# Characterization of Pretreatment Impacts on Properties of Waste Activated Sludge and Digestibility

**by**
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## Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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# Abstract

Technologies for pretreatment of waste activated sludges (WAS) prior to digestion are of increasing interest to wastewater treatment utilities because of their promise for improving sludge digestibility and reducing the mass of biosolids remaining after digestion. While there has been considerable study of pretreatment processes, a common approach to describing the impact of pretreatments on sludge biodegradability has not been developed. The overall objective of this study was to develop protocols that can be employed to characterize the impact of pretreatment processes on WAS digestion.

Sonication and ozonation were employed as models of physical and chemical pretreatment technologies respectively. A range of physical, chemical and biological responses were evaluated to assess the impact of pretreatment on WAS properties as well as digestibility. WAS samples that were generated over a range of solids residence times (SRTs) under controlled operating conditions were employed to facilitate an assessment of the interaction between pretreatment and WAS properties on digestibility.

The VS, COD and soluble TKN responses indicated that a significant fraction of the WAS solids were solublized by sonication and ozonation, however, it appeared that the types of materials which were solublized was affected by the SRT at which the WAS was generated and the level of pretreatment. The results indicated that the impact of pretreatment on biodegradability of WAS was not described by solublization values exclusively without considering the SRT of the sludge and the level and type of pretreatment. A higher level of proteinaceous materials was preferentially solublized as the result of pretreatment. Respirometry revealed that both sonication and ozonation substantially reduced the viable heterotrophs in the sludge and modestly increased the readily biodegradable fraction of COD. The ultimate yields of $CH_4$ and $NH_4$ in BMP tests and VFAs in BAP tests revealed that pretreatment marginally increased the ultimate digestibility of the sludges. Only a high dose of ozonation substantially increased the digestibility of the 15 day SRT sludge. However, both sonication and ozonation substantially increased the rate of hydrolysis which is typically the rate limiting process in WAS digestion.

The BMP test was not a useful test to evaluate the rate of methane generation due to inhibition of methanogens in the early days of BMP test for pretreated sludges. The comparison between VFA and ammonia responses in day 10 of BAP test and ultimate values of these responses after 60 days in BMP test revealed linear relationships between these responses. According to these relationships, a set of models were introduced in this study. The models can be employed to predict the ultimate methane and ammonia generation using soluble COD, VFA or ammonia responses in day 10 of BAP tests. The BAP test was determined to be a shorter test (10 days) than the BMP (55 to 60 days) test and could provide information on the rates of hydrolysis and acidification/ammonification processes. Characterization of biodegradable and non-biodegradable material in WAS samples was conducted using a simplified ADM1 model. The characterization also revealed that proteins are a substantial fraction of biodegradable materials. The estimated ammonia, VFA and methane values from the stoichiometric model were similar to the corresponding values from the experiments. This supported the validity of the simplified model for all sludges employed in this study.

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# List of Abbreviations

| | |
|---|---|
| **ADM1** | Anaerobic Digestion Model Number 1 |
| **b** | Decay Rate |
| **BAP** | Biochemical Acidogenic Potential |
| **BES** | Boromoethane Solfonate |
| **BMP** | Biochemical Methanogenic Potential |
| $\mathbf{C_b}$ | Concentration of rbCOD in bottle (mg COD $L^{-1}$) |
| **CMG** | Cumulative Methane Generation |
| **COD** | Chemical Oxygen Uptake |
| $\mathbf{COD_{ac}}$ | Acetate Added as rbCOD in Medium based on COD |
| $\mathbf{COD_{NaOH}}$ | Maximum Release of COD in Liquid Phase of Sludge |
| $\mathbf{COD_{removed}}$ | Decrease of COD in Sluble Phase of WAS |
| $\mathbf{COD_{tr}}$ | Total COD after Pretreatment |
| $\mathbf{COD_{ut}}$ | Total COD before Pretreatment |
| $C_{BAP,10}^{SCOD}$ | Soluble COD (mg COD/L) in a 10 day BAP test |
| $C_{BAP,10}^{VFA}$ | VFA concentration based on COD (mg COD/L) in a 10 day BAP test |
| $\mathbf{d_{av}}$ | Arithmetic Mean |
| $\mathbf{DD_0}$ | Degree of Inactivation |
| $\mathbf{DD_{COD}}$ | Degree of COD Released or Degree of Disintegration |
| $\mathbf{d_i}$ | Diameter of Particles |
| $\mathbf{d_s}$ | Surface Mean |
| $\mathbf{d_v}$ | Volume Mean |
| $\mathbf{d_{vs}}$ | Volume-Surface Mean |
| **EPS** | Extracellular Polymeric Substances |
| **FCOD** | Filtered COD |
| **FFCOD** | Filtered and Flocculated COD |
| **FTKN** | Filtered TKN |

| | |
|---|---|
| **HRT** | Hydraulic Residence Time |
| **I** | Inhibition Expression |
| **IWA** | International Water Association |
| **$k_{Ammon}$** | Ammonification rate constant ($d^{-1}$) |
| **$K_{I, NH3}$** | Ammonia inhibition constant |
| **LCFA** | Long Chain Fatty Acid |
| **n** | Number Frequency |
| **Odm** | Organic dry matter |
| **OUR** | Oxygen Uptake Rate |
| **$OUR_{endo}$** | Oxygen Uptake Rate in Endogenous Conditions |
| **$OUR_{total}$** | Total Oxygen Uptake Rate |
| **$OUR_{tr}$** | Oxygen Uptake Rates of Pre-treated Sludge |
| **$OUR_{ut}$** | Oxygen Uptake Rates of Raw (Untreated) Sludge |
| **pCOD** | Particulate COD |
| **PS** | Primary Sludge |
| **rbCOD** | Readily Biodegradable COD |
| **$SCOD_{NaOH}$** | Soluble COD before Pretreatment |
| **$SCOD_{tr}$** | Soluble COD after Pretreatment |
| **$SCOD_{ut}$** | Soluble COD before Pretreatment |
| **$S_o$** | Initial Concentration of Substrate |
| **SRT** | Solis Residence Time |
| **t** | Time |
| **$t_1$** | Time at point 1 when oxidation starts (d) |
| **$t_2$** | Time at point 2 when oxidation of rbCOD material ends (d) |
| **TAN** | Total Organic Nitrogen |
| **TKN** | Total Kjeldahl Nitrogen |
| **$TOC_{ss,tr}$** | Total Organic Carbon in Suspended Solid of Sludge after Pretreatment |
| **$TOC_{ss,ut}$** | Total Organic Carbon in Suspended Solid of Sludge before Pretreatment |

| | |
|---|---|
| **TS** | The Concentration of Total Solids in Sludge |
| **TVS** | Total Volatile Solids |
| $\mathbf{U_1}$ | Cumulative oxygen uptake at $t_1$ (mg $O_2$) |
| $\mathbf{U_2}$ | Cumulative oxygen uptake at $t_2$(mg $O_2$) |
| $U_{BMP}^{Methane}$ | Methane yield in a BMP test (mgCOD/mgCOD) |
| $U_{BMP}^{Methane}$ | Methane yield in a BMP test (mgCOD/mgCOD) |
| $U_{BAP,10}^{Ammon}$ | $NH_3$-N fraction of TKN in 10 day BAP test |
| $U_{BAP,10}^{SCOD}$ | SCOD fraction of TCOD in a 10 day BAP test (mg COD/mg COD) |
| $U_{BAP,10}^{VA}$ | VFA fraction in 10 day BAP test (mg COD/mg COD) |
| $U_{BAP,6}^{VFA}$ | VFA fraction in 6 day BAP test (mg COD/mg COD) |
| $U_{0}^{Ammon}$ | $NH_4$-N / TKN fraction at beginning of test |
| $U_{T}^{Ammon}$ | $NH_4$-N / TKN fraction at time t |
| $U_{BMP}^{Ammon}$ | Ultimate $NH_3$-N fraction of TCOD in BMP test |
| $U_{BMP}^{Ammon}$ | Ultimate $NH_3$-N fraction of TKN in BMP test |
| $U_{Ult}^{Ammon}$ | Maximum (ultimate) ammonia yield ($NH_4$-N / TKN) |
| **UASB** | Upflow Anaerobic Sludge Bed |
| $\mathbf{V_b}$ | Volume of liquid in batches (L) |
| $V_{O2}^{OUR\max}$ | Maximum Cumulative Oxygen uptake |
| **VDS** | Volatile Dissolved Solids |
| **VFA** | Volatile Fatty Acids |
| **VS** | Volatile Solids |
| **VSS** | Volatile Suspended Solids |
| **WAS** | Waste Activated Sludge |
| $\mathbf{X_o}$ | Initial Heterotrophic Active Biomass |
| $\mathbf{X_{rh}}$ | Readily Hydrolysable Particulate COD |
| $\mathbf{X_{sh}}$ | Slowly Hydrolysable Particulate COD |
| $\mathbf{Y_H}$ | Yield Coefficient |
| $\boldsymbol{\alpha}$ | COD Solubilization Ratio |

| | |
|---|---|
| $\beta$ | Release of Organic Carbon from the Solid Phase |
| $\mu_{max}$ | Specific Maximum Growth Rate |
| $\sigma$ | Standard Deviation |

# Chapter 1

# 1. Introduction

## 1.1. Motivations for characterization of pretreatment impact on WAS

The main by-product of biological wastewater treatment is waste activated sludge (WAS). Due to the enlargement of wastewater treatment, the generation of WAS has increased (Reynolds and Richards, 1996). The anaerobic digestion of WAS is desirable due to its reduced environmental impact, particularly reduced greenhouse gas emissions and through the generation of biogas as an alternative for fossil fuels (Gunaseelan, 1997; Chynoweth et al., 2001; Charters, 2001). The anaerobic digestion of WAS has several advantages: digested sludge is relatively stable and harmless to be disposed of, a low level of energy is required for digestion and the generated methane can be utilized as fuel. Consequently, there is a significant decrease in the energy requirements for digestion as compared to aerobic digestion. In addition, pathogenic microorganisms in WAS are reduced. However, long digestion times, requiring a large digester volumes are a significant disadvantage of the anaerobic digestion of WAS. The low rate of hydrolysis processes and the slow growth of methanogenic microorganisms which are responsible for the methanogenesis process limit the rate of anaerobic digestion (Wang et al., 1999).

The anaerobic digestion process is composed of three stages; hydrolysis, acidogenesis and methanogenesis. Studies in the literature indicate that the rate limiting stage in anaerobic digestion depends on the kind of substrate (Noike et al., 1985). The rate of anaerobic digestion of WAS is significantly slow and the hydrolytic reactions are considered to be the rate limiting

stage in the overall anaerobic digestion process (Eastman and Ferguson, 1981; Li and Noike, 1992; Shimutzu et al, 1993).Various pretreatment techniques have been introduced to improve the hydrolysis rate.

There is considerable interest in WAS pretreatments because of their potential to enhance stabilization and increase biogas production. As will be subsequently demonstrated, there have been numerous studies that have evaluated the potential of a variety of pretreatment processes to enhance anaerobic digestion. These studies have employed a variety of indicators to characterize the impact of the pretreatment on the WAS and to develop relationships between pretreatment and subsequent digestion performance. There are, however, no generally accepted indicators that have been used to characterize all pretreatment processes. In addition, there have been no attempts to examine interactions between indicator responses and sludge properties (e.g. sludges that have been developed under different SRTs).

## 1.2. Objectives

The development of knowledge of the impacts of pretreatment on WAS properties is the general objective of this research and can be divided into a number of specific objectives including:

a. The investigation of pretreatment impacts on physical, biochemical and biological properties of WAS.
b. An examination of relationships between changes in the physical, biochemical and biological characteristics of WAS and its anaerobic biodegradability.

c. An evaluation of the potential to use physical, biochemical and biological responses as indicators of the impact of pretreatment on anaerobic digestibility of WAS.
d. The development of pretreatment models to be integrated into anaerobic digestion models.
e. An examination of interactions between pretreatment and WAS SRT.

The knowledge gained in this research could potentially lead to optimization of pretreatment techniques by identifying their positive and negative effects on biodegradability of WAS.

## 1.3. Thesis Organization

This thesis is organized into seven chapters and five appendices. Chapter 1 briefly presents an introduction to sludge digestion and pretreatment of WAS as well as the motivation for characterization of pretreatment impacts on WAS. Chapter 2 presents a literature review on physical, chemical and biological properties of sludge, previous pretreatment studies and discusses the capability of different indicators to quantitatively or qualitatively characterize the impact of pretreatment technologies on WAS characteristics. In this chapter the feasibility of employing anaerobic digestion models to characterize the impacts of pretreatment on the primary process in anaerobic biodegradation (hydrolysis) is also discussed. Chapter 3 introduces an overview of the research and the methodology employed in the study. Chapters 4 and 5 present the results of applying sonication and ozonation, as models of physical and chemical pretreatment technologies, respectively. In these chapters the impacts of sonication and ozonation on physical, biochemical and biological properties of WAS are investigated and the feasibility of using these responses as indicators of the impact of pretreatment on anaerobic digestibility of WAS is evaluated. In addition, the interactions between pretreatment and WAS SRT are discussed in these chapters. The ability of the BAP test to characterize biodegradability

of raw and pretreated WAS is further evaluated in Chapter 6. Lastly, conclusions and suggestions for future work are presented in Chapter 7.

The results of preliminary experiments carried out to identify the optimal conditions for respirometry and BAP tests are discussed in Appendices A and B, respectively. Appendices C and D provide the results of experiments carried out to evaluate physical, chemical and biological impacts of sonication and ozonation on the properties of sludges respectively. A series of Excel files showing the calculation steps used in this study to determine different indicators have been included in a compact disc and attached to this thesis and a list of these Excel files are presented in Appendix E.

# Chapter 2

# 2. Background Information

A multidisciplinary approach is required to characterize pretreatment impacts on WAS. This section reviews studies related to the characteristics of WAS, previous pretreatment studies and available techniques and models for characterization of pretreatment impacts on WAS.

## 2.1. Characterization of WAS and Digestion Properties

The properties of WAS and digested sludge can be categorized as being either physical or biochemical or biological. Studies that describe the characteristics of these properties of WAS are subsequently reviewed.

### 2.1.1. Physical Properties

In this part of the literature review, the impacts of pretreatment on physical properties of sludge are discussed and the indicators for the impact of pretreatment on physical properties of sludge are introduced.

#### 2.1.1.1. Size Distribution

Hydrolysis is known as the rate limiting step in digestion of WAS (Eastman & Ferguson, 1981; Malina & Pohland, 1992). It is likely that even simple methods that reduce the size of particles can enhance hydrolysis of WAS and shorten digestion times by increasing the specific surface area of particles which are substrate for hydrolyzing bacteria. Obligator and Vogelpohl (1993) indicated that particle size had a remarkable influence on the process of anaerobic

degradation. In addition, Knocke et al. (1986), Lawler (1986), Crippe (1995) and Rodriguez et al. (2002) emphasized the effect of particle size and concentration on treatment efficiency. Palmovski et al. (2000) indicated that size reduction is effective for the improvement of anaerobic biodegradation of slowly hydrolysable material. The following describes common size indicators and descriptors and also the methods for size measurement.

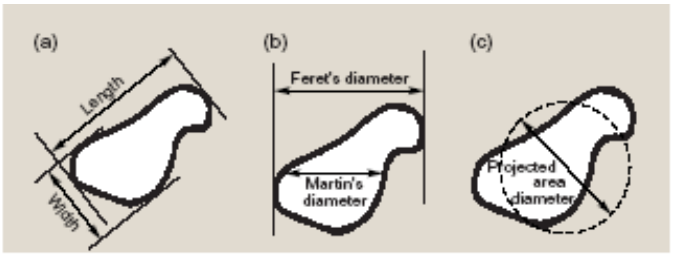


**Figure 2.1. Commonly Used Descriptors of Particle Size (Brittain, 2001)**

The most common measurement of particle size is the length (the longest dimension from edge to edge of a particle oriented parallel to the ocular scale). In addition there are two other descriptions of size, as illustrated in Figure 2.1. Ferret's diameter (the distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale) is applied to introduce the maximum size of particles in a sample taken by a syringe or needle from a serum bottle. The projected area diameter (the diameter of a circle having the same area as the particle's projected area) is another definition which can be applied to introduce the size of a particle based on its area (Brittain, 2001).

Particles in WAS generally have a wide range of sizes and shapes. Hence, particle size investigation is undertaken to obtain information about the characteristics of an ensemble of particles. A normal distribution is commonly employed to describe the distribution of particle size. In addition to the particle size distribution, the arithmetic mean of the diameter, surface

mean, volume mean and volume-surface mean of the ensemble of particles and the standard deviation of the distribution, as mentioned in Table 2.1., are valuable descriptors.

**Table 2.1. Common Size Descriptors and Calculation Methods (Brittan, 2001).**

| Arithmetic Mean | Standard Deviation | Surface Mean | Volume Mean | Volume-Surface Mean |
|---|---|---|---|---|
| $d_{av} = \frac{\sum nd_i}{\sum n}$ | $S = \left[\frac{(d_{av} - d_i)^2}{n}\right]^{\frac{1}{2}}$ | $d_s = \left[\frac{\sum nd_i^2}{\sum n}\right]^{\frac{1}{2}}$ | $d_v = \left[\frac{\sum nd_i^3}{\sum n}\right]^{\frac{1}{3}}$ | $d_{vs} = \frac{\sum nd_i^3}{\sum nd_i^2}$ |
| $d_{av}$=Arithmetic Mean n= Number Frequency $d_i$=Diameter of Particles S=Standard Deviation $d_s$=Surface Mean $d_v$=Volume Mean $d_{vs}$=Volume-Surface mean | | | | |

The analytical techniques employed to determine particle size distribution have improved in recent years (Cripps, 1995; Araya-Kroff, 2004). Methods include size fractionation using sieves and membranes, visual techniques (e.g. manual counting and image analysis) and light scattering (e.g. laser diffraction) based techniques.

Size fractionation by membrane filtration and mechanical sieving can generate isolated particles that are sorted by size by membrane with sizes from 0.45 to 200 μm (Zeeman, 1997 and Cripps, 1995) and screens with sizes 30 to 500 μm (Cripps, 1995; Wheaton, 1974) respectively. These techniques can fractionate particles according to their volume or weight. These techniques introduce a discrete size distribution which may be insufficient to investigate a specific particle size. The number of particles in each size interval can be estimated according to the average density and size of particles. Other parameters such as COD, TS, VS, and nutrient content can be measured in each fraction that is defined by this classification (Rodrigues, 2002). Such investigation for nutrient and protein content may provide some information about the biochemical and biological effects of pretreatment on the sludge immediately after pre-treating.

The other particle size analysis techniques (visual techniques and light scattering based techniques) can provide the size distribution of particles in a liquid. By these techniques, the reduction of particle dimensions due to pretreatment can be detected. Both image analysis (as a visual technique) and laser diffraction techniques can provide the size distribution of a sludge and provide some information about shape (such as circularity) of particles and flocs. In addition, image analysis can provide valuable information about the structure and the morphology of particles (Russ, 1995; Araya-Kroff et al., 2004).

The basic concept of light scattering is used in laser diffraction devices using laser and silicon detectors. The patterns of dark and bright lines generated by diffraction phenomena lines are interpreted by software to determine the particle size in a sample (Konert, 1997). Houghton and Stephenson (2002) used this technique to determine the particle size distribution of a digested sludge. Kim et al. (2003) applied this method to evaluate the impacts of different pretreatment methods on WAS. Rai et al. (2004) also used this technique for the investigation of sonication effects on WAS. The reflocculation of particles is likely to start accruing after pretreatment and a considerable change in particle size distribution may happen during some processes such as filtration or slide preparation (in sieving and visual techniques). The laser diffraction technique is therefore preferable as compared to the other techniques since sample preparation is not required in this technique and can reduce the chance of error caused by reflocculation. This technique can measure the size of particles over a wide range (0.1 to 2000 μm) and can provide additional information such as specific surface area of particles and the software can calculate other size indicators such as surface mean, volume mean and specific surface area of particles.

### 2.1.1.2. Other Physical Properties and Indicators

For further investigation of pretreatment impacts on the physical properties of sludge, other physical indicators such as circularity, viscosity and conductivity can be employed. These indicators can potentially provide additional information about the mechanisms of deflocculation and disintegration of particles and the physical characteristics of solublized materials.

Circularity is defined as the ratio between the perimeter of a circle with equivalent area to the particle and the perimeter of the particle itself. Hence, the less spherical the particle, the less its circularity is. Obviously, the maximum value of this factor is one. The ability to measure circularity is one of the features of laser diffraction analysis and image analysis techniques. Pretreatment can cause the fragmentation of flocs and the detachment of filaments as a back bone of flocs. An increase in the number of particles with low circularity and smaller diameter after pretreatment shows destruction of flocs or cells which have a relatively round shape. For example, the release of filaments from flocs can significantly increase the number of particles with smaller size and lower circularity. In addition, a decrease in the number of flocs which have large size and high circularity will be observed (Amaral et al., 2004).

The effect of pretreatment intensity can be assessed by measuring viscosity as a physical property of sludge. Goel et al. (2004) investigated pretreatment processes and showed that the viscosity of digested sludges depended on their TVS more than the TS. Since pretreatment of WAS may cause an increase in the concentration of VS in the liquid phase of sludge (VDS), the relationship between the intensity of pretreatment and viscosity of filtered sludge may be of interest.

Conductivity can be applied as another indicator of deflocculation and release of biopolymers into the liquid phase of sludge. For instance, a 16-32% increase in conductivity due to a temperature increase from 30 to 45º C and release of dominantly negatively charged biopolymers has been reported (Morgan et al., 1991).

### 2.1.2. Chemical and Biochemical Properties

The chemical properties of sludge depend on the presence of different organic or inorganic chemicals in the particulate and dissolved phases and the type and strength of bonds among chemicals, bacteria and water. Pretreatment can solublize particulate materials and change the chemical properties of sludge. The level of solublization depends on the source of the wastewater, the pretreatment technique and the intensity applied. Solublization indicators such as those employed for COD solublization can be employed to quantify the impact of pretreatment on chemical properties of sludges. Since solublized materials are potentially more susceptible to hydrolysis, as compared to particulate materials, an improvement in hydrolysis rate as the rate limiting step in digestion process is expected when pretreatment is applied. Hence solublization indicators can potentially be used to predict the impact of pretreatment on biodegradability of sludges.

Several solubilization indicators have been introduced in the literature. Kim et al. (2003) recommended the calculation of a COD solubilization ratio (α) based on Equation 2.1 to consider the effect of pretreatment on a sludge.

$$\alpha = (SCOD_{tr} - SCOD_{ut}) / COD_{tr} \times 100 \qquad 2.1.$$

Where: $SCOD_{tr}$ and $SCOD_{ut}$ are soluble COD after and before pretreatment, respectively. $COD_{tr}$ is total COD after pretreatment.

To investigate and quantify the disruption of bacterial cell walls due to pretreatment processes, the degree of COD released or degree of disintegration ($DD_{COD}$) indicating the increase of soluble COD or COD of intracellular material able to pass through a membrane with pore size of 0.45 μm was defined (Equation 2.2).

$$\mathbf{DD_{COD}\ (\%) = (SCOD_{tr}\text{-}SCOD_{ut})/(SCOD_{NaOH}\text{-}SCOD_{ut}) \times 100} \qquad 2.2.$$

Where: $SCOD_{NaOH}$ represents the maximum release of COD in liquid phase of sludge using a NaOH pretreatment method. $SCOD_{tr}$ and $SCOD_{ut}$ represent soluble COD of pre-treated and raw samples respectively (Rai et al., 2004).

As mentioned in Equation 2.1 and Equation 2.2, both the COD solublization ratio and the degree of disintegration are calculated based on the increase of soluble COD in a sludge due to disintegration of particulate matter. Equation 2.2 compares the dissolved COD to a maximum possible COD solublization which can occur using a strong pretreatment process (NaOH addition. Accordingly the maximum value of $DD_{COD}$ is one while the maximum value of α will be less than one. The measurement of $DD_{COD}$ might be employed to examine the success of a pretreatment technique in COD solublization as compared to other pretreatment techniques that are employed in different time and for different sludges. Measuring $DD_{COD}$ for a pretreatment technique is relatively difficult, since COD solublization by chemical pretreatment (NaOH addition) is also required. Hence the measuring of COD solubilization ratio (α) is preferable when the comparison between different pretreatment technologies is not required.

The release of organic carbon from the solid phase (β), as mentioned in Equation 2.3, can be an indicator for disintegration by pretreatment (Scheminski et al., 2000).

$$\beta = (TOC_{ss,ut} - TOC_{ss,tr}) / TOC_{ss,ut} \times 100 \quad 2.3.$$

Where: $TOC_{ss,ut}$ and $TOC_{ss,tr}$ are total organic carbon in suspended solids of sludge before and after pretreatment respectively.

This indicator was defined to evaluate the solubilization of organic matter by ozonation. An emphasis was put on the release of organic carbon from solids instead of the increase of total soluble organic carbon since a significant proportion of soluble organic carbon may be mineralized. The mineralization of organic carbon can also be observed as reduction in total COD of sludge. Since TOC is much harder to measure as compared to COD and does not provide information regarding the oxidation state of carbon in a sludge, the COD solublization indicators (α and $DD_{COD}$) should provide better information regarding pretreatment impact on the biochemical properties of sludge.

### 2.1.2.1. Extra Cellular Polymeric Substances (EPS)

Pretreatment of WAS results in the release of material in the soluble phase of sludges and may cause improvement in biodegradability of sludges. EPS represents a considerable fraction of the particulate materials in WAS and can be released in the soluble phase of a sludge as result of pretreatment. Knowledge of the level and composition of EPS in WAS may aid in interpreting the impact of pretreatment on properties of sludges and their biodegradability.

Almost all bacteria are able to produce EPS that participate in microbial aggregation of flocs (Geesey, 1982). Flocs consist of microbial cells enveloped in a matrix of polymers that are located outside of the cells and have a high molecular weight. The formation and composition of EPS depends on cell lysis, active secretion, shedding of cell surface material and absorption of substances. In addition to aggregation of bacterial cells, the EPS matrix can play several roles in sludge and wastewater such as formation of a protective barrier providing resistance to harmful shocks, improving adhesion of cell surfaces, accumulation of some enzymatic activities such as digestion of exogenous macromolecules for nutrients and accumulation of nutrients from the environment by sorption of exogenous organic compounds and providing a stable composition of microbial community in a highly dense condition in order to allow microorganisms to live continuously (Wingender et al., 1999).

EPS plays a key role for stabilizing aggregates structure (Laspiduo and Rittmann, 2001). Proteins in EPS can play a role as enzymes for the digestion of macromolecules and particulate materials in the microenvironment of the embedded cells. Furthermore, the extra cellular polymers are able to trap and bind the organic material having high concentrations around cells. Extra cellular enzymes located close to the cells can hydrolyze free or trapped organic matter and, consequently, provide a high concentration of hydrolyzed material with low diffusion to the surrounding material. This causes an efficient uptake of low molecular weight material (Wingender, 1999; Hoffman and Decho, 1999).

The EPS content can be considered as a chemical property of a sludge and is responsible for some physical properties of a sludge such as particle size and strength of flocs. The EPS composition and concentration and their bonds with microbial cells contribute to the physical,

chemical and biological conditions of the sludge. Therefore, investigation of the composition, concentration and extractability of EPS may introduce some valuable information about the effects of pretreatment on anaerobic digestion on sludge.

Various polymeric materials exist in EPS in activated sludge including neutral and acidic polysaccharides, lipopolysaccharides, proteins, nucleic acids and humic acids (Urbain et al., 1993; Frolund et al., 1996). Bura et al. (1998) demonstrated that polysaccharides play the most important role in flocculation and settlement as compared to proteins although proteins were found to be the dominant component of EPS. The dominance of protein in activated sludge could be due to the presence of a large quantity of exoenzymes in the flocs (Frolund et al., 1995). The complexity of the substrate present in wastewater may be the reason for the presence of exoenzymes around bacteria, while the use of synthetic substrates with pure cultures might result in simpler extracellular compounds. The substrate compounds, cell lysis and the destruction of flocs can also affect the complexity of extracellular proteins (Dignac, 1998). Bura et al. (1998) found that the COD: N: P ratio, which represents the nutrient content of substrate, is an influential parameter affecting EPS composition in microbial flocs.

The chemical nature of EPS depends on the source of the sludge and influences the physico-chemical properties of the sludge. A comparison between WAS and anaerobic sludge from a UASB showed a noticeable difference in total extractable EPS (70-90 mg EPS/g SS for WAS as compared with 10-20 mg EPS / g SS for anaerobic sludge) (Morgan et al., 1990; Karapanagiotis et al., 1989; Bowen and Keinath, 1984; Forster, 1982). Furthermore, waste activated sludge was found to be more electro negative than anaerobic sludge (Kawamura and Fanaka, 1966). When determining the composition of extracted EPS in sludge, the concentration

of amino acids is often assumed to reflect the protein content while the concentration of neutral sugars accounts for the quantity of polysaccharide present. This is only an approximation of the composition because amino acids and mono saccharides may also originate from heteropolymers (such as lipoproteins, glycoproteins, lipopolysaccharides, polysaccharides) or complex structures (such as ligno-cellulosic complexes) but this approximation is typically assumed because of the lack of analytical techniques to discriminate between these structures. Dignac et. al. (1998) observed that 75.6 percent and 5.1 percent of the TOC in EPS were from protein and sugar respectively. The remaining uncharacterized organic carbon of EPS consisted of lipids which were dominantly mono-di and triglycerides or phospholipids and sterols, nucleic acids from cell lysis and humic compounds such as fulvic and humic acids.

EPS can be quantified by extraction methods (Murthy and Novak, 1998; Wuertz et al, 2001; Liu and Fang, 2002), which can include a combination of EPS extraction, separation and measurement, and confocal laser scanning microscopy (Zhang and Fang, 2001). Liu and Fang (2002) examined different extraction techniques including formaldehyde-NaOH, EDTA and formaldehyde-ultrasound. The results revealed that the EPS composition of EPS depended on the extraction method. For instance, the concentration of carbohydrates and proteins in EPS extracted by formaldehyde-NaOH were 40.5 and 54.6 mg per g of sludge while these concentrations were 28.9 and 20.4 in EPS extracted by formaldehyde-ultrasound.

There has not been a considerable study of pretreatment impact on EPS of WAS in the literature. Pretreatment can inactivate or disrupt cells and impact on EPS loss. In addition, a pretreatment process can destroy EPS or release bonds with cells. Due to the short period of pretreatment, a comparison between the EPS content of WAS before and after pretreatment can

be employed to assess the short term impact of pretreatment on EPS including disruption and disappearance.

Pretreatment may facilitate EPS production in the anaerobic digestion process. Poxon and Darby (1997) indicated that an increase in concentration of glucose caused an increase of EPS content of anaerobic sludge in full and laboratory scale digesters. The provision of glucose as an easily assimilated substrate that is readily available for bacteria in initial stages of digestion stimulated the production of EPS. The experiments showed that acidogens were responsible for EPS production and the addition of the other materials such as propionic acid did not accelerate the rate of EPS production. Since, pretreatment can enhance the hydrolysis stage in anaerobic digestion of WAS, dissolved concentrations of carbohydrates as a suitable substrate for acidogenic bacteria may increase. Accordingly a higher formation of EPS due to pretreatment of WAS may occur in a digester.

The knowledge of EPS content of WAS and the impact of pretreatment technologies on EPS is beneficial to interpret the other pretreatment impacts such solublization or rates of biodegradability. Since a considerable fraction of organic materials in WAS are in EPS form (Liu and Fang, 2002), the composition and level of solublization might depend on the composition of EPS and the strength of bonds between EPS and microorganisms. For instance an increase in the level of colloidal COD due to pretreatment can be interpreted as the solublization of EPS and the biodegradation rate of colloidal material might depend on the chemical composition of EPS in WAS.

### 2.1.3. Biological Properties

Pretreatment of WAS can impact on the biological properties of sludge such as the activity of biomass and biodegradability of sludge. In this part of the literature review, the impacts of pretreatment on biological properties of sludge are discussed and the indicators that describe such impacts are introduced.

#### 2.1.3.1. Activity of Bacteria

Viable biomass (active cells and EPS) were found to represent the major source of $CH_4$ production during anaerobic digestion of non-pretreated WAS (Jones et al., 2007). The number of bacteria in a typical WAS is in the range of $1\text{-}10\times10^{12}$ /gr VSS (Nielson et al., 2004). Typically, about 80% of them are viable. The inactivation of bacteria in WAS, as a general biological effect of pretreatment and as an indicator representing the fraction of functionally damaged bacteria may be indicative of the conversion of biomass into a more readily biodegradable form.

Respirometry is a common method employed to measure active heterotrophic bacteria. The ratio of active biomass (measured as initial active biomass in a sample using respirometry) to particulate COD ($mgCODL^{-1}$) in WAS is defined as the active fraction. The active fraction which is measured by a combination of respirometry and COD tests can be employed as an indicator of a pretreatment's impact on the biological properties of WAS. The difference between initial active heterotrophic biomass in raw and pretreated sludges represent the fraction of bacteria that have been inactivated, died or disrupted as results of the physical or chemical stress from pretreatment. Not only disrupted biomass but dead and inactive biomass might be

disintegrated and hydrolyzed easier than active biomass. Inactivation of biomass may also impact on the quality of flocs and facilitate their disintegration.

Respirometry is employed to determine the oxygen uptake by WAS in a batch reactor. The OUR is determined by measuring the consumption of dissolved oxygen in a wastewater or sludge sample in an airtight vessel. The procedure of measurement has been introduced as a standard method (APHA, 1995). Respirometry can be employed to characterize biological properties of WAS such as active biomass content, bacterial growth and decay rates and yield coefficient.

The fraction of active heterotrophic bacteria and the kinetic parameters associated with heterotrophs were investigated by Kappler and Gujer (1992) and Wentzel et al (1998). Andreottola et al. (2002) introduced Equation 2.4. to determine the theoretical oxygen respiration rate (OUR).

$$OUR(t)=\left(\left(\frac{1-Y_H}{Y_H}\right)\mu_{max}-b\right) . X_0 . e^{(\mu_{max} - b).t} \qquad 2.4.$$

Where: t, $Y_H$, $\mu_{max}$, b and $X_0$ represent time, yield coefficient, specific maximum growth rate, decay rate and initial heterotrophic active biomass, respectively. Wentzel et al (1998) rearranged Equation 2.4 to an operational version (Equation 2.5) to determine the initial active biomass ($X_0$) in terms of mg $CODL^{-1}$ on the basis of respirometry data.

$$X_0 = \frac{e^{(y-Intercept)} \times 24}{\frac{1-Y}{Y} \times (Slope + b)} \qquad 2.5.$$

Where, "slope" is the slope of the Ln(OUR) vs. time curve. According to Equation 2.4., this slope is equal to ($\mu_{max}$-b). The y-intercept represents the intercept of the y-axis in the Ln(OUR) vs. time curve. Avcioglu et al. (1998) indicated that b values for heterotrophs are in the range of 0.1 to 0.6 $d^{-1}$ and Andreottola et al. (2002) indicated that variability of b accounted for a maximum 7% change in the calculated value of initial active biomass ($X_0$). Accordingly, the literature suggests that $X_0$ is not significantly sensitive to the value of b.

In addition to estimation of the initial active biomass in sludges, respirometry can be employed to estimate the value of $Y_H$. Rai et al. (2004) presented Equation 2.6. to determine the value of conversion yield ($Y_H$) from respirometry.

$$Y_H = 1 - \frac{\int_{t_1}^{t_2} (OUR_{total} - OUR_{endo}).dt}{COD_{removed}} \qquad 2.6.$$

Where, $Y_H$, $OUR_{total}$ and $OUR_{endo}$ represent conversion yield, total OUR and OUR in endogenous conditions respectively. $COD_{removed}$ represents the decrease of COD in soluble phase of WAS.

Pretreatment of WAS may increase the fraction of energy required for maintenance of bacteria and decrease the conversion yield ($Y_H$) of bacteria. Therefore, the yield coefficient ($Y_H$) of bacteria may be considered as an indicator of the impacts of pretreatment on biological properties (bacterial growth) of WAS (Rai et al., 2004).

### 2.1.3.2. Biodegradation Tests

Pretreatment can impact on the biodegradability of WAS. In this regard biodegradability may refer to the rate of biodegradation or the ultimate extent of biodegradation. The available tests and their capability to investigate pretreatment impacts are subsequently reviewed.

#### Biochemical Methanogenic Potential Test

The biochemical methanogenic potential (BMP) test is used to measure the potential for anaerobic biodegradability of wastewater and WAS. The test is conducted in sealed serum bottles. Before sealing, nitrogen gas is used to flush the bottle's headspace for several minute to remove oxygen. The inoculation of WAS with a sludge from an anaerobic digester is typically carried out to provide an initial population of anaerobic bacteria. While performing the test, the generated gas in the headspace is measured daily in the first weeks and occasionally later. The composition of the generated gas is examined to determine the volume of methane and other gases such as $CO_2$, $H_2$ and $N_2$.

The total methane and carbon dioxide generated in a BMP test is considered to determine the success of mineralization in an anaerobic process. In addition, the fraction of total COD which is removed during anaerobic digestion in a BMP test represents the biodegradable fraction of COD in a sample. Straub (2005) employed the profile of methane generation to characterize the hydrolysable fractions of the feed in an anaerobic digestion process using a BMP test. In addition, such a profile was employed by Jeong et al. (2005) to assess different COD fractions. The BMP test can be employed to characterize the impact of pretreatment on biodegradability of WAS. In addition, the methane generation profile can be used to fractionate COD in raw and

pre-treated sludge. The comparison of COD fractions in raw and pre-treated WAS may provide information about the impact of pretreatment on each specific fraction of COD.

## Biochemical Acidogenic Potential (BAP) Test

Pegion (1993) described a protocol for the Biochemical Acidogenic Potential (BAP) test that involved the use of bromoethane sulfonate (BES) as an inhibitor for methanogenesis. Martin Ruel et al. (2002) improved the test method for wastewater samples. In these previous studies, the biochemical acidogenic potential (BAP) test was used to evaluate the potential of wastewater for production of VFAs for use by phosphorous accumulating organisms (Barajas et al., 2000). The samples were fermented for a short period until the concentration of VFAs became stable. The maximum concentration of VFAs in the stable period was defined as the biochemical acidogenic potential. Hence, the final accumulated VFAs indicate the fermentable fraction of COD in samples (Martin Ruel et al., 2002). The rate of VFA generation, like methane production in BMP tests, indicates the results of fermentation and other complicated processes. The BAP test was found to take about 5-20 days to reach a stable level of accumulated VFAs and was significantly shorter as compared to the BMP test which may take 30-70 days. Such a reduction in test duration happens due to the elimination of the acetoclastic methanogenesis process and the measurement of accumulated VFAs which is a relatively faster response as compared to methane generation.

The previously mentioned the BAP test protocol were developed for wastewater samples. Therefore, an investigation of the best conditions to apply the test for WAS is required. This investigation would examine whether the BAP results represent the biodegradable fraction of WAS. The levels of VFAs and ammonia accumulation, the concentration of hydrogen and pH

may be significantly different from the values observed in digestion of wastewaters. The estimation of the level of inhibition due to each of these factors is beneficial to optimize the BAP test.

The literature suggests that sludge pretreatment may increase the biodegradability of WAS (Scheminski et al., 2000). Accordingly, the BAP test may be applied to investigate such improvement with an increase in BAP indicating the impact of pretreatment. In addition, BAP can be employed as a response for modeling the hydrolysis process and COD fractionation and is discussed later.

## Respirometry

Respirometry can be employed to investigate pretreatment impacts on the COD fractions of WAS and on many biokinetic parameters in aerobic biodegradation processes. Spanjers and Vanrolleghem (1995) and Mathieu and Etienne (2000) presented procedures to estimate biokinetic parameters and fractionate WAS using respirometry. By this method, the substrate was fractionated into readily hydrolysable, slowly hydrolysable and active heterotrophic biomass. Musser et al. (2009) attempted to quantify the impact of sonication on biodegradable COD fractions including readily biodegradable and slowly biodegradable COD using respirometry. The estimation of the biodegradable fractions was conducted fitting respirometry responses based on the ASM Model. They revealed that the results could be employed for incorporation of ultrasonic pretreatment into a whole-plant model. The COD fractions, which are estimated by these methods, might be indicators of a pretreatment impact on the related biodegradation stages. Such fractionation can be applied to evaluate the fractions of readily hydrolysable and slowly hydrolysable material in raw and pretreated sludge. Since pretreatment

results in the solublization of organic materials, an increase in the level of readily hydrolysable material is expected when pretreatment of WAS is applied.

## 2.2. Previous Pretreatment Studies

Pretreatment processes generally lead to the rupture of cell walls and bacterial membranes in WAS and, consequently, result in the release of organic substances to the outside of cells. These substances can be more easily hydrolyzed to their unit molecules by the extracellular enzymes of hydrolyzing bacteria. Providing easily hydrolysable organic material by pre-treating WAS remarkably increases the rate of hydrolysis and consequently improves the overall anaerobic digestion rate (Wang et al., 1999).

The pretreatment of WAS has employed different technologies including mechanical (Wang et al., 1995; Chio et al., 1997; Muller, 2001; Tiehm et al., 1997; Tiehm et al., 2001; Chu et al., 2002), chemical (Sakai et al., 1997; Deleris and Rouston, 2000; Saby et al., 2002; Lendormi et al. 2001; Chiu et al., 1997; Tanaka and Kamiyama, 2002), and thermal (Camacho et al., 2002) disintegration. Wang et al., (1995) indicated the order of pretreatment efficiency for the enhancement of methane generation as ultrasonic lysis > thermal pretreatment by autoclave > thermal pretreatment by hot water > freezing. Ultrasonication and ozonation, as examples of physical and chemical methods, and the impacts of these pretreatment techniques on physical, biochemical and biological characteristics of WAS are introduced in the following discussion.

### 2.2.1. Ultrasonic Pretreatment

Ultrasonic lysis is a cell disruption technique which has been used in the biochemical field for more than four decades. The bacterial flocs are deagglomerated and bacterial cells are

disrupted by pressure waves and cavitation generated by an ultrasound generator leading to the release of intracellular organic substances (Takatani et al., 1981). Several advantages and disadvantages of ultrasound pretreatment indicated in the literature are mentioned in Table 2.2.

**Table 2.2. Advantages and Disadvantages of Ultrasonication.**

| | | |
|---|---|---|
| **Advantages** | • Compact design and easy retrofit within existing systems.<br>• Low cost and efficient operation compared to several other pretreatments.<br>• Production of an in-situ carbon source for denitrification plants.<br>• Complete process automation.<br>• Potential to control filamentous bulking and foaming in the digester.<br>• Better digester stability.<br>• Improved VS destruction and biogas production.<br>• Better sludge dewaterability.<br>• Improved biosolids quality. | **References:**<br>Harrison and Pandit,1994;<br>Tiehm et al., 2001;<br>Hogan et al., 2004;<br>Yin et al., 2004;<br>Sandino et al., 2005<br>and<br>Khanal et al., 2006a |
| **Disadvantages** | • Capital and operating costs due to immaturity of technology.<br>• Long-term performance data of ultrasound system in full-scale are still limited. | |

### 2.2.1.1. Ultrasonication Technology

Ultrasound is a sound wave at a frequency more than 20 KHz which is above the normal hearing range of humans (Khanal et al., 2006a). The three major components of an ultrasound system are the converter (or transducer), booster and horn (or sonotrode). A converter converts electrical energy into ultrasound energy (or vibration). The booster is a mechanical amplifier that increases the amplitude (vibration) generated by the converter. The horn is a specially designed tool that delivers the ultrasonic energy to the sludge. Figure 2.2 shows the arrangement of converter, booster and horn in a typical ultrasound system (Khanal et al., 2006a).

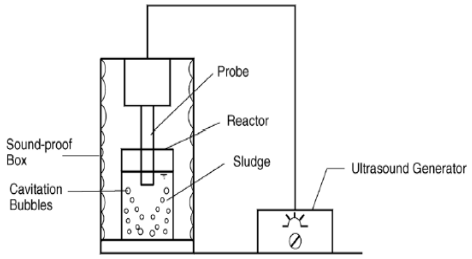


**Figure 2.2. Scheme of Ultrasonic Activated Sludge Disintegration (Khanal et al., 2006a).**

When ultrasound waves are generated in a medium such as sludge, they form a repeating pattern of compressions and rarefactions in the medium. The rarefactions are the regions of low pressure (excessively large negative pressure) in which the liquid or slurry are torn apart. As a result of the reduced pressure, microbubbles are formed in the rarefaction regions. These microbubbles also known as cavitation bubbles and contain vaporized liquid and gas that was previously dissolved in the liquid. Cavitation is the phenomenon where microbubbles are formed in the aqueous phase and expand to unstable size, and then rapidly collapse. The collapsing of the bubbles often results in localized temperatures up to 5,000 K and pressures up to 180 MPa (Suslick, 1990; Flint and Suslick, 1991). The sudden and violent collapse of a large number of microbubbles generates powerful hydro-mechanical shear forces in the bulk liquid surrounding the bubbles (Kuttruff, 1991). The bacterial cells adjacent to the collapsing bubbles are disrupted by extreme shear forces, thereby rupturing the cell wall and membranes. The localized high temperature and pressure could also assist in sludge disintegration. At high temperatures, lipids in the cytoplasmic membrane are decomposed, resulting in holes within the membrane, through which intracellular materials are leaked to the aqueous phase (Wang et al., 2005). Tiehm et al. (2001) indicated that hydro-mechanical shear forces produced by ultrasound cavitation are the main reason for sludge disintegration.

The efficiency of ultrasonication depends on many factors such as operating frequency, horn, booster and converter designs, types of sludge (proportion of WAS and PS, SRT), TS content, organic fraction, operating temperature, ultrasonic density and intensity. For example, the horn design is considered to be one of the most important factors affecting the sludge disintegration efficiency and its design is often proprietary. This is one of the reasons why it is difficult to compare the results of different studies (Khanal et al., 2006a). Accordingly, instead of investigating specific sonication devices, this study focused on the mechanisms of disintegration and the impact of sonication on biodegradability of WAS.

### 2.2.1.2. Impacts of Ultrasonication on WAS

Ultrasound pretreatment generates highly oxidative reactive radicals of hydroxyl ($OH^{\bullet}$), hydrogen ($H^{\bullet}$), and hydroperoxyl ($HO_2^{\bullet}$) and hydrogen peroxide ($H_2O_2$) that increases disintegration (Adewuyi, 2001). Wang et al. (2005) investigated the contribution of the paths which may be significantly responsible for ultrasonic activated sludge disintegration. They considered four possible paths including hydro-mechanical shear forces, oxidizing effect of $OH^{\bullet}$, $H^{\bullet}$, $N^{\bullet}$ and $O^{\bullet}$ produced under ultrasonic radiation, thermal decomposition of volatile hydrophobic substances in the sludge and increase of temperature during ultrasonic activated sludge disintegration. The control experiments conducted to determine the effect of increase of temperature revealed that the release of COD due to increase of temperature to 80ºC for 1 hour was very low (Khanal et al., 2006a). In addition, the quantity of volatile hydrophobic substances was usually very low. Accordingly, the effect of the last two paths can be ignored. As the concentration of $OH^{\bullet}$ radicals during ultrasonication is greater than that of other oxidizing materials in sludge (Hart, 1986; Hart and Henglein, 1986), the effects of $OH^{\bullet}$ was investigated

by addition of $NaHCO_3$ before pretreatment to mask the oxidizing effect of $OH^{\bullet}$. The results indicated that oxidation by $OH^{\bullet}$ was responsible for about 16 to 26 percent of WAS disintegration by ultrasound for low and high ultrasound densities (0.384 and 0.72 W/mL), respectively.

Several studies have been conducted to investigate the effect of sonication characteristics and condition on sludge disintegration (Tiehm et al., 2001; Rai et al., 2004; Bougrier et al., 2005; Grönroos, et al., 2005; Wang et al., 2005; Khanal et al., 2006a). Khanal et al. (2006a) introduced the common indicators of power or energy supplied for sludge disintegration including specific energy input, ultrasonic dose, ultrasonic density and ultrasonic intensity. A few studies have reported the sludge disintegration in terms of ultrasonic density (Chu et al., 2001; Mao et al., 2005; Wang et al., 2005). Tiehm et al. (2001) and Wang et al. (2005) also determined sludge disintegration at different ultrasonic intensities ranging from 0.1 to 1.5 $W/cm^2$ and from 30 to 230 $W/cm^2$ respectively. In general, sludge disintegration increased with intensities.

Rai et al. (2004) evaluated the effect of sonication on deflocculation of aggregates, bacterial activity and cell disruption in WAS. The results indicated that at lower levels of specific energy sonication, causes deflocculation without significant cell disruption while at higher intensity levels cell disruption was observed. Applying an excessive level of energy (more than 50000 kJ/kg) had limited effects on size reduction and was unable to destroy the remaining cells and cell debris.

Khanal et al. (2006b) investigated the impact of sonication on the structure of flocs in WAS by microscopic observation. They reported that sonication resulted in a considerable structural changes in flocs and disappearance of filaments in WAS. Khanal et al. (2006c) conducted

scanning electron micrograph (SEM) to assess the impact of sonication on biological properties of sludge. The results revealed that even short time sonication (2 minutes) disrupt structural integrity of flocs as well as filaments. They showed that longer sonication (10 to 40 minutes) could lead to disruption of majority of bacteria. The results obtained by Wang et al. (2005) indicated that protein was the predominant part of organic material which was released by sonication of WAS. Accordingly, Khanal et al. (2006a) recommended protein measurements to evaluate sonication effects.

Wang et al. (2005) evaluated the impacts of power density, sonication time and intensity and pH of sludge on properties of sludges such as soluble COD. The results showed that increase in intensity, density and time (duration) of sonication improve release of COD in liquid phase and higher pH results in higher level of COD release. They introduced a model that described the relationship between solubilization of COD and pH of sludge and power and energy applied by sonication. The model introduced in this study is not necessarily applicable for the other sonication studies with different sonication apparatuses but it is beneficial to generally understand the importance of sonication condition and their impact on the obtained results.

Wang et al. (1999) applied sonication and attempted to show a correlation between the solublization and enhancements in biogas generation with 11 day anaerobic digestion batch tests. The use of short term batch digestion tests was useful for directly comparing different pretreatments or intensities however, it was not possible to assess whether the pretreatments affected the biodegradable fraction of the WAS or the rate of biodegradation because of the short duration of the batch tests.

