hypothesized that the pre-treatment method resulted in residual chlorine dioxide or its intermediates: chlorite and chlorate, remaining in bulk solution. When introduced to the anaerobic digester, these substances may interrupt the normal operating ORP and may be toxic to the anaerobic organisms. This part of the thesis was completed in attempts to verify such hypothesis. In this phase, batch studies were completed where the pretreated sludge was treated with a dechlorinating substance to neutralize residual oxidizing substances prior to introduction into the digester. For this, sodium sulfite was selected and used in varying concentrations as the neutralizing agent.

The reaction between sulfite and chlorine dioxide produces sulfate (SO_4^{2-}) which during anaerobic decomposition is reduced biologically to sulfide (S^{2-}) by sulfate reducing bacteria (SRB). The sulfide produced may also combine with available hydrogen to form toxic hydrogen sulfide (H_2S) gas.

$$2ClO_2 + 5Na_2SO_3 + H_2O \longrightarrow 5NaSO_4 + 2HCl$$

$$\text{Organic matter} + SO_4^{2-} \longrightarrow S^{2-} + H_2O + CO_2$$

and

$$S^{2-} + 2H^+ \longrightarrow H_2S_{(g)}$$

SRBs share common substrates with many anaerobes including methanogens. SRBs may compete with methanogens, acetogens, and even fermentative microorganisms for available acetate, H_2, propionate, and butyrate in the anaerobic system (Chen, et al., 2008), hence inhibition due to metabolic competition may occur when sulfite is used. In addition to metabolic competition, inhibition may also occur from the toxicity of unionized sulfide to the various bacteria groups in the anaerobic system (Karhadkar, et al., 1987). Unionized sulfide is toxic to many organisms including methanogens and SRBs themselves since the compound can freely diffuse through the cell membrane, causing denaturation of proteins, interfering with the assimilatory metabolism of sulphur, etc. (Appels, et al., 2008). In a competing situation, sulfate reduction is energetically more favourable. SRBs have a high affinity for certain anaerobic digestion substrates and with higher growth rates than competing anaerobes, SRBs have the greater tendency to out-compete the other bacteria groups for substrate (Chen, et al., 2008).

In this batch study, it is expected that any toxic residuals following pre-treatment will be effectively neutralized by sulfite addition. Neutralization of the pre-treated sludge may result in improved digester performance depending on the concentration of sulfite used. Some inhibition may occur as sulfite concentration increases. The following results show the effect of sulfite neutralization on anaerobic digestion operations;

Solids Destruction

The initial (pre-digestion) and final (post-digestion) total and volatile solids (TS and VS, respectively) were measured for each digester unit. The solids content was determined and the average TS and VS destructions were plotted as shown in Figures 5-24 and 5-25, respectively.