Using either SCOD or the degree of COD released ($DD_{COD}$) may not be able to indicate the actual level of improvement in a digestion process due to ultrasound pretreatment. Kim et al. (2003) demonstrated that sonication caused a limited release of SCOD while it resulted in a relatively high level of VS reduction after 7 days of anaerobic digestion of WAS when compared with other pretreatments. The difference in responses may be due to partial disruption of cells by these physical methods that do not release SCOD but enhance the digestion process. This result reveals that solublization indicators may not able to describe the impact of sonication on the biodegradability of sludge.

### 2.2.2. Ozonation

Generally, ozone or ozone in combination with hydrogen peroxide is generated in an ozone generator as highly oxidizing chemicals. The cell walls of microorganisms can be disintegrated and the cytoplasm solublized by these oxidizing chemicals. The oxidation process can disintegrate water insoluble substances with high molecular weights into smaller, water soluble and biodegradable materials (Scheminski et al., 2000). Ozone, as a very reactive oxidizing agent, reacts with sludge compounds in direct and indirect reactions that occur simultaneously (Staehelin and Hoigné, 1985). The shortliving hydroxyl radicals react indirectly and rapidly with a wide range of organic material while the direct reactions occur slowly and depend on the chemical characteristics of the reactants. The capability of chemical pretreatment methods for accelerating the digestion process is of interest. Since ozonation generates the lowest chemical residuals among the chemical methods, it was chosen for this research. With an ozone dose of 0.2 gram ozone per gram organic dry matter, the degree of biodegradation of organic matter during subsequent anaerobic treatment was increased by 42% (Scheminski et al., 2000).

Like other pretreatment processes, ozonation enhances the anaerobic biodegradability of sludge and improves the anaerobic digestion process (total methane generation). In addition, this pretreatment technique results in the oxidation of a fraction of the organic material, and hence, transforms organic carbon to carbon monoxide and dioxide (Yasui and Shibata, 1995). Compared to mechanical, thermal and thermo-chemical disintegration, partial oxidation by ozone achieved the highest degree of organic matter decomposition in anaerobic biodegradation (Scheminski et al., 2000).

Ozonation can change the chemical composition of sludges. Bünning and Hempel (1996) showed that cell-walls of microorganisms are destroyed by reaction with ozone. Therefore, the intracellular proteins are released from the cells and can temporarily be found in the sludge liquor. Gel permeation chromatography investigations showed that the proteins diluted in the sludge liquor were decomposed by consecutive reactions with ozone. Due to the high reaction rates of consecutive reactions during oxidation, no noticeable protein concentration could be measured in the sludge water. Scheminski et al. (2000) measured the fraction of proteins remaining after various levels of ozonation. The protein content of the sludge was decreased by approximately 90% when high dose of ozone (0.5 g Ozone/g organic dry matter) was applied. They reported lower levels of decomposition of polysaccharides and lipids as compared to proteins. However the disappearance of these substrates did not reflect an increase in digestibility of the sludge. Due to this oxidation and consequently removal of proteins, polysaccharides and lipids, the measurement of total organic carbon (TOC) of sludge before and after ozonation was employed. Obviously, a high reduction of TOC or COD, due to ozonation, indicates a significant unwanted change in the composition of organic matter in sludge. The

biomass in anaerobic digesters may require a considerable time to be acclimated with new composition of substrate when the digesters are fed with sludges pretreated by ozonation.

The pretreatment of sludges with low dose of ozone may result in lower impacts on the chemical composition of sludges. Goel et al. (2003) evaluated the effect of low doses of ozone on solubilization and mineralization of sludge. The VSS and TVS of sludge were measured before and after ozonation to assess the ratio of solubilization and sludge mineralization during ozonation respectively. The results indicated that only a negligible amount (about 5%) of the TVS was mineralized during the pretreatment process with an ozone dose of 0.05 gr $O_3$/gr TS. However about 36% of the VSS was removed and was predominantly transformed to VDS. The results indicate that low doses of ozone cause a low level of mineralization. Scheminski et al. (2000) showed that the reduction in protein content of the sludge was minimal when a low dose of ozone was applied. For instance they showed that only 5% of proteins were oxidized when low dose of ozone (0.05 g Ozone/g organic dry matter) was applied.

A low dose of ozone can improve the biodegradability of sludge without considerable change in chemical composition of sludge. Scheminski et al. (2000) investigated the effects of different ozonation levels on the release of organic carbon from solids and the enhancement of digester gas yield. Ozone doses lower than 0.061 gr ozone/gr odm enhanced the degradability of the sludge as much as 120 $L_{gas}$ / $kg_{odm,feed}$, while increasing the dose of ozone to more than 0.061 gr ozone/gr odm resulted in a limited effect on this parameter. Goel et al. (2004) observed VSS degradation efficiencies of approximately 80% for a mixed municipal sewage sludge (77%WAS and 23%PS) with a combination of ozonation and anaerobic digestion. This efficiency was achieved by a relatively low ozone dose of 0.026 kg $O_3$/kg TVS. Accordingly, using low ozone

doses for pre-treating of WAS with low disappearance level of TOC seems to be promising for a pretreatment technology.

## 2.3. Modeling

As mentioned in the previous sections, pretreatment can impact on the physical, biological and biochemical properties of WAS. The impacts can be investigated according to changes in these sludge properties. In addition to these properties, characterization of pretreatment impacts can be done according to the biodegradability of WAS. This method of characterization can be conducted using anaerobic digestion models such as a modified ADM1.

Since pretreatment can facilitate the hydrolysis process and change the COD fractionation of the feed WAS, employing a model which focuses on the primary process of anaerobic biodegradation should improve the knowledge of this impact of pretreatment. Accordingly, the capability of anaerobic digestion models will be discussed in this section.

### 2.3.1. Anaerobic Digestion Models

Although earlier models of anaerobic digestion exist, an IWA digestion modeling task group was established in 1997 to develop a generalized anaerobic digestion model. The model, known as anaerobic digestion model 1 (ADM1) describes both biochemical and physico-chemical processes, with the exception of precipitation.

As shown in Figure 2.3., the biochemical steps are initiated by disintegration of particles to produce particulate proteins, carbohydrates and lipids in ADM1. During extracellular hydrolysis, proteins, carbohydrates and lipids are transformed to amino acids, sugars and long

chain fatty acids (LCFAs), respectively. In acidogenesis, sugars and amino acids are converted to VFAs and hydrogen. The VFAs and LCFAs are dominantly converted to acetate and hydrogen which are consumed by methanogens to produce methane (Batstone et al., 2002). The biochemical processes are combined with physico-chemical reactions including acid-base chemistry and liquid-gas exchange (e.g. gas transfer) to form the overall anaerobic digestion model (Batstone et al., 2002).

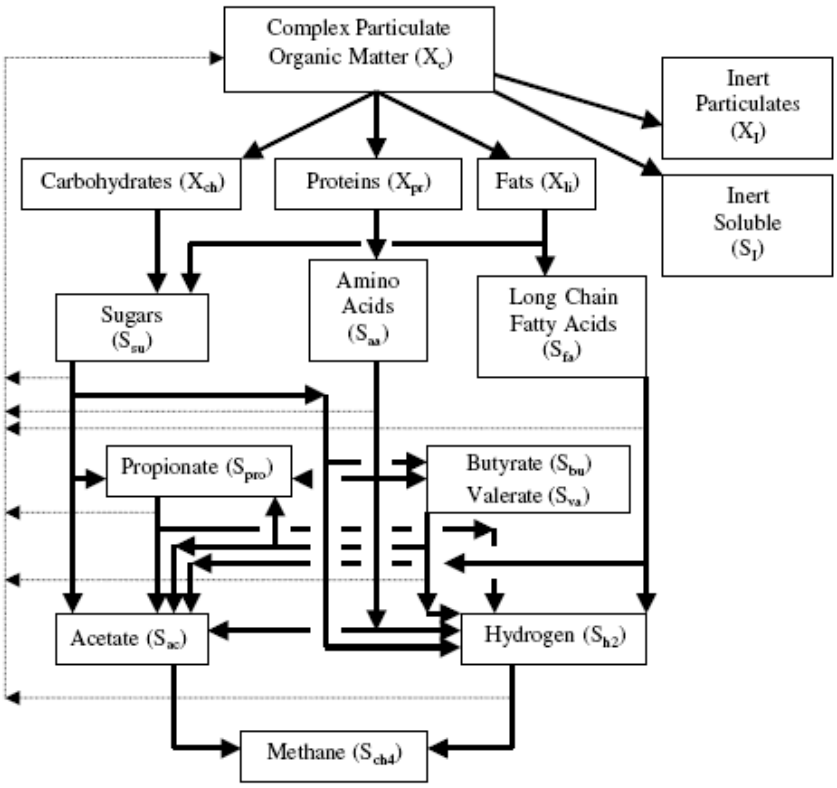


**Figure2.3. Biodegradation processes in ADM1 model (Parker, 2005)**

Generally, the modeling of anaerobic digestion of WAS is faced with several challenges. Due to the complexity of the processes and the variety of components, many parameters are involved in the digestion model. Many of these parameters are difficult to measure; consequently, the detection of compatibility of models with actual results is difficult (Jeong et

al., 2004). In addition, the investigation of model compatibility and model fitting for a targeted sample in a batch test, is highly time consuming, because methane generation is naturally a slow process. This difficulty might be solved by using the results from biochemical acidogenic potential (BAP) tests as relatively short tests which can be employed to evaluate the processes involved in the initial digestion processes such as disintegration, hydrolysis and acidogenesis.

### 2.3.2. Modeling and Hydrolysable Fractions

Anaerobic digestion of WAS consists of a complex series of interdependent reactions, a diverse consortium of bacteria and processes including disintegration of particulate material, hydrolysis, acidogenesis and methanogenesis. It is anticipated that pretreatment technologies will have the greatest influence on the initial stages of anaerobic digestion including the hydrolysis stage. Therefore, assessing the effect of pretreatment processes on the efficiency and rate of hydrolysis was one of the priorities of this research.

Model structures for the hydrolysis process that have been developed include single pathway and dual pathway structures. The single pathway model is used in almost all anaerobic models such as ADM1. The dual pathway model has been suggested recently based on batch experimental results for sludge and involves fractionation of hydrolysable material into readily hydrolysable and slowly hydrolysable material (Conklin et al., 2004 and Henze et al., 1987). Straub (2005) found that 31% of the particulate COD (pCOD) was readily hydrolysable ($X_{rh}$) and 44% of pCOD was slowly hydrolysable ($X_{sh}$) in a municipal sludge. Because of the presence of significant amounts of slowly hydrolysable material in WAS and also the release of some readily hydrolysable material after pretreatment, such fractionation may be preferable to single pathway. A pretreatment process may increase the fraction of total hydrolysable material

($X_{rh}$+$X_{sh}$), change each hydrolysable fraction ($X_{rh}$ and $X_{sh}$) and affect the degradation rate of each hydrolysable material. Each of these changes can be considered to characterize the pretreatment impacts on WAS.

Since the BAP test is a relatively short test that focuses on the initial portion of the overall process of anaerobic digestion, it is hypothesized that data from this test will be better than that from the biochemical methanogenic potential (BMP) test. For characterizing this part of digestion, the BAP test has been employed by Martin Ruel et al. (2002b) to provide a simplified model to describe hydrolysis and fermentation processes in anaerobic digestion of wastewater. In the current study, it is proposed to employ the biochemical acidogenic potential as an additional indicator of biodegradability of sludge which can be improved by pretreatment. In addition, the profile of VFA generation in the BAP test, like methane generation in a BMP test, can be employed for fractionation of hydrolysable material in WAS.

An important question about the BAP test is the sensitivity of the profile of VFA generation to substrate characteristics and the parameters in the model. As compared to the use of BMP data, an improvement in the sensitivity of the profile of VFA generation to substrate characteristics is expected due to the elimination of some sources of errors in the BAP test. The simulation and experiments carried out by Jeong et al. (2005) for the biochemical methanogenic potential of sludge revealed some information about the sensitivity of VFA generation to substrate and parameters in ADM1. The sensitivity of kinetic and stoichiometric parameters (defined in the ADM1) to the substrate (glucose), intermediate (VFAs) and final components (methane) was investigated with BMP test data. VFA generation was sensitive to limited stoichiometric parameters ($f_{Bu,Su}$, $f_{Pro,Su}$, $f_{Ac,Su}$), the anaerobic digestion pathway and

consequently the composition of substrate. The results show that model parameters related to sludge composition are identifiable from measurement of VFA generation and small uncertainty in the estimation of those parameters might happen which is a suitable sign for using the profile of VFA generation as a response for fractionation of hydrolysable fraction of substrates.

In addition to BMP and BAP tests, respirometry tests can be employed to fractionate particulate materials in WAS into readily hydrolysable, slowly hydrolysable and inert fractions. Mathieu and Etienne (2000) proposed a simplified procedure to model aerobic biodegradation of samples (according to respirogram) by focusing on adsorption of particulate substrates and hydrolysis processes.

## 2.4. Summary

There has been considerable study of pretreatment processes and their impact on WAS. A range of physical, chemical and biological responses were evaluated to assess the impact of pretreatment technologies on some properties of WAS in the literature. A majority of the studies focused on the level of solublization of particulate materials in WAS samples with respect to particle size, TS, VS, COD and TKN while the solublization of particulate materials does not necessarily results in an improvement in the biodegradability of sludges. However no attempt has been made to correlate the solublization measurements with the biodegradable fraction of the WAS or the rate of biodegradation and a common approach to describing the impact of pretreatments on sludge biodegradability has not been developed. In addition, there has also been little evaluation of the interaction between WAS characteristics and pretreatment efficiency. In many cases, the results are not comparable since the characteristics of sludges employed in the studies were different or unclear.

# Chapter 3

# 3. Approach

## 3.1. Overview

The objectives of this research were to enhance the knowledge of the impact of pretreatment on WAS properties and to identify methods that are capable of quantitatively or qualitatively predicting the anaerobic digestibility of WAS. Sonication and ozonation were employed as models of physical and chemical pretreatment techniques and thereby facilitated an assessment of the validity of indicators for different pretreatment techniques. The application of the indicators to different sludges (WAS with different SRTs) was investigated to evaluate the interaction between pretreatment and SRTs.

Figure 3.1 shows the stages of the research and series of studies that were conducted to address the objectives of the research. Three pilot scale sequencing batch reactors (SBRs) were employed to act as a consistent source of WAS with specific SRTs. The SBRs, each with a volume of 175 L, were operated on screened municipal wastewater at an hydraulic residence time (HRT) of 9.3 hours and SRT's of 1.95, 7 and 15 days. The SBRs were operated with a 6 hour cycle consisting of five basic stages including feed (30 min.), aerate (5 hr and 15 min.), mixed liquor discharge (15 min.), settle (30 min.) and decant (15 min.). The screened municipal wastewater was provided as feed during the first 30 minutes of the aeration process and mixed liquor was discharged during the last 15 minutes of the aeration process. The SBRs were employed as a source of WAS for all of the testing conducted in this study. Mixed liquor samples were taken at the end of aeration period, settled for 30 minutes and the supernatant was decanted to provide WAS samples. The results of solid tests revealed that the solids

concentrations of the thickened samples were more related to the settleability of sludge than to solids content of the original mixed liquor in the SBRs. Employing WAS samples that were generated over a range of SRTs facilitated an investigation of the interaction between pretreatment and the WAS properties.

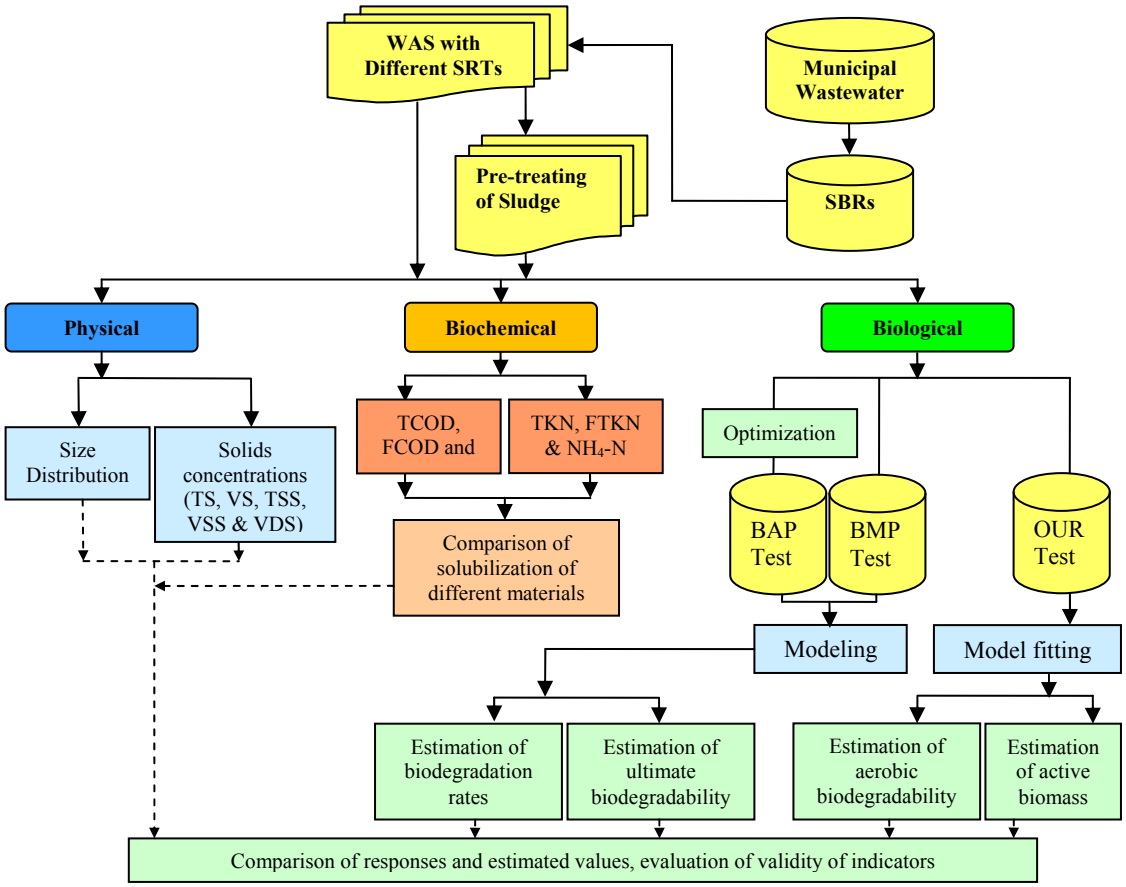


**Figure 3.1 Overall Frame Work for Characterization of the Impacts of Pretreatment on Different Sludges.**

In the ultrasound study, WAS samples from the SBRs were treated by sonication at 45°C in a bench scale apparatus with an operational frequency of 20 kHz and a maximum amplitude of 250 μm. The unit was operated in batch mode and ultrasound intensity was varied by collecting samples at different times as the treatment progressed. According to the specifications of the

ultrasound device and the batch reactor which were used as the sonication unit in this research, 1 minute of sonication time was equal to an ultrasound dose of 222.2 kJ/L.

In the ozone study, samples of the WAS were ozonated for differing time periods to achieve varying levels of pretreatment intensity. Ozonation was conducted using a Hankin Atlas ozone generator (OZOTEC, type S, model 2) at a production level of 10 Volt. The ozone generator was adjusted for a gas flow of 7.08 L/min., ozone concentration of 14666 PPM (by weight) and ozone mass flow of 0.1249 g $O_3$/min. A bench scale reactor with a 3 L volume was employed for the treatment of 1.1 L of WAS samples from the SBRs. The unit was operated in batch mode and ozonation intensity was evaluated by collecting samples at different times as the treatment progressed.

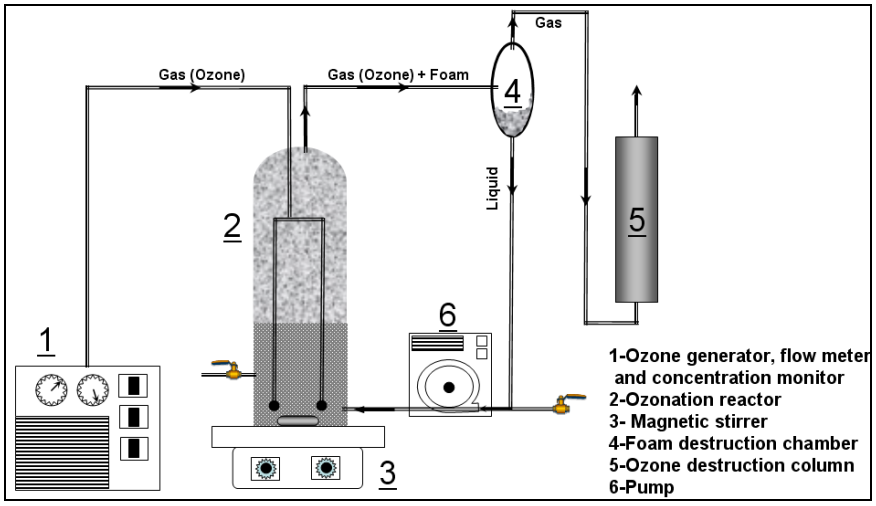


**Figure 3.2. Ozonation Apparatus**

As shown in Figure 3.2., ozone was diffused by two glass diffusers into the sludge. The sludges were stirred by a magnetic stir bar to ensure adequate mixing in the reactor. After 5 to 10 minutes of ozonation, a visible layer of foam appeared on the top of liquid phase in the reactor. The level of foam would rise and fill the headspace in about 5 minutes and then pass through the

discharge tube. Hence a glass vessel was included in this line and it acted as a foam breaker. A pump was employed to recycle the liquid from the foam breaker back to the reactor.

## 3.2. Physical Indicators and Pretreatment Impacts

The impact of pretreatment on the physical properties of WAS was characterized with respect to solids concentrations and particle size distribution. The solids concentrations including total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS) and volatile dissolved solids were measured for raw, pretreated and digested samples according to Standard Methods (APHA, 1995). Where required, samples were filtered with glass fiber filters with a nominal pore size of 1.5 μm to provide filtered samples. In some cases, the filtered samples were flocculated by adding alum, centrifuging for five minutes (4500 rpm) and then filtering with a membrane syringe filter (0.45 μm pore size) to obtain an improved estimate of the truly soluble COD (Mamais et al., 1993). These samples were used when fractionating COD and TKN into filtered COD (FCOD), flocculated-filtered COD (FFCOD) and filtered TKN (FTKN).

The size distribution of particles in samples was measured with a laser diffraction based technique (Mastersizer 2000). WAS samples (5 to 15 ml) were added to a standard 800 mL lab beaker containing 600 mL of ultra pure water. The 'dip-in' probe provided sample sonication, agitation and circulation through the flow cell for laser differaction analysis. Since there was a lag (1 to 2 hour) between pretreatment and particle size measurement, the particle size distribution of pretreated samples would likely have changed due to reflocculation of solubilized material. Low intensity sonication (provided by Mastersizer probe) was employed to disperse the weak bonds and eliminate the reflocculation effect. The volume mean size which was calculated

from the size distribution of the particles (Brittan, 2001) was employed as an indicator of the physical properties of the samples. Since all degradation processes (especially hydrolysis) in anaerobic digestion of WAS involve contact between (heterotrophic hydrolyzing) bacteria and their related substrates, particle size distribution was examined as a physical indicator to represent the availability of surface area and extent of particulate size reduction through a pretreatment. A comparison of the particle size distribution with other biochemical or biological indicators, such as SCOD, can reveal information about cell disruption and intracellular material release in each level of pretreatment intensity.

## 3.3. Biochemical Indicators

The chemical properties of a sludge depend on the presence of different organic or inorganic chemicals in the solid and soluble phases and the type and the strength of bonds among chemicals, bacteria and water. The first step of this part of the research focused on commonly used indicators such as TCOD, FCOD, FFCOD, TKN, FTKN and $NH_4$-N that where analyzed according to Standard Methods (APHA, 1995). A comparison of the FCOD and FTKN responses can provide an indication of the impact of a pretreatment on proteinaceous material as compared to other materials in sludge.

## 3.4. Biological indicators and pretreatment impacts

As shown in Figure 3.1, the biological impacts of pretreatment were investigated by conducting respirometry, BMP and BAP tests. Respirometry was employed to estimate the active biomass in raw and pretreated samples and the aerobic biodegradability of the sludges;

while BMP and BAP tests, as batch tests, were employed to estimate the ultimate anaerobic biodegradability and the rate of biodegradation of raw and pretreated sludges.

**Respirometry**

Previous studies (Jones et al., 2007) have revealed that the decay of viable organisms was found to represent the major source of $CH_4$ production during anaerobic digestion of non-pretreated WAS. Respirometry was employed to evaluate the inactivation of microorganisms during pretreatment. In this test the raw and pre-treated WAS samples were employed as inocula in a high F/M test using acetate as a substrate. In this approach, exponential growth occurs and hence an exponential increase in oxygen uptake rate was observed.

**Table 3.1. Nutrient Solution for Respirometry**

| Chemical | Concentration |
|---|---|
| Sodium Acetate ($CH_3COONa$) | 1020 mg/l |
| Ammonium Chloride ($NH_4Cl$) | 200 mg/l |
| Potassium Hydrogen Phosphate ($K_2HPO_4$) | 45 mg/l |
| Calcium Chloride ($CaCl_2 .2H_2O$) | 30 mg/l |
| Magnesium Sulfate ($MgSO_4.7H_2O$) | 25 mg/l |
| $FeSO_4$ | 20 mg/l |
| 2-Chloro-6-(Trichloromethyl) Pyridine | 0.533 mg/l |
| $ZnCl_2$ 0.05 mg/L | 1 mL of microelements solution in 1 liter of medium |
| $CuCl_2$ 0.03 mg/L | |
| $MnSO_4.H_2O$ 0.05 mg/L | |
| $(NH_4)_6 Mo_7 O_{24} .4H_2O$ 0.05 mg/L | |
| $AlCl_3$ 0.05 mg/L | |
| $CoCl_2.6H_2O$ 0.05 mg/L | |
| $NiCl_2.6H_2O$ 0.05 mg/L | |
| NaOH & HCl | To adjust pH=7 ( if required ) |

Respirometry was conducted using a Challenge Environmental Respirometer (AER-200) to separately estimate the concentrations of active heterotrophic organisms ($X_{HO}$) and readily biodegradable COD (rbCOD) in the raw and pretreated WAS samples. A series of preliminary experiments were conducted to identify the optimal operational conditions for the measurement of $X_{HO}$. The details of the experiments that were employed for identifying the optimal conditions for the respirometry test are presented in Appendix A.

Respirometry that was conducted to determine $X_{HO}$ consisted of adding 4 mL of sample (either raw or pretreated WAS) to the nutrient solution described in Table 3.1 to prepare a total test volume of 250 mL. The nutrient solution contained sodium acetate as a carbon source along with macro and micronutrients to provide a high ratio (from 10 to 20) of food to microorganisms for non-limited bacterial growth. A nitrification inhibitor (2-chloro-6-(trichloromethyl) pyridine) was added to the medium to eliminate oxygen demand by nitrifying organisms.

Respirometry was also employed to quantify the generation of readily biodegradable COD (rbCOD) by pretreatment. In these tests, a low food/microorganism (F/M) approach was employed to measure the oxygen uptake from a WAS (raw or pre-treated) sample that was inoculated (20% v/v) with raw WAS. The test involved the addition of 16 ml of samples and 4 mL of raw WAS as inocula, to water that contained the nitrification inhibitor to prepare a final volume of 250 mL. The temperature was set to 25 ºC for all respirometry tests and the pH was in the range of 7±0.2. Each set of respirometry tests was replicated one or two times to investigate the reproducibility of the results. In addition, since 8 bottles could be run in each set of respirometry tests, some bottles were run in duplicate in each respirometry test. The results of respirometry for control bottles consisting of water or medium (without sludge) revealed zero

oxygen uptake and hence it was concluded that the oxygen uptake observed in the sample bottles was due to metabolism of microorganisms.

**BMP and BAP tests**

One of the purposes of pretreatment is to improve the biodegradability of WAS. The biodegradability of WAS can be indicated either by a biochemical methanogenic potential test (BMP) or by a biochemical acidogenic potential test (BAP). In order to optimize the operational conditions for the BAP test, the total amount and rate of VFA generation were deemed to be the most important results. Methane or other gases may be produced in the BAP test and hence, gas testing was carried out to have information for completion of mass balances (with respect to COD). By conducting gas quality testing, the success of methanogenesis inhibition could be evaluated. It was deemed that in order to apply the BAP test as a reliable technique that provides a representative response of biodegradability, the test should be optimized for this application. The plan that was employed for identifying the optimal conditions for the BAP test is explained in Appendix B. The developed BAP test was employed in the pretreatment studies based on the frame work defined in this preliminary step.

In order to have an efficient biochemical reaction under mesophilic conditions, a temperature of 35 ºC for the BAP and BMP tests was chosen and both BMP and BAP tests were manually mixed once a day. According to the protocol described in Appendix B, 0.003 mM bromoethane sulfonate (BES) was added to sludges in the BAP tests to inhibit methanogenesis. In the batch tests, volumes of 50 mL of anaerobically digested wastewater treatment sludge and 250 mL of sample were added to serum bottles with a total volume of 500 mL. Before sealing, nitrogen gas was used to flush the bottle's headspace for several minutes to remove oxygen.

Liquid samples were regularly taken by a syringe with a large needle (diameter > 1mm) for measurement of pH, TCOD, filtered COD, TKN and $NH_4$-N (APHA, 1995) in BMP and BAP test bottles. The concentration of acetate, propionate, butyrate, iso butyrate, valerate and iso valerate in liquid samples taken from BAP tests were measured by ion chromatography (Dionex IonPac AS15) with conductivity detection and the total concentrations were converted from VFA mass to COD mass. The generated gas was regularly discharged from the bottles for measurement of gas volume with a manometric device. The $CH_4$ content of the gas was measured by gas chromatography and expressed as COD mass for comparison with TCOD values. Gas volume was measured every day due to considerable accumulation of biogas in bottles in the early days of digestion. Liquid sampling and all gas and liquid analysis were conducted every two days for the first 10 days of digestion. After 10 days of digestion, the measurement and analysis were conducted frequently depending on the rate of the digestion process.

The profile of ammonia generation in BAP tests was compared to that in BMP test for most of samples. The responses were quite similar (± 4% difference). Hence, the ammonia results from both BAP and BMP tests can be employed in further investigations.

It was anticipated that pretreatment may increase the fractions of total and readily hydrolysable material and affect the hydrolysis rate for readily or slowly hydrolysable material. These changes may depend on the intensity of pretreatment and can be considered to characterize pretreatment impacts on the hydrolysis of WAS. To facilitate the quantification of the impact of pretreatment on the rate of $NH_4$ generation (and hence hydrolysis of proteins) and

VFA generation an empirical model was fit to the ammonia and VFA data in BAP test. The results are presented in chapter 4 and 5.

It was proposed that the BAP test is preferred to the BMP test for describing hydrolysis and fermentation processes in anaerobic digestion of WAS, since it focuses on the initial portion of the overall process of anaerobic digestion. The modeling of digestion process based on BAP tests is more compatible with experimental results due to elimination of some causes of lags in responses such as gas-liquid transfer and also the faster reaction of acidogenic bacteria compare to methanogenic bacteria. The ability of the BAP test to characterize biodegradability of raw and pretreated WAS was evaluated in Chapter 6. A simplified stoichiometric model that was developed based on anaerobic digestion model 1 (ADM1) was employed to explore the relationship between BAP and BMP test responses.

## 3.5. Comparison of responses and estimated values

As shown in the last stage in figure 3.1, the validity of indicators for different pretreatment techniques and for different sludges (different SRTs) was investigated. A comparison of the biodegradability responses such as ultimate biodegradability and rate of biodegradation with other responses such as size distribution and solubilization (based on solid concentrations, COD and TKN) was conducted to identify the preferred indicators for predicting the impact of different pretreatment technologies on the biodegradability of WAS samples.

# Chapter 4

# 4. Characterization of sonication impacts on properties of waste activated sludge and biodegradability

## 4.1. Introduction

In this part of the study sonication was employed as a model physical pretreatment technology and a range of physical, chemical and biological responses were evaluated to assess the impact of sonication on WAS properties as well as digestibility. WAS samples that were generated at differing SRTs on municipal wastewater were employed to facilitate an assessment of the interaction between pretreatment and WAS properties on digestibility. The overall objective was to develop protocols that can be employed to characterize the impact of pretreatment processes on WAS digestion.

## 4.2. Approach

Three sequencing batch reactors (SBRs), each with a volume of 175 L, were operated on screened municipal wastewater at an hydraulic residence time (HRT) of 9.3 hours and SRT's of 1.95, 7 and 15 days respectively. The SBRs were employed as a source of WAS for all of the testing conducted in this study. WAS samples from the SBRs were mixed in 3 liter glass beakers using a magnetic stir bar prior to treatment by sonication at 45$^{o}$C in a bench scale apparatus with an operational frequency of 20 kHz and a maximum amplitude of 250 μm. The sonication unit was operated in batch mode and ultrasound intensity was varied by sonicating samples for

different times. For the unit employed in this study sonication times of 5, 10, 25 and 45 minutes corresponded to ultrasound intensities of 1111, 2222, 5555 and 9999 kJ/L respectively.

The raw and treated samples were characterized with respect to solids concentrations, TKN, COD (APHA, 1995), particle size distribution and oxygen uptake rate. A part of the samples was filtered immediately after the pretreatment process with glass fiber filters with a nominal pore of 1.5 μm to provide filtered samples. The filtered samples were flocculated by adding alum, centrifuging for five minutes (4500 rpm) and then filtering with a membrane syringe filter (0.45 μm pore size) to obtain an improved estimate of the truly soluble COD (Mamais et al., 1993). These samples were used when fractionating COD and TKN into filtered COD (FCOD), flocculated-filtered COD (FFCOD) and filtered TKN (FTKN). Particle size distribution was measured by a laser diffraction based technique (Mastersizer 2000). The volume mean size which was calculated from the size distribution of the particles (Brittan, 2001) was employed as an indicator of the physical properties of the samples.

During the period of sonication experiments, the sludges generated from all of the reactors were tested mostly on a weekly basis to evaluate possible variation in sludge quality and investigate the reproducibility of results. Therefore, COD, TKN and solid test were conducted and particle size distribution was measured for all sludges. The variation (standard deviation) of the values of mean particle size, FCOD/TCOD, FTKN/TKN, VS/TS and VDS/TS from different observation days were in the range of ±23 μm, ±1.5%, ±1%, 2% and 1% respectively. These results reveal that the reactors were producing relatively consistent sludges for further sonication experiments. In addition, sonication was replicated mostly on a weekly basis during the period of study to evaluate the reproducibility of sonication results. The variation in the results of mean

particle size, FCOD/TCOD, FTKN/TKN, TKN/TCOD, FTKN/FCOD, VS/TS and VDS/TS during the period of this study support the results in terms of reproducibility. The variability of some of these indicators was reflected in subsequent results attributed to solublization of sludges (Figure 4.1 to Figure 4.5).

Respirometry was conducted according to the protocol mentioned in Chapter 3 to separately estimate the concentrations of active heterotrophic organisms ($X_{HO}$) and readily biodegradable COD (rbCOD) in the raw and pretreated WAS samples. Each set of respirometry tests was replicated one or two times to investigate the reproducibility of the results. In addition, since 8 bottles could be run in each set of respirometry tests, some bottles were run in duplicate in each respirometry test. The results of respirometry for control bottles consisting of water or medium (without sludge) revealed zero oxygen uptake and hence it was concluded that the oxygen uptake observed in the sample bottles was due to metabolism of microorganisms.

Batch anaerobic digestion tests were conducted to assess the Biochemical Methane Potential (BMP) as well as the Biochemical Acid Potential (BAP) of the raw and pre-treated samples. The BMP and BAP tests were initiated 12 to 18 hour after pretreatment of the samples according to the protocols described in Chapter 3. Duplicate bottles were employed for each BMP or BAP test. The duplicated serum bottles were devoted for liquid sampling and gas measurements separately and sacrificed at the end of test for analysis of digested samples and investigation of the reproducibility of experiments. Liquid samples were regularly taken for measurement of pH, total and volatile suspended solids, TCOD, FCOD, FFCOD, TKN, FTKN and $NH_4$-N according to Standard Methods (APHA, 1995). The generated gas was regularly discharged from the bottles to a manometric device for measurement of gas volume. The $CH_4$

content of the gas was measured by gas chromatography and expressed as COD mass for comparison with TCOD values. Additional information on the methodology employed in the anaerobic batch tests was presented in Chapter 3.

## 4.3. Results and discussion

The testing conducted in this study characterized physical, chemical and biochemical properties of raw and pretreated WAS samples that were originally generated over a range of SRTs. The physical and chemical properties will be initially presented such that the trends in these data can be compared against the subsequently described biochemical data (respirometry, BMP and BAP tests). The overall objective of this analysis was to assess the utility of employing simple measurements to describe the impact of the ultrasound pretreatment process on biodegradability and to determine whether these relationships were consistent over a range of sludge SRTs.

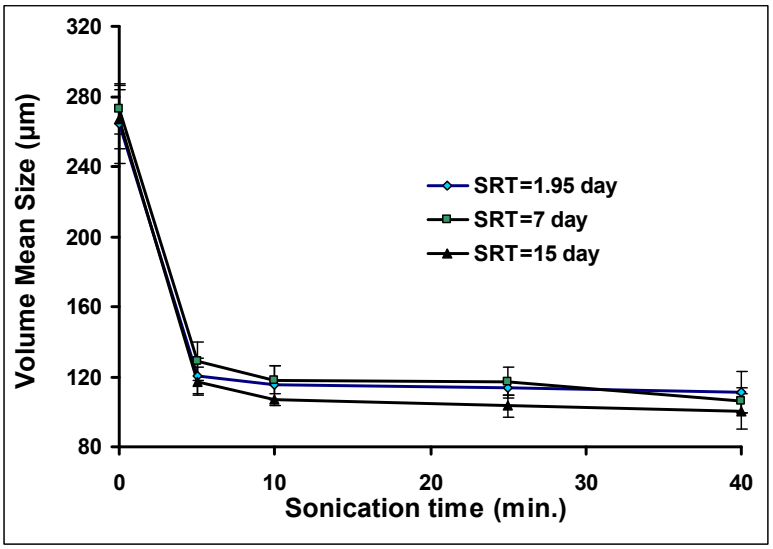


**Figure 4.1. Impact of Sonication on Particle Size**

The particle size analyzer employed in this study provided a variety of responses to describe the size distribution of particles in the raw and pretreated WAS samples. The mean volumetric particle diameter (Figure 4.1) was used as an indicator of the physical properties of the raw and pre-treated sludges since the particles were expected to have a spherical shape (Brittan, 2001). The mean particle diameter for all raw WAS samples was approximately 270 μm (from 264 to 273 μm). All of the pretreated particles had similar mean diameters (approx 110-120 μm) and there was a substantial reduction in particle size for virtually all levels of` treatment intensity and for all 3 sludge ages. The level of particle size reduction suggested that there was partial deflocculation through pretreatment and that the pre-treated sludge still contained a substantial amount of microbial aggregates.

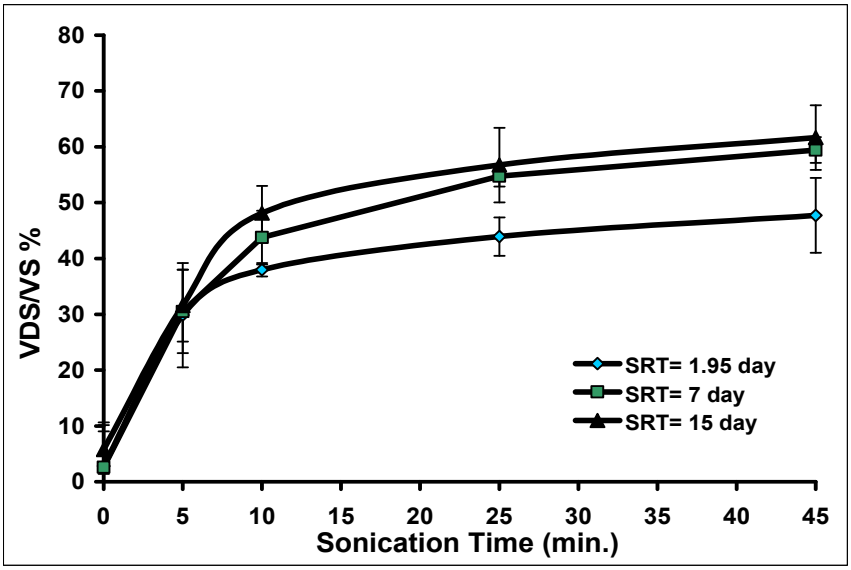


**Figure 4.2. Impact of Sonication on Soluble Fraction of VS**

Figure 4.2 presents the soluble (1.5 μm glass fibre filter) fraction of the volatile solids (VDS/VS) for sludges of different SRTs as a function of sonication time. The results represent the data collected from three separate samples that were collected from the SBRs over a one

week period. From Figure 4.2 it can be seen that the sludges generated at the 1.95 d SRT appeared to approach a maximum soluble fraction of approximately 48% while the WAS samples from the longer SRT SBRs achieved a soluble fraction of approximately 60%. These results indicate that ultrasound substantially impacted the distribution of solids between the solid and soluble phases and hence it would be expected to impact upon the digestibility of WAS streams. In addition the results indicate that the SRT at which WAS is generated can significantly impact upon the solublization of VS during pretreatment. Hence it might be expected that sonication would have a greater impact on improving the digestibility of longer SRT sludges.

The particle size data indicated a consistent reduction in particle size of about 58% for all sludges and pretreatments while the suspended solids data indicated increase solublization of solids with the level of pretreatment and differing results with sludge SRT. The particle size measurements that were recorded described only the sizes of the particles that remained after pretreatment and do not provide information on the extent of solublization. Hence particle size distribution could not be employed as an indication of the extent of particle size reduction that occurs with pretreatment.

Figure 4.3 presents the soluble fractions of the COD (FCOD and FFCOD) for the raw and pre-treated WAS samples. The FFCOD values were considered a measure of the truly soluble COD while the filtered values likely contained a significant fraction of colloidal matter. Differences between these values provide a measure of the colloidal matter and for a given sample might be indicative of the rate at which biodegradation would occur (i.e. truly soluble COD may biodegrade more rapidly than colloidal COD). From Figure 4.3 it can be observed

that the trends in the fractions of the COD that were solublized were similar to those that were observed for VS. The maximum values of the FCOD/COD ratios were 49%, 59% and 64% for the 1.95 d, 7 d and 15 d SRT WAS samples respectively. Hence it can be seen that these fractions corresponded almost exactly with the VDS/VS values.

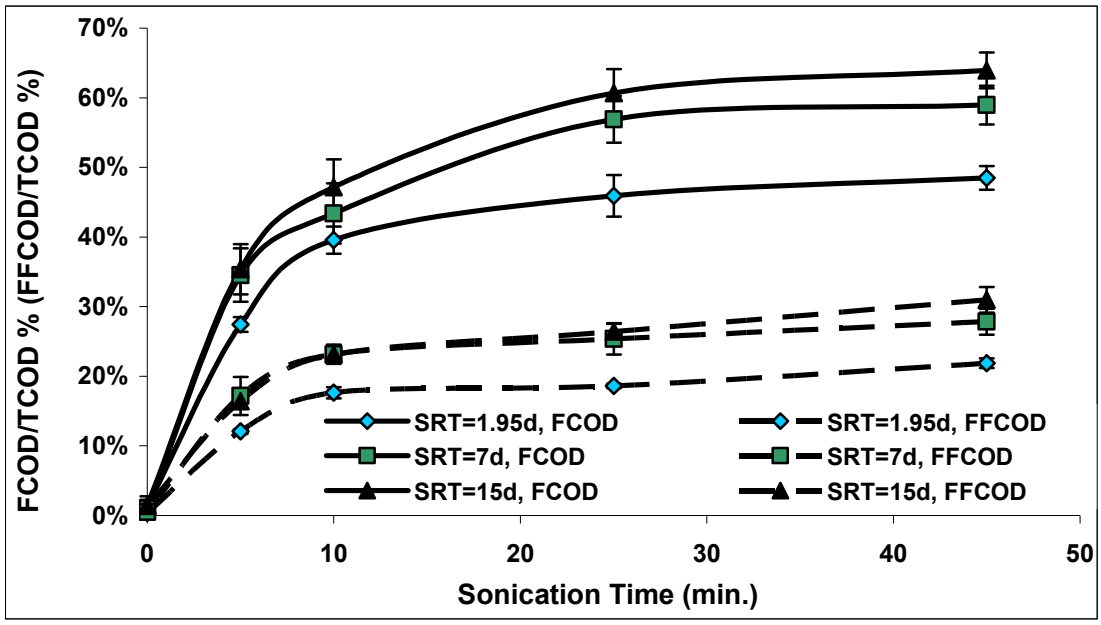


**Figure 4.3. Soluble COD Fractions vs Sonication Time**

The FFCOD/TCOD values were considerably smaller than the FCOD/TCOD values suggesting that a substantial fraction of the filtered COD was associated with colloidal matter. There was only a small increase in flocculated-filtered COD values for sonication periods greater than 25 minutes. The flocculated-filtered COD fraction approached 22% for the 1.95 day SRT and the longer SRT WAS values were approximately 30%. Hence, it can be seen that the SRT of the WAS also affected the fraction of WAS that was converted to truly soluble matter.

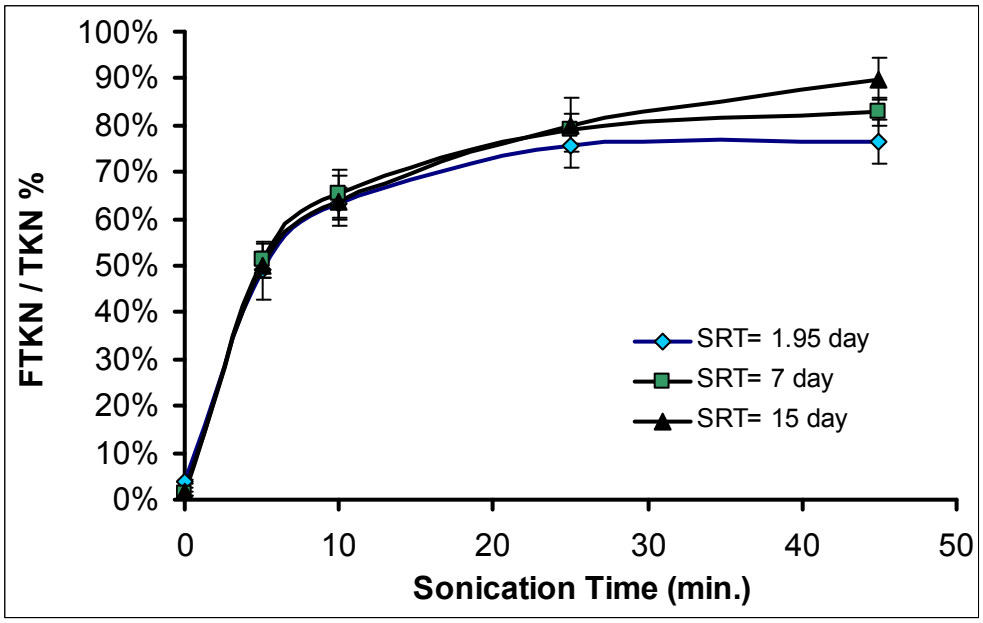


**Figure 4.4. Soluble TKN Fractions vs Sonication Time**

Figure 4.4 presents the filtered fraction of the TKN (FTKN) for sludges of different SRTs and as a function of sonication time. The FTKN responses to sonication periods shorter than 25 minutes were similar for all sludges. Sonication longer than 25 minutes resulted in some differentiation in TKN solubilization for the different WAS SRTs. At the extended sonication time the filtered TKN fraction increased with the SRT of the WAS. The filtered TKN fractions were significantly greater than the filtered VS and COD fractions for all sludges and sonication periods. Filtered COD fractions ranged from 49-64% for the longest sonication times while the corresponding filtered TKN fractions were in the range of 77-90%. These results indicate that sonication preferentially solublized proteinaceous materials such as extracellular polymers (EPS) that contain a high fraction of protein and organic nitrogen (Dignac et. al., 1998). It should be noted that the concentration of $NH_4^+$ was not increased significantly due to sonication (< 2% of TKN). As indicated by the FCOD and FFCOD values, a substantial fraction of the filtered TKN

was likely in colloids and hence the impact of the pretreatment on the rate of digestion of this material could not be directly established.

Figure 4.5 presents the TKN to COD ratio for whole and filtered (1.5 μm) sludges of different SRTs as a function of sonication time. From Figure 4.5 it can be seen that the values of the TKN/COD ratio of whole sludge varied with the SRT of the sludges from 6.2% to 8.1%. These values were consistent with the typical values reported for the nitrogen content of biomass. (i.e.7%) (Henze et al., 1999).

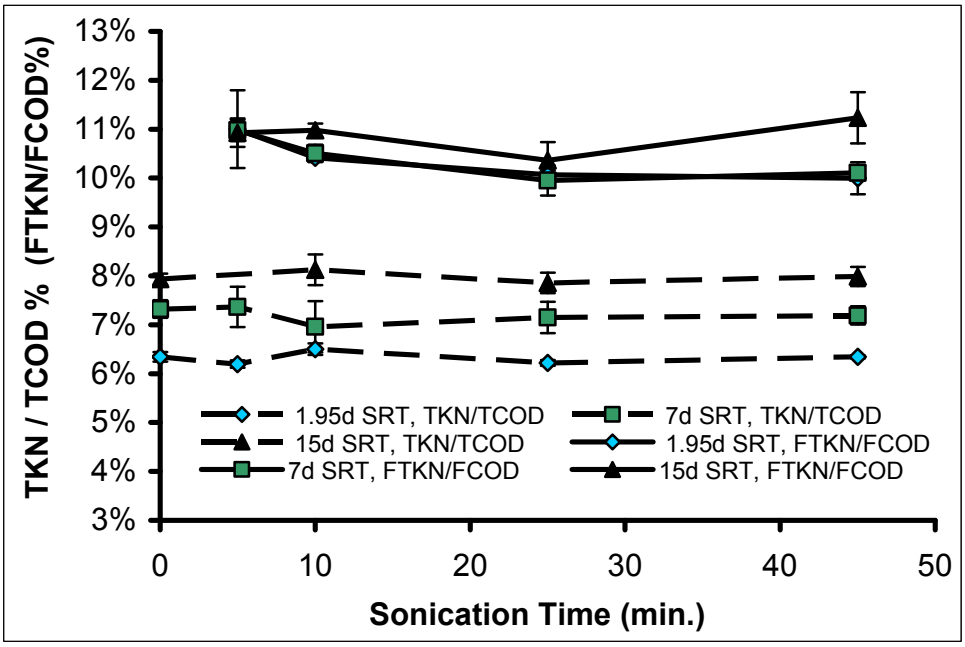


**Figure 4.5 Impact of Sonication on TKN/COD ratio**

From Figure 4.5 it can also be observed that the ratio of filtered TKN to filtered COD (FTKN/FCOD) was consistently greater than the typical value of 7% and the TKN/COD ratio of the whole sludges. These results support the hypothesis that sonication preferentially solublizes proteinaceous materials. The impact of SRT on the FTKN/FCOD ratio was less than that of the whole sludge. In addition the FTKN/FCOD value of the 15 day SRT WAS was generally greater

than that of the shorter SRT WAS samples. This might be due to solubilization of EPS as proteinaceous material (Dignac et. al., 1998) which would likely be more abundant in WAS samples that were generated at the longer SRT.

Respirometry was employed to evaluate the inactivation of microorganisms during pretreatment as, in a previous study (Jones et al., 2007), viable organisms were found to represent the major source of $CH_4$ production during anaerobic digestion of non-pretreated WAS. In this test the raw and pre-treated WAS samples were employed as inocula in a high F/M test using acetate as a substrate. In this approach, exponential growth occurs and hence an exponential increase in oxygen uptake rate was observed. Equation 4.1 (Andreottola et al., 2002) describes the oxygen uptake rate vs time for these conditions and was employed to estimate the initial heterotrophic biomass concentration ($X_o$).

$$OUR(t)=\left(\left(\frac{1-Y_H}{Y_H}\right)\mu_{max}-b\right) . X_0 . e^{(\mu_{max} - b).t} \qquad 4.1.$$

Where: OUR(t) = oxygen uptake rate at time t, mg $O_2$ $L^{-1}$ $d^{-1}$

$Y_H$ = Yield coefficient

$\mu_{max}$ = maximum specific rate of growth, $d^{-1}$

b = endogenous decay coefficient, $d^{-1}$

$X_o$ = initial concentration of heterotrophic biomass, mg COD/L,

t = time, d

According to the literature (reviewed by Avcioglu *et al*., 1998), the estimated values of b for heterotrophic bacteria has been observed to range between 0.1 to 0.6 $d^{-1}$. In this study the value of the endogenous decay rate (b) was assumed equal to 0.24 $d^{-1}$. The value of $Y_H$ was estimated according to Equation 4.2 which assumes that all of the non-oxidized COD was

employed for biomass growth. The acetate in the synthetic medium was considered as the only source of biodegradable COD in the relatively short period of the test (less than one day). Figure 4.6a confirms this assumption as it can be observed that the OUR decreased substantially after the readily biodegradable COD was depleted.

$$Y_H = 1 - \frac{V_{O2}^{OUR\max}}{COD_{ac}} \qquad 4.2.$$

Where: $Y_H$ = Yield coefficient

$V_{O2}^{OUR\max}$ = Maximum cumulative oxygen uptake (mg $O_2$)

$COD_{ac}$ = Acetate added as rbCOD in medium (mg COD)

The estimated values of $Y_H$ were 0.788± 0.002, 0.795± 0.001 and 0.775± 0.004 for the 1.95, 7 and 15 d SRT sludges. These values were similar to the $Y_H$ value of 0.78 that was reported by Insel et al. (2002) for heterotrophic biomass fed by acetate. The results revealed that the yield coefficients for all raw and pretreated samples and for all sludges with differing SRTs were similar.

Figure 4.6a presents typical respirograms that were obtained in this portion of the study. Andreottola et al. (2002) observed a 3 to 4 hour lag when using respirometry tests to estimate $\mu_{max}$ and $X_o$. From Figure 4.6a it can be observed that there was a significant time lag (2 to 6 hr) between the start of the test and when the exponential OUR was observed. The longer lag prior to exponential oxygen uptake (as compared to previous studies) might be attributed to the time required for the biomass to acclimate to acetate utilization. Hence, when fitting Equation 4.1 only the exponential portion of the OUR curves was utilized as indicated in Figure 4.6b.

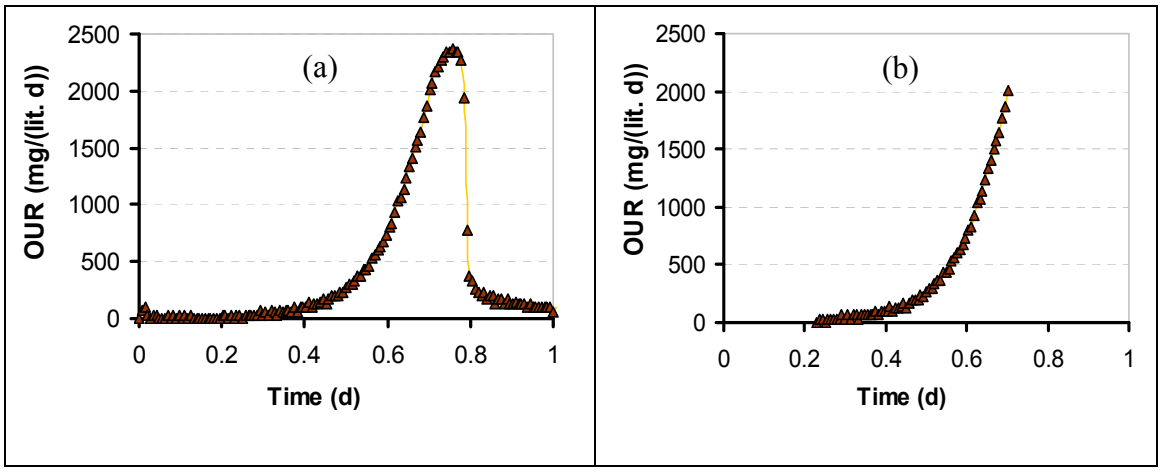


**Figure 4.6. Typical Respirometry Data Employed to Estimate $X_o$ (a) Total Respirogram; (b) Portion of Respirogram Employed for Parameter Estimation**

The values of $X_o$ and $\mu_{max}$ were estimated by fitting Equation 4.1 to the exponential portion of the OUR response. The estimated values of $\mu_{max}$ for raw sludges were in the range of 7.44±0.37 and were within the range of values (6.6 to 7.9) reported by Andreottola et al. (2002) for waste water samples in respirometry tests. In addition, these values were close to the $\mu_{max}$ value of 7.0 that was reported by Insel et al. (2002) for heterotrophic biomass fed by acetate. The estimated values for the pretreated samples were in the range of 11.68±0.31 which were greater than the values for raw sludges. The increase in $\mu_{max}$ values may have been due to a change in the composition of the remaining active heterotrophic biomass when sonication was applied.

Figure 4.7 summarizes the values of the active fraction of the particulate COD ($X_o/COD_T$) that were estimated for the raw and pretreated WAS samples for all 3 SBR sludges. From Figure 4.7 it can be observed that the active fraction of the raw sludges was considerably lower than that which might be expected for activated sludges generated over the range of SRTs in this study. Modeling of the SBRs that was conducted in a separate study (Parker et al., 2008) indicated a range of active fractions from 55-75%. The low active fractions that were measured

in this study were attributed to the use of acetate as a substrate. The values estimated likely apply to only that fraction of the biomass that can utilize acetate within the period of time employed in the respirometry testing. Hence, these values should be considered as the acetate-active fractions.

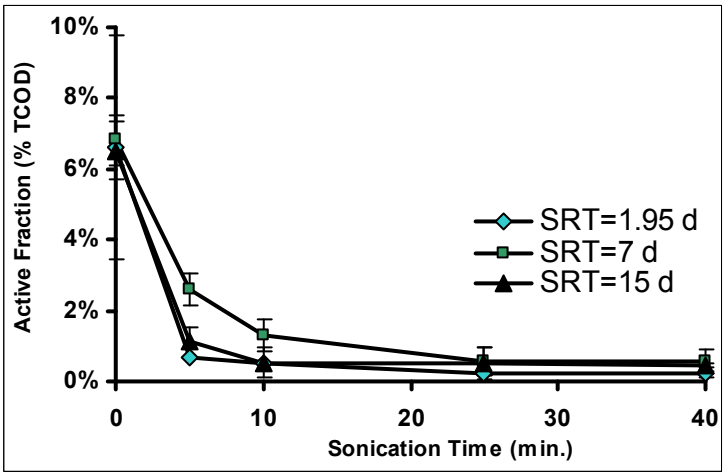


**Figure 4.7. Acetate-Active Fraction versus Sonication time**

Despite the limitations in the estimation of the active fraction it is believed that the values estimated by the technique employed in this study provide insight into the fate of the active bacteria through sonication. It might be reasonably assumed that other heterotrophic organisms would be inactivated to similar extents as those which were capable of utilizing acetate. From Figure 4.7 it can be seen that the pattern in reduction in the active fraction was similar for all of the WAS samples. After 40 minutes of sonication the active fraction was reduced by greater than 92% of the original values for all of WAS samples. The active heterotrophic biomass represents a significant fraction of the biodegradable COD in waste activated sludges. The inactivation of this biomass by sonication would likely enhance the digestability of the sludge as it would disrupt cell walls and hence increase the rate at which this material could be hydrolyzed.

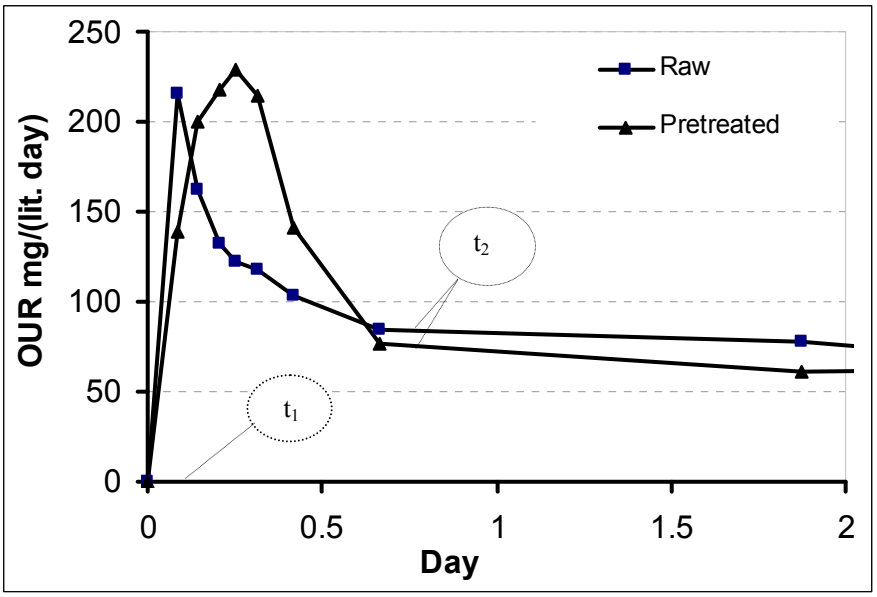


**Figure 4.8. Estimation of rbCOD from Respirometry Data**

Respirometry was also employed to quantify the generation of readily biodegradable COD (rbCOD) by pretreatment. In these tests, a low food/microorganism (F/M) approach was employed by measuring the oxygen uptake from a WAS (raw or pre-treated) sample that was inoculated (20% v/v) with raw WAS. Figure 4.8 presents typical respirograms (1.95 day SRT WAS, raw and 25 min. sonication) that were obtained in this study. In this approach, an increasing level of oxygen uptake rate was observed in the first portion of the test. This was likely due to the presence of some rbCOD in the WAS and consequently the growth of heterotrophic biomass in the respirometry batches. As can be observed from Figure 4.8, the first portion of the test was longer and the OUR was greater for pretreated sludges as compared to raw sludges since the level of rbCOD was greater in the pretreated sludges. In addition a substantial drop in the OUR was observed for all samples as the result of the depletion of rbCOD material in the batches. The results reveal that rbCOD material was biodegraded in less than one day.

Equation 4.3 was developed to estimate the concentration of rbCOD in the respirometry tests. In Equation 3, the rbCOD is calculated on the basis of the oxygen consumed during the rapid uptake portion of the test less the oxygen uptake that is attributed to endogenous decay. The latter value was estimated from the OUR that was observed immediately after the rbCOD was depleted ($t_2$) and was assumed to be constant over the relatively short period when the rbCOD was consumed. In the tests that included pretreated WAS and raw WAS as an inocula the OUR associated with the inocula was subtracted from that of the mixture on the basis of a control test that was conducted with raw WAS alone.

$$C_b = \frac{1}{1-Y_H} \times \left[\left(\frac{U_2 - U_1}{V_b}\right) - R_2 \times (t_2 - t_1)\right] \qquad 4.3$$

Where: $t_1$= Time at point 1 when oxidation starts (d)

$t_2$ = Time at point 2 when oxidation of rbCOD material ends (d)

$U_1$= Cumulative oxygen uptake at $t_1$ (mg $O_2$).

$U_2$ = Cumulative oxygen uptake at $t_2$(mg $O_2$).

$R_2$ = Oxygen uptake rate at $t_2$ (mg $O_2$ $L^{-1}d^{-1}$).

$Y_H$ = Yield coefficient

$V_b$ = Volume of liquid in batches (L)

$C_b$ = Concentration of rbCOD in bottle (mg COD $L^{-1}$)

Figure 4.9 presents the increments in rbCOD that were observed through pretreatment after normalization by the total COD for the 1.95, 7 and 15 d SRT sludges. From Figure 4.9 it can be seen that the rbCOD fraction increased only modestly with sonication time for all sludges.

The rbCOD fractions of the 1.95 day SRT sludges were consistently approximately twice the values of the 7 and 15 day SRT sludges.

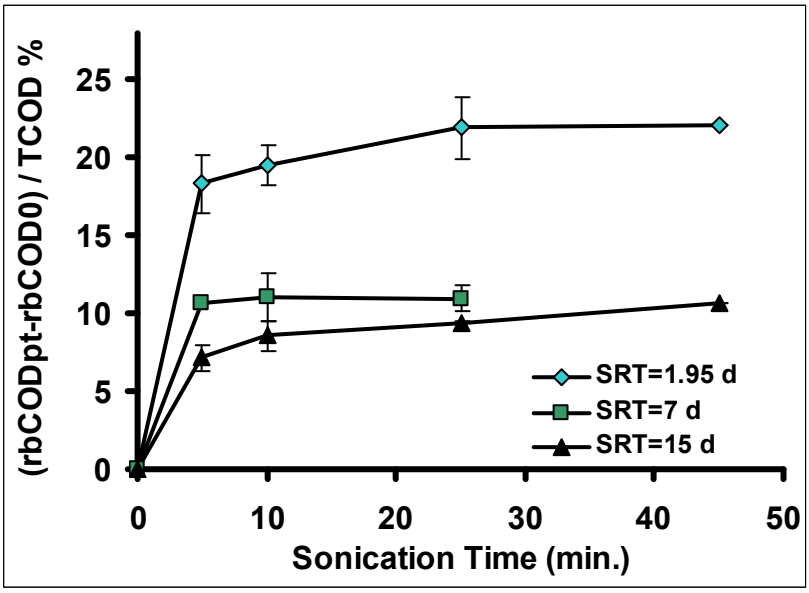


**Figure 4.9. Impact of Sonication on rbCOD**

The rbCOD fractions were inconsistent with the overall solublization of COD as indicated by the increases in the soluble COD fractions with sonication. If the ffCOD fraction were considered to be representative of the rbCOD then it would suggest that the 15 day SRT sludge would have a greater rbCOD whereas the rbCOD fractions were less in these samples. The differing responses for rbCOD and sCOD fractions suggest that differing types of materials were being solublized from the WAS samples that were generated at the different SRTs. The soluble COD that was generated from the short SRT sludges was considerably more biodegradable under aerobic conditions than that of the long SRT sludges.

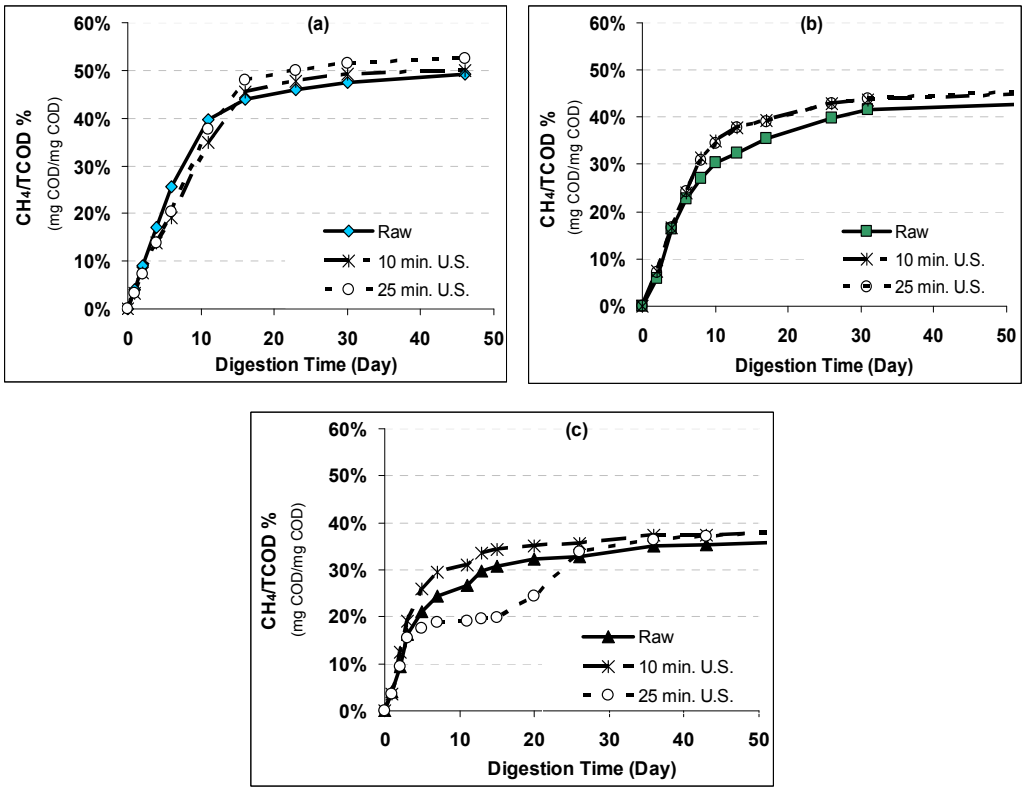


**Figure 4.10. Impact of Sonication on Methane Generation in BMP Tests for sludges with (a) 1.95 day SRT; (b) 7 day SRT; (a) 15 day SRT**

The impact of pretreatment on methane production was evaluated in BMP tests with the results obtained for 1.95, 7 and 15 day SRT sludges presented in Figure 4.10. From Figure 4.10 it can be observed that the 1.95 day SRT sludge resulted in higher $CH_4$ yields than the 7 and 15 day SRT sludges. This was expected because of the higher fraction of biodegradable organics in this sludge that would be expected to be generated at the lower SRT. It can also be noted that for the early portion of the tests, some of the pre-treated samples generated less $CH_4$ than the raw sludges. As will be subsequently demonstrated this could likely be attributed to the accumulation of volatile fatty acids in the serum bottles containing pre-treated sludges. The pH in the serum bottles was consistently greater than 7.4 and the $NH_4$-N concentration was less than 500 mg/L, hence these potential causes of inhibition were ruled out. The accumulation of the

acids likely inhibited $CH_4$ production somewhat in the early portions of the test. Towards the latter portion of the tests it appears that this inhibition was overcome as $CH_4$ production from the pre-treated samples increased and in all cases exceeded the raw sludges.

From Figure 4.10 it can be seen that pretreatment appeared to increase the ultimate methane yield only marginally (1% to 3% for all sludges) despite the significant impact on the sludge properties as indicated by the previously discussed physical, chemical and biological indicators. Hence, pretreatment did not appear to be successful in converting materials from a non-biodegradable to a biodegradable form. Pretreatment did appear to substantially impact upon the rate of methane generation in the first 20 days of the test. This factor is likely to be important for systems where the digestion capacity is limited and hence the residence times are in the low range of conventionally accepted values (i.e. 15-20 days).

The effect of pretreatment on the anaerobic biodegradation process was further investigated by examining the generation of ammonia in the BMP tests (Figure 4.11). From Figure 4.11 it can be observed that for all WAS streams, pretreatment resulted in a slight increase in the ultimate $NH_4$ yield which was consistent with the small increases in $CH_4$ yields with pretreatment. For all WAS streams pretreatment substantially increased the rate of ammonia generation during anaerobic digestion suggesting that the rate of hydrolysis was substantially enhanced. A lag in $NH_4$ generation was not observed for the pre-treated samples as was observed with $CH_4$ generation. This would confirm the conclusion that inhibition of methanogens was responsible for the lag in $CH_4$ production.

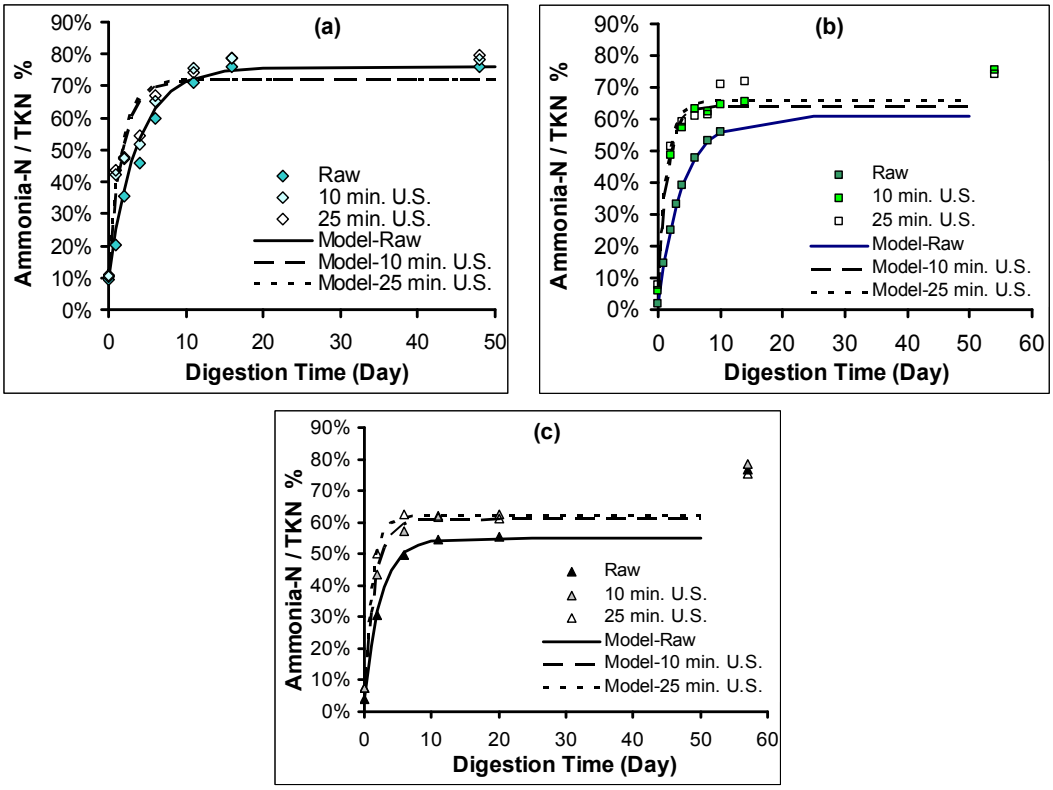


**Figure 4.11. Impact of Sonication on $NH_4$-N Generation in BMP Test for sludges with (a) 1.95 day SRT; (b) 7 day SRT; (a) 15 day SRT**

To assist in further quantifying the impact of pretreatment on the rate of $NH_4$ generation (and hence hydrolysis of proteins) an empirical model as presented in 4.4 was fit to the ammonia data for the first 10 days of the BMP.

$$\text{Ln}\left(\frac{U_{Ult}^{Ammon} - U_{t}^{Ammon}}{U_{Ult}^{Ammon} - U_{0}^{Ammon}}\right) = -k_{Ammon} \times t \qquad 4.4.$$

Where: $U_{Ult}^{Ammon}$ = Maximum (ultimate) ammonia yield ($NH_4$-N / TKN)

$U_{T}^{Ammon}$ = $NH_4$-N / TKN fraction at time t

$U_{0}^{Ammon}$ = $NH_4$-N / TKN fraction at beginning of test

$k_{Ammon}$ = Ammonification rate constant ($d^{-1}$)

t = digestion time (d).

Figure 4.11 reveals that the model described in Equation 4.4 reflected the observed values well. The $r^2$ values for the linearized form of the equation were greater than 0.92, 0.98 and 0.99 for the 1.95, 7 and 15 day SRT sludges respectively. The results of the model fitting are summarized in Figure 4.12. From Figure 4.12 it can be seen that the value of $k_{Ammon}$ were 126%, 172% and 107% of the values observed for the raw sludges with 1.95, 7 and 15 day SRT respectively. The $k_{Ammon}$ value for the 15 day SRT sludge increased incrementally with sonication intensity while the $k_{Ammon}$ values for the pretreated 1.95 and 7 day SRT sludges were similar and considerably higher than that observed for the raw sludge. These results would suggest that the impact of sonication on the rate of hydrolysis of proteins was not the same for sludges that were generated at differing SRTs.

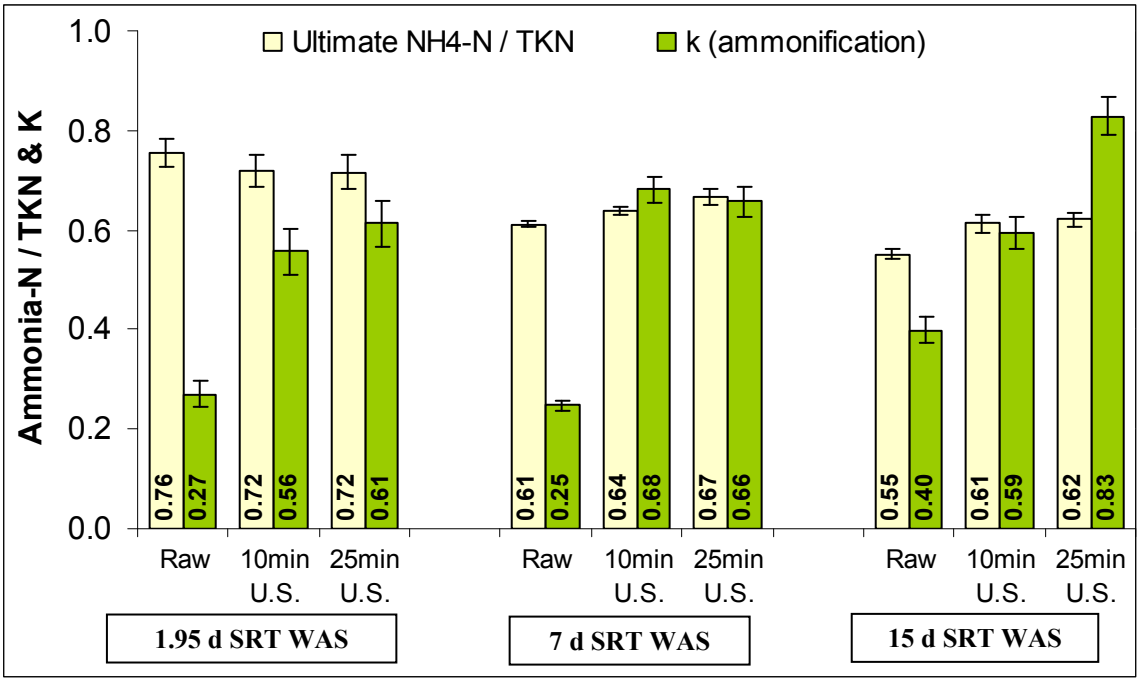


**Figure 4.12. Ultimate Ammonia Generation and Ammonification Rate Constant**

BAP tests were conducted to obtain additional insight into the impact of pretreatment on the hydrolysis and acidification processes. The fractions of the initial COD that were measured as VFAs in the BAP tests are presented versus time in Figure 4.13. From Figure 4.13 it can be observed that, as expected, the yields of VFAs at the end of BAP test were greater for the 1.95 day SRT sludges (50 to 65%) as compared to that for 7 and 15 day SRT sludges (28.8 to32.6% and 28.7 to 44%).

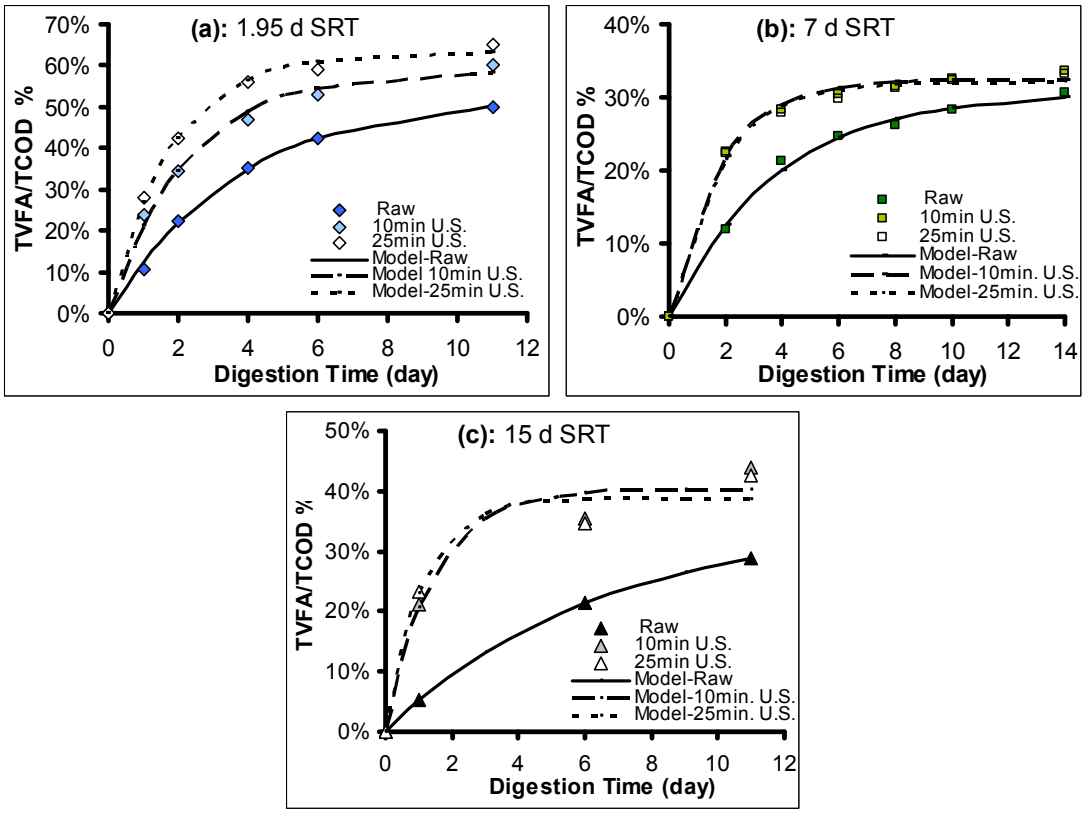


**Figure 4.13. Impact of Sonication on VFA Production in BAP Tests**

An empirical model, similar to the model presented in Equation 4.4, was fit to the VFA data for the first 10 days of the BAP test that is presented in Figure 4.13. This facilitated quantification of the impact of pretreatment on ultimate VFA yield ($U_{Ult}^{VFA}$) and the rate constant for VFA generation ($k_{VFA}$). Figure 4.13 reveals that the model reflected the observed values well

($r^2$ values for linearized model were greater than 0.993, 0.995 and 0.97 for 1.95, 7 and 15 day SRT sludges respectively). The results of the model fitting are summarized in Figure 4.14. Figure 4.14 reveals that the improvement in estimated ultimate yield of VFAs was marginal (maximum 10%) for all sludges. This was consistent with the ultimate ammonia and methane yields shown in Figures 4.10 and 4.12.

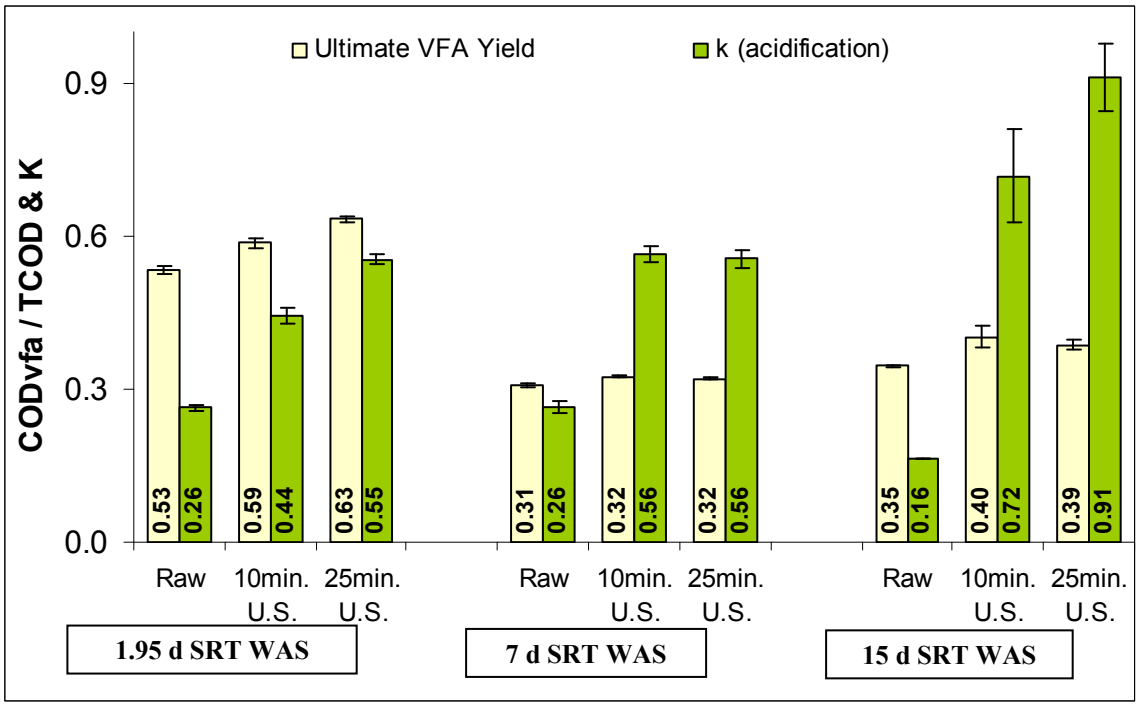


**Figure 4.14. Maximum VFA Concentration and Rate Constant of VFA generation**

From Figure 4.14 it can be seen that the value of $k_{vfa}$ was greater for the 15 day SRT sludge as compared to that for 1.95 and 7 SRT sludges with similar sonication intensities. The rate of acidification was improved by 111%, 115% and 468% for 1.95, 7 and 15 day SRT sludges. This result was consistent with the results of COD solubilization that were presented in Figure 4.3. As expected, these results would suggest that the impact of sonication on the rate of hydrolysis was not the same for sludges that were generated at differing SRTs. The rapid

generation of VFAs in the pre-treated samples likely resulted in some inhibition of the methanogens during the early portion of the BMP tests and hence was responsible for the observed lag in these responses.

The ultimate yield of ammonia from TKN was greater than the yield of VFAs from COD for samples with the same pretreatment intensities. This might be due to higher solubilization level for proteinaceous materials as compared to that for other material (shown in Figure 4.5). The $NH_4$ and VFA responses can be considered as separate indicators of the impact of sonication on the rate of hydrolysis and acidification during anaerobic digestion. While both responses suggested an increase in the rate of generation there was somewhat of an inconsistency in the trend between intensity and rate enhancement. The results indicate that, as previously suggested by the VS, COD and TKN analyses sonication impacted the sludges that were generated at differing SRTs differently. There appears to be a difference in the relative release of proteins and carbohydrates which subsequently affected the relative rates at which $NH_4$ and VFAs were generated.

In summary, despite considerable solublization of WAS with sonication, the ultimate digestibility of WAS was only marginally improved. There did not appear to be any relationship between the extent of solublization and ultimate digestibility. Pretreatment with ultrasound did however result in a substantial increase in the rate of digestion of WAS as indicated by both BMP and BAP tests. The improvements in these rates were more closely related to the information provided by solublization data. In anaerobic systems that are operating at traditional solids residence times the increased rates of digestion might be interpreted as an improvement of

the digestibility of the WAS. The BAP test has proved to be an effective short term technique for determining the improvement in the rates of digestion that might be achieved with sonication.

## 4.4. Conclusion

Pretreatment of waste activated sludges with ultrasound resulted in substantial reduction in particle size for sludges of differing solids residence time and for all sonication intensities examined. However, particle size analysis did not provide insight into the extent of solublization of WAS. The VS, COD and soluble TKN responses indicated that a significant fraction of the WAS solids were solublized by sonication, however, it appeared that the types of materials which were solublized was affected by the SRT at which the WAS was generated and the level of sonication. The solublization of COD was greater for WAS streams that were generated at longer SRTs while a greater fraction of rbCOD was generated from the 1.95 day SRT WAS as compared to the 7 and 15 day SRT WAS streams. The generation of $CH_4$ and $NH_4$ in BMP tests and VFAs in BAP tests revealed that sonication only marginally increased the yield of these materials but substantially increased the rate of hydrolysis which is often the rate limiting process in WAS digestion. The relative trends in the rates of $NH_4$ generation in the BMP tests and VFA generation in BAP tests versus sonication intensity differed for the sludges generated at differing SRTs. Sonication appeared to differentially affect the availability of carbohydrates and proteins depending upon the WAS SRT.

# Chapter 5

# 5. Characterization of Ozonation impacts on properties of waste activated sludge and biodegradability

## 5.1. Introduction

In this part of the study ozonation was employed as a model of chemical pretreatment technology and a range of physical, chemical and biological responses were evaluated to assess the impact of ozonation on WAS properties as well as sludge digestibility. WAS that was generated at differing SRTs on municipal wastewater was employed to facilitate an assessment of the interaction between pretreatment and WAS properties on digestibility. The overall objective was to develop protocols that can be employed to characterize the impact of pretreatment processes on WAS digestion.

## 5.2. Approach

Three sequencing batch reactors (SBRs), each with a volume of 175 L, were operated on screened municipal wastewater at an hydraulic residence time (HRT) of 9.3 hours and SRT's of 1.95, 7 and 15 days respectively. The SBRs were employed as a source of WAS for all of the testing conducted in this study. Samples of the WAS were ozonated for differing time periods to achieve varying levels of pretreatment intensity. Ozonation was conducted using a Hankin Atlas ozone generator (OZOTEC, type S, model 2) at a production level of 10 Volt. The ozone generator was adjusted for a gas flow of 7.1 L/min., ozone concentration of 14700 PPM (by weight) and ozone mass flow of 0.125 g $O_3$/min. WAS samples from the SBRs were mixed in 4

liter glass containers using magnetic stir bar to provide a consistent source of sludge to the pretreatment apparatus. A bench scale reactor with a 3 L volume was employed for contacting 1.1 L of WAS samples with ozone. The unit was operated in batch mode and ozonation intensity was evaluated by treating samples for different times (10, 15, 20 and 30 min).

Raw and treated samples were characterized with respect to particle size distribution, solids, COD and nitrogen species. Particle size distribution was measured by a laser diffraction based technique (Mastersizer 2000). The volume mean size which was calculated from the size distribution of particles (Brittan, 2001) was employed as an indicator of the physical properties of the samples. A part of the samples was filtered immediately after the pretreatment process with glass fiber filters with a nominal pore of 1.5 μm to provide filtered samples. A part of the filtered samples was flocculated by adding alum, centrifuged for five minutes (in 4500 rpm) and then filtered with a membrane syringe filter (0.45 μm pore size) to provide flocculated-filtered samples. These samples were created to provide information on the truly soluble materials. Samples that were only filtered with the 1.5 μm filters were expected to contain a substantial amount of colloidal matter. These samples were used when fractionating COD and TKN to filtered COD (FCOD), flocculated-filtered COD (FFCOD) and filtered TKN (FTKN).

During the period of ozonation experiments, the sludges generated from all of the reactors were tested mostly on a weekly basis to evaluate possible variation in sludge quality and investigate the reproducibility of results. Therefore, COD, TKN and solid test were conducted and particle size distribution was measured for all sludges. The variation (standard deviation) of the values of mean particle size, FCOD/TCOD, FTKN/TKN from different observation days were in the range of ±19 μm, ±3.5% and ±0.5% respectively. These results reveal that the

reactors were producing relatively consistent sludges for further ozonation experiments. In addition, ozonation was replicated mostly on a weekly basis during the period of study to evaluate the reproducibility of sonication results. The variation of mean particle size, FCOD/TCOD, FTKN/TKN, TKN/TCOD and FTKN/FCOD during the period of this study support the results in terms of reproducibility. The variability of some of these indicators was reflected in subsequent results attributed to solublization of sludges (Figure 5.1 to Figure 5.4).

Respirometry was conducted according to the protocol mentioned in Chapter 3 to separately estimate the concentrations of active heterotrophic organisms ($X_{HO}$) and readily biodegradable COD (rbCOD) in the raw and pretreated WAS samples. Each set of respirometry tests was replicated one or two times to investigate the reproducibility of the results. In addition, since 8 bottles could be run in each set of respirometry tests, some bottles were run in duplicate in each respirometry test. The results of respirometry for control bottles consisting of water or medium (without sludge) revealed zero oxygen uptake and hence it was concluded that the oxygen uptake observed in the sample bottles was due to metabolism of microorganisms.

Batch anaerobic digestion tests were conducted to assess the Biochemical Methane Potential (BMP) as well as the Biochemical Acid Potential (BAP) of the raw and pre-treated samples. The BMP and BAP tests were initiated 12 to 18 hour after the pretreatment of the samples according to the protocols described in Chapter 3. Duplicate bottles were employed for each BMP or BAP test. The duplicated serum bottles were devoted for liquid sampling and gas measurements separately and sacrificed at the end of test for analysis of digested samples and investigation of the reproducibility of experiments. Liquid samples were regularly taken for measurement of pH, total and volatile suspended solids, TCOD, FCOD, FFCOD, TKN, FTKN

and $NH_4$-N according to Standard Methods (APHA, 1995). The generated gas was regularly discharged from the bottles to a manometric device for measurement of gas volume. The $CH_4$ content of the gas was measured by gas chromatography and expressed as COD mass for comparison with TCOD values. Additional information for the methodology of the anaerobic batch tests was mentioned in Chapter 3.

## 5.3. Results and discussion

The testing conducted in this study characterized physical, chemical and biochemical properties of raw and pretreated WAS samples that were originally generated over a range of SRTs. The physical and chemical properties will be initially presented such that the trends in these data can be compared against the subsequently described biochemical data (respirometry, BMP and BAP tests). The overall objective of this analysis was to assess the utility of employing simple measurements to describe the impact of the ozonation pretreatment process on biodegradability and to determine whether these relationships were consistent over a range of sludge SRTs.

The particle size analyzer employed in this study provided a variety of responses to describe the size distribution of particles in the raw and pretreated WAS samples. The mean volumetric particle diameter (Figure 5.1) was used as an indicator of the physical properties of the raw and pre-treated sludges since the particles were expected to have a spherical shape (Brittan, 2001). The mean particle diameter for all raw WAS samples was approximately 250 μm (from 248 to 255 μm). All of the pretreated particles had similar mean diameters (approx 214-242 μm) and there was only a slight reduction in particle size for virtually all levels of` ozonation intensity and for all 3 sludge ages. The level of particle size reduction suggested that there was

limited level of deflocculation through pretreatment and that the pre-treated sludge still contained a substantial amount of microbial aggregates. As will be subsequently demonstrated, there was considerable solublization of solids and COD that were not reflected in the particle size distribution. Measurements of particle size distribution were not found to provide useful information on the ozonation of sludge since they only describe the distribution of particles that remain after treatment and do not quantify components that have been solublized.

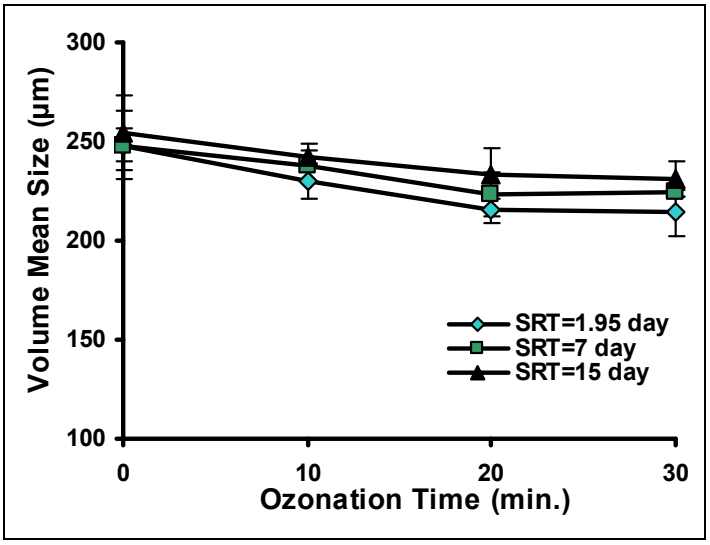


**Figure 5.1. Results of Particle Size Analysis**

The concentrations of TCOD, FCOD and FFCOD were measured for all raw and pretreated samples (Figure 5.2). The FFCOD values were considered as a measure of the truly soluble COD while the FCOD values likely contained a significant fraction of colloidal matter. Differences in these values for a given sample might be considered as indicative of the rate at which biodegradation would occur (i.e. truly soluble COD will biodegrade more rapidly than colloidal COD). Despite the minimal differences in particle size, soluble COD fractions were observed to increase with pretreatment intensity (ozonation time). There was a substantial difference in COD solublization between the sludges that were generated at different SRTs. The

soluble fractions (FCOD & FFCOD) of the pre-treated 1.95 day SRT sludge were observed to increase linearly with ozone dose (43% and 26% COD solublization after 30 minutes of ozonation) while these fractions of the 7 and 15 day SRT sludges were found to increase by only 5-10% after 15 minutes of ozone dosing and were in the range of 15-24% for the 30 minute ozone doses.

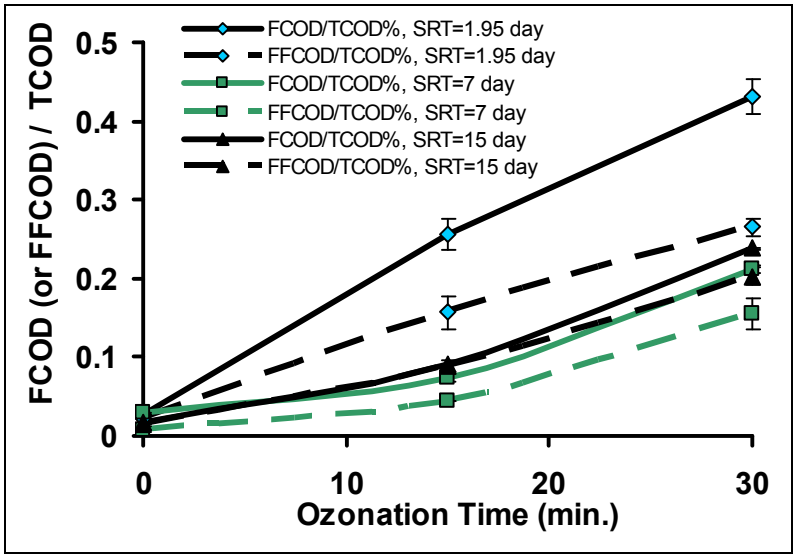


**Figure 5.2. Soluble COD Fractions vs Ozonation Time**

The difference between the FCOD and FFCOD measurements of each sludge sample provide an indication of the amount of colloidal solids that are present. From Figure 5.2 it can be seen that the difference in these measurements increased with the level of ozonation and decreased as the SRT increased. Hence it would appear that ozonation resulted in degradation of floc to form colloidal solids and that this occurred to a greater extent with the short SRT sludge. It is known that as SRT increases the composition of the WAS with respect to active cells, decay products and inert materials will change (Metcalf and Eddy, 2003). It would appear that these different fractions have an influence on the release of colloidal solids by ozonation.

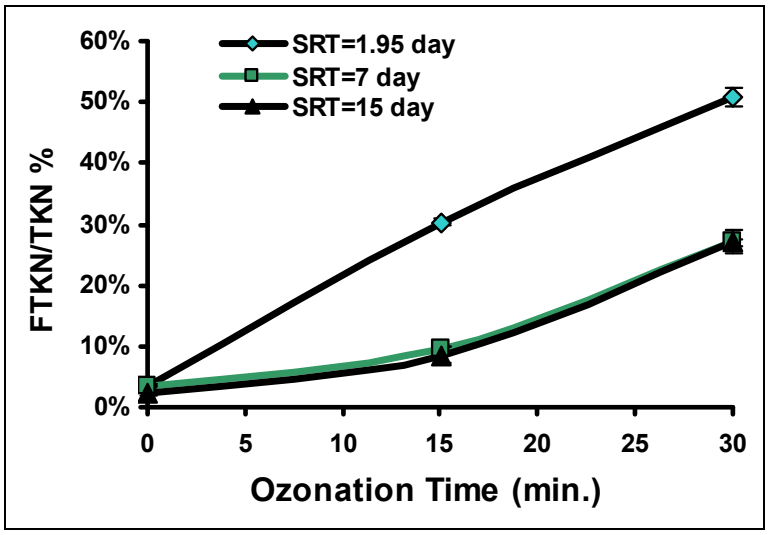


**Figure 5.3. Soluble TKN Fractions vs Ozonation Time**

The solublization of TKN by ozonation followed a similar trend to that of FCOD (Figure 5.3) in that the solublization of the 1.95 day SRT sludge was substantially greater than that of the longer SRT sludges. The COD and TKN results suggest that there are components present in short SRT sludges that are more susceptible to solubilization by ozone than those present in longer SRT sludges. Biodegradable particulate COD present in raw wastewater that is enmeshed in the floc may represent this fraction since some amount of this material would be expected to be present in the 1.95 day SRT sludge while it would be expected to have been hydrolyzed in the SBRs with longer SRTs. As indicated by the FCOD and FFCOD values, a substantial fraction of the filtered TKN was likely in colloids which would require a longer time for digestion as compared to truly soluble material. Hence, the impact of the pretreatment on the rate of digestion of this material could not be directly established without measuring colloidal and truly soluble fractions of FTKN. The concentration of $NH_4^+$ was not increased significantly due to sonication (< 2.7% of TKN) indicating that the ozonation did not degrade protein to the extent that $NH_4^+$ was released.