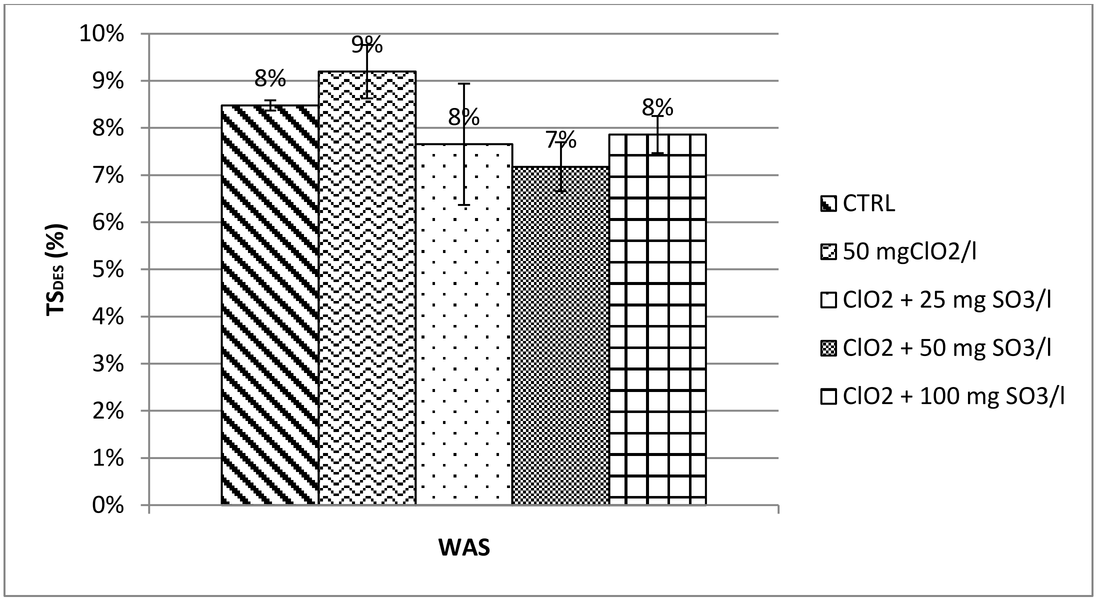

Figure 5- 24: Total solids destruction obtained in batch operated anaerobic reactors with feed conditions as follows; CTRL – digester receiving untreated WAS, 4hr – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time, 25 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 25 mg/l sulfite solution, 50 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 50 mg/l sulfite solution, 100 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 100 mg/l sulfite solution. Comparison revealed no significant differences between the conditions tested (*P* = >0.05).

Total solids destruction was 8%, 9%, 8%, 7%, 8% in digester receiving untreated WAS, pre-treated WAS, pre-treated WAS with 25 mg/l sulfite, pre-treated WAS with 50 mg/l sulfite, pre-treated WAS with 100 mg/l sulfite, respectively. Volatile solids destruction was 20%, 22%, 22%, 22%, 20% in digester receiving untreated WAS, pre-treated WAS, pre-treated WAS with 25 mg/l sulfite, pre-treated WAS with 50 mg/l sulfite, pre-treated WAS with 100 mg/l sulfite, respectively. Statistical comparisons between the untreated, pre-treated, and neutralized pre-treated sludge revealed no significant differences in both TS_{DES} and VS_{DES} (P = >0.05) regardless of the sulfite concentration used.

This observation confirms that chlorine dioxide is used up during pre-treatment so that little to no residuals remain thereby not negatively impacting the digester performance.

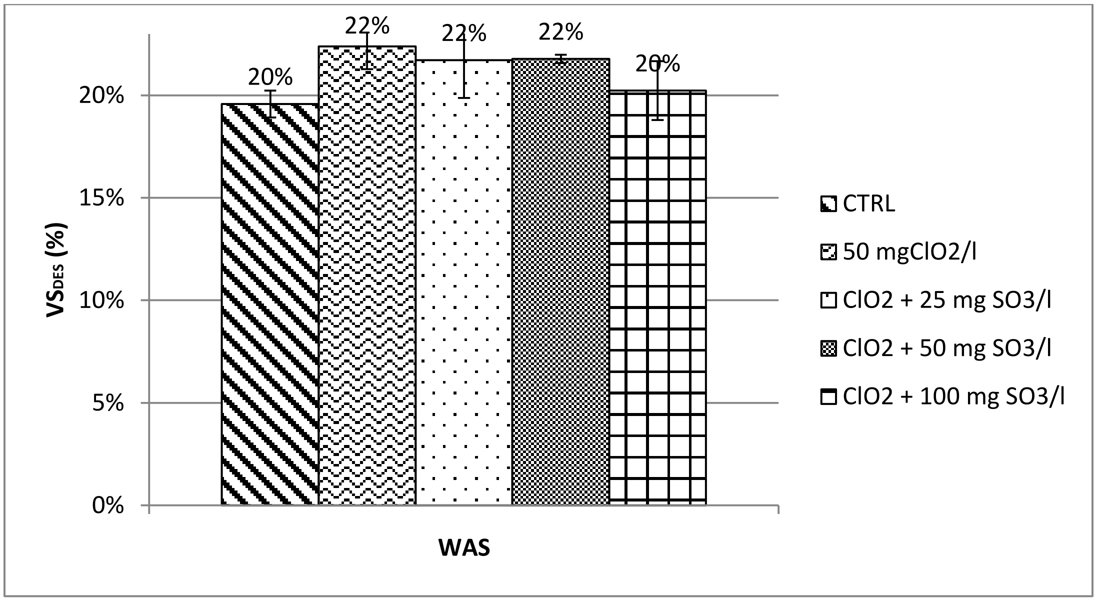

Figure 5- 25: Volatile solids destruction obtained in batch operated anaerobic reactors with feed conditions as follows; CTRL – digester receiving untreated WAS, 4hr – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time, 25 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 25 mg/l sulfite solution, 50 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 50 mg/l sulfite solution, 100 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 100 mg/l sulfite solution. Comparison revealed no significant differences between the conditions tested (*P* = >0.05).