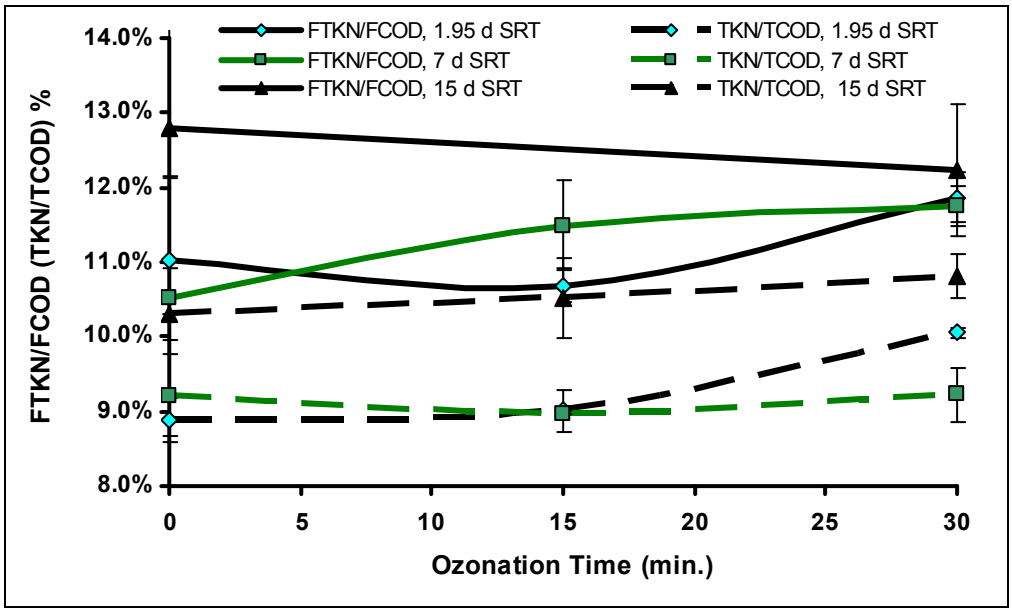


**Figure 5.4. TKN/COD Ratio vs Ozonation Time**

Figure 5.4 presents the TKN to COD ratio for whole and filtered sludges of different SRTs as a function of ozonation time. From Figure 5.4 it can be seen that the values of the TKN/COD ratio of the whole sludge (TKN/TCOD) varied from 8.9% to 10.8% and increased with the SRT of the sludges. This was likely due to the accumulation of extracellular substances in the sludge that contained a high TKN/COD ratio (Dignac et. al., 1998). From Figure 5.4 it can also be observed that the ratio of filtered TKN to filtered COD (FTKN/FCOD) depended on the SRT of sludge and was consistently greater than the TKN/COD ratio of whole ozonated sludges. It appeared that the types of materials which were solubilized was affected by the SRT at which the WAS was generated. These results also support the hypothesis that ozonation preferentially solubilizes proteinaceous materials.

Respirometry was employed to evaluate the inactivation of microorganisms during the pretreatment as in a previous study (Jones et al., 2007) viable organisms were found to represent the major source of $CH_4$ production during anaerobic digestion of non-pretreated WAS. In this

test the raw and pre-treated WAS samples were employed as inocula in a high F/M test using acetate as a substrate. In this approach, exponential growth occurs and hence an exponential increase in oxygen uptake rate is observed. Equation 5.1 which was described by Andreottola et al. (2002) was employed to estimate the initial heterotrophic biomass concentration ($X_o$).

$$OUR(t)=\left(\left(\frac{1-Y_H}{Y_H}\right)\mu_{max}-b\right) . X_0 . e^{(\mu_{max} - b).t} \qquad 5.1.$$

Where: OUR(t) = oxygen uptake rate at time t, mg $O_2$ $L^{-1}$ $d^{-1}$

$Y_H$ = Yield coefficient

$\mu_{max}$ = maximum specific rate of growth, $d^{-1}$

b = endogenous decay coefficient, $d^{-1}$

$X_o$ = initial concentration of heterotrophic biomass, mg COD/L,

t = time, d

According to the literature (reviewed by Avcioglu *et al*., 1998), the estimated values of b for heterotrophic bacteria has been observed to range between 0.1 to 0.6 $d^{-1}$. In this study the value of the endogenous decay rate (b) was assumed equal to 0.24 $d^{-1}$. The value of $Y_H$ was assumed equal to 0.78 as reported by Insel et al. (2002) for heterotrophic biomass fed by acetate.

Figure 5.5a presents typical respirograms that were obtained in this portion of the study. Andreottola et al. (2002) observed a 3 to 4 hour lag when using respirometry tests to estimate $\mu_{max}$ and $X_o$. From Figure 5.5a it can be observed that there was a significant time lag (3 to 6 hr) between the start of the test and when the exponential OUR was observed. The longer lag prior to exponential oxygen uptake (as compared to previous studies) might be attributed to the time

required for the biomass to acclimate to acetate utilization. Hence, when fitting Equation 5.1 only the exponential portion of the OUR curves was utilized as indicated in Figure 5.5b.

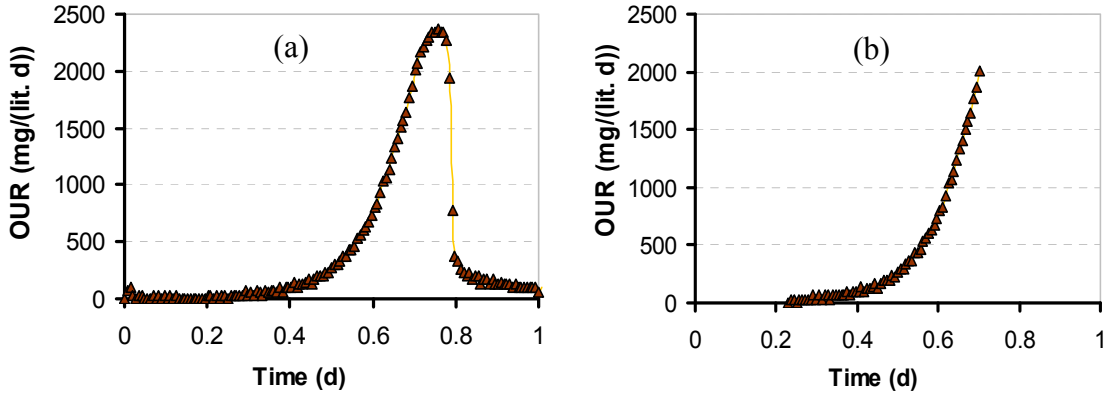


**Figure 5.5. Typical Respirometry Data Employed to Estimate $X_o$ (a) total respirogram; (b) Portion of Respirogram Employed for Parameter Estimation**

The values of $X_o$ and $\mu_{max}$ were estimated by fitting Equation 5.1 to the exponential portion of the OUR response. The estimated values of $\mu_{max}$ for raw sludges were in the range of 6.6±0.26 and were equal to the lower range of values (6.6 to 7.9) reported by Andreottola et al. (2002) for waste water samples in respirometry tests. In addition, these values were close to the $\mu_{max}$ value of 7.0 that was reported by Insel et al. (2002) for heterotrophic biomass fed by acetate. The estimated values of $\mu_{max}$ for the pretreated samples were in the range of 9.08±0.47 which were greater than the values for raw sludges. The increase in $\mu_{max}$ values may have been due to a change in the composition of the remaining active heterotrophic biomass when sonication was applied.

Figure 5.6 summarizes the values of the active fractions of the particulate COD ($X_o/TCOD_{raw}$) that were estimated for the raw and pretreated WAS samples for all 3 SBR sludges. From Figure 5.6 it can be observed that the active fraction of the raw sludges was 5.2,

6.9 and 4.2 % for the 1.95, 7 and 15 day SRT sludges. These values were considerably lower than that which might be expected for activated sludges generated over the range of SRTs in this study. Modeling of the SBRs that was conducted in a separate study (Parker et al., 2008) indicated the active fraction of the raw sludges was 65.4 and 52.8% for 1.95 and 15 day SRT sludges. The estimated values in this study were a consistent fraction of the values reported by Parker et al. (2008) and were in the range of 7.9 to 8.0% of the previous values. The consistency of the value of this fraction suggests that changes in the viable biomass that were measured with the current technique could be extrapolated to changes in the total viable biomass.. The low active fractions that were measured in this study were attributed to the use of acetate as a substrate. The estimated values likely apply to only that fraction of the biomass that can utilize acetate within the period of time employed in the respirometry testing. Hence, these values should be considered as the acetate-active fractions.

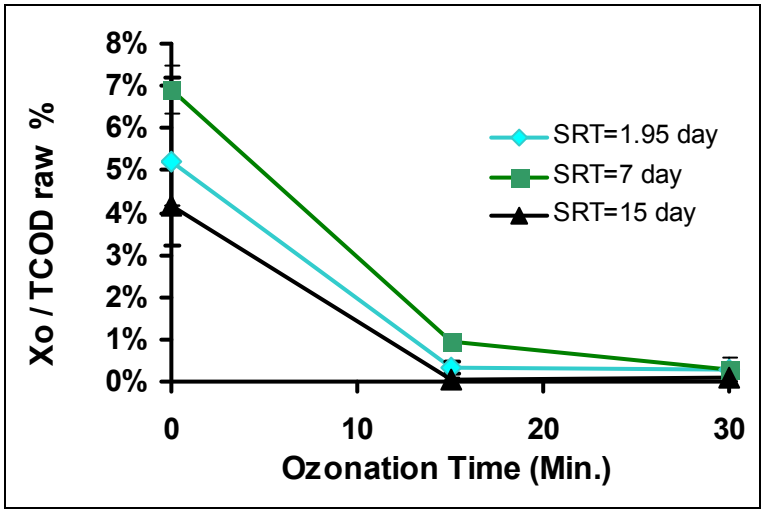


**Figure 5.6. Acetate-Active Fraction versus Ozonation time**

Despite the limitations in the estimation of the active fraction it is believed that the values estimated by the technique employed in this study provide insight into the fate of the active

bacteria through ozonation. It can be reasonably assumed that other heterotrophic organisms would be inactivated to similar extents as those which were capable of utilizing acetate. From Figure 5.6 it can be seen that the pattern in reduction in the active fraction was similar for all of the WAS samples. After 30 minutes of ozonation the active fraction was reduced by greater than 95% of the original values for all the WAS samples. The active heterotrophic biomass represents a significant fraction of the biodegradable COD in waste activated sludges. The inactivation of this biomass by ozonation would likely enhance the digestibility of the sludge as it would oxidize and disrupt cell walls and hence increase the rate at which this material could be hydrolyzed.

Respirometry was also employed to quantify the generation of readily biodegradable COD (rbCOD) by ozonation according to the protocol explained in chapter 4 to measure the generation of rbCOD from sludges by ultrasound treatment. The results of the respirometry tests revealed a reduction in the rbCOD fraction as the level of ozonation increased (Figure 5.7). The reduction of rbCOD may have been due to either the oxidation of rbCOD with ozonation or due to a change in the chemical composition of the solubilized material that would result in a low rbCOD in the respirometer due to a need for acclimation of the biomass to the modified substrate. The TCOD values of the raw and pretreated samples indicated that the TCOD of the samples were not significantly reduced (less than 3%). The limited oxidation of sludge suggested the loss of COD was not the reason for the reduction of rbCOD. A set of acclimated respirometry tests was therefore carried out to investigate whether differing results might be obtained with a biomass that was acclimated to the pretreated WAS. In these tests WAS was fed with ozonated samples and were allowed to acclimate for 3 days in the respirometer to generate a seed for further experiments. This acclimated seed was directly used to measure the rbCOD of raw and 15 min pretreated samples. The results of the acclimated respirometry (Figure 5.7) showed an

increase in the fraction of rbCOD with ozonation. Hence, it would appear that an acclimated respirometry protocol is required for measurement of rbCOD in WAS samples that are pretreated by ozonation.

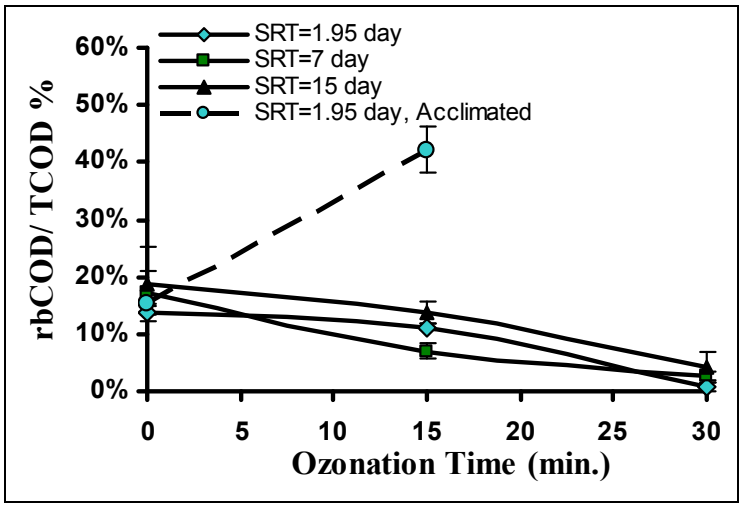


**Figure 5.7. Impact of Ozonation on rbCOD**

The impact of ozonation on methane production was evaluated in BMP tests. The tests were conducted with and without pH-buffer addition (0.8 g/l $NaHCO_3$ + 0.8 g/l $KHCO_3$) for raw and pretreated sludges. The pH was observed to decrease to about 6 and methane generation stopped in all non-buffered pre-treated sludges after about 2 days. This pH drop was likely due to the high concentration of accumulated VFAs in the bottles containing pretreated sludge. The low pH and high VFA concentrations would have inhibited methanogenesis in the batch tests. Such inhibition would be less likely to occur in continuous flow digesters that are fed pretreated sludge due to the dilution of feed sludge into the digester contents and the accumulation of alkalinity in the digesters.

BMP tests (with pH buffer) demonstrated lags in $CH_4$ production for the pre-treated sludges as compared to the raw sludges (Figure 5.8). The estimation of rbCOD with respirometry

tests revealed that ozonation can change the chemical composition of sludge and that results in inhibition of aerobic heterotrophs. Such inhibition may also occur in anaerobic digestion processes; especially for methanogens since they are more sensitive to chemical changes. As will be subsequently demonstrated this could also be attributed to the accumulation of volatile fatty acids in the serum bottles containing pre-treated sludges. The pH in the serum bottles with pH buffer was consistently greater than 7.2 and $NH_4$-N was less than 500 mg/L, hence these potential causes of inhibition were ruled out. The accumulation of the acids likely inhibited $CH_4$ production somewhat in the early portions of the test. Towards the latter portion of the tests it appears that this inhibition was overcome as $CH_4$ production from the pre-treated samples increased and in the most of cases exceeded the raw sludges.

The BMP tests were employed to assess the effect of ozonation on the ultimate digestibility of sludges. The impact of ozonation was found to depend upon the sludge SRT and the ozone dose. From Figure 5.8 it can be seen that ozonation marginally increased the ultimate digestibility of the 1.95 and 7 day SRT sludges (from 39.7% to 41.1% and from 32.2% to 32.7) while a high dose of ozonation increased the digestibility of the 15 day SRT sludge from 31.8 % to 39% of the initial COD. As previously demonstrated the soluble fraction of COD for the 15 day SRT sludge was smaller than the soluble COD fraction for the 1.95 day sludge. However, a greater improvement in biodegradability was observed for the long SRT sludge when a high dose of ozone was applied. These results reveal an inconsistency between the solubilization data and the improvement in the ultimate digestibility of sludge.

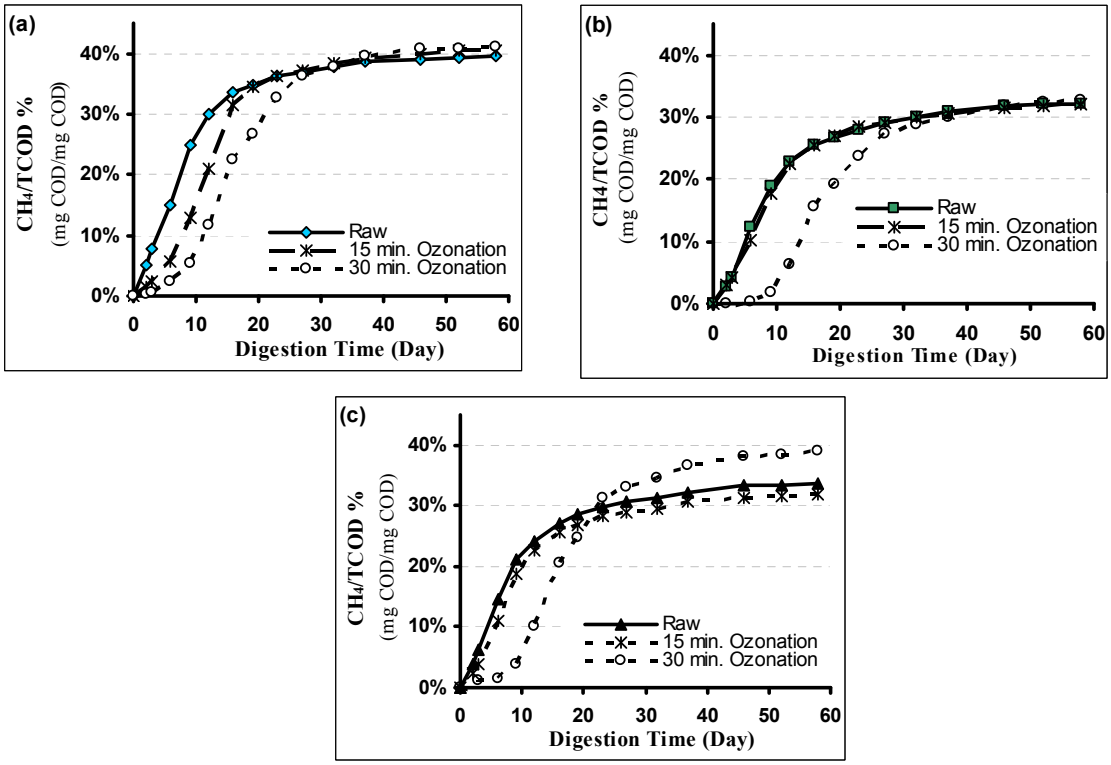


**Figure 5.8. Impact of Ozonation on Methane Generation in BMP Tests for sludges with (a) 1.95 day SRT; (b) 7 day SRT; (a) 15 day SRT**

The effect of ozonation on the anaerobic biodegradation process was further investigated by examining the generation of $NH_4$-N in the BMP tests (Figure 5.9). From Figure 5.9 it can be observed that for the 15 day SRT sludge, applying a high dose of ozone resulted in a substantial increase in the ultimate $NH_4$ yield. This result was consistent with the increases in ultimate $CH_4$ yields with ozonation. For all WAS streams pretreatment substantially increased the rate of $NH_4$-N generation during anaerobic digestion suggesting that the rate of hydrolysis was substantially enhanced. A lag in $NH_4$ generation was not observed for the pre-treated samples as was observed with $CH_4$ generation. This would support the conclusion that inhibition of methanogens was responsible for the lag in $CH_4$ production.

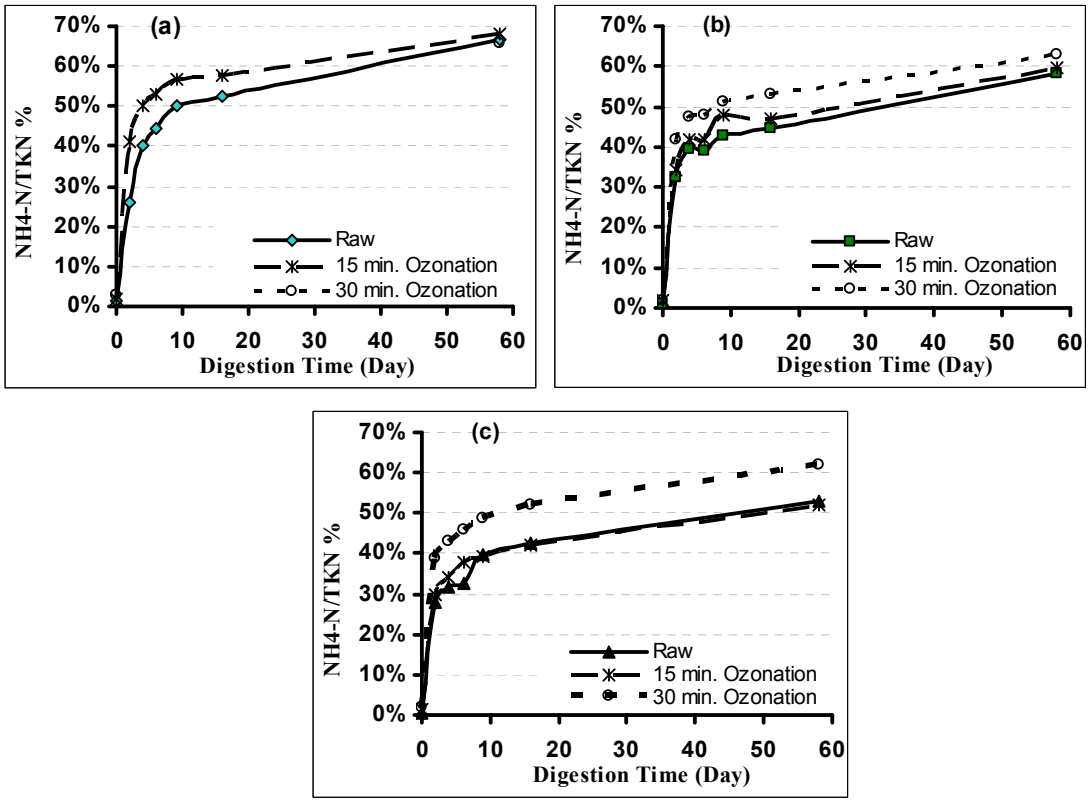


**Figure 5.9. Impact of Ozonation on $NH_4$-N Generation in BMP Test for sludges with (a) 1.95 day SRT; (b) 7 day SRT; (a) 15 day SRT**

To assist in further quantifying the impact of pretreatment on the rate of $NH_4$ generation (and hence hydrolysis of proteins) an empirical model was fit to the data presented in Figure 5.10 as per:

$$\text{Ln}\left(\frac{U_{Ult}^{Ammon} - U_{t}^{Ammon}}{U_{Ult}^{Ammon} - U_{0}^{Ammon}}\right) = -k_{Ammon} \times t \qquad 5.2.$$

Where: $U_{Ult}^{Ammon}$ = Maximum (ultimate) ammonia yield ($NH_4$-N / TKN)

$U_{T}^{Ammon}$ = $NH_4$-N / TKN fraction at time t

$U_{0}^{Ammon}$ = $NH_4$-N / TKN fraction at beginning of test

$k_{Ammon}$ = Ammonification rate constant ($d^{-1}$)

t = digestion time (d).

Figure 5.10 reveals that the model described in Equation 5.4 reflected the observed values well. The $r^2$ values for the linearized form of the equation were greater than 0.996, 0.991 and 0.96 for the 1.95, 7 and 15 day SRT sludges respectively. The results showed that the $U_{Ult}^{Ammon}$ values for raw and pretreated sludges with a 1.95 day SRT were greater than those of the sludges with 7 and 15 day SRTs and the same level of ozonation. The value of $U_{Ult}^{Ammon}$ increased incrementally (between 0.01 to 0.10) for all sludges with ozonation intensity. From Figure 5.11 it can be seen that for most of the ozonation intensities (except for the sludge with 7 day SRT and 15 min. ozonation) the value of *k* increased by 24% to 77% of the values observed for the raw sludges. These results would suggest that ozonation improved the rate of hydrolysis of proteins. The extent of this impact was not the same for sludges that were generated at differing SRTs.

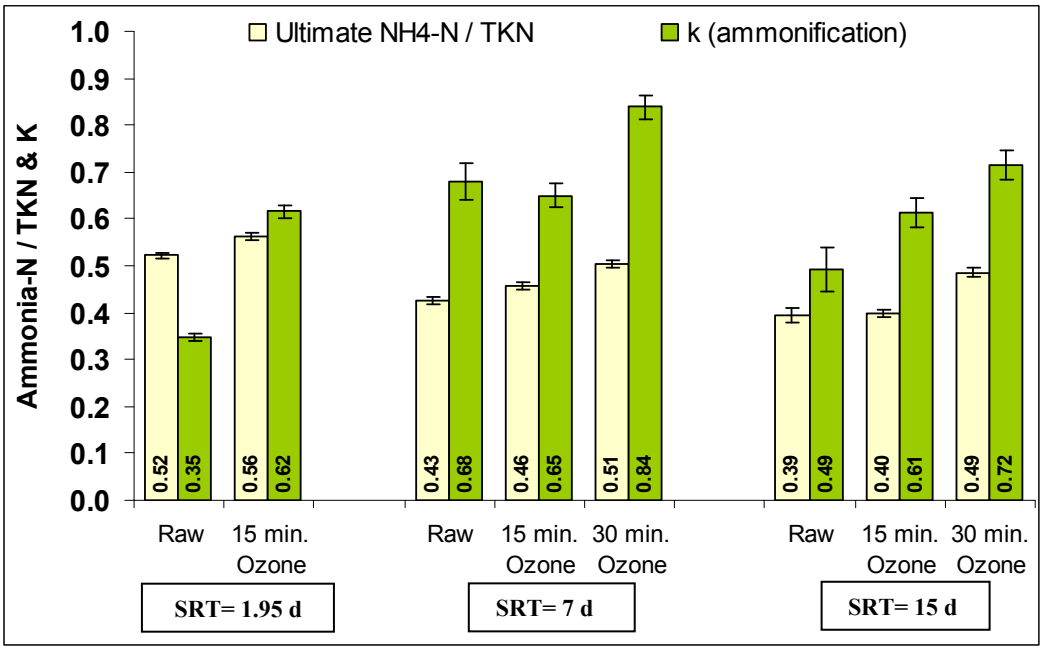


**Figure 5.10. Ultimate Ammonia Generation and Ammonification Rate Constant**

BAP tests were conducted to obtain additional insight into the impact of ozonation on the hydrolysis and acidification processes. The results of the BAP tests for all WAS streams are

presented in Figure 5.11. From Figure 5.11, it can be observed that, as expected, the yields of VFAs from shorter SRT sludges were higher than that observed for the longer SRT sludge with the same level of ozonation. It can also be observed that the rate of VFA generation in the BAP tests increased as the level of pretreatment increased. A substantial increase in the rate of VFA generation and the amount of accumulated VFAs in the BAP tests were observed for the 15 day SRT sludge with a high dose of ozone. This result supports the observations with respect to ultimate biodegradability of samples that were made in the BMP tests. The results also confirmed the prior conclusion that inhibition of methanogens was responsible for the lag in $CH_4$ production.

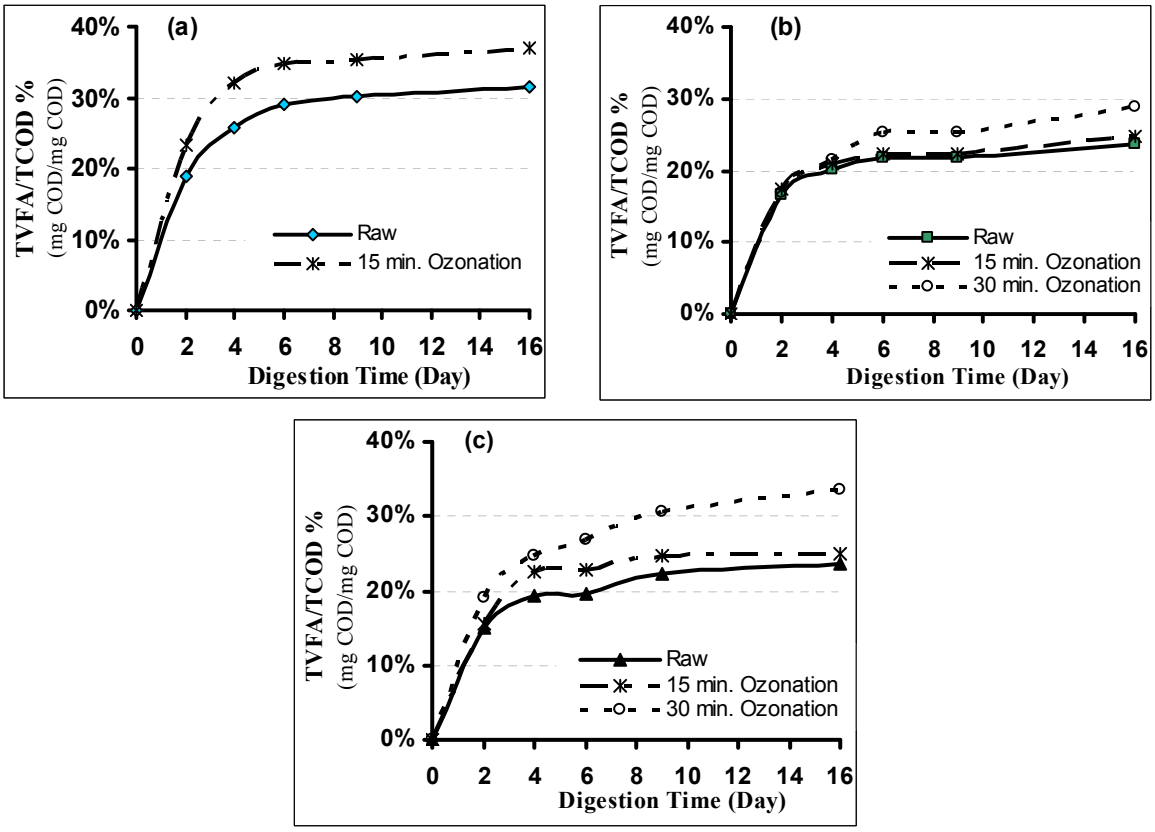


**Figure 5.11. Impact of Ozonation on VFA Production in BAP Tests**

An empirical model, similar to the model presented in Equation 5.2, was fit to the VFA data for the first 10 days of the BAP test that is presented in Figure 5.11. This facilitated quantification of the impact of pretreatment on ultimate VFA yield ($U_{Ult}^{VFA}$) and the rate constant for VFA generation ($k_{VFA}$). The model reflected the observed values well and $r^2$ values for linearized model were greater than 0.999, 0.99 and 0.985 for 1.95, 7 and 15 day SRT sludges respectively. The results of the model fitting are summarized in Figure 5.12. Figure 5.12 reveals that the improvement in estimated ultimate yield of VFAs was marginal (0 to 6%) for all sludges except for the 15 day SRT sludge at the high dose of ozone (10%). This was consistent with the ultimate ammonia and methane yields shown in Figures 5.8 and 5.10.

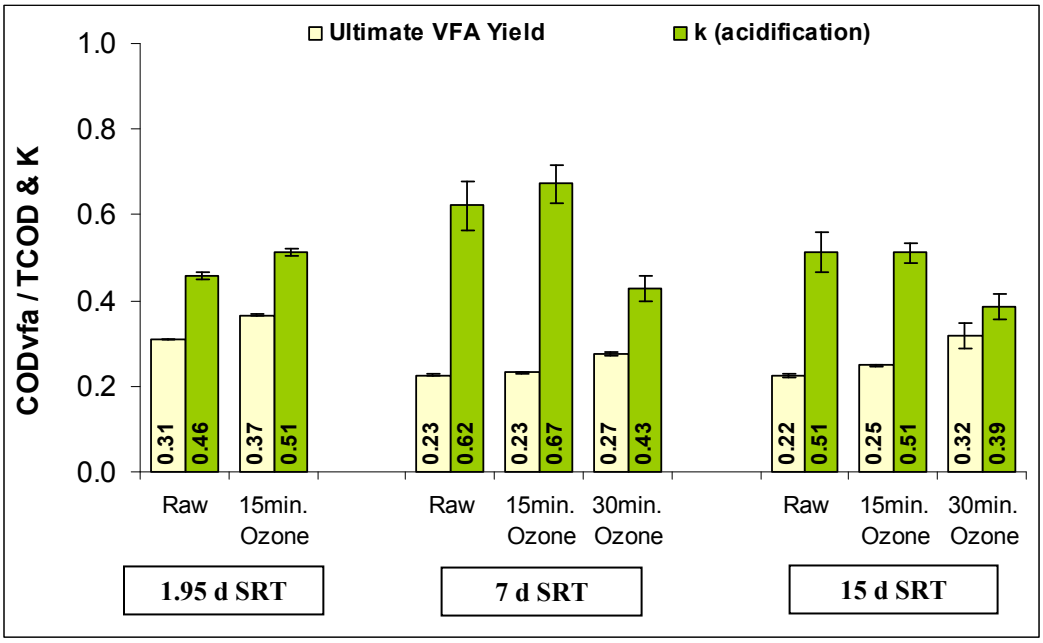


**Figure 5.12. Maximum VFA Concentration and Rate Constant of VFA generation**

The results shown in Figure 5.12 would suggest that the impact of sonication on the rate of hydrolysis was different for sludges that were generated at differing SRTs. From Figure 5.12 it can be seen that the value of $k_{vfa}$ was greater for pretreated samples as compared to the raw

samples for the 1.95 day SRT sludge. However it can be seen that the $k_{vfa}$ values for the 7 and 15 day SRT sludges decreased for the samples that were ozonated for 30 minutes; while their ultimate VFA yield increased. The results suggest that the decrease in $k_{vfa}$ was due to release of some level of slowly biodegradable materials from non-biodegradable material when the highest level of ozonation (30 minute) was applied. As previously described the respirometry results revealed a change in composition of the readily biodegradable material required a 3 day acclimation was required for aerobic degradation. Such acclimation was resulted in a reduction of the rate of biodegradation. Hence either a conversion of non biodegradable material to slowly biodegradable material or a change in the chemical composition of the readily biodegradable material may have resulted in the decrease in the values of $k_{vfa}$.

The ultimate yield of ammonia from TKN was greater than the yield of VFAs from COD for samples with the same pretreatment intensities. This might be due to higher solubilization of proteinaceous materials as compared to that for other materials (Figure 5.4). The $NH_4$ and VFA responses can be considered as separate indicators of the impact of sonication on the rate of hydrolysis and acidification during anaerobic digestion. The results indicate that, as previously suggested by the COD and TKN analyses, ozonation impacted the sludges that were generated at differing SRTs differently. There appears to be a difference in the relative release of proteins and carbohydrates which subsequently affected the relative rates at which $NH_4$ and VFAs were generated.

In summary, despite considerable solublization of WAS with ozonation, with one exception (15 day SRT sludge with highest ozone dose), the ultimate digestibility of WAS was only marginally improved. There did not appear to be any relationship between the extent of

solublization and ultimate digestibility. Pretreatment with ozone did however result in a substantial increase in the rate of digestion of WAS as indicated by both BMP and BAP tests. The improvements in these rates were more closely related to the information provided by solublization data. In anaerobic systems that are operating at traditional solids residence times the increased rates of digestion might be interpreted as an improvement of the digestibility of the WAS. The BAP test has proved to be an effective short term technique for determining the improvement in the rates of digestion that might be achieved with ozonation.

## 5.4. Conclusion

Ozonation of waste activated sludges resulted in only a slight reduction in particle size for sludges of differing solids residence time and for all ozone doses examined. Measurements of particle size distribution were not found to provide useful information on sludge pretreatment since they only describe the distribution of particles that remain after treatment and do not quantify components that have been solubilized.

There was an inconsistency between solubilization indicators (fCOD, ffCOD) and improvement in biodegradability of sludge when comparing soluble COD values to ultimate biodegradability of samples. It appeared that the types of materials which were solubilized was affected by the dose of ozone and the SRT at which the WAS was generated. Ozonation appeared to preferentially solublize proteinaceous materials.

Respirometry revealed that ozonation substantially reduced the viable heterotrophs in the sludge. Unacclimated respirometry revealed a reduction in rbCOD as ozonation does increased. However, an acclimated respirometric approach revealed an increase in rbCOD with ozone dose.

Hence, it was concluded that ozonation modifies the chemical composition of WAS such that biomass adaption is required before the biodegradable fraction can be established.

Ozonation marginally increased the ultimate digestibility of shorter SRT sludges while a high dose of ozonation increased the digestibility of the 15 day SRT sludge substantially. Ozonation substantially increased the rate of hydrolysis which is the rate limiting process in WAS digestion. The relative trends in the rates of $NH_4$-N generation in the BMP tests and VFA generation in BAP tests versus ozone dose differed for the sludges generated at differing SRTs.

The BMP test was not a useful test to evaluate the rate of methane generation due to inhibition of methanogens in the early days of BMP test for pretreated sludges. The BAP test is a shorter term test (10 days) than the BMP (55 to 60 days) test and could provide information on the rates of hydrolysis and acidification/ammonification processes.

# Chapter 6

# 6. Feasibility of Employing BAP Tests for Characterizing Anaerobic Biodegradability of Raw and Pretreated WAS

## 6.1. Introduction

The rate of anaerobic digestion of WAS is generally believed to be limited by the rate of decay/hydrolysis of biomass while the ultimate biodegradability is defined by the viable heterotrophic fraction. Processes that treat WAS to enhance digestibility may impact either or both of these factors however a commonly accepted test for measuring enhancement has not been defined. The biochemical acidogenic potential (BAP) is a short term anaerobic digestion test (5-15 days) with methanogenesis inhibited. The BAP test includes the decay/hydrolysis processes and the products of the BAP test (VFA) should be an indication of the availability of substrates for the methanogenesis process that is also active in the commonly used biochemical methanogenic potential (BMP) test.

Pegion (1993) presented a protocol for the BAP test that involved the use of bromoethane sulfonate (BES) as an inhibitor for methanogenesis. The BAP test has been used to evaluate the potential of wastewater or sludge for production of VFAs for use by phosphorous accumulating organisms (Barajas et al., 2000). The samples were fermented for a short period until the concentration of VFAs became stable. Martin Ruel et al. (2002b) optimized the BAP test for wastewater samples to measure the maximum concentration of VFAs in the stable period as the biochemical acidogenic potential. However, this condition may not necessarily be the optimum condition for digestion of WAS samples with different composition of biodegradable material.

The accumulation of digestion byproducts such as VFAs and $NH_3$ that are potentially inhibitory might influence the results obtained in a BAP test as compared to a BMP test. Biostatic inhibition of methanogens is normally reversible and can result in product inhibition and extreme pH or acid concentrations. The free form of weak acids and bases can disrupt protein motive force homeostasis after passing through the cell membrane and subsequent dissociation (Snoeyink and Jenkins, 1980). The levels of VFA and ammonia accumulation, the concentration of hydrogen and pH may significantly differ from the values observed in digestion of wastewaters. An estimation of the level of inhibition due to each of these factors is beneficial to examine whether the BAP test results can represent the biodegradable fraction of WAS.

The concentration of hydrogen ion (pH) can substantially affect the rate of VFA generation. Eastman et al. (1981) and Elefsiniotis and Oldham (1994) identified pH values in the range of 5 to 6 as optimal for VFA generation in digesters. Ghosh et al. (1995) indicated that a pH of 5.8 was optimal for VFA generation during the anaerobic digestion of WAS. A model introduced by Siegrist et al. (2002) includes the inhibitory effect of pH for the digestion processes. However, prediction of the pH of sludge in BAP tests is challenging because the composition of the WAS, the conversion rate of the WAS and the accumulation of intermediate materials highly affect the pH that is established in the test. However, this model can be employed to evaluate the significance of pH inhibition for each anaerobic digestion process over the range of pH values that are commonly observed in BAP tests.

High concentrations of accumulated acetate and hydrogen can potentially result in a considerable level of inhibition in BAP tests. The model developed by Siegrist et al. (2002) also

describes these inhibitions. Since the accumulation of acetate and hydrogen is significant in BAP tests, the level of these inhibitions should be investigated in BAP tests.

High concentrations of free ammonia are inhibitory for the anaerobic digestion of WAS (Fujishima et al., 2000). The concentration of free ammonia is a function of concentration of total ammonia nitrogen (TAN) and the pH of the digester. Siegrist et al. (2002) introduced a model for ammonia inhibition that can be employed to evaluate the significance of free ammonia inhibition for each anaerobic digestion process over the range of TAN and pH values observed in BAP tests.

In addition to the application of BAP tests for the measurement of the maximum concentration of VFAs as an indicator for biodegradability of WAS, the BAP test can be applied to provide information about biodegradation rates for the initial processes in anaerobic digestion of WAS. The BAP test has been applied by Martin Ruel et al. (2002a) to provide a simplified model to describe hydrolysis and fermentation processes in anaerobic digestion of wastewater. Since the BAP test focuses on the initial portion of the overall process of anaerobic digestion, it is desirable to be applied instead of BMP tests in order to provide information about hydrolysis and fermentation processes in anaerobic digestion of WAS.

In the current study, the BMP test was found to take about 50-70 days to reach a stable level of accumulated methane and was significantly longer as compared to the BAP test which is expected to take 5-15 days. This is due to the low growth rates of methanogenic bacteria as compared to those of acidogens. The consumption of acetate which is absorbed on the surface of acetoclastic methanogenic bacteria and stored as the cell internal materials takes considerable time. In addition, gas-liquid transfer and the separation of methane are time consuming

processes. These steps can cause a variable time lag between substrate disappearance and methane appearance (Jeong et al., 2005) and the time lag can be a significant source of error between experimental results and predicted values from models.

As discussed in chapters 4 and 5, Inhibition of $CH_4$ generation was observed in early stages of digestion when the BMP test was employed to evaluate the rate of hydrolysis during digestion of pretreated WAS samples. Further investigations revealed that inhibition of the methanogenesis process was attributed to the high concentration of volatile fatty acids in the serum bottles containing pre-treated sludges. In such cases, BAP test could still be employed instead of BMP test to characterize the impact of pretreatment on biodegradability of sludges.

The modeling of digestion processes based on BAP test data can enhance the compatibility of experimental and analytical results due to elimination of some causes of time lags such as gas-liquid transfer and also faster reaction of acidogenic bacteria compare to methanogenic bacteria. An improvement in the sensitivity of the profile of VFA generation to substrate characteristics is expected due to the elimination of some sources of errors in the BAP test. The simulation and experiments carried out by Jeong et al. (2005) for the biochemical methanogenic potential of sludge revealed information about the sensitivity of VFA generation to substrate and parameters in ADM1. The sensitivity of kinetic and stoichiometric parameters (defined in ADM1 model) to the substrate (glucose), intermediate products (VFAs) and final components (methane) was investigated in a BMP test. VFA generation was sensitive to a limited number of stoichiometric parameters ($f_{Bu,Su}$, $f_{Pro,Su}$, $f_{Ac,Su}$) the anaerobic digestion pathway and consequently the composition of the substrate. Hence a better fractionation of parameters is expected when employing BAP tests instead of BMP tests.

The overall objective of this study was to investigate the ability of the BAP test to characterize biodegradability of raw and pretreated WAS. A simplified stoichiometric model that was developed based on anaerobic digestion model 1 (ADM1) was employed to explore the relationship between BAP and BMP test responses.

## 6.2. Approach

Three sequencing batch reactors (SBRs), each with a volume of 175 L, were operated on screened municipal wastewater at an hydraulic residence time (HRT) of 9.3 hours and SRTs of 1.95, 7 and 15 days respectively. The SBRs were employed as a source of WAS for all of the testing conducted in this study. WAS samples that were generated with different SRTs were employed to examine the validity of the BAP test for a range of sludge properties. The pretreatment of WAS samples can provide sludges with different fractions of readily and slowly biodegradable material. Sonication and ozonation were applied as models of physical and chemical pretreatment to examine the validity of the BAP test to characterize pretreated sludges with different rates of biodegradation.

WAS samples from the SBRs were treated by sonication at 45$^{o}$C in a bench scale apparatus with an operational frequency of 20 kHz and a maximum amplitude of 250 μm. For the unit employed in this study sonication times of 5, 10, 25 and 45 minutes corresponded to ultrasound intensities of 1111, 2222, 5555 and 9999 kJ/L respectively. Ozonation was achieved by diffusing ozone into a sealed glass reactor containing 1100 ml of sludge. Both of the units were operated in a batch mode and for each pretreatment process a range of treatment intensities were evaluated by collecting samples at different times as the treatment progressed. Additional

information about changes in the levels of readily biodegradable material and rate of biodegradation with these pretreatment technologies are discussed in chapters 4 and 5.

As previously mentioned, the BAP test protocol was originally optimized for wastewater samples and an investigation of the optimum condition for digestion of WAS samples in BAP test has been required. A set of preliminary experiments were conducted to find the best condition with regard to mixing condition, dilution of samples, inhibitor concentration, the portion of inoculums and pretreatment intensities and the results are discussed in chapter 3.

Batch anaerobic digestion tests were conducted to assess the Biochemical Methane Potential (BMP) as well as the Biochemical Acid Potential (BAP) of the raw and pre-treated samples. The BMP and BAP tests were conducted according to the protocols described in Chapter 3. Duplicate bottles were employed for each BMP or BAP test. The duplicated serum bottles were devoted for liquid sampling and gas measurements separately and sacrificed at the end of test for analysis of digested samples and investigation of the reproducibility of experiments. Liquid samples were regularly taken for measurement of pH, total and volatile suspended solids, TCOD, FCOD, FFCOD, TKN, FTKN and $NH_4$-N according to Standard Methods (APHA, 1995). The generated gas was regularly discharged from the bottles to a manometric device for measurement of gas volume. The $CH_4$ content of the gas was measured by gas chromatography and expressed as COD mass for comparison with TCOD values. Additional information for the methodology of the anaerobic batch tests was presented in Chapter 3.

The profile of ammonia generation in BAP tests was compared to that in BMP tests for most of samples. The responses were quite similar (± 4% difference). Hence the ammonia results

from both the BAP and BMP tests were employed when investigating the relationship between ammonia and other responses.

## 6.3. Results and discussions

A comparison of the relationships between ultimate methane and ammonia generation in BMP tests and responses from BAP tests is presented in section 6.3.1. In section 6.3.2, the details of a model for fractionation of composites in WAS is introduced to facilitate the interpretation of the relationship between the BMP and BAP responses. The significance of inhibition in the BAP test and its impacts on sludge characterization results was investigated.

### 6.3.1. Prediction of biodegradability from BAP Tests

The relationship between the VFA and ammonia responses in the early period of the BAP tests and the ultimate ammonia and methane yields in the BMP tests were investigated. A consistent relationship between these responses for the BAP and BMP tests would suggest that the BAP test could be employed as a short test for estimating the ultimate biodegradability of WAS.

In this part of study, the VFA and ammonia responses from the BAP tests were compared with the ultimate biodegradability of sludge as measured by the BMP tests. A range of raw and pretreated WAS samples with different SRTs were employed to investigate relationships for sludges with different rates of biodegradation and ultimate biodegradability.

The concentrations of accumulated VFAs after 6 and 10 days in the BAP test were compared to the ultimate generation of methane in BMP tests (Figure 6.1). Both responses are

presented as fractions of the total COD of the sludge. As shown in Figure 6.1, a linear relationship between the concentrations of accumulated VFAs and ultimate methane generation was observed for both data sets. The best fit linear model can be described by Equations 6.1 and 6.2.

$$U_{BMP}^{Methane} = 0.52 \times U_{BAP,6}^{VFA} + 0.23 \qquad 6.1.$$

$$U_{BMP}^{Methane} = 0.50 \times U_{BAP,10}^{VFA} + 0.22 \qquad 6.2.$$

Where: $U_{BAP,6}^{VFA}$ = VFA fraction in 6 day BAP test (mg COD/mg COD)

$U_{BAP,10}^{VA}$ = VFA fraction in 10 day BAP test (mg COD/mg COD)

$U_{BMP}^{Methane}$ = Methane yield in a BMP test (mgCOD/mgCOD).

The values of $r^2$ for the models described by Equation 6.1 and 6.2 were 0.81 and 0.90 respectively. The values of standard error for estimated slopes and intercepts were ±0.06 and ±0.02 in the 6 day model and ±0.04 and ±0.015 in the 10 day model. According to these results, the model based on the 10 day BAP test that is described by Equation 6.2 was a better fit of the model to the data as compared to the model described by Equation 6.1.

The adequacy of the linear models for predicting $U_{BMP}^{Methane}$ by the concentration of accumulated VFAs ($U_{BAP,6}^{VA}$) in day 6 and day 10 of the BAP tests was investigated by statistical tests (Montgomery, 2001) and the calculation and details are mentioned in appendix E. The differences between the predicted and experimental $U_{BMP}^{Methane}$ (residual) were plotted for both models in a cumulative normal probability test. The results confirmed that the residuals in both models were normally and independently distributed with a mean of zero and constant variance.

A plot of the residuals versus the fit values also revealed that the residuals in both models were structureless. A t-test (paired two samples for means) was applied to check if the mean of the residuals was equal to zero and the differences between experimental and predicted values were not significant. The results of this test also supported the previous results about the adequacy of both models. Standardized residuals were examined to investigate if there were any outliers among the residuals. The results show that the errors between predicted values and experimental values were greater in the day 6 model as compared to day 10 model responses. The standardized residuals test revealed that there were outliers among the residuals in the model for the 6 day responses. The lack of fit of the day 6 model may have been due to the presence of slowly biodegradable materials which were biodegraded after 6 days. Hence, the model based on the 10 day BAP test was deemed to be acceptable and was preferable to the model based on the shorter BAP test.

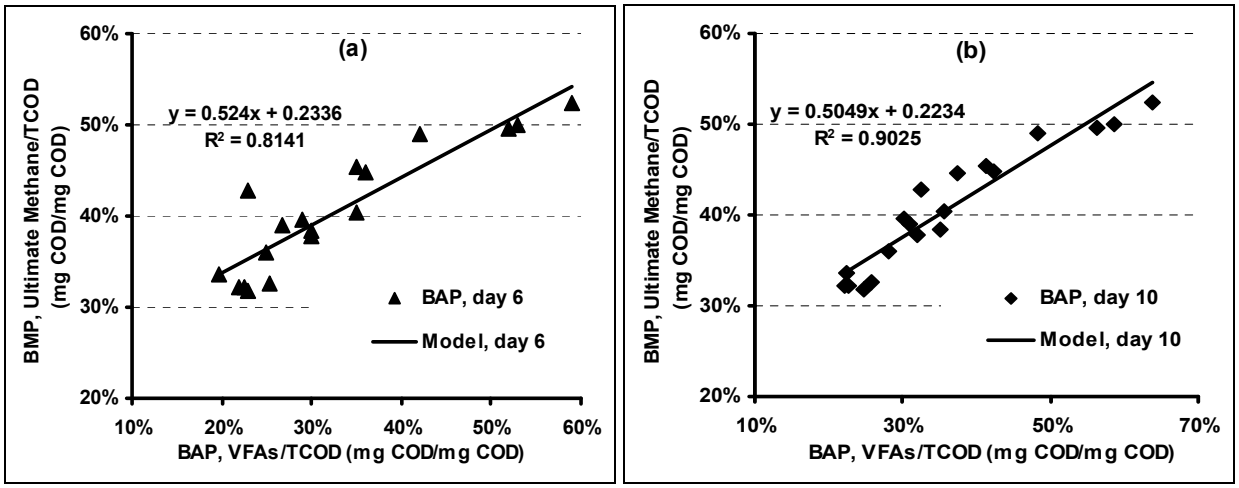


**Figure 6.1. Relationship between methane in BMP tests and VFA responses in (a) 6 day BAP test and (b) 10 day BAP test**

As shown in Figure 6.1, a non linear model could be employed to describe the relationship between the concentrations of accumulated VFAs and ultimate methane generation for both data sets. However, the statistical investigation revealed that linear model could simply and adequately describe the relationship between the VFAs and ultimate methane generation without significant loss in accuracy. The investigation of relationships between the other responses from BAP and BMP tests that are subsequently presented revealed that, linear models could better describe the observed experimental values as compared to non linear models. Hence, the linear model that was presented in Equation 6.2 was deemed to be more consistent with the other models introduced in this study.

Figure 6.1b and equation 6.2 reveal that $U_{BAP,10}^{VFA}$ values were significantly less than $U_{BMP}^{Methane}$ values for all samples with $U_{BMP}^{Methane}$ values smaller than 50%. The difference in responses could be due to the presence of slowly biodegradable material that took longer than 10 days to biodegrade. It also could be due to inhibition of some steps or pathways during digestion of the sludge, errors in the test or the lack of measurement of responses such as hydrogen in the BAP tests. The values of $U_{BAP,10}^{VFA}$ were greater than $U_{BMP}^{Methane}$ for sludges with $U_{BMP}^{Methane}$ values greater than 50%. If the VFAs generated in the BAP test were converted to $CH_4$ in the BMP tests, it would be expected that the values would be similar. The differences between the VFA and $CH_4$ values could be partially due to uptake of VFAs and conversion of them to non-biodegradable decay products in BMP tests. Section 6.3.2 presents an investigation of the mechanisms, errors or conditions to further explore the underlying reasons of the differences between $U_{BAP,10}^{VFA}$ values and $U_{BMP}^{Methane}$ values.

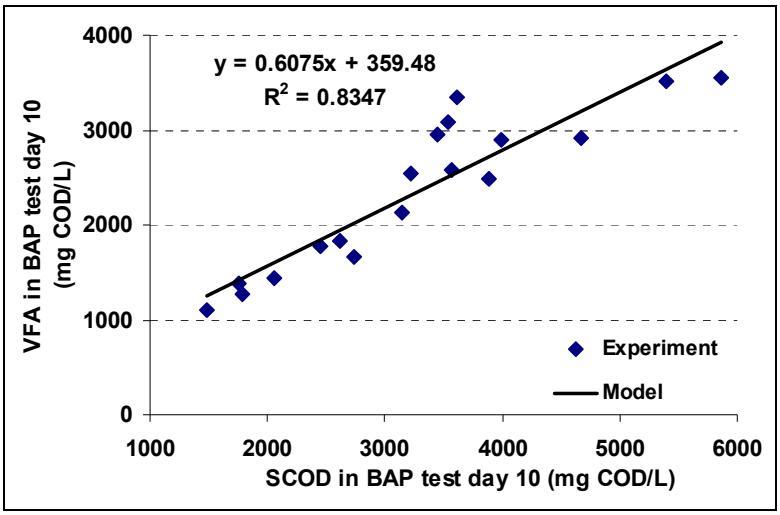


**Figure 6.2. Relationship between soluble COD and VFA responses in BAP test**

The measurement of COD is much simpler than measurement of VFAs and hence the prediction of ultimate methane generation on the basis of soluble COD in day 10 of the BAP tests was investigated. Figure 6.2 shows the relationship between accumulated VFA and SCOD in day 10 of the BAP tests. The results reveal that there was a linear relationship between VFA and SCOD in day 10 (Figure 6.2). The best fit linear model is shown in Equation 6.3.

$$C_{BAP,10}^{VA} = 0.61 \times C_{BAP,10}^{SCOD} + 359.5 \qquad 6.3.$$

Where: $C_{BAP,10}^{VFA}$ = VFA concentration based on COD (mg COD/L) in a 10 day BAP test

$C_{BAP,10}^{SCOD}$ = Soluble COD (mg COD/L) in a 10 day BAP test

The value of $r^2$ for the model described by Equation 6.3 was 0.83 and the values of standard error for estimated slope and intercept in the model were ±0.07 and ±236 respectively. These results indicate a reasonable fit of the model to the data.

Figure 6.2 and Equation 6.3 reveal that the $C_{BAP,10}^{SCOD}$ values were consistently greater than $C_{BAP,10}^{VFA}$ values for all sludges. The difference in responses could be partially due to the presence

of soluble material that required more than 10 days to biodegrade. It also could be due to inhibition of some steps or pathways during digestion of sludge, errors in the test or lack of measurement of generated hydrogen in BAP tests. The investigation in Section 6.3.2 was conducted to explore the underlying reasons of such differences between $C_{BAP,10}^{SCOD}$ and $C_{BAP,10}^{VFA}$ values.

Figure 6.3a shows the relationship between accumulated SCOD in day 10 of the BAP tests and ultimate methane yield in BMP tests. The best fit linear model is shown in Equation 6.4.

$$U_{BMP}^{Methane} = 0.51 \times U_{BAP,10}^{SCOD} + 0.15 \qquad 6.4.$$

Where: $U_{BAP,10}^{SCOD}$ = SCOD fraction of TCOD in a 10 day BAP test (mg COD/mg COD).

$U_{BMP}^{Methane}$ = Methane yield in a BMP test (mgCOD/mgCOD).

The value of $r^2$ for the model described by Equation 6.4 was 0.90 and the values of standard error for estimated slope and intercept in the model were ±0.04 and ±0.02 respectively. These results indicate a reasonable fit of the model to the data. The adequacy of the linear models for predicting $U_{BMP}^{Methane}$ by $U_{BAP,10}^{SCOD}$ was investigated with employing the statistical tests and procedures that were previously employed for the models described in Equation 6.1 and 6.2. The statistical investigation revealed that the model was acceptable and could adequately describe the relationship between $U_{BMP}^{Methane}$ and $U_{BAP,10}^{SCOD}$ values.

Figure 6.3a and Equation 6.4 reveal that the $U_{BMP}^{Methane}$ values were 1.4% to 17.3% smaller than $U_{BAP,10}^{SCOD}$ values. Pretreatment may solubilize particulate inert COD and impact on the value of

$U_{BAP,10}^{SCOD}$ while the improvement in ultimate methane generation was marginal (chapters 4 and 5). This could be the source of variability of data when plotting $U_{BMP}^{Methane}$ versus $U_{BAP,10}^{SCOD}$. Figure 6.3b shows the linear relationship between $U_{BMP}^{Methane}$ and $U_{BAP,10}^{SCOD}$ for the raw sludges. As can be seen in Figure 6.3b, $U_{BMP}^{Methane}$ values still were 1.4% to 12.3% smaller than $U_{BAP,10}^{SCOD}$ values and the $r^2$ value (0.937) for the linear model in Figure 6.3b was not much better than $r^2$ value (0.9) for the model described in Equation 6.4. These results reveal that solubilization of inert material by pretreatment is partially responsible for the difference in responses and the variability of data. The difference in responses could also be due to inhibition of some steps or pathways during digestion of sludge, errors in the test or lack of measurement of responses in BAP tests such as hydrogen generation. The investigation in 6.3.2 was conducted to explore the underlying reasons of the differences between $U_{BMP}^{Methane}$ and $U_{BAP,10}^{SCOD}$ values.

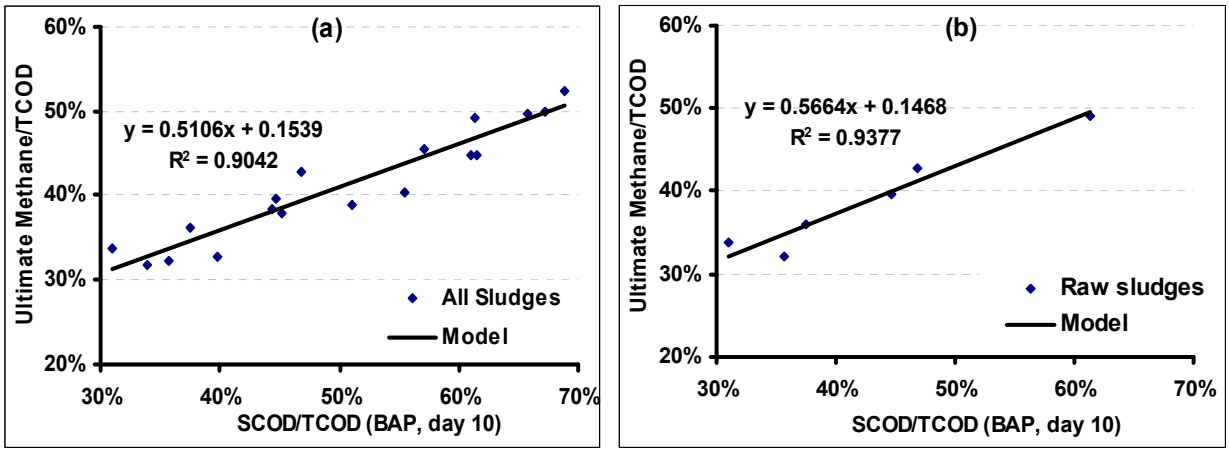


**Figure 6.3. Relationship between soluble COD and methane responses in BAP and BMP tests for (a) all sludges, (b) raw sludges**

A comparison of the $r^2$ values, and the standard errors attributed to estimated slopes and intercepts in the models described in Equations 6.2 and 6.4 revealed that there was no clear

evidence to suggest that VFA responses were better than SCOD data for predicting ultimate methane generation. Therefore, measurement of SCOD in the BAP tests and employing the linear model described in Equation 6.4 is a short, simple and widely applicable test to predict the anaerobic biodegradability of sludges as compared to using methane and VFA responses in BMP and BAP tests.

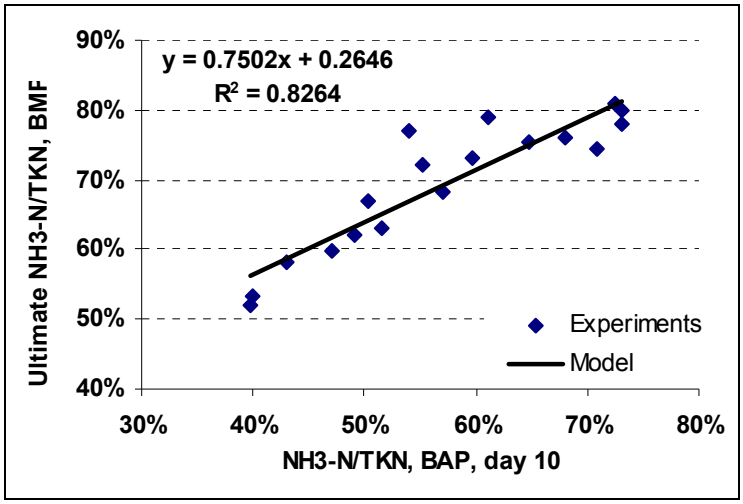


**Figure 6.4. Relationship between $NH_3$-N fraction of TKN in 10 day BAP test and at the end of BMP test**

A substantial portion of the organic material that is biodegradable in WAS is proteinaceous. Hence the ammonia and TKN data from the BAP tests were employed to assess the use of BAP tests to predict the ultimate biodegradability of proteinaceous material. Figure 6.4 shows the ammonia concentrations in day 10 of the BAP tests versus the ultimate ammonia concentrations in the BMP tests. Both responses were normalized with respect to the TKN in the samples. The results indicate that there was a linear relationship between the responses and the best fit linear model is described by Equation 6.5.

$$U_{BMP}^{Ammon} = 0.75 \times U_{BAP,10}^{Ammon} + 0.26 \qquad 6.5.$$

Where: $U_{BAP,10}^{Ammon}$ = $NH_3$-N fraction of TKN in 10 day BAP test

$U_{BMP}^{Ammon}$ = Ultimate $NH_3$-N fraction of TKN in BMP test

Figure 6.5 shows the ammonia concentrations in day 10 of the BAP tests versus ultimate ammonia concentration in BMP test when both responses were normalized with respect to the TCOD of the sludges. These responses are easier to measure since TCOD already should be measured when predicting ultimate methane generation and the additional measurement of TKN is not required. The best fit linear model is presented in Equation 6.6.

$$U_{BMP}^{Ammon} = 0.74 \times U_{BAP,10}^{Ammon} + 0.026 \qquad 6.6.$$

Where: $U_{BAP,10}^{Ammon}$ = $NH_3$-N fraction of TCOD in 10 day BAP test

$U_{BMP}^{Ammon}$ = Ultimate $NH_3$-N fraction of TCOD in BMP test

The values of $r^2$ for the models described by Equations 6.5 and 6.6 were 0.83 and 0.88 respectively. The values of the standard errors for the estimated slopes and intercepts of the models described by Equation 6.5 and 6.6 were ±0.08 and ±0.07 for the 6 day model and ±0.05 and ±0.004 for the 10 day model respectively. The generally good fit and high $r^2$ values suggested that $NH_4$ release in BAP tests could be employed to predict the ultimate $NH_4$ release in BMP tests. All of the statistical procedures that were previously described were employed to assess the adequacy of the models presented in Equations 6.5 and 6.6. The investigation revealed that both sets of residuals were normally and independently distributed with mean zero and constant and unknown variance. There was no indication of outliers among both sets of residuals and they were structureless with respect to the predicted values. Hence, these results demonstrated the adequacy of the models (Appendix E).

Both Figure 6.5 and Equation 6.5 reveal that all $U_{BAP,10}^{Ammon}$ values were less than the $U_{BMP}^{Ammon}$ values. These differences were 7.8% to 16.5% of the TKN. The difference in values might be due to the presence of slowly biodegradable proteinaceous material that took longer than 10 days to biodegrade.

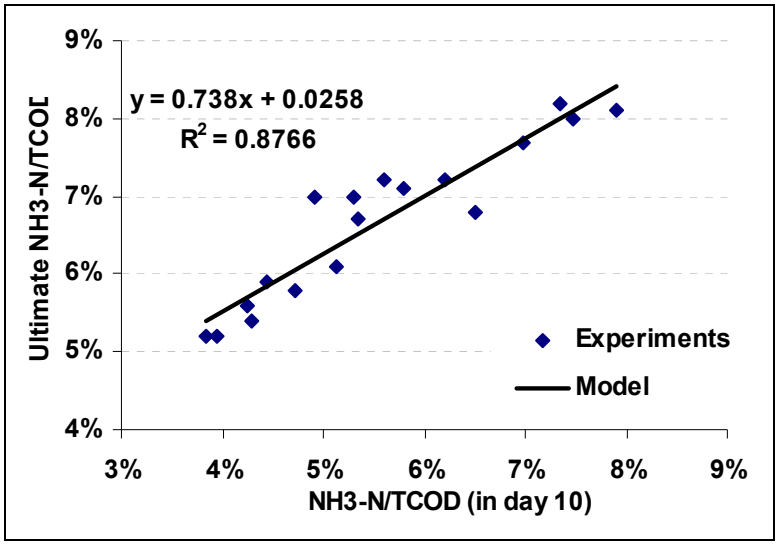


**Figure 6.5. Relationship between ammonia to TCOD ratio in 10 day BAP test and at the end of BMP test**

The models described in Equation 6.2, 6.4, 6.5 and 6.6 reveal that the BAP responses could be employed to predict the ultimate biodegradability of sludge. A comparison of trends in the described models reveals that the relationship between BAP and BMP responses was not a direct one to one relationship. The comparison of models for prediction of ultimate ammonia yield (Figure 6.4 and 6.5) and ultimate methane yield (Figure 6.2) suggest some inconsistencies in the responses. In Figure 6.4 and 6.5 it can be observed that the yields of $NH_3$-N in the BMP tests were consistently less than those observed in the BAP tests. This trend was expected as the longer duration of the BMP tests would allow for increased hydrolysis of proteins and hence increased $NH_3$-N release when compared to the shorter term BAP tests. In contrast, Figure 6.2

indicates that while the ultimate $CH_4$ yield in the BMPs was greater than the VFA yields in the BAP test for WAS samples with low digestibility, the opposite was observed for WAS samples with higher digestibility. The change in the relationship between the $CH_4$ and VFA responses suggested that differing biodegradation pathways were occurring in the BMP and BAP tests. The inhibition of some steps or pathways due to accumulation of VFAs is likely and could partially explain such inconsistencies.

### 6.3.2. Fractionation of composites in WAS

The composition of biodegradable materials may vary in sludges with differing biodegradability. Such variation could potentially impact on the BAP and BMP responses and may be the reason for the inconsistency observed among the models. The potential to use the data from BAP and BMP tests for fractionation of TCOD to into non biodegradable materials and biodegradable fractions (carbohydrates, proteins and lipids) in the WAS samples is investigated in this section. In addition, the relationship between ammonia and VFA responses in the BAP test and ultimate ammonia and methane generation as described by Equations 6.2, 6.4 and 6.5 are compared to the fractionation results. It was suspected that inhibition of some steps or pathways is likely during digestion of sludge in BAP test. An estimation of the level of inhibition for every digestion process in BAP tests was examined to evaluate whether the BAP test responses represent the biodegradable fraction of WAS. This investigation was conducted to identify if inhibition was the underlying reason for the differences between the BAP and BMP responses.

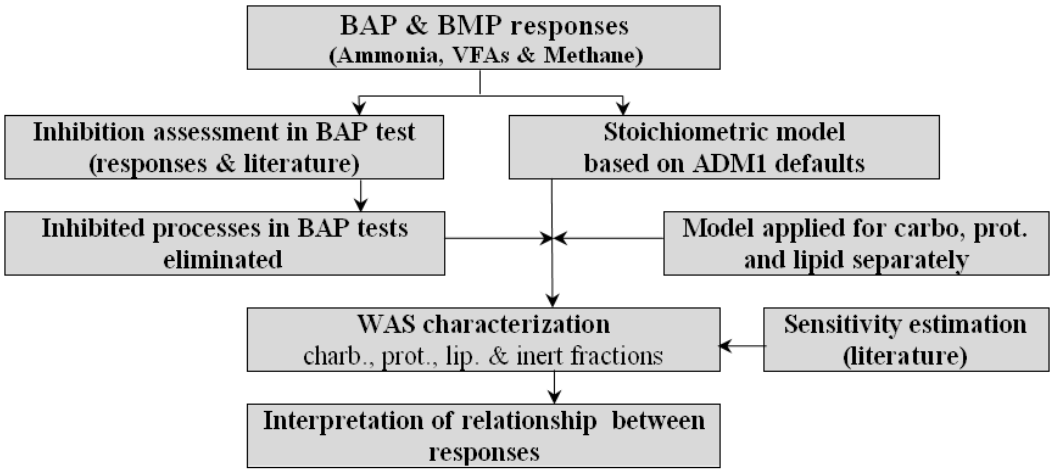


**Figure 6.6. Overview of sludge characterization with BAP test.**

A simplified stoichiometric model was developed to fractionate the TCOD of the feed sludges based on the flow of COD through the digestion process. The model was built according to the ADM1 model employing default values and inhibited digestion processes were eliminated from the pathways. An improvement in the sensitivity of VFA responses to substrate characteristics was expected as compared to methane responses in BMP tests due to the elimination of some sources of errors. Hence an improvement in the characterization of biodegradable materials might be observed when employing BAP responses. An overview of this investigation is shown in Figure 6.6. Each of these steps is explained in subsequent sections.

### 6.3.2.1. Inhibition assessment of BAP tests

In the BAP tests elevated concentrations of acetate and $H^+$, as potentially inhibitory chemicals, were expected and observed in BAP tests due to accumulation of VFAs. Although $H_2$ was not directly measured, methane generation was not observed in the BAP tests indicating that hydrogenotrophic methanogenesis was inhibited. Hence hydrogen accumulation was expected in the BAP tests. Ammonia is another potential inhibitory chemical that is produced as a result of

fermentation of amino acids. In this section the potential for inhibition of anaerobic digestion processes by these substances was investigated.

Inhibition models for acetate, hydrogen ($H_2$) and hydrogen ion (pH) were introduced by Siegrist et al. (2002) for the processes in the ADM1 model. A preliminary estimation of the level of inhibition which may have occurred in the worst possible conditions was conducted to understand the importance of inhibition for each process of anaerobic digestion. The results are summarized in Table 6.1. The values of "I" as the inhibition expression are multiplied with the process rates and hence a value of 1 suggests that inhibition was not expected to occur in the tests.

The values of the expression for acetate inhibition ($I_{ac}$) in the different steps of anaerobic digestion were calculated for the minimum and maximum acetate concentrations (700 and 3600 mg COD/L) that were observed in the 10 day BAP tests and are presented in Table 6.1. The results indicate that the anaerobic oxidation of LCFAs ($I_{ac,5}$) and also propionate ($I_{pro,6}$) were partially inhibited. Depending on the amount of lipids in the WAS, the inhibition of LCFA oxidation may cause a significant decrease in the rate of VFA generation in the BAP test. Since the BAP test is considered as a short test, a significant decrease in the rate of a digestion process would influence the final responses at the end of such a short test. Hence, the responses in such conditions would not represent the biodegradability of sludge and may result in the underestimation of biodegradability of samples.

Hydrogen accumulation was expected in the BAP test due to the inhibition of hydrogenotrophic methanogenesis. The levels of inhibition by hydrogen gas were estimated and are summarized in Table 6.1. The results show that high concentrations of hydrogen gas up to a

maximum fraction of 7% (Feitkenhauer, 2003) in the gas phase can inhibit anaerobic oxidation of LCFAs ($I_{H2,5}$= 0.03 and $I_{H2,6}$= 0.01) in the BAP test.

**Table 6.1. Estimation of Inhibition of Sludge Digestion Processes in Mesophilic (35ºc) Conditions Based on ADM1.**

| Process | Acetate inhibition $I_{ac,i}=K_{I,ac,i}/(K_{I,ac,i}+Sac)$ for $K_{I,ac,j}$=1500 gr COD/$m^3$ | $H_2$ inhibition $I_{H2,j}=K_{I,H2,j}/(K_{I,H2,j}+S_{H2})$ (max. possible fraction of $H_2$ in gas phase= 7%) | $H^+$ inhibition $I_{H,i}=K_{I,H,i}^2/(K_{I,H,i}^2+S_H^2)$ $S_H=[H^+]=10^{-pH}$ | $NH_3$ inhibition $I_{H,i}=K_{I,NH3,i}^2/(K_{I,NH3,i}^2+S_{NH3}^2)$ ($S_{NH3}=10^{(pH-9.3)}\times$ TAN, pH=7.35) |
|---|---|---|---|---|
| **Fermentation of Amino Acids** ***Process Code (3)*** | **Not Inhibitory** | **Not Inhibitory** | $K_{I,H,3}=10^{-2}$ mol/$m^3$ & pH=5 → **$I_{H,3}$=1** **Not Inhibitory** | **Not Inhibitory** |
| **Fermentation of Sugar** ***Process code (4)*** | **Not Inhibitory** | **Not Inhibitory** | $K_{I,H,4}=10^{-2}$ mol/$m^3$& pH=5 →**$I_{pH,4}$=1** **Not Inhibitory** | **Not Inhibitory** |
| **Anaerobic Oxidation of LCFAs** ***Process Code (5)*** | For $S_{ac}$=700 gr COD/$m^3$ →**$I_{ac,5}$= 0.68** **Inhibitory** ---------- For $S_{ac}$=3600 grCOD/$m^3$ →**$I_{ac,5}$= 0.29)** **Inhibitory** | Max $H_{2liq}$ conc.=102mgCOD/lit $K_{I,H2,5}$=3 mgCOD/lit →**$I_{H2,5}$= 0.03** **Completely Inhibitory** | $K_{I,H,5}=5\times10^{-4}$ mol/$m^3$& pH=5 →**$I_{pH,5}$= 0.999** **Not Inhibitory** | **Not Inhibitory** |
| **Anaerobic Oxidation of Propionate** ***Process Code (6)*** | For $S_{ac}$=700 gr COD/$m^3$ →$I_{ac,6}$=0.68 **Inhibition** ---------- (And for Sac=3600 gr COD/$m^3$ →**$I_{ac,6}$= 0.29** **High Inhibitory** | Max $H_{2liq}$ conc.=102mgCOD/lit $K_{I,H2,6}$=1 mgCOD/lit →**$I_{H2,6}$= 0.01** **Completely Inhibitory** | $K_{I,H,6}=5\times10^{-4}$mol/$m^3$& pH=5 →**$I_{pH,6}$= 0.999** **Not Inhibitory** | In first 10 days TAN (g-N $m^3$)=543, $K_{I,NH3}$=25 g-N $m^{-3}$ → $S_{NH3}$ (g-N$m^3$)=6.09 → **$I_{NH3}$= 0.94** **Not Inhibitory** ---------- After 10 days TAN(g-N $m^3$)=702, $K_{I,NH3}$=17 g-N $m^{-3}$ → $S_{NH3}$ (g-N$m^3$)=7.87 → **$I_{NH3}$= 0.91** **Not Inhibitory** |
| **Acetotrophic Methano genesis** ***Process code (7)*** | **Already Inhibited in BAP Test by BES Addition** | **Already Inhibited in BAP Test by BES Addition** | **Already Inhibited in BAP Test by BES Addition** | **Already Inhibited in BAP Test by BES Addition** |
| **Hydrogeno trophic Methano genesis** ***Process Code (8)*** | **Not Inhibitory** | **Not Inhibitory** | $K_{I,H,8}=5\times10^{-4}$ mol/$m^3$& pH=5 →$I_{pH,8}$= 0.999 **Not Inhibitory** | **Not Inhibitory** |

$\rho_3 = \mu_{max,3}\times [S_{aa}/(K_{S,aa}+S_{aa})]\times I_{pH,3}\times X_{aa}$ $\rho_4 = \mu_{max,4}\times [S_{su}/(K_{S,su}+S_{su})]\times I_{pH,4}\times X_{su}$

$\rho_5 = \mu_{max,5}\times [S_{fa}/(K_{S,fa}+S_{fa})]\times I_{ac,5}\times I_{H2,5}\times I_{pH,5}\times X_{fa}$ $\rho_6 = \mu_{max,6}\times [S_{pro}/(K_{S,pro}+S_{pro})]\times I_{ac,6}\times I_{H2,6}\times I_{pH,6}\times I_{NH3,6}\times X_{pro}$

$\rho_7 = \mu_{max,7}\times [S_{ac}/(K_{S,ac}+S_{ac})]\times I_{pH,7}\times I_{NH3,7}\times X_{ac}$ $\rho_8 = \mu_{max,8}\times [S_{H2}/(K_{S,H2}+S_{H2})]\times I_{pH,8}\times I_{NH3,8}\times X_{H2}$

The values of pH were greater than 6.5 in all BAP tests that were conducted in this study. According to Table 6.1, pH values of 5 and higher are not significantly inhibitory. This suggests that pH did not cause extreme inhibition of any digestion process in the BAP tests in this study. It can be deduced that the control of pH may not be essential in BAP tests. It should be noted that the release of free ammonia ($NH_3$) due to the fermentation of amino acids may compensate for the presence of accumulated VFAs and control the pH of the sludge in the BAP test.

A preliminary estimation of ammonia inhibition which may occur in the worst possible condition was conducted to understand the importance of inhibition for each process of anaerobic digestion. Free ammonia is inhibitory to the anaerobic oxidation of propionate (Fujishima et al., 2000). As indicated in Equation 6.7 (Snoeyink and Jenkins, 1980), for pH values lower than 9.3, the dominant part of total ammonia exists in ammonium form which is not inhibitory to the digestion processes.

Free ammonia ($NH_3$): $NH_4^+ \leftrightarrow NH_3 + H^+$ pKa=9.3 6.7.

According to the model indicated by Siegrist (2002) the inhibition expression ($I_{NH3}$) can be calculated by equation 6.8.

$$I_{NH\#} = \frac{K_{I,NH3}^{\ 2}}{K_{I,NH3}^{\ 2} + S_{NH3}^{\ 2}} \quad 6.8.$$

Where,

$$S_{NH3} = 10^{(pH-9.3)} \times TAN \quad 6.9.$$

$K_{I,\ NH3}$ is a constant equal to 25 g of $Nm^{-3}$ for anaerobic oxidation of propionate and equal to 17 g of $Nm^{-3}$ for acetotrophic methanogenesis. A 50% inhibition is reached if $S_{I,NH3}$ is equal to $K_{I,NH3}$ .

High concentrations of ammonia can potentially inhibit anaerobic oxidation of propionate, acetotrophic methanogenesis and hydrogenotrophic methanogenesis during anaerobic digestion of WAS. The ammonia inhibition expression for these steps was calculated. The summary of results for different possible pH and TAN values is presented in Table 6.1. The results show that ammonia may have caused a limited level of inhibition for these three important processes in BMP tests. It should be noted that inhibition of acetotrophic methanogenesis and hydrogenotrophic methanogenesis is not an issue in BAP tests since they were inhibited by BES addition. Fujishima et al. (2000) indicated that acclimation of digesters can decrease ammonia inhibition. In this particular study, the inhibition of this process in BAP and BMP tests could be less than the calculated values since the seed used for the tests had an ammonia concentration greater than 750 mg-N.$m^{-3}$ and probably was acclimated to this concentration of ammonia.

The theoretical estimation of inhibition showed that the rate of anaerobic oxidation of LCFAs and rate of anaerobic oxidation of propionate and butyrate may have been inhibited in the BAP tests. This issue should be considered when building a stoichiometric model and interpreting the results of BAP tests.

Figure 6.7 shows an example of the profile of individual VFAs generated in a BAP test (7 day SRT WAS with 5 min. sonication). From Figure 6.7 can be observed that concentrations of acetate, butyrate and propionate increased substantially with time. This revealed that the

uptake of these intermediate products was inhibited. The theoretical estimation of inhibition showed that this could be due to inhibitory levels of acetate and dissolved hydrogen in the BAP test (Table 2). The experiments showed that the concentrations of valerate were very low for many sludge samples. In some cases the concentration of valerate decreased after several days of digestion. These results indicated that the valerate oxidizing bacteria were not inhibited or were acclimated to elevated concentration of VFAs after several days in the BAP test. The concentration of acetate did not decrease in the BAP test confirming that the BES dose was sufficient to inhibit methanogenesis.

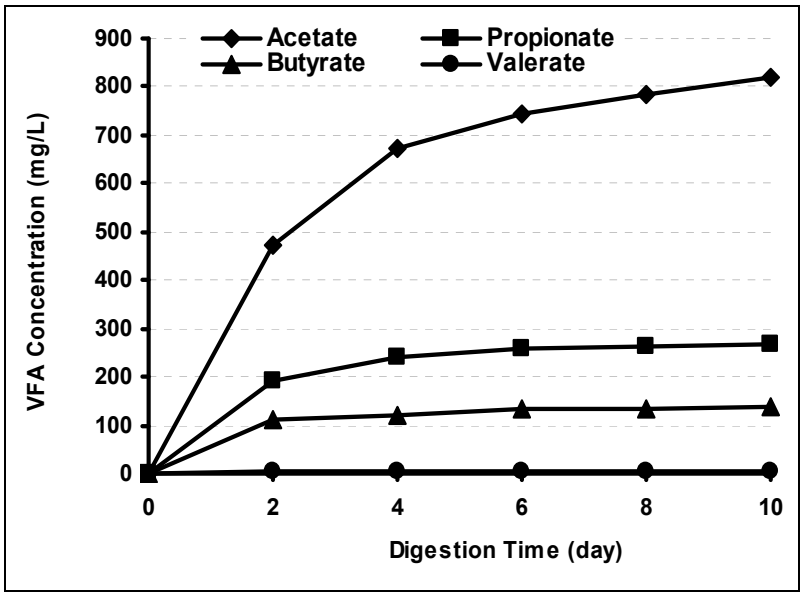


**Figure 6.7. Concentration of Individual VFAs in BAP test**

## 6.3.2.2. Stoichiometric model based on ADM1 defaults

The use of the ultimate methane responses in the BMP tests and VFA and ammonia responses in the BAP tests for substrate characterization by using a simplified stoichiometric model, such as a simplified ADM1, was of interest. Generally, the characterization of sludge by ADM1 is faced with several challenges due to the complexity of the processes and the variety of

components which were difficult to measure in this study. The digestion processes in the model that were substantially inhibited were eliminated from the model. For ultimate methane generation, it was assumed that all readily and slowly biodegradable COD would flow through the digestion processes and eventually appear as methane or non-biodegradable decay products regardless of their rate of biodegradation. According to this assumption, the reaction kinetics were eliminated to simplify the model to a stoichiometric model.

**Table 6.2. Parameters in ADM1 and recommended values**

| | Symbol | Description | Value | | Symbol | Description | Value |
|---|---|---|---|---|---|---|---|
| **Stoichiometric Parameters** | $^{*}f_{xi,xc}$ $+f_{si,xc}$ | Particulate inert+soluble inert from composites | Variable | **Stoichiometric Parameters** | $f_{bu,aa}$ | Butyrate from amino acids | 0.26 |
| | $^{*}f_{ch,xc}$ | Carbohydrates from composites | Variable | | $f_{pro,aa}$ | Propionate from amino acids | 0.05 |
| | $^{*}f_{pr,xc}$ | Proteins fraction from composites | Variable | | $f_{ac,aa}$ | Acetate from amino acids | 0.4 |
| | $^{*}f_{li,xc}$ | Lipids from composites | Variable | **Yield Parameters** Yield of biomass on … | $Y_{su}$ | Yield on sugar | 0.1 |
| | $f_{fa,li}$ | Fatty acids from lipids | 0.95 | | $Y_{aa}$ | Amino acids | 0.08 |
| | $f_{h2,su}$ | Hydrogen from sugars | 0.19 | | $Y_{fa}$ | Fatty acids | 0.06 |
| | $f_{bu,su}$ | Butyrate from sugars | 0.13 | | $Y_{c4+}$ | Butyrate/valerate | 0.06 |
| | $f_{pro,su}$ | propionate from sugars | 0.27 | | $Y_{pro}$ | Propionate | 0.04 |
| | $f_{ac,su}$ | Acetate from sugars | 0.41 | | $Y_{ac}$ | Acetate | 0.05 |
| | $f_{h2,aa}$ | Hydrogen from amino acids | 0.06 | | $Y_{h2}$ | Hydrogen | 0.06 |
| | $f_{va,aa}$ | Valerate from amino acids | 0.23 | | $^{*}N_{aa}$ | N in amino acids | 0.007 |

*-Parameters that were considered as variable were estimated by stoichiometric model.

The parameters involved in the pathway of anaerobic biodegradation of WAS are indicated in Table 6.2. The default values of parameters presented in ADM1 were employed. According to these values, the biomass was assumed to decay once on average during the digestion process and hence 65% of the biomass decay products were assumed to degrade. This means that all biomass growth that was calculated based on stoichiometry decayed and 65 percent of it was returned to the digestion process as biodegradable carbohydrates, proteins and lipids (20%, 20% and 25%). The flow of COD in the stoichiometric model showed that,

depending on the composition of WAS samples, about 5.3% to 10.4% of the TCOD of WAS was used for bacterial growth. Hence, the biodegradable fraction of the decay products was about 3.4% to 6.8% of TCOD. This fraction was added to the values of VFA and methane generation in the model. It should be noted that while adding the biodegradable fraction of biomass to the responses in the model improved the accuracy of the model, this correction had limited impact on the final results for sludge characterization.

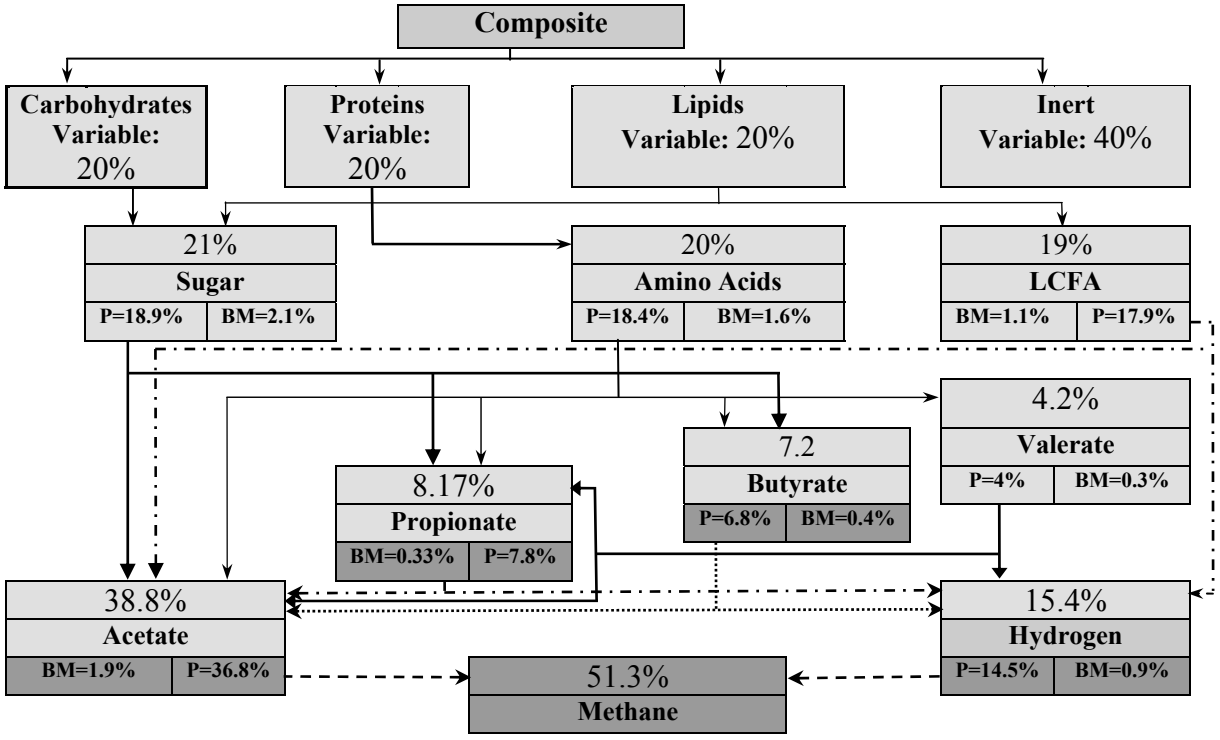


**Figure 6.8. Example of COD distribution in BMP tests**

Figure 6.8 shows the flow of COD through the digestion process as described by the default ADM1 model with the biodegradable carbohydrates, proteins and lipids each contributing 20% of the TCOD. The non-biodegradable COD was 40% of the TCOD in this example. The solid lines represent the pathways that are common between BMP and BAP tests. The dashed lines represent those reactions that occur in the BMP test which are inhibited in the

BAP test as discussed in Section 6.3.2.1. The accumulated value of each intermediate or final product in the BMP test is described in the related box in Figure 6.8. In this example 38.8% acetate was generated due to the six fermentation and oxidation processes mentioned in Figure 6.8. As a result of the acetoclastic methanogenesis process, acetate was converted to biomass (1.9% shown as BM) and methane as byproduct (36.8% shown as P). The values for the BAP test (not shown in Figure 6.8) can be calculated by subtracting the values attributed to inhibited processes.

VFA and ammonia generation in the BAP tests and methane generation in BMP tests were considered as experimental responses. VFA, ammonia and methane responses were divided by total COD to make the responses from different sludge samples comparable. In all cases, both VFA and ammonia concentrations essentially plateaued by day 10 of the BAP test. An empirical model based on first order reaction in a batch test was fit to the VFA profiles in the first 10 days to estimate the ultimate ammonia or VFA generation. The results revealed that the ultimate responses were 0.5 to 3.8 percent greater than the corresponding responses on day 10 of digestion. Further information about this empirical model has been described in Appendix E.

The best fit values for the COD fractions were determined as those that minimized the value of the objective function described in Equation 6.10.

$$Y=\frac{\left|U_{BMP}^{Meth}-U_{Model}^{Meth}\right|}{U_{BMP}^{Meth}}+\frac{\left|U_{BAP}^{Ammon}-U_{Model}^{Ammon}\right|}{U_{BAP}^{Ammon}}+\frac{\left|U_{BAP}^{TVFA}-U_{Model}^{TVFA}\right|}{U_{BAP}^{TVFA}}+\frac{\left|U_{BAP}^{Act}-U_{Model}^{Act}\right|+\left|U_{BAP}^{Pro}-U_{Model}^{Pro}\right|+\left|U_{BAP}^{But}-U_{Model}^{But}\right|+\left|U_{BAP}^{Val}-U_{Model}^{Val}\right|}{U_{BAP}^{TVFA}} \quad 6.10.$$

Where, Y is the summation of the absolute values of errors between BAP and BMP responses from model and those from experiments. $U_{BMP}^{Methane}$, $U_{BAP}^{Ammon}$, $U_{BAP}^{Acet}$, $U_{BAP}^{Prop}$, $U_{BAP}^{But}$and $U_{BAP}^{Val}$ are the values of methane, ammonia, acetate, propionate, butyrate and valerrate respectively in BMP or

BAP tests for each sample. $U_{Model}^{Methane}$, $U_{Model}^{Ammon}$, $U_{Model}^{Acet}$, $U_{Model}^{Prop}$, $U_{Model}^{But}$ and $U_{Model}^{Val}$ are the values of methane, ammonia, acetate, propionate, butyrate and valerrate respectively from the stoichiometric model for BMP or BAP tests for each sample. Every model response was a function of the COD fractions of the raw sludge. The fractionation of COD in the raw sludge ($f_{xi,xc}$, $f_{si,xc}$, $f_{ch,xc}$, $f_{pr,xc}$ and $f_{li,xc}$) will depend on the composition of sludge which was considered as variable during the fitting exercise. The summation of $f_{xi,xc}$ and $f_{si,xc}$ represents the non-biodegradable fraction of WAS. Using the data available, the sum of these two parameters could be estimated but they could not be identified separately.

#### 6.3.2.3. Identifiability of the model and measurements for each COD fraction

In the testing conducted in this study, $CH_4$ was measured using the offgas while VFAs were measured in the liquid phase. LCFAs were not measured and hence the accumulation of LCFAs in the BAP tests where their degradation was likely inhibited may explain the differing trends between the BAP VFA yield and BMP $CH_4$ yields. The simplified stoichiometric model was employed to characterize the end products that would be expected from the three major carbon sources (carbohydrates, proteins and lipids) when present alone. The simplified model was then employed to estimate the COD fractions that were present in the feed sludges based upon the results of the BAP and BMP tests.

The VFA and ammonia generation in BAP tests and methane generation in BMP tests for 1 unit of carbohydrate, protein and lipid are presented in Table 6.3. The results show that 83.6% and 72.9% of the COD would be detected in BMP and BAP tests when one unit of carbohydrate is applied as substrate; while 84.1% and 82.2% COD were predicted in the BMP and BAP tests from protein as a substrate. The model predicted 17.1% and 8.5% of TCOD would be the

generated hydrogen and not measured in the BAP test with carbohydrates and proteins as substrates respectively.

**Table 6.3. Estimated responses by simplified model for each type of composites**

| Composition of Sludge (1 unit of COD) | BAP test | | | | BMP test |
|---|---|---|---|---|---|
| | **VFA** (measured) | **Hydrogen** (not measured) | **LCFA** (not measured) | **Ammonia** (g ammon/ g COD) | **Methane** (measured) |
| **Carbohydrates** | 0.729 | 0.171 | 0 | -0.0034 * | 0.836 |
| **Proteins** | 0.822 | 0.085 | 0 | 0.1163 | 0.841 |
| **Lipids** | 0.036 | 0.009 | 0.95 | -0.0017 * | 0.887 |

*Negative ammonia values indicate the amount of ammonia nitrogen required when digesting 1 COD unit of a substrate.

While the results for carbohydrates and proteins reveal relatively small differences between the fractions that would be measured in the BAP and BMP tests, there were substantial differences for lipids due to the inhibition of LCFA oxidation pathway. Table 6.3 indicates that 88.7% and 3.6% of COD would be measured in the BMP and BAP tests when lipids are employed as substrate. As already mentioned in Table 6.1, the inhibition of anaerobic oxidation of LCFA (LCFA uptake) in BAP test may occur due to the presence of inhibitory levels of hydrogen gas and inhibitory concentration of accumulated acetate in batches. This results in the accumulation of LCFAs as intermediate products. The digestion of lipids results in the generation of LCFAs as much as 95% of TCOD in BAP tests that was not detected when VFAs were measured by ion chromatography.

According to the model, the digestion of 1 unit of protein eventually generates 0.116 g ammonia/g COD. It should be noted that bacteria required nitrogen for their growth and eventually a portion of nitrogen remains as non-biodegradable nitrogen in non biodegradable byproducts and decay products. The values of ammonia generation were negative for digestion of carbohydrates and lipids, since these substrates do not include nitrogen for growth of bacteria,

and the negative values show the amount of ammonia that is required in a digester as source of nitrogen for bacterial growth.

The deviation between BAP and BMP tests would depend on the relative contribution of carbohydrates, proteins and lipids to the COD in the raw sludge. The estimated values of carbohydrates ($f_{ch,xc}$), proteins ($f_{pr,xc}$) and lipids ($f_{li,xc}$) and non-biodegradable fractions ($f_{si} + f_{xi}$) versus the observed biodegradable fraction of TCOD are shown in Figure 6.9. The result showed that the total biodegradable fraction of the sludge varied from 33.6% to 83.5% of total COD.

The results reveal that proteins contributed a substantial fraction of biodegradable materials. In addition, an incremental increase of $f_{pr,xc}$ and $f_{ch,xc}$ was observed as the biodegradability of the sludge improved. The biodegradation of carbohydrates increased from 0% to 28.4% for sludges with biodegradable fraction greater than 40% and the value of $f_{ch,xc}$ was zero for sludges with biodegradable fraction lower than that 40%.

The value of $f_{li,xc}$ was about 7% for low levels of biodegradability and it decreased to zero for sludges with a biodegradability of 50% and higher. As shown in Table 6.3, 88.7% and 3.6% of COD would be detected in BMP and BAP tests respectively when lipids are employed as substrate hence lipids are not detected as VFAs in BAP tests and higher fraction of lipids in the sludge could result in greater differences between BAP and BMP results. As discussed earlier, a lower level of VFA generation was observed in BAP test as compared to methane generation in BMP tests when the biodegradability of the samples was smaller than 45% (Figure 6.1). A considerable part of this difference might be due to the presence of lipids in the composition of sludges with low level of biodegradability.

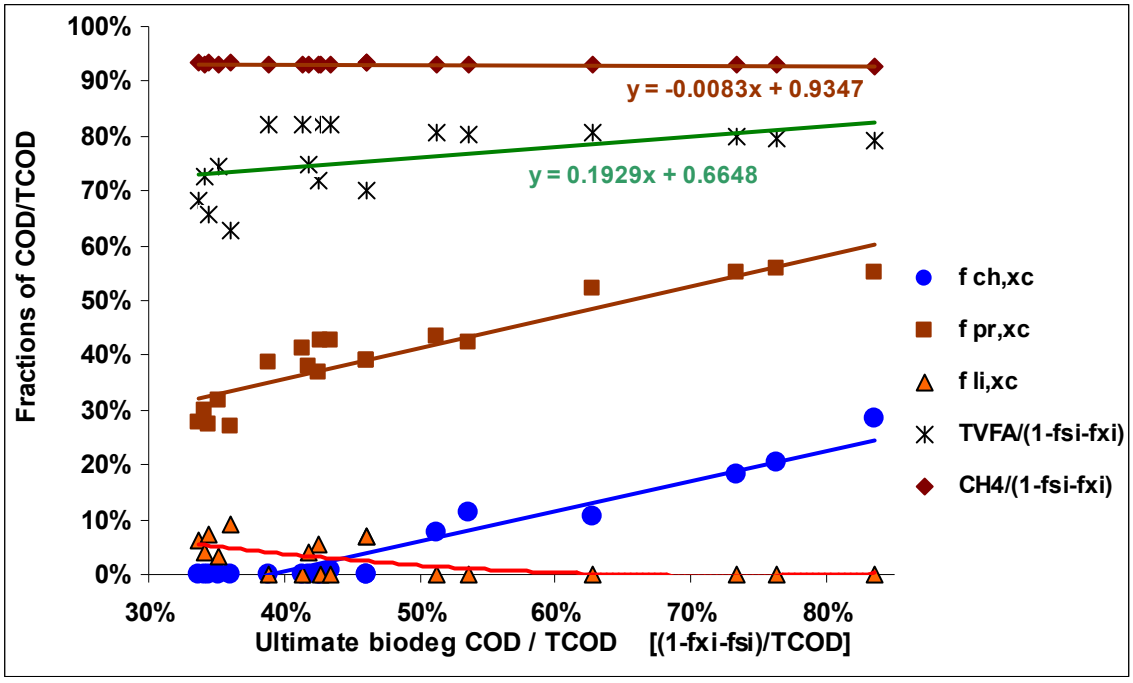


**Figure 6.9. COD fractionation versus biodegradable COD by simplified model**

From Figure 6.9, it can be observed that the ratio of ultimate methane generation to biodegradable fraction of TCOD in the BMP tests (shown in the figure as $CH_4$/(1-fsi-fxi)) was relatively constant for all raw and pretreated sludges regardless of the SRT of the samples or the pretreatment technology (mean and standard error of 0.931 and 0.0004 respectively). According to this result, ultimate methane generation described about 93.1% of the ultimate biodegradability. The difference between ultimate methane generation and ultimate biodegradability was due to the portion of biodegradable material that was converted into cellular mass and eventually resulted in non-biodegradable decay products.