COD Removal

The initial and final COD were measured for each reactor. Average COD reductions were analyzed and reported in Figure 5-26.

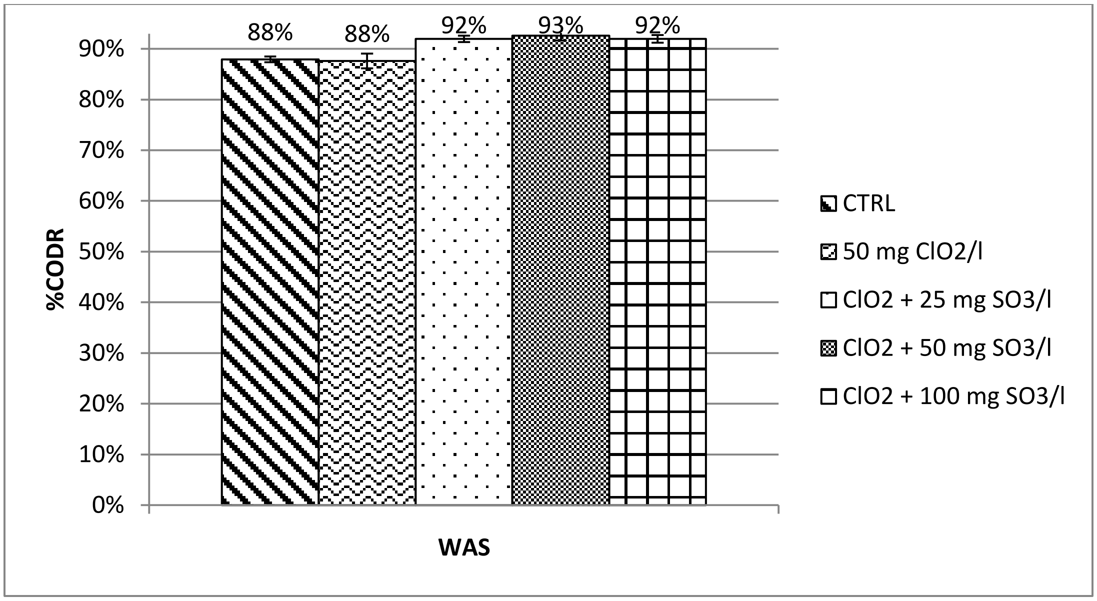

Figure 5- 26: COD removal obtained in batch operated anaerobic reactors with feed conditions as follows; CTRL – digester receiving untreated WAS, 4hr – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time, 25 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 25 mg/l sulfite solution, 50 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 50 mg/l sulfite solution, 100 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 100 mg/l sulfite solution. Comparison revealed some significant differences between the conditions tested (*P* = <0.05).

Here slight improvements to COD removal were observed when sulfite was used to neutralize the pre-treated sludge. Comparisons between the untreated, pre-treated, and neutralized pre-treated sludge reveal there were significant differences in the COD removal between the pre-treated sludge feed and the 50 and 100 mg SO_3/L neutralized sludge feed ($P = <0.05$). Based on previous observations, it is believed that this slight increase in COD_R is due to the additional COD from the sulfite addition and not from the dechlorination effect of sulfite. No significant changes were observed between the different concentrations of sulfite used.

Biogas and methane production

The total biogas production (by volume) was measured throughout the experimental period and the average values are shown in Figure 5-27.