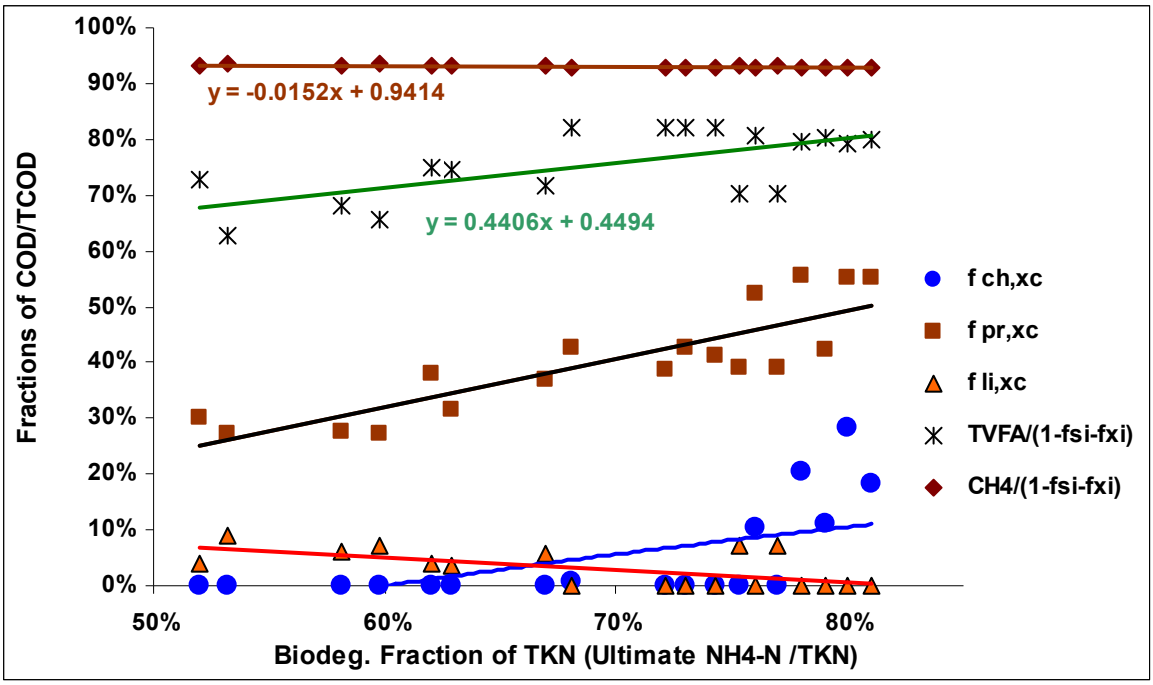


**Figure 6.10. COD fractionation versus biodegradable TKN by simplified model**

The estimated values of carbohydrates ($f_{ch,xc}$), proteins ($f_{pr,xc}$) and lipids ($f_{li,xc}$) and non-biodegradable fractions ($f_{si}$ + $f_{xi}$) versus biodegradable fraction of TKN (shown in the figure as $NH_4$-N/TKN) are shown in Figure 6.10. The result showed that the biodegradable fraction of the TKN varied from 53% to 81%. An increase of $f_{pr,xc}$ and $f_{ch,xc}$ was observed as the biodegradability of the sludge improved. The biodegradation of carbohydrates increased from 0% to 28.4% for sludges with biodegradable fraction greater than 60% and the value of $f_{ch,xc}$ was zero for sludges with biodegradable fraction lower than 60%. The value of $f_{li,xc}$ was about 7% for low levels of biodegradability and it decreased to zero for sludges with a biodegradability of 70% and higher.

The variation of the biodegradable fractions including proteins, carbohydrates and lipids with biodegradability of sludges was similar in both Figure 6.9 and Figure 6.10. In both figures, the fractions of protein and carbohydrates increased and the fraction of lipids decreased as

biodegradability of sludges increased. The slopes of lines attributed to carbohydrates, proteins and lipids fraction (0.57±0.06, 0.55±0.04 and -0.12±0.04) in Figure 6.9 were similar to the slopes of the lines in Figure 6.10 (0.87±0.13, 0.58±0.02 and -0.22±0.07) respectively; while the values of intercepts of the lines attributed to carbohydrates, proteins and lipids (-0.21±0.02, +0.13±0.03 and 0.09±0.02) in Figure 6.9 was different from the values (-0.35±0.13, -0.2±0.09 and 0.18±0.05) in Figure 6.10 respectively. According to the values of measured or estimated biodegradable fractions in chapter 4 and 5, the biodegradable fractions of proteinaceous material (based on TKN) was 17%±3%, 26%±2%, and 19%±2% greater than biodegradable fractions of all materials (based on COD) in 1.95, 7 and 15 day SRT sludges respectively. This might be the reason for the differences between the values of intercepts in Figure 6.9 and Figure 6.10.

Despite the relatively constant ratio of ultimate methane generation to biodegradable fraction of TCOD in the BMP tests, the ratio of total VFA generation to the biodegradable fraction of TCOD (shown in Figure 6.9 as TVFA/(1-fsi-fxi)) depended on the ultimate biodegradability of samples. The relationship between this ratio and ultimate biodegradability of samples has been mentioned in Equation 6.11.

$$U_{BAP}^{VFAs} = \mathbf{0.193 \times (1-f_{xi}-f_{si}) + 0.665} \qquad 6.11.$$

Figure 6.11 presents the methane generation in the BMP tests versus the VFA generation in BAP tests for sludges with a biodegradable fraction from 33.6% to 62.7% (ultimate methane generation from 31.4% to 58.2%). The ultimate methane generation in the BMP tests and TVFA generation in the BAP tests were estimated according to the estimated fraction of carbohydrates, proteins and lipids. It can be seen that the estimated values from the stoichiometric model were similar to the corresponding values from the experiments. This indicates that the model could

successfully identify the biodegradable and non-biodegradable composites in a wide range of raw and pretreated samples.

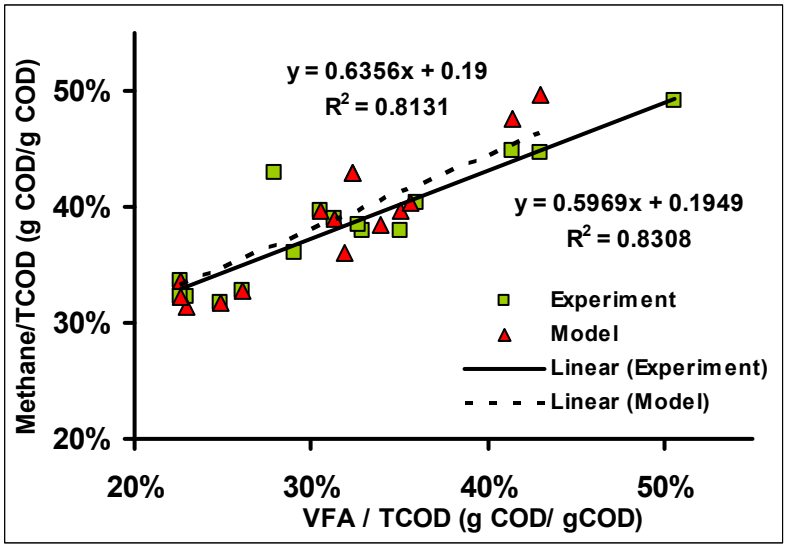


**Figure 6.11. Comparison of experimental and model results**

Preliminary modeling of the N values revealed that estimated $NH_3$-N values were highly sensitive to the value of $N_{aa}$ which has a recommended value is 0.007 in literature (Table 4). In this part of the investigation, $N_{aa}$ was considered as a variable parameter along with the variable stoichiometric parameters to find the best value of it for each specific WAS sample. This investigation could potentially reveal some information about the nitrogen to COD ratio in different sludges.

From Figure 6.12 it can be observed that $N_{aa}$ decreased as the biodegradability of the samples increased. This suggests that proteins in sludges with high biodegradability had smaller nitrogen to COD ratio. Such variation of $N_{aa}$ may be due to a change in the population and composition of bacterial from one operational condition to another. For example, the increase of SRT in a reactor causes a reduction of the F/M ratio. The scarcity of substrate in this condition can substantially reduce the population of bacteria present and subsequently change the

composition of decay products and byproducts such as EPS. The results of COD fractionation by this procedure (not shown here) were similar to the results presented in Figure 6.9 and Figure 6.10.

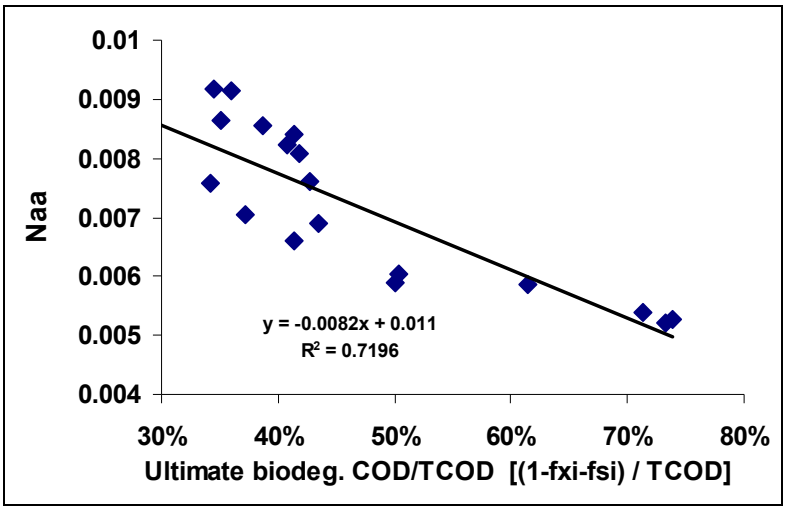


**Figure 6.12. Values of $N_{aa}$ as a variable parameter**

## 6.4. Conclusion

A set of preliminary experiments were conducted and a protocol was established for BAP test for digestion of WAS samples with regard to the best condition for mixing and dilution of samples, inhibitor concentration and the portion of inoculums. A comparison of the digestion responses such as ammonia generation showed that BAP test that was carried out according to the established protocol was valid for a wide range of raw and pretreated sludges.

The experimental results and theoretical estimation of inhibition suggested the anaerobic oxidation of LCFAs (LCFA uptake) was inhibited in the BAP tests. This inhibition resulted in a significant difference between COD attributed to accumulated VFA in BAP test and COD attributed to ultimate methane generation in BMP test. The comparison between VFA and ammonia responses in day 10 of BAP test and ultimate values of these responses after 60 days in

BMP test revealed linear relationships between these responses. According to these relationships, a set of models were introduced in this study. The models can be employed to predict the ultimate methane and ammonia generation using soluble COD, VFA or ammonia responses in day 10 of BAP tests. The BAP test appeared to be a good indicator of WAS digestibility and is much more cost effective than performing long term BMP tests.

Characterization of biodegradable and non-biodegradable material in WAS samples was conducted using a simplified ADM1 model. The characterization indicated that the significant difference between VFA in BAP tests and ultimate methane generation in BMP tests was due to the presence of lipids in the sludges. The characterization also revealed that proteins are a substantial fraction of biodegradable materials. The estimated ammonia, VFA and methane values from the stoichiometric model were similar to the corresponding values from the experiments. This supported the validity of the simplified model for all sludges employed in this study.

# Chapter 7

# 7. Conclusions

Pretreatment of waste activated sludges with ultrasound resulted in substantial reduction in particle size for sludges of differing solids residence time and for all sonication intensities examined; While ozonation of waste activated sludges resulted in only a slight reduction in particle size for all ozone doses examined. Particle size distribution only describes the distribution of particles that remain after treatment and do not quantify components that have been solubilized. Hence, particle size analysis did not provide insight into either the extent of solublization or biodegradability of WAS.

The VS, COD and soluble TKN responses indicated that a significant fraction of the WAS solids were solublized by sonication and ozonation, however, it appeared that the types of materials which were solublized was affected by the SRT at which the WAS was generated and the level of pretreatment. Sonication resulted in a greater solublization of solids, COD and TKN for WAS streams that were generated at longer SRTs while a greater fraction of rbCOD was generated from the 1.95 day SRT WAS as compared to the 7 and 15 day SRT WAS streams. Ozonation resulted in greater solublization of COD and TKN for WAS streams that were generated from the 1.95 day SRT WAS as compared to the 7 and 15 day SRT WAS streams. The results indicated an inconsistency between solublization indicators (VDS, fCOD, ffCOD and FTKN) and improvement in biodegradability of sludge. Hence, the impact of pretreatment on biodegradability of WAS was not described by solublization values exclusively without considering the SRT of the sludge and the level and type of pretreatment.

Both pretreatment technologies appeared to differentially affect the availability of carbohydrates and proteins depending upon the WAS SRT. A higher level of proteinaceous materials was preferentially solublized as the result of both sonication and ozonation pretreatment.

Respirometry revealed that sonication and ozonation substantially reduced the viable heterotrophs in the sludge. Respirometry that was employed to measure rbCOD in WAS revealed that the rbCOD fraction was increased modestly with sonication time for all sludges. The rbCOD fractions of the 1.95 day SRT sludges were consistently approximately twice the values of the 7 and 15 day SRT sludges. Unacclimated respirometry revealed a reduction in rbCOD when ozonation was employed and ozone dose increased. However, an acclimated respirometric approach revealed an increase in rbCOD with ozone dose. Hence, it was concluded that ozonation modifies the chemical composition of WAS such that biomass adaption is required before the biodegradable fraction can be established in short term tests.

The ultimate yields of $CH_4$ and $NH_4$ in BMP tests and VFAs in BAP tests revealed that sonication only marginally increased the ultimate digestibility of the sludges. A high dose of ozonation increased the digestibility of the 15 day SRT sludge substantially while ozonation marginally increased the ultimate digestibility of shorter SRT sludges. Both sonication and ozonation substantially increased the rate of hydrolysis which is the rate limiting process in WAS digestion. The relative trends in the rates of $NH_4$-N generation in the BMP tests and VFA generation in BAP tests versus pretreatment intensity differed for the sludges generated at differing SRTs.

The BMP test was not a useful test to evaluate the rate of methane generation due to inhibition of methanogens in the early days of BMP test for pretreated sludges. The BAP test is a shorter term test (10 days) than the BMP (55 to 60 days) test and could provide information on the rates of hydrolysis and acidification/ammonification processes.

A comparison between VFA and ammonia responses in day 10 of the BAP tests and ultimate values of these responses after 60 days in BMP test revealed that there are linear relationships between responses. According to these relationships, a set of models were introduced in this study. Statistical investigations support that models can be employed to predict the ultimate methane and ammonia generation using soluble COD, VFA or ammonia responses in day 10 of BAP tests. The BAP test appeared to be a good indicator of WAS digestibility and is much more cost effective than performing long term BMP tests.

The biodegradable and non-biodegradable materials in WAS samples were characterized using a simplified ADM1 model to understand the underlying cause of the linear relationships between responses. The characterization indicated that the difference between VFAs in BAP tests and ultimate methane generation in BMP tests was due to the presence of lipids in the sludges. A major fraction of the lipids is converted to LCFAs and accumulated in BAP test as a result of the inhibition of LCFAs uptake. The characterization also revealed that proteins are substantial fraction of biodegradable materials in all WAS samples.

## 7.1. Recommendations for Future Work

The results of this study indicated that simple batch tests can be employed to predict the impact of physical and chemical pretreatments on the ultimate biodegradability of sludges. However, it is recommended that bench or pilot scale continuous flow digesters be operated along with batch tests to further develop relationships that are capable of predicting the performance of full size digester. Such investigation could be employed to validate the results obtained in this study.

A set of models were introduced in this study according to the linear relationships between VFA and ammonia responses in day 10 of the BAP tests and ultimate values of these responses after 60 days in the BMP tests. The models can be employed to predict the ultimate methane and ammonia generation using soluble COD, VFA or ammonia responses in day 10 of BAP tests. The BAP test appeared to be a good indicator of WAS digestibility and is much more cost effective than performing long term BMP tests. However a considerable difference between COD attributed to accumulated VFA in BAP test and COD attributed to ultimate methane generation in BMP test was observed. This might be attributed to the lack of measurement of LCFAs that were accumulated due to the inhibition of anaerobic oxidation of LCFAs (LCFA uptake). It also could be due to the lack of measurement of hydrogen gas in the BAP tests. The investigation conducted in this study could theoretically estimate the level of accumulated VFA and hydrogen generated in BAP test for different sludge composition. However, repeating the BAP and BMP tests with the measurement of VFAs and hydrogen gas is recommended to validate the theoritical results and the models presented in this study.

This study suggested the models predicting the ultimate digestibility responses in BMP test using BAP test for raw and pretreated sludges by sonication and ozonation. Since sonication and ozonation are models of physical and chemical pretreatment technologies respectively, the models are expected to be applicable for other physical or chemical pretreatment technologies. Hence applying other physical or chemical pretreatment technologies is recommended to validate the correlation between BAP and BMP responses. Thermal pretreatment technologies can also be employed to investigate the feasibility of employing BAP tests for characterization of impact of a wide range of pretreated technologies on digestibility.

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# Appendix A

# Optimization of Conditions for Respirometry Tests

The details of the testing that was employed for identifying the optimal conditions for the respirometry test is explained as follow.

The optimal condition was evaluated for Wheaton media lab bottle (capacity 250 ml, 67 mm diameter, 152 mm height without cap and 33-430 screw cap size). A limitation in oxygen transfer from gas phase to liquid phase and limitation in absorption of the generated COD gas by the liquid surface of KOH column in the bottles was observed. Since a significant level of readily biodegradable COD (acetate) added in the respirometry bottles, conducting high concentration of sludge requires a great level of oxygen consumption and generated a higher level of COD generation in batch tests; while both the rate of oxygen transfer to liquid phase and absorption of generated COD was deemed to be limited to characteristics of respirometry apparatus such as the surface area of liquid phase (liquid surface area about 27 $cm^2$ before vortex) and KOH column (13 x 100mm Pyrex test tube with beaded rim). As shown in Figure A.1, high levels of uncertainty were observed when using the responses of respirometry tests consisting of low concentration of WAS. According to these preliminary experiments, the optimal range of sludge concentration in the media was found to be 50 to 160 mg COD/L.

In the preliminary experiment, peptone and proteins were added as source of carbon and nutrient. The respirometry responses revealed that as expected their biodegradation (hydrolysis) requited a considerable time while readily biodegradable source of carbon and nutrient was needed. Accordingly, acetate and a series of chemical were chosen to provide sources of energy, carbon and nutrient in the batch reactors.

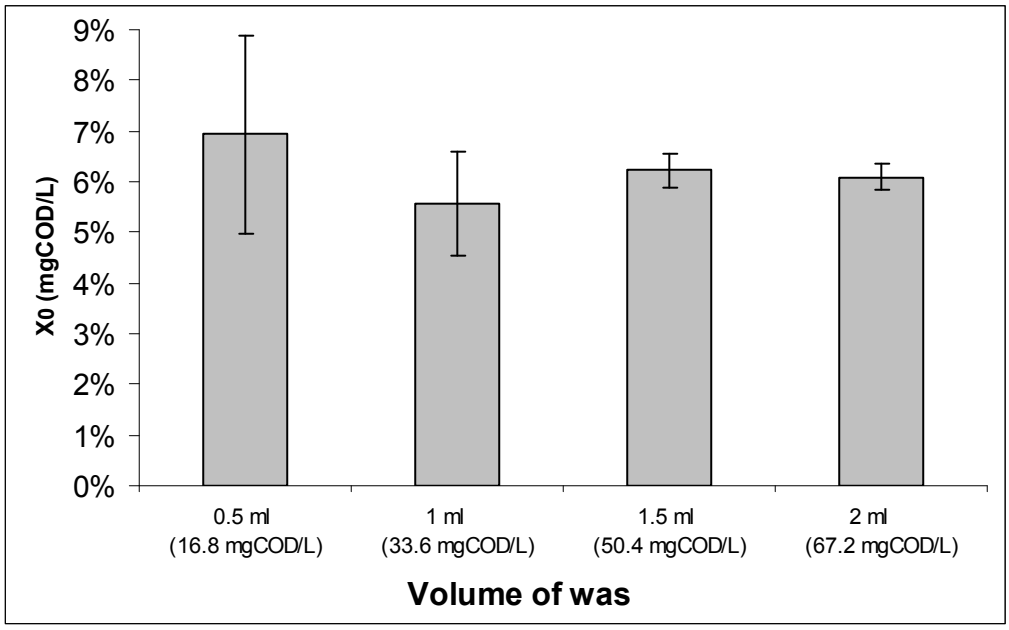


**Figure A.1. Estimation of $X_0$ for different volumes of sludge**

Preliminary experiments showed that the optimal concentration of acetate was 900 to 1100 mg/l. When low concentration of acetate was added as substrate, a short exponential portion in respirograms was observed that was not enough for estimation of $X_{HO}$ by curve fitting. High concentration of acetate results a flat response after exponential portion in respirograms. This was likely to be because of limitation of bacterial growth due to some limitation in oxygen transfer from gas phase to liquid phase while acetate was available as substrate in the batch reactors.

# Appendix B

# Optimization of Conditions for BAP Tests

In order to optimize the operational conditions for the BAP test, the total amount and rate of VFA generation were deemed to be the most important results. Methane or other gases may be produced in the BAP test and hence, gas testing was carried out to have information for completion of mass balances (with respect to COD). By conducting gas quality testing, the success of methanogenesis inhibition could be evaluated. It was deemed that in order to apply the BAP test as a reliable technique that provides a representative response of biodegradability, the test should be optimized for this application.

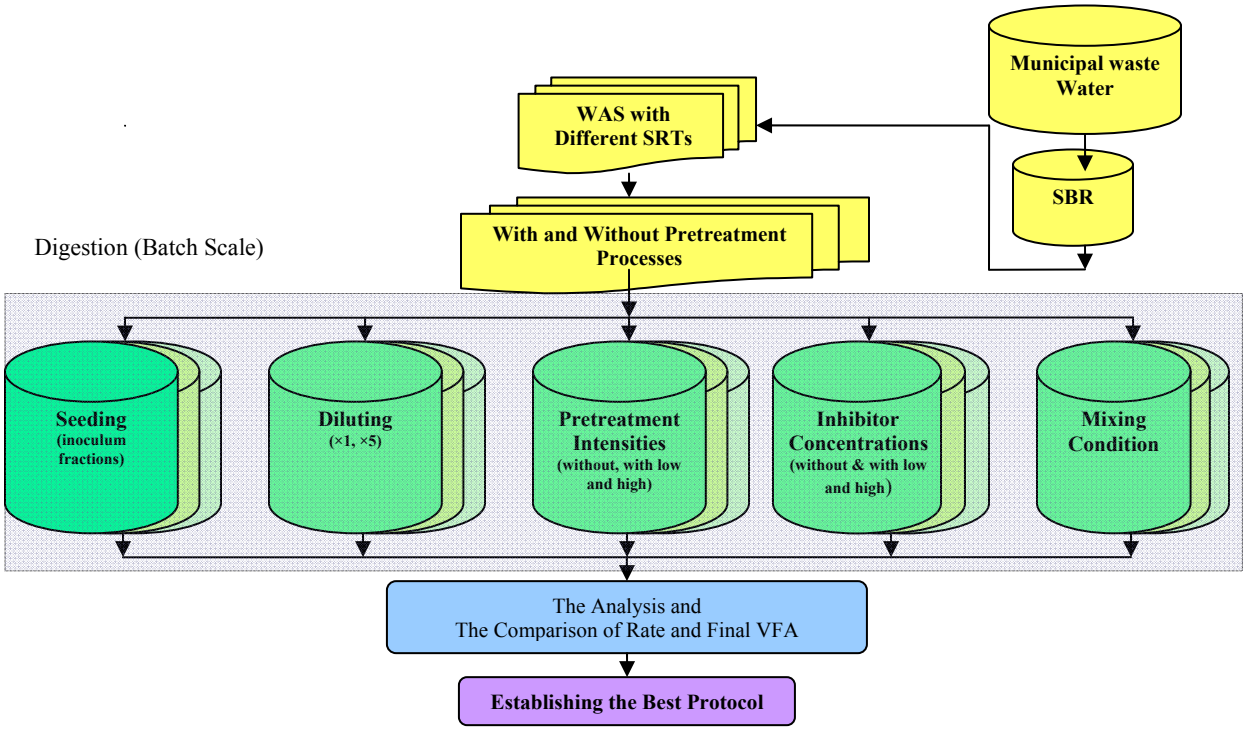


**Figure B.1. BAP Test Development Testing**

Figure A.1 depicts the plan that was employed for identifying the optimal conditions for the BAP test. The developed BAP test was employed in the pretreatment studies based on the frame work defined in this preliminary step.

Maintaining a vortex condition in the BAP bottles during incubation was recommended for wastewater samples (Martin Ruel et al., 2002) and was examined for WAS in this research. Therefore, BAP test bottles were incubated in a shaker incubator under vortex mixing condition and the results were compared to similar bottles in an incubator that were manually mixed once a day. As shown in Figure B.2, the results revealed that a similar level of VFA and methane generation (based on COD) was observed in manually mixed BAP and BMP test bottles respectively; While, in early days of digestion, the level of VFA generation in the BAP test under vortex mixing condition was greater than the responses in the BAP test without shaking. Hence, like the BMP tests, the BAP tests were conducted without shaking. This condition is desirable in terms of simplicity of operational conditions.

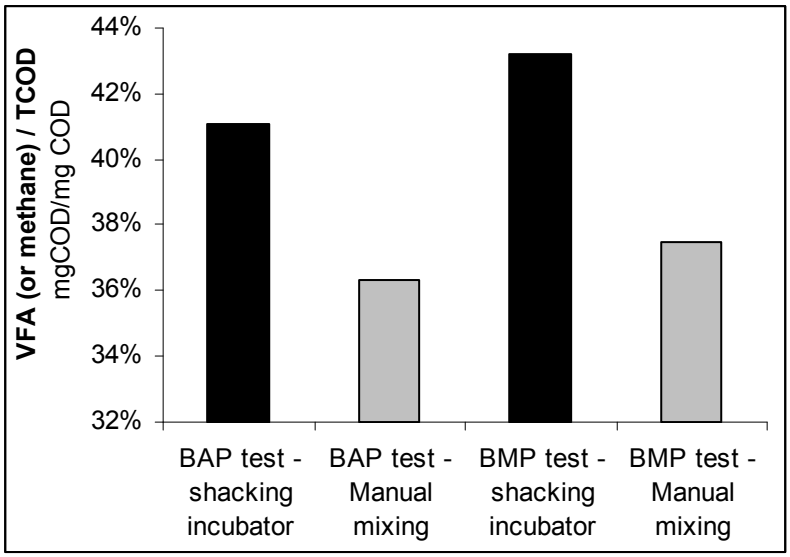


**Figure B.2. Impact of mixing condition on digestion process in BAP and BMP tests**

In order to inhibit acetoclastic methanogens, 1 mM of bromoethane sulfonate (BES) has been recommended in the literature for wastewater samples (Martin Ruel et al., 2002b). However, due to the higher availability of substrate as compared to wastewater, especially for pre-treated WAS, the required amount of inhibitor was examined for different levels of WAS pretreatment. In the testing, the recommended concentration (e.g. 0.001 M/L) of BES for wastewater samples did not completely inhibit methanogenesis in the early stages of digestion. As shown in Figure B.3, it was found that 0.003 M/L of BES was the optimum concentration of inhibitor required in the BAP test and methanogenesis was inhibited by this concentration of BES. It should be noted that a limited amount of methane was generated in early hours of digestion in BAP tests with 0.003 M/L of BES. This might be attributed to the time that is required to inhibit methanogens in seeds.

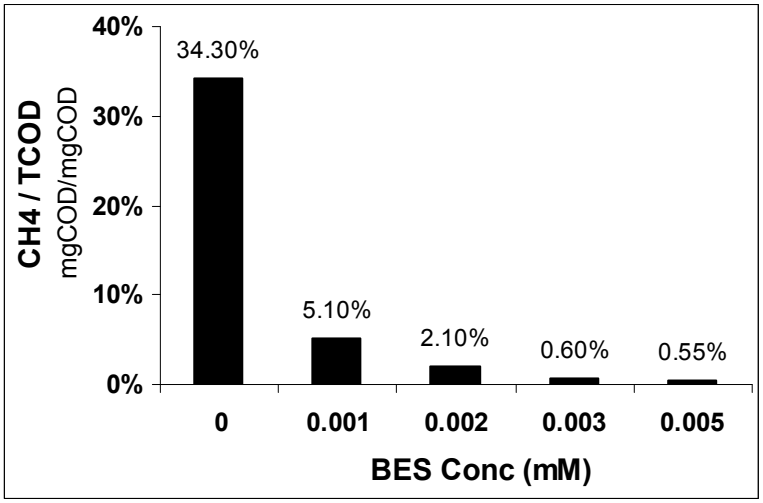


**Figure B.3. Impact of BES Concentration on methane generation**

The addition of BES could result in the accumulation of VFAs and a decrease in pH to an inhibitory level. Hence some samples were diluted to examine VFA generation to reduce inhibitory conditions. A significant improvement in VFA generation from diluted samples would indicate inhibition in the BAP test without dilution. A comparison between VFA generation in diluted and non-diluted WAS samples in BAP test revealed that the dilution of samples were not required for WAS samples.

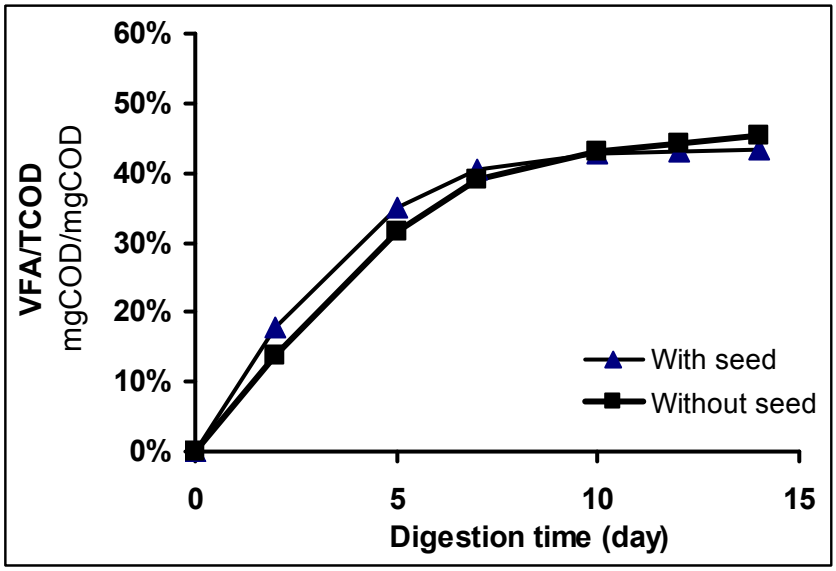


**Figure B.4. Impact of Seeding on VFA generation in BAP tests**

In a BMP test, methanogens are the most important bacteria that should be added to WAS because of their low rate of growth and their limited population in WAS. The inhibition of methanogens is required in the BAP test. Hence, inoculation in BAP tests may not be as necessary as in BMP tests. As shown in Figure B.4, BAP tests that were conducted for raw sludges with 50 ml of seed from a large scale digester and without seeding provided similar results after subtracting results attributed to the seed. The results indicated that the facultative bacteria in WAS can quickly start hydrolysis and acidification in anaerobic conditions. As shown

in figure 3.2, WAS under several pretreatment intensities should be digested in BAP test frame to evaluate digestion rates. In addition, widely acceptable operational conditions should be introduced for a wide level of pretreatment intensities. The experiments revealed that digestion process is relatively slower in BAP test without seeding as compared to the other for some pretreated WAS samples. This could be due to inactivation of bacteria by intensive pretreatment. In further BAP experiments, seeding was applied for consistency between BAP and BMP test and having comparable results for samples pretreated in a wide range of intensity.

# Appendix C:

# Results of Sonication Experiments:

Table C.1.Particles mean volume size for sonication

| Sample | | Pretreatment Intensity (min.) | Volume mean size | | | |
|---|---|---|---|---|---|---|
| SRT (day) | Pretreatment Type | | First replicate | Second replicate | Average | Std Error |
| 1.95 | Sonication | 0 | 241.4 | 287.4 | 264.4 | 23 |
| | | 5 | 102.7 | 138.7 | 120.7 | 18 |
| | | 10 | 104 | 126 | 115 | 11 |
| | | 25 | 109.3 | 117.3 | 113.3 | 4 |
| | | 40 | 99.3 | 123.3 | 111.3 | 12 |
| 7 | | 0 | 258.6 | 286.6 | 272.6 | 14 |
| | | 5 | 117.6 | 139.6 | 128.6 | 11 |
| | | 10 | 110.3 | 126.3 | 118.3 | 8 |
| | | 25 | 107.7 | 125.7 | 116.7 | 9 |
| | | 40 | 99.4 | 113.4 | 106.4 | 7 |
| 15 | | 0 | 250 | 284 | 267 | 17 |
| | | 5 | 109.4 | 125.4 | 117.4 | 8 |
| | | 10 | 107.3 | N/A | 107.3 | 0 |
| | | 25 | 97.2 | 109.2 | 103.2 | 6 |
| | | 40 | 90.4 | 110.4 | 100.4 | 10 |

Table C.2. Measurement of solid concentrations (run1).

| Sample (for 5 ml volume) | | WAS | | | filtered WAS (1. 5 µ) | | | VS (mg/L) | VSS (mg/L) | $VDS/VS_0$ (VS-VSS)/VS | Run |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SRT (day) | Pretreatment Type | Tare (g) | Dried (g) | Fired (g) | Tare (g) | Dried (g) | Fired (g) | | | | |
| 1.95 days | Raw | 2.5386 | 2.6468 | | | 1.4362 | 1.4270 | 8,460 | 9,200 | | Run 1 |
| | | 2.5380 | 2.6443 | 2.6028 | 1.4134 | 1.4362 | 1.4270 | 8,300 | 9,200 | 0% | |
| | 5 min Sonication | | | | | | | | | N/A | |
| | | | | | | | | | | | |
| | 10 min Sonication | 2.5369 | 2.6177 | 2.5762 | 1.4069 | 1.4187 | 1.4137 | 8,300 | 5,000 | | |
| | | 2.5547 | 2.6350 | 2.5942 | 1.4180 | 1.4317 | 1.4265 | 8,160 | 5,200 | 38.0% | |
| | 25 min Sonication | 2.5403 | 2.6020 | 2.5684 | 1.3989 | 1.4070 | 1.4030 | 6,720 | 4,000 | | |
| | | 2.5170 | 2.5780 | 2.5432 | 1.4263 | 1.4354 | 1.4310 | 6,960 | 4,400 | 38.6% | |
| | 45 min Sonication | 2.5296 | 2.6027 | 2.5627 | 1.4031 | 1.4126 | 1.4075 | 8,000 | 5,100 | | |
| | | 2.5504 | 2.6227 | 2.5826 | 1.4162 | 1.4264 | 1.4215 | 8,020 | 4,900 | 37.6% | |
| 7 days | Raw | 2.5337 | 2.6109 | 2.5630 | 1.4048 | 1.4217 | 1.4118 | 9,580 | 9,900 | | Run 1 |
| | | 2.5375 | 2.6144 | 2.5657 | 1.4092 | 1.4262 | 1.4165 | 9,740 | 9,700 | -1.4% | |
| | 5 min Sonication | 2.5353 | 2.6088 | 2.5632 | 1.4089 | 1.4219 | 1.4152 | 9,120 | 6,700 | | |
| | | 2.5585 | 2.6338 | 2.5868 | 1.4045 | 1.4174 | 1.4100 | 9,400 | 7,400 | 23.9% | |
| | 10 min Sonication | 2.5215 | 2.5874 | 2.5438 | 1.4055 | 1.4151 | 1.4103 | 8,720 | 4,800 | 42.8% | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2.5242 | 2.5903 | 2.5473 | 1.4036 | 1.4119 | 1.4068 | **8,600** | **5,100** | | |
| | 25 min Sonication | 2.5561 | 2.6233 | 2.5800 | 1.3981 | 1.4060 | 1.4016 | **8,660** | **4,400** | | |
| | | 2.5223 | 2.5929 | 2.5497 | 1.4101 | 1.4168 | 1.4129 | **8,640** | **3,900** | **52.0%** | |
| | 45 min Sonication | 2.5663 | 2.6288 | 2.5884 | 1.4043 | 1.4107 | 1.4073 | **8,080** | **3,400** | | |
| | | 2.5436 | 2.6065 | 2.5661 | 1.4096 | 1.4156 | 1.4123 | **8,080** | **3,300** | **58.5%** | |
| **15 days** | Raw | 2.5450 | 2.5935 | 2.5652 | 1.4120 | 1.4220 | 1.4162 | **5,660** | **5,800** | | **Run 1** |
| | | 2.5371 | 2.5839 | 2.5560 | 1.4208 | 1.4310 | 1.4254 | **5,580** | **5,600** | **-1.4%** | |
| | 5 min Sonication | 2.5552 | 2.5984 | 2.5709 | 1.3924 | 1.3993 | 1.3952 | **5,500** | **4,100** | | |
| | | 2.5565 | 2.5991 | 2.5720 | 1.3924 | 1.3993 | 1.3952 | **5,420** | **4,100** | **24.9%** | |
| | 10 min Sonication | 2.5280 | 2.5645 | 2.5397 | 1.4130 | 1.4172 | 1.4147 | **4,960** | **2,500** | | |
| | | 2.5410 | 2.5783 | 2.5536 | 1.4301 | 1.4350 | 1.4322 | **4,940** | **2,800** | **46.5%** | |
| | 25 min Sonication | 2.5283 | 2.5643 | 2.5397 | 1.3998 | 1.4038 | 1.4018 | **4,920** | **2,000** | | |
| | | 2.5470 | 2.5841 | 2.5589 | 1.4084 | 1.4119 | 1.4097 | **5,040** | **2,200** | **57.8%** | |
| | 45 min Sonication | 2.5303 | 2.5639 | 2.5419 | 1.4010 | 1.4036 | 1.4021 | **4,400** | **1,500** | | |
| | | 2.5253 | 2.5557 | 2.5342 | 1.4073 | 1.4109 | 1.4088 | **4,300** | **2,100** | **58.6%** | |

Table C.3. Summary of results of solid concentrations measurement (run1, run2 and run3).

| Sludge source | Pretreatment Intensity Duration (minute) | VDS / VS% | | | | |
|---|---|---|---|---|---|---|
| | | run1 | run2 | run3 | Avg | Std Error |
| SRT= 1.95 day | 0 | 0.0% | 1.1% | 17.2% | **6.1%** | 8.1% |
| | 5 | | 20.5% | 39.2% | **29.9%** | 9.3% |
| | 10 | 38.0% | 35.0% | 40.9% | **38.0%** | 1.7% |
| | 25 | 38.6% | 42.8% | 50.3% | **43.9%** | 3.4% |
| | 45 | 37.6% | 45.2% | 60.3% | **47.7%** | 6.7% |
| SRT= 7 day | 0 | -1.4% | -5.9% | 15.2% | **2.6%** | 6.4% |
| | 5 | 23.9% | 22.2% | 45.4% | **30.5%** | 7.5% |
| | 10 | 42.8% | 35.9% | 52.5% | **43.8%** | 4.8% |
| | 25 | 52.0% | 53.9% | 58.3% | **54.7%** | 1.8% |
| | 45 | 58.5% | 55.9% | 63.8% | **59.4%** | 2.3% |
| SRT= 15 day | 0 | -1.4% | 5.1% | 13.7% | **5.8%** | 4.4% |
| | 5 | 24.9% | 25.4% | 44.4% | **31.6%** | 6.4% |
| | 10 | 46.5% | 40.7% | 57.3% | **48.1%** | 4.9% |
| | 25 | 57.8% | 44.7% | 67.7% | **56.7%** | 6.7% |
| | 45 | 58.6% | 53.5% | 72.8% | **61.6%** | 5.8% |

Table C.4. Measurement of COD concentrations (run1, run2 and run3).

| Sample | Sonication time | Type | x Dilution | Abs@ 600nm | COD | COD (mg/L) | | $COD_{avg}$ x Dil | FCOD/TCOD & FFCOD/TCOD |
|---|---|---|---|---|---|---|---|---|---|
| Run1, T=1.95 day | 0 min | TCOD | 20 | 0.262 | 0.249 | 655.0 | 622.5 | 12775 | |
| | | FCOD | 10 | 0.012 | 0.013 | 30.0 | 32.5 | 313 | 2% |
| | | FFCOD | 10 | | | | | | |
| | 5 min | TCOD | 20 | | | | | | |
| | | FCOD | 10 | | | | | | |
| | | FFCOD | 10 | | | | | | |
| | 10 min | TCOD | 20 | 0.270 | 0.269 | 675.0 | 672.5 | 13475 | |
| | | FCOD | 10 | 0.230 | 0.226 | 575.0 | 565.0 | 5700 | 42% |
| | | FFCOD | 10 | | | | | | |
| | 25 min | TCOD | 20 | 0.253 | 0.260 | 632.5 | 650.0 | 12825 | |
| | | FCOD | 10 | 0.267 | 0.268 | 667.5 | 670.0 | 6688 | 52% |
| | | FFCOD | 10 | | | | | | |
| | 45 min | TCOD | 20 | 0.264 | 0.269 | 660.0 | 672.5 | 13325 | |
| | | FCOD | 10 | 0.274 | 0.275 | 685.0 | 687.5 | 6863 | 52% |
| | | FFCOD | 10 | | | | | | |
| Run1, SRT=7 day | 0 min | TCOD | 20 | 0.294 | 0.298 | 735.0 | 745.0 | 14800 | |
| | | FCOD | 10 | 0.012 | 0.008 | 30.0 | 20.0 | 250 | 2% |
| | | FFCOD | 10 | | | | | | |
| | 5 min | TCOD | 20 | 0.294 | 0.296 | 735.0 | 740.0 | 14750 | |
| | | FCOD | 10 | 0.210 | 0.206 | 525.0 | 515.0 | 5200 | 35% |
| | | FFCOD | 10 | | | | | | |
| | 10 min | TCOD | 20 | 0.314 | 0.283 | 785.0 | 707.5 | 14925 | |
| | | FCOD | 10 | 0.280 | 0.285 | 700.0 | 712.5 | 7063 | 47% |
| | | FFCOD | 10 | | | | | | |
| | 25 min | TCOD | 20 | 0.277 | 0.275 | 692.5 | 687.5 | 13800 | |
| | | FCOD | 10 | 0.326 | 0.324 | 815.0 | 810.0 | 8125 | 59% |
| | | FFCOD | 10 | | | | | | |
| | 45 min | TCOD | 20 | 0.259 | 0.254 | 647.5 | 635.0 | 12825 | |
| | | FCOD | 10 | 0.316 | 0.313 | 790.0 | 782.5 | 7863 | 61% |
| | | FFCOD | 10 | | | | | | |
| Run1, SRT=15 day | 0 min | TCOD | 20 | 0.169 | 0.155 | 422.5 | 387.5 | 8100 | |
| | | FCOD | 10 | 0.003 | 0.007 | 7.5 | 17.5 | 125 | 2% |
| | | FFCOD | 10 | | | | | | |
| | 5 min | TCOD | 20 | 0.162 | 0.168 | 405.0 | 420.0 | 8250 | |
| | | FCOD | 10 | 0.133 | 0.140 | 332.5 | 350.0 | 3413 | 41% |
| | | FFCOD | 10 | | | | | | |
| | 10 min | TCOD | 20 | 0.162 | 0.145 | 405.0 | 362.5 | 7675 | |
| | | FCOD | 10 | 0.161 | 0.175 | 402.5 | 437.5 | 4200 | 55% |
| | | FFCOD | 10 | | | | | | |
| | 25 min | TCOD | 20 | 0.152 | 0.151 | 380.0 | 377.5 | 7575 | |
| | | FCOD | 10 | 0.201 | 0.193 | 502.5 | 482.5 | 4925 | 65% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **FFCOD** | 10 | | | | | | |
| | **45 min** | **TCOD** | 20 | 0.131 | 0.135 | 327.5 | 337.5 | **6650** | |
| | | **FCOD** | 10 | 0.188 | 0.175 | 470.0 | 437.5 | **4538** | **68%** |
| | | **FFCOD** | 10 | | | | | | |
| **Run2, SRT=1.95 day** | **0 min** | **TCOD** | 20 | 0.177 | 0.167 | 442.5 | 417.5 | **8600** | |
| | | **FCOD** | 10 | 0.006 | 0.005 | 15.0 | 12.5 | **138** | **2%** |
| | | **FFCOD** | 10 | 0.002 | 0.003 | 5.0 | 7.5 | **63** | **1%** |
| | **5 min** | **TCOD** | 20 | 0.179 | 0.179 | 447.5 | 447.5 | **8950** | |
| | | **FCOD** | 10 | 0.096 | 0.093 | 240.0 | 232.5 | **2363** | **26%** |
| | | **FFCOD** | 10 | 0.046 | 0.043 | 115.0 | 107.5 | **1113** | **12%** |
| | **10 min** | **TCOD** | 20 | 0.183 | 0.183 | 457.5 | 457.5 | **9150** | |
| | | **FCOD** | 10 | 0.131 | 0.131 | 327.5 | 327.5 | **3275** | **36%** |
| | | **FFCOD** | 10 | 0.066 | 0.069 | 165.0 | 172.5 | **1688** | **18%** |
| | **25 min** | **TCOD** | 20 | 0.177 | 0.174 | 442.5 | 435.0 | **8775** | |
| | | **FCOD** | 10 | 0.146 | 0.148 | 365.0 | 370.0 | **3675** | **42%** |
| | | **FFCOD** | 10 | 0.068 | 0.065 | 170.0 | 162.5 | **1663** | **19%** |
| | **45 min** | **TCOD** | 20 | 0.172 | 0.169 | 430.0 | 422.5 | **8525** | |
| | | **FCOD** | 10 | 0.153 | 0.158 | 382.5 | 395.0 | **3888** | **46%** |
| | | **FFCOD** | 10 | 0.077 | 0.077 | 192.5 | 192.5 | **1925** | **23%** |
| **Run 2, SRT=7 day** | **0 min** | **TCOD** | 20 | 0.268 | 0.266 | 670.0 | 665.0 | **13350** | |
| | | **FCOD** | 10 | 0.010 | 0.005 | 25.0 | 12.5 | **188** | **1%** |
| | | **FFCOD** | 10 | 0.006 | 0.005 | 15.0 | 12.5 | **138** | **1%** |
| | **5 min** | **TCOD** | 20 | 0.282 | 0.282 | 705.0 | 705.0 | **14100** | |
| | | **FCOD** | 10 | 0.155 | 0.156 | 387.5 | 390.0 | **3888** | **28%** |
| | | **FFCOD** | 10 | 0.082 | 0.081 | 205.0 | 202.5 | **2038** | **14%** |
| | **10 min** | **TCOD** | 20 | 0.282 | 0.282 | 705.0 | 705.0 | **14100** | |
| | | **FCOD** | 10 | 0.193 | 0.196 | 482.5 | 490.0 | **4863** | **34%** |
| | | **FFCOD** | 10 | 0.125 | 0.121 | 312.5 | 302.5 | **3075** | **22%** |
| | **25 min** | **TCOD** | 20 | 0.262 | 0.261 | 655.0 | 652.5 | **13075** | |
| | | **FCOD** | 10 | 0.262 | 0.265 | 655.0 | 662.5 | **6588** | **50%** |
| | | **FFCOD** | 10 | 0.121 | 0.121 | 302.5 | 302.5 | **3025** | **23%** |
| | **45 min** | **TCOD** | 20 | 0.249 | 0.248 | 622.5 | 620.0 | **12425** | |
| | | **FCOD** | 10 | 0.263 | 0.268 | 657.5 | 670.0 | **6638** | **53%** |
| | | **FFCOD** | 10 | 0.129 | 0.129 | 322.5 | 322.5 | **3225** | **26%** |
| **Run 2, SRT=15 day** | **0 min** | **TCOD** | 20 | 0.124 | 0.130 | 310.0 | 325.0 | **6350** | |
| | | **FCOD** | 10 | 0.010 | 0.004 | 25.0 | 10.0 | **175** | **3%** |
| | | **FFCOD** | 10 | 0.006 | 0.008 | 15.0 | 20.0 | **175** | **3%** |
| | **5 min** | **TCOD** | 20 | 0.140 | 0.140 | 350.0 | 350.0 | **7000** | |
| | | **FCOD** | 10 | 0.081 | 0.081 | 202.5 | 202.5 | **2025** | **29%** |
| | | **FFCOD** | 10 | 0.043 | 0.043 | 107.5 | 107.5 | **1075** | **15%** |
| | **10 min** | **TCOD** | 20 | 0.121 | 0.121 | 302.5 | 302.5 | **6050** | |
| | | **FCOD** | 10 | 0.100 | 0.099 | 250.0 | 247.5 | **2488** | **41%** |
| | | **FFCOD** | 10 | 0.055 | 0.055 | 137.5 | 137.5 | **1375** | **23%** |
| | **25 min** | **TCOD** | 20 | 0.148 | 0.142 | 370.0 | 355.0 | **7250** | |
| | | **FCOD** | 10 | 0.155 | 0.157 | 387.5 | 392.5 | **3900** | **54%** |
| | | **FFCOD** | 10 | 0.073 | 0.074 | 182.5 | 185.0 | **1838** | **25%** |
| | **45 min** | **TCOD** | 20 | 0.142 | 0.137 | 355.0 | 342.5 | **6975** | |
| | | **FCOD** | 10 | 0.166 | 0.165 | 415.0 | 412.5 | **4138** | **59%** |
| | | **FFCOD** | 10 | 0.089 | 0.094 | 222.5 | 235.0 | **2288** | **33%** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SRT=1.95 d | 0 min | TCOD | 20 | 0.085 | 0.091 | 212.5 | 227.5 | 4400 | |
| | | FCOD | 10 | 0.000 | 0.000 | 0.0 | 0.0 | 0 | 0% |
| | | FFCOD | 10 | 0.000 | 0.000 | 0.0 | 0.0 | 0 | 0% |
| | 5 min | TCOD | 20 | 0.083 | 0.096 | 207.5 | 240.0 | 4475 | |
| | | FCOD | 10 | 0.050 | 0.052 | 125.0 | 130.0 | 1275 | 28% |
| | | FFCOD | 10 | 0.016 | 0.026 | 40.0 | 65.0 | 525 | 12% |
| | 10 min | TCOD | 20 | 0.102 | 0.100 | 255.0 | 250.0 | 5050 | |
| | | FCOD | 10 | 0.081 | 0.083 | 202.5 | 207.5 | 2050 | 41% |
| | | FFCOD | 10 | 0.033 | 0.035 | 82.5 | 87.5 | 850 | 17% |
| | 25 min | TCOD | 20 | 0.096 | 0.087 | 240.0 | 217.5 | 4575 | |
| | | FCOD | 10 | 0.087 | 0.082 | 217.5 | 205.0 | 2113 | 46% |
| | | FFCOD | 10 | 0.033 | 0.034 | 82.5 | 85.0 | 838 | 18% |
| | 45 min | TCOD | 20 | 0.106 | 0.104 | 265.0 | 260.0 | 5250 | |
| | | FCOD | 10 | 0.101 | 0.102 | 252.5 | 255.0 | 2538 | 48% |
| | | FFCOD | 10 | 0.045 | 0.044 | 112.5 | 110.0 | 1113 | 21% |
| Run 3, SRT=7 day | 0 min | TCOD | 20 | 0.183 | 0.184 | 457.5 | 460.0 | 9175 | |
| | | FCOD | 10 | 0.000 | 0.000 | 0.0 | 0.0 | 0 | 0% |
| | | FFCOD | 10 | 0.000 | 0.000 | 0.0 | 0.0 | 0 | 0% |
| | 5 min | TCOD | 20 | 0.173 | 0.174 | 432.5 | 435.0 | 8675 | |
| | | FCOD | 10 | 0.143 | 0.140 | 357.5 | 350.0 | 3538 | 41% |
| | | FFCOD | 10 | 0.069 | 0.069 | 172.5 | 172.5 | 1725 | 20% |
| | 10 min | TCOD | 20 | 0.172 | 0.164 | 430.0 | 410.0 | 8400 | |
| | | FCOD | 10 | 0.160 | 0.160 | 400.0 | 400.0 | 4000 | 48% |
| | | FFCOD | 10 | 0.084 | 0.081 | 210.0 | 202.5 | 2063 | 25% |
| | 25 min | TCOD | 20 | 0.170 | 0.165 | 425.0 | 412.5 | 8375 | |
| | | FCOD | 10 | 0.206 | 0.206 | 515.0 | 515.0 | 5150 | 61% |
| | | FFCOD | 10 | 0.094 | 0.091 | 235.0 | 227.5 | 2313 | 28% |
| | 45 min | TCOD | 20 | 0.175 | 0.175 | 437.5 | 437.5 | 8750 | |
| | | FCOD | 10 | 0.217 | 0.218 | 542.5 | 545.0 | 5438 | 62% |
| | | FFCOD | 10 | 0.104 | 0.104 | 260.0 | 260.0 | 2600 | 30% |
| Run 3, SRT=15 day | 0 min | TCOD | 20 | 0.111 | 0.104 | 277.5 | 260.0 | 5375 | |
| | | FCOD | 10 | 0.000 | 0.000 | 0.0 | 0.0 | 0 | 0% |
| | | FFCOD | 10 | 0.000 | 0.000 | 0.0 | 0.0 | 0 | 0% |
| | 5 min | TCOD | 20 | 0.119 | 0.121 | 297.5 | 302.5 | 6000 | |
| | | FCOD | 10 | 0.083 | 0.089 | 207.5 | 222.5 | 2150 | 36% |
| | | FFCOD | 10 | 0.042 | 0.042 | 105.0 | 105.0 | 1050 | 18% |
| | 10 min | TCOD | 20 | 0.113 | 0.117 | 282.5 | 292.5 | 5750 | |
| | | FCOD | 10 | 0.103 | 0.107 | 257.5 | 267.5 | 2625 | 46% |
| | | FFCOD | 10 | 0.053 | 0.055 | 132.5 | 137.5 | 1350 | 23% |
| | 25 min | TCOD | 20 | 0.106 | 0.103 | 265.0 | 257.5 | 5225 | |
| | | FCOD | 10 | 0.132 | 0.132 | 330.0 | 330.0 | 3300 | 63% |
| | | FFCOD | 10 | 0.062 | 0.053 | 155.0 | 132.5 | 1438 | 28% |
| | 45 min | TCOD | 20 | 0.090 | 0.092 | 225.0 | 230.0 | 4550 | |
| | | FCOD | 10 | 0.118 | 0.116 | 295.0 | 290.0 | 2925 | 64% |
| | | FFCOD | 10 | 0.052 | 0.054 | 130.0 | 135.0 | 1325 | 29% |