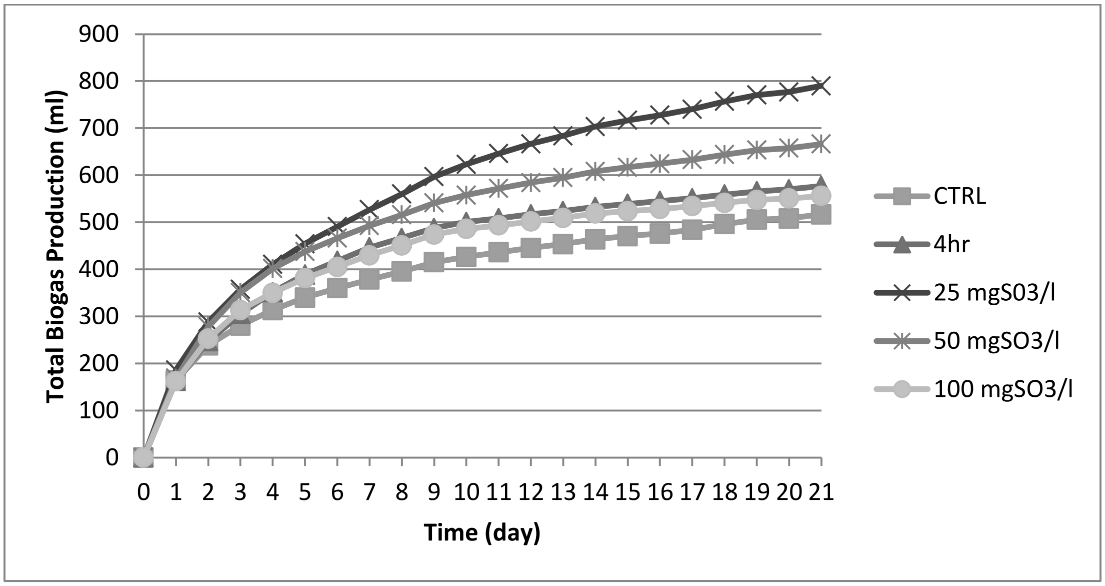

Figure 5- 27: Total gas production observed in batch operated anaerobic reactors with feed conditions as follows; CTRL – digester receiving untreated WAS, 4hr – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time, 25 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 25 mg/l sulfite solution, 50 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 50 mg/l sulfite solution, 100 mg SO_3/l – digester receiving WAS pre-treated with 50 mg/l chlorine dioxide in a 4 hour reaction time and then neutralized with 100 mg/l sulfite solution. Comparison revealed some significant differences between the conditions tested (*P* = <0.05).

Comparisons between the untreated, pre-treated, and neutralized pre-treated sludge reveal there were no significant differences in the biogas production except where 25 mg SO_3/L was used (*P* = <0.05). Based on previous observations, it is also believed that this slight increase is due to the additional COD from the sulfite addition and not from the dechlorination effect of sulfite. If such is the case, indeed sulfite dechlorination is not necessary here. No significant differences were observed where 50 and 100 mg SO_3/L were used (P = >0.05).

From this batch study, it was confirmed that chlorine dioxide is either used up during sludge pre-treatment so that no residual remains and/or if remaining, does not negatively impact anaerobic digester performance. Sulfite addition to neutralize any residual chlorine dioxide did not improve digester performance significantly. The solids destruction, COD removal and biogas production remained the same for all condition tested.

5.2.6 Sludge Biodegradability Assay

Chlorine dioxide is a disinfectant and powerful oxidizing agent. It is possible that by-products or residuals from the pre-treatment agent may cause toxic interactions within the digester unit so that improve solubilization following pre-treatment does not translate to improved performance within the digester unit. Although it was shown previously that sulfite neutralization had little impact on improving digester performance, it is likely that the additional COD interaction and / or presence of unionized sulfide impacted these result.

So here a BOD_5 study was completed to verify the impact of chlorine dioxide during pre-treatment by way of assessing the biodegradability of the solubilized compounds. It is expected that the pre-treated sludge will be determined as the least bioavailable, not because it actually is but because of the nature of the test where the seed is directly exposed to the chlorinating substance. It is also expected that when neutralized, the bioavailability of the sludge will increase accordingly.

This postulation is confirmed as seen in Figure 5-28; the pre-treated sludge is shown as least bioavailable due to direct inhibition of the organisms present including the seed organism. Following sulfite addition, the bioavailability of the pre-treated sludge is shown to increase.