Table C.5. Summary of measurement of COD concentrations (run1, run2 and run3).

| Sludge source | Sonication time (min.) | FCOD/TCOD% | | | | | FFCOD/TCOD % | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Run1 | Run2 | Run3 | Avg | Std Error | Run2 | Run3 | Avg | Std Error |
| SRT= 1.95 day | 0 | 2% | 1.6% | 0.0% | **1.3%** | 0.7% | 0.7% | 0.0% | **0.4%** | 0.36% |
| | 5 | | 26.4% | 28.5% | **27.4%** | 1.0% | 12.4% | 11.7% | **12.1%** | 0.35% |
| | 10 | 42% | 35.8% | 40.6% | **39.6%** | 1.9% | 18.4% | 16.8% | **17.6%** | 0.81% |
| | 25 | 52% | 41.9% | 46.2% | **45.9%** | 3% | 18.9% | 18.3% | **18.6%** | 0.32% |
| | 45 | 52% | 45.6% | 48.3% | **48.5%** | 1.7% | 22.6% | 21.2% | **21.9%** | 0.70% |
| SRT= 7 day | 0 | 2% | 1.4% | 0.0% | **1.0%** | 0.5% | 1.0% | 0.0% | **0.5%** | 0.51% |
| | 5 | 35% | 27.6% | 40.8% | **34.5%** | 3.8% | 14.5% | 19.9% | **17.2%** | 2.72% |
| | 10 | 47% | 34.5% | 47.6% | **43.4%** | 4.3% | 21.8% | 24.6% | **23.2%** | 1.37% |
| | 25 | 59% | 50.4% | 61.5% | **56.9%** | 3.4% | 23.1% | 27.6% | **25.4%** | 2.24% |
| | 45 | 61% | 53.4% | 62.1% | **59.0%** | 2.8% | 26.0% | 29.7% | **27.8%** | 1.88% |
| SRT= 15 day | 0 | 2% | 2.8% | 0.0% | **1.4%** | 0.8% | 2.8% | 0.0% | **1.4%** | 1.38% |
| | 5 | 41% | 28.9% | 35.8% | **35.4%** | 3.6% | 15.4% | 17.5% | **16.4%** | 1.07% |
| | 10 | 55% | 41.1% | 45.7% | **47.2%** | 4.0% | 22.7% | 23.5% | **23.1%** | 0.38% |
| | 25 | 65% | 53.8% | 63.2% | **60.7%** | 3.5% | 25.3% | 27.5% | **26.4%** | 1.08% |
| | 45 | 68% | 59.3% | 64.3% | **63.9%** | 2.6% | 32.8% | 29.1% | **31.0%** | 1.84% |

Table C.6. Measurement of TKN concentrations (run1, run2 and run3).

| Sample | Sonication Time (min) | Type | x Dilution | Raw TKN data | | Average | Average of Blank Values | Raw TKN -Blank | Correction factor | Corrected TKN | FTKN/TTKN% | TTKN/TCOD% or FTKN/FCOD % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Run 1-SRT=1.95 d | 0 | TTKN | 100 | 9.889 | 9.889 | 9.889 | -0.095 | 9.98 | 1.195 | 835.5 | | 6.5% |
| | | FTKN | 100 | 0.477 | 0.477 | 0.477 | 0.058 | 0.42 | 1.195 | 35.1 | 4.2% | 11.2% |
| | 5 | TTKN | 100 | | | 0.000 | 0.000 | 0.00 | 1.195 | 0.0 | | |
| | | FTKN | 100 | | | 0.000 | 0.000 | 0.00 | 1.195 | 0.0 | | |
| | 10 | TTKN | 100 | 10.013 | 10.013 | 10.013 | -0.095 | 10.11 | 1.195 | 845.8 | | 6.3% |
| | | FTKN | 100 | 6.919 | 7.148 | 7.034 | 0.058 | 6.98 | 1.195 | 583.7 | 69.0% | 10.2% |
| | 25 | TTKN | 100 | 9.581 | 9.581 | 9.581 | -0.095 | 9.68 | 1.195 | 809.7 | | 6.3% |
| | | FTKN | 100 | 8.159 | 8.159 | 8.159 | 0.058 | 8.10 | 1.195 | 677.9 | 83.7% | 10.1% |
| | 45 | TTKN | 100 | 9.911 | 9.911 | 9.911 | -0.095 | 10.01 | 1.195 | 837.3 | | 6.3% |
| | | FTKN | 100 | 8.061 | 8.703 | 8.382 | 0.058 | 8.32 | 1.195 | 696.6 | 83.2% | 10.2% |
| Run 1-SRT=7 d | 0 | TTKN | 100 | 13.028 | 13.028 | 13.028 | -0.063 | 13.09 | 1.195 | 1095.5 | | 7.4% |
| | | FTKN | 100 | 0.525 | 0.525 | 0.525 | 0.251 | 0.27 | 1.195 | 22.9 | 2.1% | 9.2% |
| | 5 | TTKN | 100 | 12.581 | 12.581 | 12.581 | -0.063 | 12.64 | 1.195 | 1058.1 | | 7.2% |
| | | FTKN | 100 | 6.865 | 6.865 | 6.865 | 0.251 | 6.61 | 1.195 | 553.5 | 52.3% | 10.6% |
| | 10 | TTKN | 100 | 12.008 | 12.008 | 12.008 | -0.063 | 12.07 | 1.195 | 1010.1 | | 6.8% |
| | | FTKN | 100 | 9.274 | 9.555 | 9.415 | 0.251 | 9.16 | 1.195 | 766.8 | 75.9% | 10.9% |
| | 25 | TTKN | 100 | 12.126 | 12.126 | 12.126 | -0.063 | 12.19 | 1.195 | 1020.0 | | 7.4% |
| | | FTKN | 100 | 10.465 | 10.465 | 10.465 | 0.251 | 10.21 | 1.195 | 854.7 | 83.8% | 10.5% |
| | 45 | TTKN | 100 | 11.100 | 11.100 | 11.100 | -0.063 | 11.16 | 1.195 | 934.1 | | 7.3% |
| | | FTKN | 100 | 9.821 | 10.208 | 10.015 | 0.251 | 9.76 | 1.195 | 817.0 | 87.5% | 10.4% |
| Run 1-SRT=15 d | 0 | TTKN | 100 | 7.429 | 7.429 | 7.429 | -0.047 | 7.48 | 1.195 | 625.6 | | 7.7% |
| | | FTKN | 100 | 0.634 | 0.634 | 0.634 | 0.399 | 0.24 | 1.195 | 19.7 | 3.1% | 15.7% |
| | 5 | TTKN | 100 | 7.892 | 7.892 | 7.892 | -0.047 | 7.94 | 1.195 | 664.4 | | 8.1% |
| | | FTKN | 100 | 4.669 | 4.669 | 4.669 | 0.399 | 4.27 | 1.195 | 357.3 | 53.8% | 10.5% |
| | 10 | TTKN | 100 | 7.151 | 7.151 | 7.151 | -0.047 | 7.20 | 1.195 | 602.3 | | 7.8% |
| | | FTKN | 100 | 5.690 | 5.862 | 5.776 | 0.399 | 5.38 | 1.195 | 450.0 | 74.7% | 10.7% |
| | 25 | TTKN | 100 | 6.940 | 6.940 | 6.940 | -0.047 | 6.99 | 1.195 | 584.7 | | 7.7% |
| | | FTKN | 100 | 6.793 | 6.793 | 6.793 | 0.399 | 6.39 | 1.195 | 535.1 | 91.5% | 10.9% |
| | 45 | TTKN | 100 | 6.234 | 6.234 | 6.234 | -0.047 | 6.28 | 1.195 | 525.6 | | 7.9% |
| | | FTKN | 100 | 6.407 | 6.231 | 6.319 | 0.399 | 5.92 | 1.195 | 495.4 | 94.3% | 10.9% |
| Run 2-SRT=1.95 d | 0 | TTKN | 100 | 6.820 | 6.340 | 6.580 | -0.057 | 6.64 | 1.228 | 540.5 | | 6.3% |
| | | FTKN | 100 | 0.125 | 0.125 | 0.125 | -0.072 | 0.20 | 1.228 | 16.0 | 3.0% | 11.7% |
| | 5 | TTKN | 100 | 6.835 | 6.835 | 6.835 | -0.057 | 6.89 | 1.228 | 561.2 | | 6.3% |
| | | FTKN | 100 | 2.888 | 2.888 | 2.888 | -0.072 | 2.96 | 1.228 | 241.0 | 42.9% | 10.2% |
| | 10 | TTKN | 100 | 7.303 | 7.303 | 7.303 | -0.057 | 7.36 | 1.228 | 599.3 | | 6.6% |
| | | FTKN | 100 | 4.153 | 4.170 | 4.162 | -0.072 | 4.23 | 1.228 | 344.7 | 57.5% | 10.5% |
| | 25 | TTKN | 100 | 6.526 | 6.526 | 6.526 | -0.057 | 6.58 | 1.228 | 536.1 | | 6.1% |
| | | FTKN | 100 | 4.390 | 4.390 | 4.390 | -0.072 | 4.46 | 1.228 | 363.4 | 67.8% | 9.9% |
| | 45 | TTKN | 100 | 6.611 | 6.611 | 6.611 | -0.057 | 6.67 | 1.228 | 543.0 | | 6.4% |
| | | FTKN | 100 | 4.489 | 4.308 | 4.399 | -0.072 | 4.47 | 1.228 | 364.0 | 67.0% | 9.4% |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Run 2-SRT=7 d | 0 | TTKN | 100 | 11.556 | 11.316 | 11.436 | 0.000 | 11.44 | 1.228 | 931.3 | | 7.0% |
| | | FTKN | 100 | 0.002 | 0.002 | 0.002 | -0.057 | 0.06 | 1.228 | 4.8 | 0.5% | 2.6% |
| | 5 | TTKN | 100 | 11.711 | 11.711 | 11.711 | 0.000 | 11.71 | 1.228 | 953.7 | | 6.8% |
| | | FTKN | 100 | 5.146 | 5.146 | 5.146 | -0.057 | 5.20 | 1.228 | 423.7 | 44.4% | 10.9% |
| | 10 | TTKN | 100 | 10.813 | 10.513 | 10.663 | 0.000 | 10.66 | 1.228 | 868.3 | | 6.2% |
| | | FTKN | 100 | 5.972 | 6.191 | 6.082 | -0.057 | 6.14 | 1.228 | 499.9 | 57.6% | 10.3% |
| | 25 | TTKN | 100 | 10.459 | 10.459 | 10.459 | 0.000 | 10.46 | 1.228 | 851.7 | | 6.5% |
| | | FTKN | 100 | 7.595 | 7.595 | 7.595 | -0.057 | 7.65 | 1.228 | 623.1 | 73.2% | 9.5% |
| | 45 | TTKN | 100 | 10.420 | 10.423 | 10.422 | 0.000 | 10.42 | 1.228 | 848.7 | | 6.8% |
| | | FTKN | 100 | 7.984 | 7.982 | 7.983 | -0.057 | 8.04 | 1.228 | 654.7 | 77.1% | 9.9% |
| Run 2-SRT=15 d | 0 | TTKN | 100 | 6.050 | 6.435 | 6.243 | 0.018 | 6.22 | 1.228 | 506.9 | | 8.0% |
| | | FTKN | 100 | 0.020 | 0.020 | 0.020 | -0.024 | 0.04 | 1.228 | 3.6 | 0.7% | 2.0% |
| | 5 | TTKN | 100 | 6.420 | 6.420 | 6.420 | 0.018 | 6.40 | 1.228 | 521.3 | | 7.4% |
| | | FTKN | 100 | 2.827 | 2.827 | 2.827 | -0.024 | 2.85 | 1.228 | 232.2 | 44.5% | 11.5% |
| | 10 | TTKN | 100 | 5.757 | 5.836 | 5.797 | 0.018 | 5.78 | 1.228 | 470.6 | | 7.8% |
| | | FTKN | 100 | 3.384 | 3.384 | 3.384 | -0.024 | 3.41 | 1.228 | 277.5 | 59.0% | 11.2% |
| | 25 | TTKN | 100 | 6.768 | 6.768 | 6.768 | 0.018 | 6.75 | 1.228 | 549.7 | | 7.6% |
| | | FTKN | 100 | 5.050 | 5.050 | 5.050 | -0.024 | 5.07 | 1.228 | 413.2 | 75.2% | 10.6% |
| | 45 | TTKN | 100 | 6.467 | 6.755 | 6.611 | 0.018 | 6.59 | 1.228 | 536.9 | | 7.7% |
| | | FTKN | 100 | 5.324 | 5.324 | 5.324 | -0.024 | 5.35 | 1.228 | 435.5 | 81.1% | 10.5% |
| Run 3-SRT=1.95 d | 0 | TTKN | 100 | 3.440 | 3.440 | 3.440 | 0.097 | 3.34 | 1.222 | 273.6 | | 6.2% |
| | | FTKN | 100 | 0.265 | 0.125 | 0.195 | 0.047 | 0.15 | 1.161 | 12.7 | 4.7% | |
| | 5 | TTKN | 100 | 3.443 | 3.443 | 3.443 | 0.097 | 3.35 | 1.222 | 273.8 | | 6.1% |
| | | FTKN | 100 | 1.793 | 1.793 | 1.793 | 0.047 | 1.75 | 1.161 | 150.4 | 54.9% | 11.8% |
| | 10 | TTKN | 100 | 4.297 | 4.140 | 4.219 | 0.097 | 4.12 | 1.222 | 337.3 | | 6.7% |
| | | FTKN | 100 | 2.560 | 2.524 | 2.542 | 0.047 | 2.50 | 1.161 | 214.9 | 63.7% | 10.5% |
| | 25 | TTKN | 100 | 3.580 | 3.580 | 3.580 | 0.097 | 3.48 | 1.222 | 285.0 | | 6.2% |
| | | FTKN | 100 | 2.543 | 2.543 | 2.543 | 0.047 | 2.50 | 1.161 | 215.0 | 75.4% | 10.2% |
| | 45 | TTKN | 100 | 4.146 | 4.226 | 4.186 | 0.097 | 4.09 | 1.222 | 334.6 | | 6.4% |
| | | FTKN | 100 | 3.126 | 3.134 | 3.130 | 0.047 | 3.08 | 1.161 | 265.5 | 79.4% | 10.5% |
| Run 3-SRT=7 d | 0 | TTKN | 100 | 8.662 | 8.662 | 8.662 | 0.159 | 8.50 | 1.222 | 695.8 | | 7.6% |
| | | FTKN | 100 | 0.214 | 0.214 | 0.214 | 0.107 | 0.11 | 1.161 | 9.2 | 1.3% | |
| | 5 | TTKN | 100 | 8.804 | 8.804 | 8.804 | 0.159 | 8.65 | 1.222 | 707.4 | | 8.2% |
| | | FTKN | 100 | 4.782 | 4.782 | 4.782 | 0.107 | 4.68 | 1.161 | 402.7 | 56.9% | 11.4% |
| | 10 | TTKN | 100 | 8.321 | 8.321 | 8.321 | 0.159 | 8.16 | 1.222 | 667.9 | | 8.0% |
| | | FTKN | 100 | 4.937 | 4.916 | 4.927 | 0.107 | 4.82 | 1.161 | 415.1 | 62.2% | 10.4% |
| | 25 | TTKN | 100 | 7.878 | 7.878 | 7.878 | 0.159 | 7.72 | 1.222 | 631.7 | | 7.5% |
| | | FTKN | 100 | 6.007 | 6.007 | 6.007 | 0.107 | 5.90 | 1.161 | 508.2 | 80.5% | 9.9% |
| | 45 | TTKN | 100 | 8.136 | 8.136 | 8.136 | 0.159 | 7.98 | 1.222 | 652.8 | | 7.5% |
| | | FTKN | 100 | 6.512 | 6.409 | 6.461 | 0.107 | 6.35 | 1.161 | 547.2 | 83.8% | 10.1% |
| Run 1-SRT=15 d | 0 | TTKN | 100 | 5.520 | 5.520 | 5.520 | 0.198 | 5.32 | 1.222 | 435.5 | | 8.1% |
| | | FTKN | 100 | 0.254 | 0.254 | 0.254 | 0.184 | 0.07 | 1.161 | 6.0 | 1.4% | |
| | 5 | TTKN | 100 | 5.649 | 5.649 | 5.649 | 0.198 | 5.45 | 1.222 | 446.1 | | 7.4% |
| | | FTKN | 100 | 2.891 | 2.891 | 2.891 | 0.184 | 2.71 | 1.161 | 233.2 | 52.3% | 10.8% |
| | 10 | TTKN | 100 | 6.347 | 6.347 | 6.347 | 0.198 | 6.15 | 1.222 | 503.2 | | 8.8% |
| | | FTKN | 100 | 3.576 | 3.539 | 3.558 | 0.184 | 3.37 | 1.161 | 290.6 | 57.7% | 11.1% |
| | 25 | TTKN | 100 | 5.478 | 5.478 | 5.478 | 0.198 | 5.28 | 1.222 | 432.1 | | 8.3% |
| | | FTKN | 100 | 3.875 | 3.875 | 3.875 | 0.184 | 3.69 | 1.161 | 317.9 | 73.6% | 9.6% |
| | 45 | TTKN | 100 | 4.821 | 4.871 | 4.846 | 0.198 | 4.65 | 1.222 | 380.4 | | 8.4% |
| | | FTKN | 100 | 4.346 | 4.343 | 4.345 | 0.184 | 4.16 | 1.161 | 358.4 | 94.2% | 12.3% |

Table C.7. Calculation of average and error for FTKN/ TKN values (run1, run2 and run3).

| Sludge source | Pretreatment Intensity Duration (minute) | FTKN/TKN% | | | | |
|---|---|---|---|---|---|---|
| | | run1 | run2 | run3 | Avg | Std Error |
| SRT= 1.95 day | 0 | 4% | 3.0% | 4.7% | **3.9%** | 0.5% |
| | 5 | | 42.9% | 54.9% | **48.9%** | 6.0% |
| | 10 | 69% | 57.5% | 63.7% | **63.4%** | 3.3% |
| | 25 | 84% | 67.8% | 75.4% | **75.6%** | 4.6% |
| | 45 | 83% | 67.0% | 79.4% | **76.5%** | 4.9% |
| SRT= 7 day | 0 | 2% | 0.5% | 1.3% | **1.3%** | 0.5% |
| | 5 | 52% | 44.4% | 56.9% | **51.2%** | 3.6% |
| | 10 | 76% | 57.6% | 62.2% | **65.2%** | 5.5% |
| | 25 | 84% | 73.2% | 80.5% | **79.1%** | 3.1% |
| | 45 | 87% | 77.1% | 83.8% | **82.8%** | 3.0% |
| SRT= 15 day | 0 | 3% | 0.7% | 1.4% | **1.7%** | 0.7% |
| | 5 | 54% | 44.5% | 52.3% | **50.2%** | 2.9% |
| | 10 | 75% | 59.0% | 57.7% | **63.8%** | 5.5% |
| | 25 | 92% | 75.2% | 73.6% | **80.1%** | 5.7% |
| | 45 | 94% | 81.1% | 94.2% | **89.9%** | 4.4% |

Table C.8. Calculation of average and error for TKN/ TCOD values (run1, run2 and run3).

| Sludge source | Pretreatment Intensity Duration (minute) | TKN/TCOD% | | | | |
|---|---|---|---|---|---|---|
| | | run1 | run2 | run3 | Avg | Std Error |
| SRT= 1.95 day | 0 | 7% | 6.3% | 6.2% | **6.3%** | 0.1% |
| | 5 | | 6.3% | 6.1% | **6.2%** | 0.1% |
| | 10 | 6% | 6.6% | 6.7% | **6.5%** | 0.1% |
| | 25 | 6% | 6.1% | 6.2% | **6.2%** | 0.1% |
| | 45 | 6% | 6.4% | 6.4% | **6.3%** | 0.0% |
| SRT= 7 day | 0 | 7% | 7.0% | 7.6% | **7.3%** | 0.2% |
| | 5 | 7% | 6.8% | 8.2% | **7.4%** | 0.4% |
| | 10 | 7% | 6.2% | 8.0% | **7.0%** | 0.5% |
| | 25 | 7% | 6.5% | 7.5% | **7.1%** | 0.3% |
| | 45 | 7% | 6.8% | 7.5% | **7.2%** | 0.2% |
| SRT= 15 day | 0 | 8% | 8.0% | 8.1% | **7.9%** | 0.1% |
| | 5 | 8% | 7.8% | 8.8% | **8.1%** | 0.3% |
| | 10 | 8% | 7.6% | 8.3% | **7.9%** | 0.2% |
| | 25 | 8% | 7.7% | 8.4% | **8.0%** | 0.2% |
| | 45 | 7% | 6.3% | 6.2% | **6.3%** | 0.1% |

Table C.9. Calculation of average and error for FTKN/ FCOD values (run1, run2 and run3).

| Sludge source | Pretreatment Intensity Duration (minute) | FTKN/FCOD% | | | | |
|---|---|---|---|---|---|---|
| | | run1 | run2 | run3 | Avg | Std Error |
| SRT= 1.95 day | 0 | 11.2% | 11.7% | | **11.4%** | 0.2% |
| | 5 | | 10.2% | 11.8% | **11.0%** | 0.8% |
| | 10 | 10.2% | 10.5% | 10.5% | **10.4%** | 0.1% |
| | 25 | 10.1% | 9.9% | 10.2% | **10.1%** | 0.1% |
| | 45 | 10.2% | 9.4% | 10.5% | **10.0%** | 0.3% |
| SRT= 7 day | 0 | 9.2% | 2.6% | | **5.9%** | 3.3% |
| | 5 | 10.6% | 10.9% | 11.4% | **11.0%** | 0.2% |
| | 10 | 10.9% | 10.3% | 10.4% | **10.5%** | 0.2% |
| | 25 | 10.5% | 9.5% | 9.9% | **9.9%** | 0.3% |
| | 45 | 10.4% | 9.9% | 10.1% | **10.1%** | 0.2% |
| SRT= 15 day | 0 | 15.7% | 2.0% | | **8.9%** | 6.8% |
| | 5 | 10.5% | 11.5% | 10.8% | **10.9%** | 0.3% |
| | 10 | 10.7% | 11.2% | 11.1% | **11.0%** | 0.1% |
| | 25 | 10.9% | 10.6% | 9.6% | **10.4%** | 0.4% |
| | 45 | 10.9% | 10.5% | 12.3% | **11.2%** | 0.5% |

Table C.10. Anaerobic digestion batch test, 1.95 day SRT WAS, Methane generation

| Sample | Day | Sludge Vol.(ml) | Gas production | Comul. Gas Prod. | CH4 fraction% | Accumulated CH4 | Corrected CH4 | TCOD mg/l | Accumulated $CH_4$/TCOD% |
|---|---|---|---|---|---|---|---|---|---|
| SRT=1.95d-Raw | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 5250 | 0% |
| | 1 | 280 | 78.0 | 78.0 | 12.2 | 41.2 | 136.17 | | 4% |
| | 2 | 280 | 76.0 | 154.0 | 24.4 | 91.6 | 314.31 | | 9% |
| | 4 | 280 | 101.0 | 255.0 | 38.3 | 166.3 | 595.9 | | 16% |
| | 6 | 280 | 92.0 | 347.0 | 52.7 | 252.3 | 924.87 | | 25% |
| | 11 | 280 | 171.0 | 518.0 | 60.7 | 376.7 | 1401.2 | | 38% |
| | 16 | 280 | 68.0 | 586.0 | 60.3 | 416.8 | 1545.03 | | 42% |
| | 23 | 280 | 39.0 | 625.0 | 59.4 | 437.5 | 1614.5 | | 44% |
| | 30 | 280 | 22.0 | 647.0 | 58.6 | 448.3 | 1650.89 | | 45% |
| | 46 | 280 | 13.0 | 660.0 | 58.6 | 455.9 | 1668.95 | | 45% |
| SRT=1.95d- 5 min. sonication | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 5250 | 0% |
| | 1 | 280 | 82.0 | 82.0 | 11.5 | 39.3 | 128.54 | | 4% |
| | 2 | 280 | 72.0 | 154.0 | 22.3 | 83.3 | 281.10 | | 8% |
| | 4 | 280 | 77.0 | 231.0 | 33.5 | 138.4 | 484.04 | | 13% |
| | 6 | 280 | 47.0 | 278.0 | 44.5 | 187.8 | 666.9 | | 18% |
| | 11 | 280 | 184.0 | 462.0 | 56.5 | 323.0 | 1186.51 | | 32% |
| | 16 | 280 | 147.0 | 609.0 | 61.1 | 424.6 | 1576.36 | | 43% |
| | 23 | 280 | 41.0 | 650.0 | 61.1 | 449.6 | 1662.96 | | 45% |
| | 30 | 280 | 27.0 | 677.0 | 59.4 | 461.5 | 1703.5 | | 46% |
| | 46 | 280 | 16.0 | 693.0 | 59.4 | 471.0 | 1729.14 | | 47% |
| SRT=1.95 d -10 min. sonication | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 5250 | 0% |
| | 1 | 280 | 78.0 | 78.0 | 11.2 | 37.9 | 122.65 | | 3% |
| | 2 | 280 | 73.0 | 151.0 | 21.2 | 79.4 | 265.36 | | 7% |
| | 4 | 280 | 81.0 | 232.0 | 33.2 | 137.5 | 480.66 | | 13% |
| | 6 | 280 | 61.0 | 293.0 | 44.7 | 194.5 | 693.78 | | 19% |
| | 11 | 280 | 186.0 | 479.0 | 56.8 | 331.5 | 1220.56 | | 33% |
| | 16 | 280 | 138.0 | 617.0 | 61.6 | 429.0 | 1594.17 | | 43% |
| | 23 | 280 | 41.0 | 658.0 | 60.7 | 451.5 | 1670.44 | | 45% |
| | 30 | 280 | 25.0 | 683.0 | 58.73 | 461.2 | 1702.43 | | 46% |
| | 46 | 280 | 18.0 | 701.0 | 58.73 | 471.8 | 1732.32 | | 47% |
| SRT=1.95 d-25 min. sonication | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 5250 | 0% |
| | 1 | 280 | 82 | 82.0 | 11.0 | 37.6 | 121.76 | | 3% |
| | 2 | 280 | 76 | 158.0 | 20.2 | 77.0 | 255.61 | | 7% |
| | 4 | 280 | 90 | 248.0 | 32.7 | 138.8 | 485.98 | | 13% |
| | 6 | 280 | 69 | 317.0 | 45.9 | 205.0 | 735.64 | | 20% |
| | 11 | 280 | 215 | 532.0 | 57.0 | 356.2 | 1319.33 | | 36% |
| | 16 | 280 | 137 | 669.0 | 61.1 | 450.7 | 1680.78 | | 46% |
| | 23 | 280 | 37 | 706.0 | 60.3 | 470.9 | 1747.97 | | 48% |
| | 30 | 280 | 24 | 730.0 | 59.91 | 484.2 | 1794.61 | | 49% |
| | 46 | 280 | 20 | 750.0 | 59.91 | 496.2 | 1830.14 | | 50% |
| Seed (control)- SRT= 1.95 d | 0 | 280 | 0 | 0 | 0.00 | 0 | N/A | | |
| | 1 | 280 | 113 | 113.0 | 18.00 | 67.1 | | | |
| | 2 | 280 | 69 | 182.0 | 30.86 | 121.9 | | | |
| | 4 | 280 | 61 | 243.0 | 37.45 | 161.8 | | | |
| | 6 | 280 | 41 | 284.0 | 43.85 | 196.5 | | | |
| | 11 | 280 | 81 | 365.0 | 48.04 | 246.3 | | | |
| | 16 | 280 | 58 | 423.0 | 51.34 | 284.6 | | | |
| | 23 | 280 | 50 | 473.0 | 53.24 | 316.2 | | | |
| | 30 | 280 | 44.0 | 517.0 | 50.76 | 332.1 | | | |
| | 46 | 280 | 57.0 | 574.0 | 50.76 | 361.0 | | | |

Table C.11. Anaerobic digestion batch test, 7 day SRT WAS, Methane generation

| Sample | Day | Sludge Vol.(ml) | Gas production | Comul. Gas Prod. | CH4 fraction% | Accumulated CH4 | Corrected CH4 | TCOD mg/l | Accumulated $CH_4$/TCOD% |
|---|---|---|---|---|---|---|---|---|---|
| SRT=7d -Raw | 0 | 280 | 0 | 0 | 0 | 0 | 0.00 | 4400 | 0% |
| | 2 | 280 | 85.0 | 85.0 | 15.2 | 52.5 | 180.18 | | 6% |
| | 4 | 280 | 105.0 | 190.0 | 34.4 | 138.6 | 504.82 | | 16% |
| | 6 | 280 | 67.0 | 257.0 | 43.1 | 190.1 | 701.39 | | 23% |
| | 8 | 280 | 47.0 | 304.0 | 48.0 | 225.4 | 831.47 | | 27% |
| | 10 | 280 | 33.0 | 337.0 | 52.3 | 253.7 | 934.60 | | 30% |
| | 13 | 280 | 23.0 | 360.0 | 54.4 | 271.7 | 996.98 | | 32% |
| | 17 | 280 | 40.5 | 400.5 | 56.3 | 299.3 | 1091.49 | | 35% |
| | 26 | 280 | 59.0 | 459.5 | 58.5 | 339.5 | 1226.05 | | 40% |
| | 31 | 280 | 17.0 | 476.5 | 60.6 | 355.3 | 1277.21 | | 42% |
| | 54 | 280 | 25.0 | 501.5 | 61.5 | 373.2 | 1319.52 | | 42.9% |
| SRT=7 d-5 min. sonication | 0 | 280 | 0 | 0 | 0 | 0 | 0.00 | 4450 | 0% |
| | 2 | 280 | 87.0 | 87.0 | 14.7 | 50.9 | 173.65 | | 6% |
| | 4 | 280 | 98.0 | 185.0 | 30.4 | 121.6 | 436.89 | | 14% |
| | 6 | 280 | 68.0 | 253.0 | 41.1 | 177.3 | 650.36 | | 21% |
| | 8 | 280 | 76.0 | 329.0 | 51.1 | 242.1 | 898.26 | | 29% |
| | 10 | 280 | 37.0 | 366.0 | 57.5 | 280.1 | 1040.40 | | 33% |
| | 13 | 280 | 28.0 | 394.0 | 59.3 | 301.2 | 1115.05 | | 36% |
| | 17 | 280 | 41.5 | 435.5 | 59.6 | 326.8 | 1201.42 | | 39% |
| | 26 | 280 | 51.0 | 486.5 | 62.4 | 366.0 | 1331.90 | | 43% |
| | 31 | 280 | 17.0 | 503.5 | 62.7 | 377.5 | 1365.92 | | 44% |
| | 54 | 280 | 25.5 | 529.0 | 62.0 | 391.5 | 1392.44 | | 44.7% |
| SRT=7d -10 min. sonication | 0 | 280 | 0 | 0 | 0 | 0 | 0.00 | 4300 | 0% |
| | 2 | 280 | 95.0 | 95.0 | 17.7 | 62.9 | 221.94 | | 7% |
| | 4 | 280 | 102.0 | 197.0 | 33.1 | 136.8 | 497.64 | | 17% |
| | 6 | 280 | 72.0 | 269.0 | 43.5 | 195.0 | 721.17 | | 24% |
| | 8 | 280 | 72.0 | 341.0 | 52.0 | 254.6 | 948.12 | | 32% |
| | 10 | 280 | 37.0 | 378.0 | 55.3 | 283.6 | 1054.46 | | 35% |
| | 13 | 280 | 27.0 | 405.0 | 58.0 | 306.3 | 1135.26 | | 38% |
| | 17 | 280 | 26.5 | 431.5 | 57.9 | 321.5 | 1180.08 | | 39% |
| | 26 | 280 | 43.0 | 474.5 | 60.6 | 354.6 | 1286.18 | | 43% |
| | 31 | 280 | 13.0 | 487.5 | 61.0 | 363.6 | 1310.32 | | 44% |
| | 54 | 280 | 22.0 | 509.5 | 62.5 | 381.1 | 1350.88 | | 44.9% |
| SRT= 7 d- 25 min. sonication | 0 | 280 | 0 | 0 | 0 | 0 | 0.00 | 4300 | 0% |
| | 2 | 280 | 96 | 96.0 | 17.3 | 61.6 | 216.38 | | 7% |
| | 4 | 280 | 104 | 200.0 | 33.4 | 138.0 | 502.51 | | 17% |
| | 6 | 280 | 74 | 274.0 | 43.5 | 196.6 | 727.37 | | 24% |
| | 8 | 280 | 67 | 341.0 | 51.1 | 250.4 | 931.42 | | 31% |
| | 10 | 280 | 38 | 379.0 | 54.0 | 278.4 | 1033.74 | | 34% |
| | 13 | 280 | 35 | 414.0 | 57.0 | 306.4 | 1135.57 | | 38% |
| | 17 | 280 | 25.5 | 439.5 | 57.0 | 320.9 | 1177.75 | | 39% |
| | 26 | 280 | 44 | 483.5 | 59.9 | 354.6 | 1286.36 | | 43% |
| | 31 | 280 | 20 | 503.5 | 59.8 | 366.3 | 1321.16 | | 44% |
| | 54 | 280 | 23 | 526.5 | 61.4 | 384.7 | 1365.16 | | 45.4% |
| Seed (control)- SRT= 7 d | 0 | 280 | 0 | 0 | 0 | 0 | N/A | | |
| | 2 | 280 | 96 | 96.0 | 19.55 | 69.6 | | | |
| | 4 | 280 | 69 | 165.0 | 29.37 | 115.4 | | | |
| | 6 | 280 | 30 | 195.0 | 33.95 | 137.5 | | | |
| | 8 | 280 | 33 | 228.0 | 39.11 | 163.8 | | | |
| | 10 | 280 | 31 | 259.0 | 42.84 | 186.8 | | | |
| | 13 | 280 | 22 | 281.0 | 47.61 | 209.7 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 17 | 280 | 50 | 331.0 | 51.95 | 246.9 | |
| | 26 | 280 | 85.5 | 416.5 | 56.8 | 308.2 | |
| | 31 | 280 | 42.0 | 458.5 | 58.2 | 336.1 | |
| | 54 | 280 | 84.0 | 542.5 | 63.9 | 404.8 | |

Table C.12. Anaerobic digestion batch test, 15 day SRT WAS, Methane generation

| Sample | Day | Sludge Vol.(ml) | Gas production | Comul. Gas Prod. | CH4 fraction% | Accumulated CH4 | Corrected CH4 | TCOD mg/l | Accumulated $CH_4$/TCOD% |
|---|---|---|---|---|---|---|---|---|---|
| SRT=15 d -Raw | 3 | 300 | 64.0 | 188.0 | 36.3 | 132.5 | 447.9 | 3950 | 16.2% |
| | 5 | 300 | 52.0 | 240.0 | 43.0 | 171.0 | 579.72 | | 21.0% |
| | 7 | 300 | 35.5 | 275.5 | 48.3 | 200.9 | 676.30 | | 24.5% |
| | 11 | 300 | 26.0 | 301.5 | 51.8 | 222.8 | 740.04 | | 27% |
| | 13 | 300 | 34.0 | 335.5 | 54.3 | 247.2 | 821.28 | | 30% |
| | 15 | 300 | 11.0 | 346.5 | 55.7 | 256.7 | 847.12 | | 31% |
| | 20 | 300 | 24.0 | 370.5 | 57.3 | 274.3 | 893.64 | | 32% |
| | 26 | 300 | 12.0 | 382.5 | 58.0 | 282.9 | 906.38 | | 33% |
| | 36 | 300 | 30.0 | 412.5 | 60.1 | 305.9 | 967.61 | | 35% |
| | 43 | 300 | 12.0 | 424.5 | 59.8 | 312.5 | 978.39 | | 35% |
| | 57 | 300 | 17.0 | 441.5 | 59.8 | 322.7 | 996.56 | | 36.1% |
| SRT=15d -10 min. sonication | 3 | 300 | 56.0 | 187.0 | 39.3 | 152.5 | 527.69 | 3950 | 19.1% |
| | 5 | 300 | 68.0 | 255.0 | 47.7 | 205.1 | 715.81 | | 25.9% |
| | 7 | 300 | 38.0 | 293.0 | 52.3 | 235.8 | 815.87 | | 29.5% |
| | 11 | 300 | 22.0 | 315.0 | 54.3 | 252.6 | 859.15 | | 31% |
| | 13 | 300 | 26.0 | 341.0 | 56.6 | 272.8 | 923.55 | | 33% |
| | 15 | 300 | 11.5 | 352.5 | 57.6 | 281.8 | 947.53 | | 34% |
| | 20 | 300 | 16.0 | 368.5 | 58.5 | 293.4 | 970.14 | | 35% |
| | 26 | 300 | 12.0 | 380.5 | 59.2 | 302.1 | 983.40 | | 36% |
| | 36 | 300 | 25 | 405.5 | 61.087 | 322.0 | 1031.84 | | 37% |
| | 43 | 300 | 10 | 415.5 | 60.58 | 326.8 | 1035.57 | | 37% |
| | 57 | 300 | 14 | 429.5 | 60.58 | 335.3 | 1046.98 | | 37.9% |
| SRT= 15 d- 25 min. sonication | 3 | 300 | 57 | 187.0 | 35.1 | 127.0 | 425.63 | 3950 | 15.4% |
| | 5 | 300 | 30 | 217.0 | 39.0 | 148.0 | 487.69 | | 17.7% |
| | 7 | 300 | 18 | 235.0 | 41.4 | 161.1 | 516.87 | | 18.7% |
| | 11 | 300 | 14 | 249.0 | 42.1 | 168.7 | 523.60 | | 19% |
| | 13 | 300 | 15 | 264.0 | 42.8 | 176.7 | 539.28 | | 20% |
| | 15 | 300 | 8 | 272.0 | 43.2 | 181.2 | 545.34 | | 20% |
| | 20 | 300 | 49 | 321.0 | 49.2 | 219.7 | 675.26 | | 24% |
| | 26 | 300 | 74 | 395.0 | 59.6 | 288.9 | 930.65 | | 34% |
| | 36 | 300 | 36 | 431.0 | 61.36 | 315.1 | 1004.41 | | 36% |
| | 43 | 300 | 17 | 448.0 | 60.67 | 323.8 | 1023.44 | | 37% |
| | 57 | 300 | 25 | 473.0 | 60.67 | 339.0 | 1061.59 | | 38.4% |
| Seed (control)-SRT= 15 d | 3 | 300 | 37 | 177.0 | 34 | 123.3 | N/A | | |
| | 5 | 300 | 45 | 222.0 | 40.33 | 156.7 | | | |
| | 7 | 300 | 42 | 264.0 | 46.533 | 191.1 | | | |
| | 11 | 300 | 49 | 313.0 | 50.93 | 226.6 | | | |
| | 13 | 300 | 33 | 346.0 | 53.9 | 251.5 | | | |
| | 15 | 300 | 23 | 369.0 | 55.97 | 269.4 | | | |
| | 20 | 300 | 51 | 420.0 | 58.56 | 305.5 | | | |
| | 26 | 300 | 44 | 464.0 | 60.799 | 337.6 | | | |
| | 36 | 300 | 61 | 525.0 | 63.98 | 384.2 | | | |
| | 43 | 300 | 38 | 563.0 | 63.67 | 407.7 | | | |
| | 57 | 300 | 53 | 616.0 | 63.68 | 441.5 | | | |

Table C.13. Anaerobic digestion batch test, 1.95 day SRT WAS, Ammonia generation

| Sample | Day | ×Dilution | Conc. | Conc.×Dilution | Corrected Ammonia conc. (280/250 × (Bottles - (3/28)*Seed)) | TCOD mg/l | TKN, FTKN and NH4-N conc. (before digestion) | Ammonia Conc. / Total TKN (mg ammon/l)/(mg-N/l) | Ammonia Con / TCOD % (mg ammon/l)/(mgCOD/l) |
|---|---|---|---|---|---|---|---|---|---|
| SRT=1.95d-Raw | 0 | 26 | 4.685 | 121.81 | 50.46 | 5255 | TKN: | 9.5% | 0.96% |
| | 1 | 26 | 6.675 | 173.55 | 107.57 | | 534.4 | 20.1% | 2.05% |
| | 2 | 26 | 9.618 | 250.068 | 189.36 | | | 35.5% | 3.60% |
| | 4 | 26 | 11.69 | 303.94 | 245.19 | | FTKN: | 45.9% | 4.67% |
| | 6 | 31 | 12.614 | 391.034 | 318.61 | | 111.7 | 59.7% | 6.06% |
| | 11 | 31 | 14.656 | 454.336 | 380.09 | | | 71.2% | 7.23% |
| | 16 | 31 | 15.454 | 479.074 | 406.10 | | Ammonia: | 76.0% | 7.73% |
| | 48 | 31 | 15.55 | 482.05 | 406.60 | | 50.466 | 76.1% | 7.74% |
| | | | | | | | | | |
| SRT=1.95d- 5 min. sonication | 0 | 26 | 4.723 | 122.798 | 51.57 | 5255 | TKN: | 9.7% | 0.98% |
| | 1 | 26 | 10.138 | 263.588 | 208.41 | | 534.4 | 39.0% | 3.97% |
| | 2 | 26 | 11.538 | 299.988 | 245.27 | | | 45.9% | 4.67% |
| | 4 | 26 | 12.863 | 334.438 | 279.35 | | FTKN: | 52.3% | 5.32% |
| | 6 | 31 | 12.914 | 400.334 | 329.02 | | 203 | 61.6% | 6.26% |
| | 11 | 31 | 15.216 | 471.696 | 399.54 | | | 74.8% | 7.60% |
| | 16 | 31 | 15.658 | 485.398 | 413.18 | | Ammonia: | 77.4% | 7.86% |
| | 48 | 31 | 16.221 | 502.851 | 429.90 | | 51.58 | 80.5% | 8.18% |
| | | | | | | | | | |
| SRT=1.95 d -10 min. sonication | 0 | 26 | 4.842 | 125.892 | 55.04 | 5255 | TKN: | 10.3% | 1.05% |
| | 1 | 26 | 10.759 | 279.734 | 226.49 | | 534.4 | 42.4% | 4.31% |
| | 2 | 26 | 11.782 | 306.332 | 252.37 | | | 47.3% | 4.80% |
| | 4 | 26 | 12.774 | 332.124 | 276.75 | | FTKN: | 51.8% | 5.27% |
| | 6 | 31 | 13.473 | 417.663 | 348.43 | | 232.6 | 65.2% | 6.63% |
| | 11 | 31 | 15.315 | 474.765 | 402.97 | | | 75.5% | 7.67% |
| | 16 | 31 | 15.859 | 491.629 | 420.16 | | Ammonia: | 78.7% | 8.00% |
| | 48 | 31 | 15.89 | 492.59 | 418.41 | | 55.042 | 78.4% | 7.96% |
| | | | | | | | | | |
| SRT=1.95 d-25 min. sonication | 0 | 26 | 4.91196 | 127.71096 | 57.07 | 5255 | TKN: | 10.7% | 1.09% |
| | 1 | 26 | 11.01 | 286.26 | 233.80 | | 534.4 | 43.8% | 4.45% |
| | 2 | 26 | 11.826 | 307.476 | 253.66 | | | 47.5% | 4.83% |
| | 4 | 26 | 13.236 | 344.136 | 290.21 | | FTKN: | 54.3% | 5.52% |
| | 6 | 31 | 13.737 | 425.847 | 357.60 | | 295.4 | 67.0% | 6.80% |
| | 11 | 31 | 15.127 | 468.937 | 396.45 | | | 74.2% | 7.54% |
| | 16 | 31 | 15.841 | 491.071 | 419.54 | | Ammonia: | 78.6% | 7.98% |
| | 48 | 31 | 16.105 | 499.255 | 425.87 | | 57.194 | 79.8% | 8.10% |
| | | | | | | | | | |
| Seed SRT=1.95d | 0 | 26 | 27.552 | 716.352 | | 5255 | TKN: | | |
| | 1 | 26 | 27.824 | 723.424 | | | 2237.5 | | |
| | 2 | 26 | 29.076 | 755.976 | | | | | |
| | 4 | 26 | 30.521 | 793.546 | | | FTKN: | | |
| | 6 | 31 | 32.084 | 994.604 | | | 1141.2 | | |
| | 11 | 31 | 34.614 | 1073.034 | | | | | |
| | 16 | 31 | 35.071 | 1087.201 | | | Ammonia: | | |
| | 48 | 31 | 35.831 | 1110.761 | | | 716.35 | | |
| | | | | | | | | | |

Table C.14. Anaerobic digestion batch test, 7 day SRT WAS, Ammonia generation

| Sample | Day | ×Dilution | Conc. | Conc.×Dilution | Corrected Ammonia conc. (280/250 × (Bottles - (3/28)*Seed)) | TCOD mg/l | TKN, FTKN and NH4-N conc. (before digestion) | Ammonia Conc. / Total TKN (mg ammon/l)/(mg-N/l) | Ammonia Con / TCOD % (mg ammon/l)/(mgCOD/l) |
|---|---|---|---|---|---|---|---|---|---|
| SRT=7d-Raw | 0 | 30 | 3.4825 | 104.475 | 6.96 | 4400 | TKN: | 1.6% | 0.2% |
| | 2 | 30 | 6.794 | 203.82 | 108.28 | | 424 | 25.5% | 2.5% |
| | 4 | 30 | 8.298 | 248.94 | 156.75 | | | 37.0% | 3.6% |
| | 6 | 30 | 9.847 | 295.41 | 207.17 | | FTKN: | 48.9% | 4.7% |
| | 8 | 30 | 10.34 | 310.2 | 218.07 | | 10.1 | 51.4% | 5.0% |
| | 10 | 30 | 10.833 | 324.99 | 234.17 | | | 55.2% | 5.3% |
| | 14 | 30 | 11.715 | 351.45 | 256.22 | | Ammonia: | 60.4% | 5.8% |
| | 54 | 30 | 13.525 | 405.75 | 305.88 | | 6.96 | 72.1% | 7.0% |
| | | | | | | | | | |
| SRT=75d- 5 min. sonication | 0 | 30 | 3.831 | 114.94 | 18.7 | 4450 | TKN: | 4.3% | 0.4% |
| | 2 | 30 | 9.325 | 279.75 | 193.3 | | 435.5 | 44.4% | 4.3% |
| | 4 | 30 | 10.283 | 308.49 | 223.4 | | | 51.3% | 5.0% |
| | 6 | 30 | 10.799 | 323.97 | 239.2 | | FTKN: | 54.9% | 5.4% |
| | 8 | 30 | 11.293 | 338.79 | 250.1 | | 226.7 | 57.4% | 5.6% |
| | 10 | 30 | 11.6 | 348 | 259.9 | | | 59.7% | 5.8% |
| | 14 | 30 | 12.205 | 366.15 | 272.7 | | Ammonia: | 62.6% | 6.1% |
| | 54 | 30 | 13.889 | 416.67 | 318.1 | | 18.69 | 73.0% | 7.1% |
| | | | | | | | | | |
| SRT=7 d -10 min. sonication | 0 | 30 | 3.969 | 119.07 | 23.3 | 4300 | TKN: | 5.7% | 0.5% |
| | 2 | 30 | 9.519 | 285.57 | 199.8 | | 409.8 | 48.8% | 4.6% |
| | 4 | 30 | 10.621 | 318.63 | 234.8 | | | 57.3% | 5.5% |
| | 6 | 30 | 11.372 | 341.16 | 258.4 | | FTKN: | 63.1% | 6.0% |
| | 8 | 30 | 11.442 | 343.26 | 255.1 | | 281.8 | 62.2% | 5.9% |
| | 10 | 30 | 11.763 | 352.89 | 265.4 | | | 64.8% | 6.2% |
| | 14 | 30 | 12.056 | 361.68 | 267.7 | | Ammonia: | 65.3% | 6.2% |
| | 54 | 30 | 13.601 | 408.03 | 308.4 | | 23.31 | 75.3% | 7.2% |
| | | | | | | | | | |
| SRT=7 d-25 min. sonication | 0 | 30 | 4.2 | 126.23 | 31.3 | 4300 | TKN: | 7.9% | 0.7% |
| | 2 | 30 | 9.586 | 287.58 | 202.1 | | 394.3 | 51.3% | 4.7% |
| | 4 | 30 | 10.584 | 317.52 | 233.6 | | | 59.2% | 5.4% |
| | 6 | 30 | 10.84 | 325.2 | 240.5 | | FTKN: | 61.0% | 5.6% |
| | 8 | 30 | 11.068 | 332.04 | 242.5 | | 336.4 | 61.5% | 5.6% |
| | 10 | 30 | 12.176 | 365.28 | 279.3 | | | 70.8% | 6.5% |
| | 14 | 30 | 12.507 | 375.21 | 282.8 | | Ammonia: | 71.7% | 6.6% |
| | 54 | 30 | 13.141 | 394.23 | 293.0 | | 31.32 | 74.3% | 6.8% |
| | | | | | | | | | |
| Seed SRT=7d | 0 | 30 | 30.57 | 917.1 | | | TKN: | | |
| | 2 | 30 | 33.334 | 1000.02 | | | 2118.4 | | |
| | 4 | 30 | 33.907 | 1017.21 | | | | | |
| | 6 | 30 | 34.358 | 1030.74 | | | FTKN: | | |
| | 8 | 30 | 35.933 | 1077.99 | | | 1189.3 | | |
| | 10 | 30 | 36.06 | 1081.8 | | | | | |
| | 14 | 30 | 38.168 | 1145.04 | | | Ammonia: | | |
| | 54 | 30 | 41.268 | 1238.04 | | | 917.1 | | |
| | | | | | | | | | |
| | | | | | | | | | |