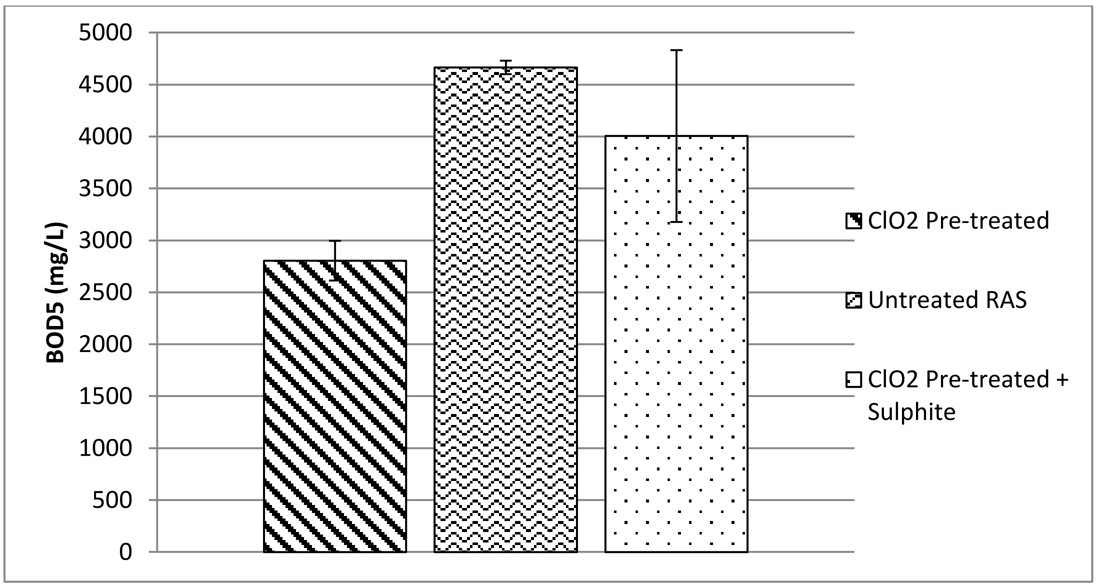

Figure 5- 28: A BOD_5 comparison of three sludge types showing Untreated WAS > ClO2 Pre-treated + Sulphite > ClO2 Pre-treated. ClO2 Pre-treated: WAS pre-treated with 50 mg/l chlorine dioxide, Untreated WAS: untreated WAS, and ClO2 Pre-treated + Sulphite: WAS pre-treated with 50 mg/l chlorine dioxide and then neutralized with sulfite solution.

Based on this and previous findings, it is has been shown that chlorine dioxide and its by-products are indeed used-up during pre-treatment hence why the anaerobic digester is not negatively affected by the pre-treatment method.

Chapter 6. Conclusions

The purpose of this research was to evaluate the feasibility of using chlorine dioxide as a pre-treatment input on waste activated sludge (WAS) to: a) determine its effect on pre-existing filamentous sludge bulking, b) evaluate its potential at improving the solubility of sludge, and c) to investigate its effect on the anaerobic digestion process.

Parameters including the sludge volume indices, capillary suction time value, solids destruction, COD removal and biogas production were measured and analyzed as efficiency indicators between untreated and pre-treated sludge inputs. The conclusions drawn from the results of this experiment are:

- **Chlorine dioxide pre-treatment may be used to alleviate filamentous sludge bulking.**

The WAS obtained from the SEWPCC is significantly affected by filamentous organisms. The presence of filamentous organisms was confirmed by microscopic observations. Gram stain and wet mount observation indicated the presence of *Microthrix parvicella* in abundance. Pre-treatment of the sludge with chlorine dioxide showed a tendency to decrease the sSVI by as much as 57%. The decrease in sSVI ranged between 28 to 49% and 42 to 57% for reactors maintained at 50 and 100 mg ClO_2/L, respectively. The decrease in sSVI was directly proportional to the specific chlorine dioxide dosage (per VSS) present in the reactors.

- **Chlorine dioxide pre-treatment greatly increases the solubility of sludge.**

Chlorine dioxide pre-treatment was shown to solubilize particulate organic matter in the WAS by as much as 85% in terms of soluble COD (sCOD) production from untreated sludge. Also here, the sCOD production was directly proportional to the specific chlorine dioxide dosage (per VSS) present in the reactors.

- **Chlorine dioxide pre-treatment of sludge does not negatively affect digester performance.**

Chlorine dioxide dissociates into the chlorite and chlorate ion, both of which can be toxic. It is possible that the use of chlorine dioxide as a pre-treatment agent may result in disinfection by-products or residual entering into the anaerobic digester and producing inhibition to normal function of the digester.

Following evaluation from both semi-continuously and batch digester operations, it was shown that pre-treatment of sludge using chlorine dioxide does not negatively impact anaerobic digester performance The solids destruction, COD removal and biogas production remained the same for digesters receiving untreated WAS and digesters receiving either fermented or chlorine dioxide pre-treated WAS. This observation was regardless of the operating SRT or the sludge type used i.e. WAS only or PS/WAS mixture. It is therefore possible to reduce the digester operating SRT to as low as 6-days without significant negative impacts to the digester performance.

Chapter 7. Engineering Significance

In this thesis we have evaluated the use of chlorine dioxide as a pre-treatment agent for the alleviation of filamentous bulking and enhanced solubilization of sludge. The following explains the significance of this thesis for wastewater treatment facilities;

1) Chlorine dioxide has been shown to fragment protruding filaments in sludge experiencing bulking due to the presence of filamentous organisms. Sludge bulking of waste activated sludge (WAS) or return activated sludge (RAS), if not resolved in a timely manner, can lead to a multitude of problems for the treatment facility. When introduce into the anaerobic digester, filamentous bulking sludge may cause excessive foaming in the digester unit causing blockage of vent pipes and buildup of internal pressure. Not to mention the health and safety risk associated with excessive foaming. Temporary control measures using chlorine is readily utilized for RAS but rarely for WAS possibly for fear of any adverse impacts to the anaerobic digesters. In this thesis, we have shown chlorine dioxide to be effective at mitigating filamentous bulking in WAS without negative impacts to the anaerobic digester. By alleviating sludge bulking, there is also potential for improving digester operation and the sludge settling and dewatering characteristics.
2) In this thesis, we have also shown vast improvement in the solubilization of WAS following chlorine dioxide pre-treatment. For treatment plants that are challenged by complex sludge types, a separate pre-treatment stage is particularly beneficial. Complex sludge or sludge with a particularly low hydrolysis rate will require a long hydraulic retention time (HRT) to disintegrate. This is more costly for the treatment facility. Pre-treatment allows for improved solubility and subsequently more efficient operation of the anaerobic digester. Chlorine

dioxide was shown here to increase solubilization of WAS by as much as 85% in terms of soluble COD (sCOD) production from an untreated sludge.

3) For any sludge pre-treatment method, it is important that the pre-treatment agent of choice does not inhibit digester performance. In this thesis, it has been shown that the use of chlorine dioxide as a pre-treatment agent does not negatively impact digester performance for volatile solids destruction, COD removal and biogas production.

4) As mentioned before, a long hydraulic or solids retention time imposes additional operating cost to the treatment facility. In this thesis, we were able to show the potential to reduce the SRT without negative impact to digester performance. Typically, conventional anaerobic digesters require a 15 – 30 day SRT. With pre-treatment, the digester SRT could be shortened to as low as 6 days while maintaining the solids destruction and COD removal capability. The only limitation shown in this thesis was a slight decrease in biogas production. The pre-treatment of sludge prior to digestion means the potential to reduce the digester SRT, increase substrate turnover, smaller environmental foot print; all of which lead to cost savings for the treatment facility.

5) Although not directly evaluated in this thesis, chlorine dioxide was also observed to reduce odours in the sludge. Chlorine dioxide is known to alleviate odours; mainly those generated by decaying organic matter, hydrogen sulfide and phenolic compounds (US EPA, OW, Office of Ground Water and Drinking Water, April 1999). Another significance of a separate pre-treatment stage is the potential to reduce toxic shock in the digester unit. A separate pre-treatment process allows for the physical separation of the acid-forming phase and the methane-forming phase (Cohen, et al., 1979) of anaerobic digestion; thereby providing optimal environmental conditions for both microbial groups to thrive.

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