Table C.15. Anaerobic digestion batch test, 15 day SRT WAS, Ammonia generation

| Sample | Day | ×Dilution | Conc. | Conc.×Dilution | Corrected Ammonia conc. | TCOD mg/l | TKN, FTKN and NH4-N conc. (before digestion) | Ammonia Conc. / Total TKN (mg ammon/l)/(mg-N/l) | Ammonia Con / TCOD % (mg ammon/l)/(mgCOD/l) |
|---|---|---|---|---|---|---|---|---|---|
| SRT=15d-Raw | 0 | 31 | 4.367 | 135.38 | 14.97 | | TKN: | 4.1% | 0.4% |
| | 2 | 31 | 7.21 | 223.45 | 110.61 | | 362 | 30.6% | 2.8% |
| | 6 | 31 | 9.39 | 291.18 | 180.04 | | FTKN: | 49.7% | 4.6% |
| | 11 | 31 | 10.04 | 311.18 | 197.44 | 3950 | --- | 54.5% | 5.0% |
| | 20 | 31 | 10.7 | 331.7 | 200.45 | | Ammonia: | 55.4% | 5.1% |
| | 57 | 31 | 13.36 | 414.25 | 277.88 | | 14.973 | 76.8% | 7.0% |
| | | | | | | | | | |
| SRT=15 d -10 min. sonication | 0 | 31 | 4.705 | 145.87 | 27.56 | | TKN: | 7.6% | 0.7% |
| | 2 | 31 | 8.44 | 261.73 | 156.55 | | 362 | 43.2% | 4.0% |
| | 6 | 31 | 10.11 | 313.5 | 206.83 | | FTKN: | 57.1% | 5.2% |
| | 11 | 31 | 10.74 | 333.06 | 223.70 | 3950 | --- | 61.8% | 5.7% |
| | 20 | 31 | 11.39 | 353.21 | 226.26 | | Ammonia: | 62.5% | 5.7% |
| | 57 | 31 | 13.54 | 419.77 | 284.51 | | 27.559 | 78.6% | 7.2% |
| | | | | | | | | | |
| SRT=15 d-25 min. sonication | 0 | 31 | 4.68 | 145.09 | 26.63 | | TKN: | 7.4% | 0.7% |
| | 2 | 31 | 9.12 | 282.59 | 181.59 | | 362 | 50.2% | 4.6% |
| | 6 | 31 | 10.62 | 329.13 | 225.57 | | FTKN: | 62.3% | 5.7% |
| | 11 | 31 | 10.78 | 334.21 | 225.08 | 3950 | --- | 62.2% | 5.7% |
| | 20 | 31 | 11.28 | 349.77 | 222.13 | | Ammonia: | 61.4% | 5.6% |
| | 57 | 31 | 13.21 | 409.45 | 272.12 | | 26.629 | 75.2% | 6.9% |
| | | | | | | | | | |
| Seed SRT=15d | 0 | 31 | 23.79 | 737.43 | | | TKN: | | |
| | 2 | 31 | 25.41 | 787.65 | | | 1824.5 | | |
| | 6 | 31 | 27.32 | 846.89 | | | FTKN: | | |
| | 11 | 31 | 28.38 | 879.87 | | | 1032.3 | | |
| | 20 | 31 | 31.87 | 987.97 | | | Ammonia: | | |
| | 57 | 31 | 35.35 | 1096.1 | | | 737.43 | | |
| | | | | | | | | | |

Table C.16. Anaerobic digestion batch test, 1.95 day SRT WAS, VFA generation

| Sample | Day | ×Dilution | Acetate | Propionate | Butyrate | Iso Butyrate | Corrected values | | | TVFA (mg COD/L) | TCOD (mgCOD/L) | TVFA/TCOD (mg COD/l) / (mgCOD/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Acetate | Propionate | Butyrate+ Iso Butyrate | | | |
| SRT=1.95d-Raw | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 60.4 | 25 | 0 | 0 | 331.6 | 133.6 | 0.0 | 556 | | 11% |
| | 2 | 5 | 121 | 54.7 | 0 | 0 | 671.4 | 298.5 | 0.0 | 1168 | | 22% |
| | 4 | 5 | 173 | 68.6 | 10.6 | 14.5 | 962.4 | 377.7 | 140.6 | 1854 | 5250 | 35% |
| | 6 | 5 | 224 | 74.7 | 10.8 | 14.3 | 1254.4 | 418.3 | 140.6 | 2227 | | 42% |
| | 11 | 4 | 281 | 82.1 | 13.8 | 13.1 | 1562.9 | 453.6 | 150.6 | 2628 | | 50% |
| | | | | | | | | | | | | |
| | Correction e.g. for acetate: $(Act-Act_{seed} \times V_{seed}/V_T) \times V_T /( V_T - V_{seed}) \times Dil$ | | | | | | | | | | | |
| SRT=1.95d- 5 min. sonication | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 98 | 42.5 | 0 | 17 | 542.1 | 231.6 | 95.2 | 1102 | | 21% |
| | 2 | 5 | 156 | 67.2 | 9.36 | 17.3 | 867.4 | 368.5 | 149.3 | 1754 | | 33% |
| | 4 | 5 | 225 | 80.9 | 13 | 22.1 | 1253.6 | 446.6 | 196.6 | 2370 | 5250 | 45% |
| | 6 | 5 | 269 | 88.5 | 13.5 | 21.7 | 1506.4 | 495.6 | 197.1 | 2715 | | 52% |
| | 11 | 5 | 319 | 92.3 | 15.4 | 20.7 | 1775.7 | 510.8 | 202.2 | 3035 | | 58% |
| | | | | | | | | | | | | |
| SRT=1.95 d -10 min. sonication | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 115 | 48.2 | 0 | 17.1 | 637.3 | 263.6 | 95.8 | 1253 | | 24% |
| | 2 | 5 | 175 | 70.5 | 0 | 17.7 | 973.8 | 387.0 | 99.1 | 1805 | | 34% |
| | 4 | 5 | 234 | 84.1 | 13.7 | 22.1 | 1304.0 | 464.5 | 200.5 | 2459 | 5250 | 47% |
| | 6 | 5 | 276 | 90.2 | 14.6 | 21.9 | 1545.6 | 505.1 | 204.4 | 2785 | | 53% |
| | 11 | 5 | 333 | 94.9 | 16.6 | 21.3 | 1854.1 | 525.3 | 212.2 | 3159 | | 60% |
| | | | | | | | | | | | | |
| SRT=1.95 d-25 min. sonication | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 135 | 58.1 | 0 | 18.1 | 749.3 | 319.0 | 101.4 | 1466 | | 28% |
| | 2 | 5 | 199 | 88.8 | 11.1 | 18.9 | 1108.2 | 489.5 | 168.0 | 2228 | | 42% |
| | 4 | 5 | 272 | 107 | 15.8 | 25.2 | 1516.8 | 592.8 | 229.6 | 2933 | 5250 | 56% |
| | 6 | 5 | 295 | 111 | 14.1 | 24.3 | 1652.0 | 621.6 | 215.0 | 3094 | | 59% |
| | 11 | 5 | 344 | 117 | 15.7 | 23.5 | 1915.7 | 649.1 | 219.5 | 3425 | | 65% |
| | | | | | | | | | | | | |
| Seed SRT=1.95d | 0 | - | - | - | - | - | | | | | | |
| | 1 | 5 | 11.1 | 10.6 | 0 | 0 | | | | | | |
| | 2 | 5 | 10.4 | 13 | 0 | 0 | | | | | | |
| | 4 | 5 | 10.7 | 10.7 | 0 | 0 | | | | | | |
| | 6 | 5 | 0 | 0 | 0 | 0 | | | | | | |
| | 11 | 5 | 17.9 | 10.2 | 0 | 0 | | | | | | |
| | | | | | | | | | | | | |

Table C.17. Anaerobic digestion batch test, 7 day SRT WAS, VFA generation

| Sample | Day | ×Dilution | Acetate | Propionate | Butyrate | Iso Butyrate | Valerate | Corrected values | | | | TCOD(mgCOD/L) | TVFA/TCOD (mg COD/l) / (mgCOD/L) | TVFA/TCOD (mg COD/l) / (mgCOD/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Acetate | Propionate | Butyrate+ Iso Butyrate | Valerate | | | |
| SRT=7d-Raw | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 4400 | 0 | 0% |
| | 2 | 5 | 46.7 | 18.9 | 3.69 | 4.37 | 2.87 | 259.5 | 105.8 | 45.1 | 2.9 | | 525 | 12% |
| | 4 | 5 | 91.1 | 30 | 6.15 | 6.42 | 3.63 | 509.3 | 168.0 | 70.4 | 3.6 | | 933 | 21% |
| | 6 | 5 | 108 | 33.5 | 7.31 | 7.27 | 5.19 | 603.5 | 187.6 | 81.6 | 5.2 | | 1087 | 25% |
| | 8 | 5 | 117 | 34.8 | 7.88 | 6.99 | 4.92 | 654.0 | 194.9 | 83.3 | 4.9 | | 1154 | 26% |
| | 10 | 5 | 129 | 36.9 | 8.99 | 7.11 | 6.22 | 714.1 | 205.2 | 90.2 | 6.2 | | 1249 | 28% |
| | 14 | 5 | 143 | 39.3 | 10.2 | 6.91 | 6.81 | 775.3 | 217.2 | 95.8 | 6.8 | | 1344 | 31% |
| | Correction e.g. for acetate: $(Act-Act_{seed} \times V_{seed}/V_T) \times V_T / (V_T - V_{seed}) \times Dil$ | | | | | | | | | | | | | |
| SRT=7d- 5 min. sonication | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 4450 | 0 | 0.0% |
| | 2 | 5 | 84.9 | 34.5 | 7.98 | 11.6 | 3.69 | 473.4 | 193.2 | 109.6 | 3.7 | | 1004 | 22.6% |
| | 4 | 5 | 120 | 43.1 | 9.17 | 12.7 | 2.76 | 671.1 | 241.4 | 122.5 | 2.8 | | 1309 | 29.4% |
| | 6 | 5 | 133 | 45.8 | 10.2 | 13.5 | 3.78 | 743.5 | 256.5 | 132.7 | 3.8 | | 1430 | 32.1% |
| | 8 | 5 | 140 | 46.7 | 10.5 | 13 | 4.34 | 782.8 | 261.5 | 131.6 | 4.3 | | 1479 | 33.2% |
| | 10 | 5 | 148 | 47.7 | 11.3 | 13.2 | 4.81 | 820.5 | 265.7 | 137.2 | 4.8 | | 1537 | 34.5% |
| | 14 | 5 | 160 | 48 | 12.1 | 12.4 | 5.18 | 870.5 | 265.9 | 137.2 | 5.2 | | 1591 | 35.8% |
| SRT=7 d -10 min. sonication | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 4300 | 0 | 0% |
| | 2 | 5 | 82.9 | 32.9 | 8.24 | 10.9 | 2.77 | 462.2 | 184.2 | 107.2 | 2.8 | | 972 | 23% |
| | 4 | 5 | 112 | 39.2 | 9.39 | 11.7 | 2.44 | 626.3 | 219.5 | 118.1 | 2.4 | | 1220 | 28% |
| | 6 | 5 | 122 | 40.8 | 10.2 | 12.1 | 2.89 | 681.9 | 228.5 | 124.9 | 2.9 | | 1306 | 30% |
| | 8 | 5 | 130 | 41.2 | 10.2 | 11.4 | 3.24 | 726.8 | 230.7 | 121.0 | 3.2 | | 1351 | 31% |
| | 10 | 5 | 137 | 41.8 | 11 | 11.4 | 3.86 | 758.9 | 232.6 | 125.4 | 3.9 | | 1398 | 33% |
| | 14 | 5 | 148 | 42.2 | 11.6 | 10.6 | 4.6 | 803.3 | 233.4 | 124.3 | 4.6 | | 1446 | 34% |
| SRT=7 d-25 min. sonication | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 4300 | 0 | 0% |
| | 2 | 5 | 84.3 | 31.6 | 8.06 | 10.3 | 2.72 | 470.0 | 177.0 | 102.8 | 2.7 | | 962 | 22% |
| | 4 | 5 | 112 | 37.4 | 9.44 | 11.3 | 2.21 | 626.3 | 209.4 | 116.1 | 2.2 | | 1201 | 28% |
| | 6 | 5 | 121 | 39.1 | 10.2 | 11.7 | 2.71 | 676.3 | 219.0 | 122.6 | 2.7 | | 1281 | 30% |
| | 8 | 5 | 130 | 40.1 | 10.4 | 11.5 | 3.41 | 726.8 | 224.6 | 122.6 | 3.4 | | 1345 | 31% |
| | 10 | 5 | 137 | 40.6 | 11.1 | 11.5 | 3.08 | 758.9 | 225.9 | 126.6 | 3.1 | | 1388 | 32% |
| | 14 | 5 | 148 | 40.7 | 11.6 | 10.6 | 3.8 | 803.3 | 225.0 | 124.3 | 3.8 | | 1431 | 33% |
| Seed SRT=7d | 0 | 5 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| | 2 | 5 | 3.4 | 0 | 0 | 0 | 0 | | | | | | | |
| | 4 | 5 | 1.5 | 0 | 0 | 0 | 0 | | | | | | | |
| | 6 | 5 | 2.17 | 0 | 0 | 0 | 0 | | | | | | | |
| | 8 | 5 | 1.99 | 0 | 0 | 0 | 0 | | | | | | | |
| | 10 | 5 | 13.8 | 2.43 | 0 | 0 | 0 | | | | | | | |
| | 14 | 5 | 42.5 | 4.8 | 0 | 0 | 0 | | | | | | | |

Table C.18. Anaerobic digestion batch test, 15 day SRT WAS, VFA generation

| Sample | Day | ×Dilution | Acetate | Propionate | Butyrate | Iso Butyrate | Corrected values | | | TVFA (mg COD/L) | TCOD (mgCOD/L) | TVFA/TCOD (mg COD/l) / (mgCOD/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Acetate | Propionate | Butyrate+ Iso Butyrate | | | |
| RT=15d-Raw | 0 | 5 | | | | | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 14 | 13.1 | 0 | 0 | 84 | 78.6 | 0 | 209 | | 5.3% |
| | 6 | 5 | 78 | 38.2 | 0 | 0 | 468 | 229.2 | 0 | 846 | | 21.4% |
| | 11 | 5 | 127 | 35.5 | 0 | 0 | 762 | 213 | 0 | 1135 | | 28.7% |
| | | | | | | | | | | | | |
| | Correction e.g. for acetate: $(Act-Act_{seed} \times V_{seed}/V_T) \times V_T / (V_T - V_{seed}) \times Dil$ | | | | | | | | | | | |
| SRT=15 d - 10 min. sonication | 0 | 0 | | | | | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 61.9 | 48.7 | 0 | 0 | 371.4 | 292.2 | 0 | 838 | | 21% |
| | 6 | 5 | 109 | 62.7 | 12.5 | 0 | 654 | 376.2 | 75 | 1403 | | 36% |
| | 11 | 5 | 175 | 53 | 12.2 | 0 | 1050 | 318 | 73.2 | 1734 | | 44% |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| SRT=15 d- 25 min. sonication | 0 | 5 | | | | | 0 | 0 | 0 | 0 | | 0% |
| | 1 | 5 | 68.8 | 53.2 | 0 | 0 | 412.8 | 319.2 | 0 | 923 | | 23% |
| | 6 | 5 | 106 | 60.5 | 12.5 | 0 | 636 | 363 | 75 | 1364 | | 35% |
| | 11 | 5 | 167 | 52.2 | 12.5 | 0 | 1002 | 313.2 | 75 | 1679 | | 43% |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Seed SRT=15 d | 0 | 5 | 0 | 0 | 0 | 0 | | | | | | |
| | 1 | 5 | 0 | 0 | 0 | 0 | | | | | | |
| | 6 | 5 | 0 | 0 | 0 | 0 | | | | | | |
| | 11 | 5 | 0 | 0 | 0 | 0 | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

# Appendix D:

# Results for ozonation experiments:

Table D.1. Particles mean volume size for ozonation

| Sample | | Pretreatment Intensity (min.) | Volume mean size | | | |
|---|---|---|---|---|---|---|
| SRT (day) | Pretreatment Type | | First replicate | Second replicate | Average | Std Error |
| 1.95 | Ozonation | 0 | 231.31 | 265.31 | 248.31 | 17 |
| | | 10 | 220.66 | 238.66 | 229.66 | 9 |
| | | 20 | 208.84 | 222.84 | 215.84 | 7 |
| | | 30 | 202.211 | 226.211 | 214.211 | 12 |
| 7 | | 0 | 240.21 | 256.21 | 248.21 | 8 |
| | | 10 | 229.45 | 245.45 | 237.45 | 8 |
| | | 20 | 212.45 | 234.45 | 223.45 | 11 |
| | | 30 | 221.89 | 225.89 | 223.89 | 2 |
| 15 | | 0 | 235.414 | 273.414 | 254.414 | 19 |
| | | 10 | 236.48 | 248.48 | 242.48 | 6 |
| | | 20 | 220.85 | 246.85 | 233.85 | 13 |
| | | 30 | 222.16 | 240.16 | 231.16 | 9 |

Table D.2. Measurement of COD concentrations

| Sample | Ozonation time | Type | x Dilution | Abs@ 600nm | | COD (mg/L) | | $COD_{avg}$ x Dil | FCOD/TCOD & FFCOD/TCOD |
|---|---|---|---|---|---|---|---|---|---|
| T=1.95 day | 0 min | **TCOD** | 20 | 0.262 | 0.249 | 655.0 | 622.5 | **7025** | |
| | | **FCOD** | 10 | 0.008 | 0.007 | 20.0 | 17.5 | **188** | **2.7%** |
| | | **FFCOD** | 10 | 0.008 | 0.005 | 20.0 | 12.5 | **163** | **2.3%** |
| | 15 min | **TCOD** | 20 | 0.150 | 0.130 | 375.0 | 325.0 | **7000** | |
| | | **FCOD** | 10 | 0.066 | 0.077 | 165.0 | 192.5 | **1788** | **25.5%** |
| | | **FFCOD** | 10 | 10 | 0.050 | 0.038 | 125.0 | **1100** | **15.7%** |
| | 30 min | **TCOD** | 20 | 0.130 | 0.130 | 325.0 | 325.0 | **6500** | |
| | | **FCOD** | 10 | 0.066 | 0.077 | 165.0 | 192.5 | **2800** | **43.1%** |
| | | **FFCOD** | 10 | 0.066 | 0.072 | 165.0 | 180.0 | **1725** | **26.5%** |
| SRT=7 day | 0 min | **TCOD** | 20 | 0.253 | 0.271 | 632.5 | 677.5 | **13100** | |
| | | **FCOD** | 10 | 0.014 | 0.016 | 35.0 | 40.0 | **375** | **2.9%** |
| | | **FFCOD** | 10 | 0.004 | 0.004 | 10.0 | 10.0 | **100** | **0.8%** |
| | 15 min | **TCOD** | 20 | 0.278 | 0.250 | 695.0 | 625.0 | **13200** | |
| | | **FCOD** | 10 | 0.037 | 0.041 | 92.5 | 102.5 | **975** | **7.4%** |
| | | **FFCOD** | 10 | 0.023 | 0.024 | 57.5 | 60.0 | **588** | **4.5%** |
| | 30 min | **TCOD** | 20 | 0.273 | 0.269 | 682.5 | 672.5 | **13550** | |
| | | **FCOD** | 10 | 0.118 | 0.112 | 295.0 | 280.0 | **2875** | **21.2%** |
| | | **FFCOD** | 10 | 0.095 | 0.073 | 237.5 | 182.5 | **2100** | **15.5%** |
| SRT=15 day | 0 min | **TCOD** | 20 | 0.235 | 0.235 | 587.5 | 587.5 | **11750** | |
| | | **FCOD** | 10 | 0.009 | 0.008 | 22.5 | 20.0 | **213** | **1.8%** |
| | | **FFCOD** | 10 | 0.006 | 0.007 | 15.0 | 17.5 | **163** | **1.4%** |
| | 15 min | **TCOD** | 20 | 0.230 | 0.230 | 575.0 | 575.0 | **11500** | |
| | | **FCOD** | 10 | 0.042 | 0.042 | 105.0 | 105.0 | **1050** | **9.1%** |
| | | **FFCOD** | 10 | 0.037 | 0.044 | 92.5 | 110.0 | **1013** | **8.8%** |
| | 30 min | **TCOD** | 20 | 0.230 | 0.229 | 575.0 | 572.5 | **11475** | |
| | | **FCOD** | 10 | 0.109 | 0.110 | 272.5 | 275.0 | **2738** | **23.9%** |
| | | **FFCOD** | 10 | 0.098 | 0.088 | 245.0 | 220.0 | **2325** | **20.3%** |

Table D.3. Summary of measurement of COD concentrations

| Sludge source | Ozonation time (min.) | FCOD/TCOD% | | | FFCOD/TCOD % | | |
|---|---|---|---|---|---|---|---|
| | | Replicate 1 | Replicate 2 | Std Error | Replicate 1 | Replicate 2 | Std Error |
| SRT= 1.95 day | 0 | 2.8% | 2.5% | **0.2%** | 2.8% | 1.8% | **0.5%** |
| | 15 | 23.6% | 27.5% | **2.0%** | 17.9% | 13.6% | **2.1%** |
| | 30 | 45.4% | 40.8% | **2.3%** | 25.4% | 27.7% | **1.2%** |
| SRT= 7 day | 0 | 2.7% | 3.1% | **0.2%** | 0.8% | 0.8% | **0.0%** |
| | 15 | 7.0% | 7.8% | **0.4%** | 4.4% | 4.5% | **0.1%** |
| | 30 | 21.8% | 20.7% | **0.6%** | 17.5% | 13.5% | **2.0%** |
| SRT= 15 day | 0 | 1.9% | 1.7% | **0.1%** | 1.3% | 1.5% | **0.1%** |
| | 15 | 9.1% | 9.1% | **0.0%** | 8.0% | 9.6% | **0.8%** |
| | 30 | 23.7% | 24.0% | **0.1%** | 21.4% | 19.2% | **1.1%** |

Table D.4. Measurement of TKN concentrations.

| Sample | Ozonation Time (min) | Type | x Dilution | Run1 and run2 (diluted) | | Average | Average × Dil |
|---|---|---|---|---|---|---|---|
| SRT=1.95 d | 0 | TTKN | 100 | 6.03 | 6.45 | 6.24 | 624.35 |
| | | FTKN | 10 | 1.868 | 2.272 | 2.070 | 20.70 |
| | 15 | TTKN | 100 | 6.11 | 6.49 | 6.30 | 630.55 |
| | | FTKN | 10 | 18.69 | 19.53 | 19.11 | 191.10 |
| | 30 | TTKN | 100 | 6.49 | 657.5 | 6.53 | 653.2 |
| | | FTKN | 10 | 32.28 | 34.18 | 33.23 | 332.30 |
| SRT=7 d | 0 | TTKN | 100 | 11.34 | 12.79 | 12.07 | 1206.7 |
| | | FTKN | 10 | 3.32 | 4.56 | 3.94 | 39.40 |
| | 15 | TTKN | 100 | 1183 | 1183.9 | 11.84 | 1183.8 |
| | | FTKN | 10 | 10.61 | 11.79 | 11.200 | 112.00 |
| | 30 | TTKN | 100 | 12.02 | 12.97 | 12.49 | 1249.3 |
| | | FTKN | 10 | 32.95 | 34.51 | 33.78 | 337.80 |
| SRT=15 d | 0 | TTKN | 100 | 12.11 | ----- | 12.12 | 1211.7 |
| | | FTKN | 10 | 2.32 | 3.12 | 2.72 | 27.20 |
| | 15 | TTKN | 100 | 11.47 | 12.73 | 12.10 | 1210 |
| | | FTKN | 10 | 8.57 | 12.09 | 10.33 | 103.30 |
| | 30 | TTKN | 100 | 12.05 | 12.74 | 12.40 | 1240 |
| | | FTKN | 10 | 31.07 | 35.89 | 33.48 | 334.80 |

Table D.5. Calculation of average and error for FTKN/ TKN, TKN/TCOD and FTKN/FCOD ratios

| Sludge source | Ozonation Time (min.) | FTKN/TKN% | | TKN/TCOD% | | FTKN/FCOD% | |
|---|---|---|---|---|---|---|---|
| | | Avg | Std Error | Avg | Std Error | Avg | Std Error |
| SRT= 1.95 day | 0 | 3.3% | 0.3% | 8.9% | 0.3% | 11.0% | 1.1% |
| | 15 | 30.3% | 0.7% | 9.0% | 0.3% | 10.7% | 0.2% |
| | 30 | 50.9% | 1.5% | 10.0% | 0.1% | 11.9% | 0.3% |
| SRT= 7 day | 0 | 3.3% | 0.5% | 9.2% | 0.6% | 10.5% | 1.7% |
| | 15 | 9.5% | 0.5% | 9.0% | 0.001% | 11.5% | 0.6% |
| | 30 | 27.0% | 0.6% | 9.2% | 0.3% | 11.7% | 0.3% |
| SRT= 15 day | 0 | 2.2% | 0.3% | 10.3% | 0.0% | 12.8% | 1.9% |
| | 15 | 8.5% | 1.5% | 10.5% | 0.5% | --- | --- |
| | 30 | 27.0% | 1.9% | 10.8% | 0.3% | 12.2% | 0.9% |

Table D.6. Anaerobic digestion batch test, 1.95 day SRT WAS, Methane generation

| Sample | Day | Sludge Vol.(ml) | Gas production | Comul. Gas Prod. | CH4 fraction% | Accumulated CH4 | Corrected CH4 | TCOD mg/l | Accumulated $CH_4$/TCOD% |
|---|---|---|---|---|---|---|---|---|---|
| SRT=1.95d-Raw | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 7025 | **0%** |
| | 2 | 280 | 142 | 142.0 | 18.0 | 72.4 | 252.7 | | **5%** |
| | 3 | 280 | 66 | 208.0 | 25.4 | 108.4 | 385.2 | | **8%** |
| | 6 | 280 | 108 | 316.0 | 43.6 | 202.8 | 738.4 | | **15%** |
| | 9 | 280 | 158 | 474.0 | 57.3 | 328.9 | 1227.7 | | **25%** |
| | 12 | 280 | 100 | 574.0 | 59.6 | 394.5 | 1475.3 | | **30%** |
| | 16 | 280 | 59 | 633.0 | 63.6 | 442.4 | 1649.3 | | **34%** |
| | 19 | 280 | 28 | 661.0 | 63.4 | 459.7 | 1708.6 | | **35%** |
| | 23 | 280 | 39 | 700.0 | 63.0 | 483.2 | 1790.1 | | **36%** |
| | 27 | 280 | 19 | 719.0 | 61.7 | 491.5 | 1816.0 | | **37%** |
| | 32 | 280 | 22 | 741.0 | 61.9 | 505.7 | 1861.8 | | **38%** |
| | 37 | 280 | 21 | 762.0 | 62.1 | 519.2 | 1900.3 | | **39%** |
| | 46 | 280 | 12 | 774.0 | 62.1 | 526.7 | 1916.0 | | **39%** |
| | 52 | 280 | 9 | 783.0 | 62.1 | 532.3 | 1932.8 | | **39%** |
| | 58 | 280 | 8 | 791.0 | 62.1 | 537.2 | 1948.2 | | **39.7%** |
| SRT=1.95d-15 min Ozonation | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 7000 | **0%** |
| | 2 | 280 | 92.0 | 92.0 | 8.0 | 28.2 | 75.9 | | **2%** |
| | 3 | 280 | 42.0 | 134.0 | 11.0 | 40.6 | 114.0 | | **2%** |
| | 6 | 280 | 74.0 | 208.0 | 23.1 | 89.1 | 283.9 | | **6%** |
| | 9 | 280 | 107.0 | 315.0 | 41.3 | 180.6 | 634.5 | | **13%** |
| | 12 | 280 | 133.0 | 448.0 | 53.3 | 282.7 | 1028.2 | | **21%** |
| | 16 | 280 | 168.0 | 616.0 | 63.8 | 417.2 | 1548.4 | | **32%** |
| | 19 | 280 | 60.0 | 676.0 | 63.6 | 454.9 | 1689.4 | | **35%** |
| | 23 | 280 | 39.0 | 715.0 | 63.5 | 479.4 | 1774.8 | | **36%** |
| | 27 | 280 | 25.0 | 740.0 | 63.2 | 494.4 | 1827.3 | | **37%** |
| | 32 | 280 | 25.0 | 765.0 | 63.4 | 510.8 | 1882.1 | | **38%** |
| | 37 | 280 | 21.0 | 786.0 | 63.6 | 524.6 | 1921.8 | | **39%** |
| | 46 | 280 | 20.0 | 806.0 | 63.6 | 537.3 | 1958.7 | | **40%** |
| | 52 | 280 | 9.0 | 815.0 | 63.6 | 543.1 | 1976.0 | | **40%** |
| | 58 | 280 | 3.0 | 818.0 | 63.6 | 545.0 | 1979.1 | | **40.4%** |
| SRT=1.95d-30 min Ozonation | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 6525 | **0%** |
| | 2 | 280 | 70 | 70.0 | 4.0 | 13.2 | 16.0 | | **0%** |
| | 3 | 280 | 22 | 92.0 | 5.7 | 18.9 | 27.2 | | **1%** |
| | 6 | 280 | 74 | 166.0 | 12.5 | 45.8 | 110.5 | | **2%** |
| | 9 | 280 | 61 | 227.0 | 22.0 | 83.9 | 247.7 | | **5%** |
| | 12 | 280 | 90 | 317.0 | 37.5 | 158.0 | 529.1 | | **12%** |
| | 16 | 280 | 156 | 473.0 | 54.0 | 285.1 | 1020.0 | | **22%** |
| | 19 | 280 | 71 | 544.0 | 58.0 | 336.7 | 1216.8 | | **27%** |
| | 23 | 280 | 97 | 641.0 | 62.5 | 409.0 | 1493.4 | | **33%** |
| | 27 | 280 | 62 | 703.0 | 64.1 | 452.9 | 1661.5 | | **36%** |
| | 32 | 280 | 26 | 729.0 | 64.3 | 470.2 | 1719.7 | | **38%** |
| | 37 | 280 | 41 | 770.0 | 64.6 | 497.4 | 1813.0 | | **40%** |
| | 46 | 280 | 22 | 792.0 | 64.6 | 511.6 | 1855.8 | | **41%** |
| | 52 | 280 | 6 | 798.0 | 64.6 | 515.5 | 1865.8 | | **41%** |
| | 58 | 280 | 6 | 804.0 | 64.6 | 519.4 | 1876.8 | | **41.1%** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Seed for SRT=1.95d** | 0 | 280 | 0 | 0 | 0 | 0 | | | |
| | 2 | 280 | 130 | 130.0 | 22 | 85.8 | | | |
| | 3 | 280 | 34 | 164.0 | 28.6 | 112.7 | | | |
| | 6 | 280 | 80 | 244.0 | 38.6 | 169.6 | | | |
| | 9 | 280 | 51 | 295.0 | 43.8 | 205.4 | | | |
| | 12 | 280 | 54 | 349.0 | 47.2 | 239.8 | | | |
| | 16 | 280 | 56 | 405.0 | 51.9 | 281.0 | | | |
| | 19 | 280 | 34 | 439.0 | 53.5 | 303.4 | | | |
| | 23 | 280 | 42.0 | 481.0 | 55.8 | 332.8 | | | |
| | 27 | 280 | 28.0 | 509.0 | 56.5 | 350.4 | | | |
| | 32 | 280 | 40.0 | 549.0 | 57.3 | 375.4 | | | |
| | 37 | 280 | 57.0 | 606.0 | 58.6 | 412.2 | | | |
| | 46 | 280 | 56.0 | 662.0 | 58.6 | 445.0 | | | |
| | 52 | 280 | 22.0 | 684.0 | 58.6 | 457.9 | | | |
| | 58 | 280 | 18.0 | 702.0 | 58.6 | 468.5 | | | |

Table D.7. Anaerobic digestion batch test, 7 day SRT WAS, Methane generation

| Sample | Day | Sludge Vol.(ml) | Gas production | Comul. Gas Prod. | CH4 fraction% | Accumulated CH4 | Corrected CH4 | TCOD mg/l | Accumulated $CH_4$/TCOD% |
|---|---|---|---|---|---|---|---|---|---|
| SRT=7d-Raw | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 13100 | 0% |
| | 2 | 280 | 162.0 | 162.0 | 17.0 | 71.7 | 250.2 | | 3% |
| | 3 | 280 | 82.0 | 244.0 | 24.0 | 109.6 | 390.2 | | 4% |
| | 6 | 280 | 237.0 | 481.0 | 50.2 | 296.7 | 1114.2 | | 12% |
| | 9 | 280 | 213.0 | 694.0 | 61.6 | 457.6 | 1742.2 | | 19% |
| | 12 | 280 | 128.0 | 822.0 | 63.8 | 544.9 | 2077.0 | | 23% |
| | 16 | 280 | 97.0 | 919.0 | 65.5 | 612.9 | 2331.2 | | 25% |
| | 19 | 280 | 48.0 | 967.0 | 64.8 | 642.2 | 2438.7 | | 27% |
| | 23 | 280 | 60.0 | 1027.0 | 63.8 | 677.9 | 2568.8 | | 28% |
| | 27 | 280 | 39.0 | 1066.0 | 63.5 | 701.9 | 2657.2 | | 29% |
| | 32 | 280 | 36.0 | 1102.0 | 63.6 | 725.0 | 2739.1 | | 30% |
| | 37 | 280 | 42.0 | 1144.0 | 63.7 | 752.0 | 2831.4 | | 31% |
| | 46 | 280 | 35.0 | 1179.0 | 63.7 | 774.3 | 2906.5 | | 32% |
| | 52 | 280 | 12.0 | 1191.0 | 63.7 | 782.0 | 2931.6 | | 32% |
| | 58 | 280 | 9.0 | 1200.0 | 63.7 | 787.7 | 2950.0 | | 32.2% |
| SRT=7d-15 min Ozonation | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 13200 | 0% |
| | 2 | 280 | 160.0 | 160.0 | 19.0 | 79.8 | 282.4 | | 3% |
| | 3 | 280 | 58.0 | 218.0 | 24.7 | 108.9 | 387.5 | | 4% |
| | 6 | 280 | 200.0 | 418.0 | 46.0 | 256.3 | 952.6 | | 10% |
| | 9 | 280 | 223.0 | 641.0 | 60.4 | 428.5 | 1625.8 | | 18% |
| | 12 | 280 | 174.0 | 815.0 | 63.5 | 547.0 | 2085.3 | | 23% |
| | 16 | 280 | 103.0 | 918.0 | 65.9 | 621.1 | 2364.1 | | 26% |
| | 19 | 280 | 53.0 | 971.0 | 65.4 | 654.5 | 2487.9 | | 27% |
| | 23 | 280 | 58.0 | 1029.0 | 64.7 | 690.2 | 2618.1 | | 28% |
| | 27 | 280 | 34.0 | 1063.0 | 64.0 | 710.1 | 2690.3 | | 29% |
| | 32 | 280 | 35.0 | 1098.0 | 63.9 | 732.2 | 2768.0 | | 30% |
| | 37 | 280 | 33.0 | 1131.0 | 63.8 | 753.0 | 2835.5 | | 31% |
| | 46 | 280 | 33.0 | 1164.0 | 63.7 | 773.8 | 2904.4 | | 31% |
| | 52 | 280 | 14.0 | 1178.0 | 63.7 | 782.7 | 2934.6 | | 32% |
| | 58 | 280 | 15.0 | 1193.0 | 63.7 | 792.3 | 2968.3 | | 32.2% |
| SRT=7d-30 min Ozonation | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 13550 | 0% |
| | 2 | 280 | 70 | 70.0 | 1.8 | 5.9 | -13.0 | | 0% |
| | 3 | 280 | 26 | 96.0 | 2.2 | 7.6 | -18.1 | | 0% |
| | 6 | 280 | 67 | 163.0 | 7.1 | 25.0 | 27.5 | | 0% |
| | 9 | 280 | 68 | 231.0 | 17.9 | 65.3 | 173.2 | | 2% |
| | 12 | 280 | 144 | 375.0 | 39.1 | 176.7 | 604.2 | | 6% |
| | 16 | 280 | 275 | 650.0 | 60.5 | 398.7 | 1474.5 | | 16% |
| | 19 | 280 | 123 | 773.0 | 63.3 | 483.9 | 1805.5 | | 19% |
| | 23 | 280 | 156 | 929.0 | 66.7 | 596.8 | 2244.5 | | 24% |
| | 27 | 280 | 120 | 1049.0 | 68.4 | 683.3 | 2582.9 | | 27% |
| | 32 | 280 | 61 | 1110.0 | 67.2 | 721.1 | 2723.7 | | 29% |
| | 37 | 280 | 57 | 1167.0 | 66 | 755.6 | 2845.9 | | 30% |
| | 46 | 280 | 66 | 1233.0 | 66 | 799.2 | 3006.1 | | 32% |
| | 52 | 280 | 26 | 1259.0 | 66 | 816.4 | 3069.2 | | 32% |
| | 58 | 280 | 14 | 1273.0 | 66 | 825.6 | 3101.6 | | 32.7% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Seed for SRT=7d** | 0 | 280 | 0 | 0 | 0 | 0 | | | |
| | 2 | 280 | 130 | 130.0 | 22 | 85.8 | | | |
| | 3 | 280 | 34 | 164.0 | 28.6 | 112.7 | | | |
| | 6 | 280 | 80 | 244.0 | 38.6 | 169.6 | | | |
| | 9 | 280 | 51 | 295.0 | 43.8 | 205.4 | | | |
| | 12 | 280 | 54 | 349.0 | 47.2 | 239.8 | | | |
| | 16 | 280 | 56 | 405.0 | 51.9 | 281.0 | | | |
| | 19 | 280 | 34 | 439.0 | 53.5 | 303.4 | | | |
| | 23 | 280 | 42.0 | 481.0 | 55.8 | 332.8 | | | |
| | 27 | 280 | 28.0 | 509.0 | 56.5 | 350.4 | | | |
| | 32 | 280 | 40.0 | 549.0 | 57.3 | 375.4 | | | |
| | 37 | 280 | 57.0 | 606.0 | 58.6 | 412.2 | | | |
| | 46 | 280 | 56.0 | 662.0 | 58.6 | 445.0 | | | |
| | 52 | 280 | 22.0 | 684.0 | 58.6 | 457.9 | | | |
| | 58 | 280 | 18.0 | 702.0 | 58.6 | 468.5 | | | |

Table D.8. Anaerobic digestion batch test, 15 day SRT WAS, Methane generation

| Sample | Day | Sludge Vol.(ml) | Gas production | Comul. Gas Prod. | CH4 fraction% | Accumulated CH4 | Corrected CH4 | TCOD mg/l | Accumulated $CH_4$/TCOD% |
|---|---|---|---|---|---|---|---|---|---|
| SRT=15d-Raw | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 11525 | 0% |
| | 2 | 280 | 166.0 | 166.0 | 22.0 | 93.7 | 319.2 | | 4% |
| | 3 | 280 | 81.0 | 247.0 | 30.9 | 141.9 | 497.3 | | 6% |
| | 6 | 280 | 234.0 | 481.0 | 51.8 | 317.4 | 1165.2 | | 14% |
| | 9 | 280 | 198.0 | 679.0 | 60.3 | 458.9 | 1709.3 | | 21% |
| | 12 | 280 | 109.0 | 788.0 | 60.7 | 526.1 | 1955.0 | | 24% |
| | 16 | 280 | 91.0 | 879.0 | 63.6 | 591.6 | 2192.6 | | 27% |
| | 19 | 280 | 48.0 | 927.0 | 63.1 | 620.5 | 2294.0 | | 28% |
| | 23 | 280 | 55.0 | 982.0 | 62.6 | 653.7 | 2408.5 | | 30% |
| | 27 | 280 | 34.0 | 1016.0 | 62.2 | 673.8 | 2477.0 | | 31% |
| | 32 | 280 | 29.0 | 1045.0 | 62.2 | 691.8 | 2532.0 | | 31% |
| | 37 | 280 | 38.0 | 1083.0 | 62.1 | 715.2 | 2600.9 | | 32% |
| | 46 | 280 | 41.0 | 1124.0 | 62.1 | 740.6 | 2678.8 | | 33% |
| | 52 | 280 | 12.0 | 1136.0 | 62.1 | 748.1 | 2699.1 | | 33% |
| | 58 | 280 | 9.0 | 1145.0 | 62.1 | 753.7 | 2713.8 | | 33.7% |
| SRT=15d-15 min Ozonation | 0 | 280 | 0 | 0 | 0 | 0 | 0 | 11750 | 0% |
| | 2 | 280 | 148.0 | 148.0 | 15.0 | 61.2 | 189.1 | | 2% |
| | 3 | 280 | 66.0 | 214.0 | 23.9 | 100.1 | 330.2 | | 4% |
| | 6 | 280 | 202.0 | 416.0 | 46.5 | 252.8 | 906.6 | | 11% |
| | 9 | 280 | 216.0 | 632.0 | 59.5 | 415.1 | 1534.1 | | 19% |
| | 12 | 280 | 136.0 | 768.0 | 61.8 | 505.2 | 1871.0 | | 23% |
| | 16 | 280 | 96.0 | 864.0 | 62.9 | 568.4 | 2100.0 | | 26% |
| | 19 | 280 | 46.0 | 910.0 | 63.1 | 597.9 | 2203.6 | | 27% |
| | 23 | 280 | 56.0 | 966.0 | 63.3 | 633.9 | 2329.5 | | 28% |
| | 27 | 280 | 30.0 | 996.0 | 62.5 | 650.6 | 2384.2 | | 29% |
| | 32 | 280 | 27.0 | 1023.0 | 62.4 | 667.2 | 2433.4 | | 30% |
| | 37 | 280 | 42.0 | 1065.0 | 62.3 | 693.1 | 2512.6 | | 31% |
| | 46 | 280 | 35.0 | 1100.0 | 62.3 | 714.9 | 2575.8 | | 31% |
| | 52 | 280 | 13.0 | 1113.0 | 62.3 | 723.0 | 2598.8 | | 32% |
| | 58 | 280 | 10.0 | 1123.0 | 62.3 | 729.2 | 2616.0 | | 31.8% |
| SRT=15d-30 min Ozonation | 0 | 280 | 0 | 0 | 0 | 0 | 0.0 | 11475 | 0% |
| | 2 | 280 | 82 | 82.0 | | 0.0 | -36.8 | | 0% |
| | 3 | 280 | 29 | 111.0 | 11.7 | 33.8 | 87.0 | | 1% |
| | 6 | 280 | 88 | 199.0 | 12.0 | 45.2 | 107.9 | | 1% |
| | 9 | 280 | 84 | 283.0 | 24.9 | 99.6 | 310.4 | | 4% |
| | 12 | 280 | 168 | 451.0 | 45.7 | 230.5 | 819.1 | | 10% |
| | 16 | 280 | 270 | 721.0 | 62.0 | 440.2 | 1640.5 | | 20% |
| | 19 | 280 | 129 | 850.0 | 64.6 | 530.3 | 1991.3 | | 25% |
| | 23 | 280 | 182 | 1032.0 | 67.9 | 662.5 | 2507.4 | | 31% |
| | 27 | 280 | 60 | 1092.0 | 66.6 | 699.1 | 2646.1 | | 33% |
| | 32 | 280 | 60 | 1152.0 | 65.7 | 736.2 | 2783.7 | | 35% |
| | 37 | 280 | 71 | 1223.0 | 64.8 | 779.8 | 2942.6 | | 37% |
| | 46 | 280 | 48 | 1271.0 | 64.8 | 810.9 | 3053.0 | | 38% |
| | 52 | 280 | 15 | 1286.0 | 64.8 | 820.6 | 3086.3 | | 38% |
| | 58 | 280 | 18 | 1304.0 | 64.8 | 832.3 | 3128.5 | | 39.0% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Seed for SRT=15** | 0 | 280 | 0 | 0 | 0 | 0 | | | |
| | 2 | 280 | 130 | 130.0 | 22 | 85.8 | | | |
| | 3 | 280 | 34 | 164.0 | 28.6 | 112.7 | | | |
| | 6 | 280 | 80 | 244.0 | 38.6 | 169.6 | | | |
| | 9 | 280 | 51 | 295.0 | 43.8 | 205.4 | | | |
| | 12 | 280 | 54 | 349.0 | 47.2 | 239.8 | | | |
| | 16 | 280 | 56 | 405.0 | 51.9 | 281.0 | | | |
| | 19 | 280 | 34 | 439.0 | 53.5 | 303.4 | | | |
| | 23 | 280 | 42.0 | 481.0 | 55.8 | 332.8 | | | |
| | 27 | 280 | 28.0 | 509.0 | 56.5 | 350.4 | | | |
| | 32 | 280 | 40.0 | 549.0 | 57.3 | 375.4 | | | |
| | 37 | 280 | 57.0 | 606.0 | 58.6 | 412.2 | | | |
| | 46 | 280 | 56.0 | 662.0 | 58.6 | 445.0 | | | |
| | 52 | 280 | 22.0 | 684.0 | 58.6 | 457.9 | | | |
| | 58 | 280 | 18.0 | 702.0 | 58.6 | 468.5 | | | |

Table D.9. Anaerobic digestion batch test, 1.95 day SRT WAS, Ammonia generation

| Sample | Day | ×Dilution | Conc. | Conc.×Dilution | Corrected Ammonia conc. (280/250 × (Bottles - (3/28)*Seed)) | TCOD mg/l | TKN mg/l | Ammonia Conc. / Total TKN (mg ammon/l)/(mg-N/l) | Ammonia Con / TCOD % (mg ammon/l)/(mgCOD/l) |
|---|---|---|---|---|---|---|---|---|---|
| SRT=1.95d-Raw | 0 | 30 | 3.523 | 105.69 | 9.2 | 7025 | 624.35 | **1.5%** | **0.13%** |
| | 2 | 30 | 8.433 | 252.99 | 163.0 | | | **26.1%** | **2.32%** |
| | 4 | 30 | 11.127 | 333.81 | 250.8 | | | **40.2%** | **3.57%** |
| | 6 | 30 | 11.935 | 358.05 | 277.4 | | | **44.4%** | **3.95%** |
| | 9 | 30 | 13.117 | 393.51 | 312.4 | | | **50.0%** | **4.45%** |
| | 16 | 30 | 13.996 | 419.88 | 327.1 | | | **52.4%** | **4.66%** |
| | 58 | 30 | 17.453 | 523.59 | 417.5 | | | **66.9%** | **5.94%** |
| | | | | | | | | | |
| SRT=1.95d- 15 min. Ozonation | 0 | 30 | 3.598 | 107.94 | 11.7 | 7000 | 630.55 | **1.9%** | **0.17%** |
| | 2 | 30 | 11.281 | 338.43 | 258.7 | | | **41.0%** | **3.70%** |
| | 4 | 30 | 13.078 | 392.34 | 316.4 | | | **50.2%** | **4.52%** |
| | 6 | 30 | 13.615 | 408.45 | 333.8 | | | **52.9%** | **4.77%** |
| | 9 | 30 | 14.515 | 435.45 | 359.4 | | | **57.0%** | **5.13%** |
| | 16 | 30 | 15.058 | 451.74 | 362.7 | | | **57.5%** | **5.18%** |
| | 58 | 30 | 17.808 | 534.24 | 429.5 | | | **68.1%** | **6.14%** |
| | | | | | | | | | |
| SRT=1.95d- 30 min. Ozonation | 0 | 30 | 3.777 | 113.31 | 17.7 | 6525 | 653.2 | **2.7%** | **0.27%** |
| | 2 | 30 | | | | | | | |
| | 4 | 30 | | | | | | | |
| | 6 | 30 | | | | | | | |
| | 9 | 30 | | | | | | | |
| | 16 | 30 | | | | | | | |
| | 58 | 30 | 17.852 | 535.56 | 430.9 | | | **66.0%** | **6.60%** |
| | | | | | | | | | |
| Seed SRT=1.95d | 0 | 30 | 30.333 | 909.99 | | | | | |
| | 2 | 30 | 33.418 | 1002.54 | | | | | |
| | 4 | 30 | 34.181 | 1025.43 | | | | | |
| | 6 | 30 | 34.339 | 1030.17 | | | | | |
| | 9 | 30 | 35.652 | 1069.56 | | | | | |
| | 16 | 30 | 39.779 | 1193.37 | | | | | |
| | 58 | 30 | 46.916 | 1407.48 | | | | | |
| | | | | | | | | | |

Table D.10. Anaerobic digestion batch test, 7 day SRT WAS, Ammonia generation

| Sample | Day | ×Dilution | Conc. | Conc.×Dilution | Corrected Ammonia conc. (280/250 × (Bottles - (3/28)*Seed)) | TCOD mg/l | TKN mg/l | Ammonia Conc. / Total TKN (mg ammon/l)/(mg-N/l) | Ammonia Con / TCOD % (mg ammon/l)/(mgCOD/l) |
|---|---|---|---|---|---|---|---|---|---|
| SRT=7d-Raw | 0 | 30 | 3.5705 | 107.115 | 10.8 | 13400 | 1026.2 | **0.9%** | **0.08%** |
| | 2 | 30 | 15.208 | 456.24 | 390.7 | | | **32.4%** | **2.92%** |
| | 4 | 30 | 17.783 | 533.49 | 474.5 | | | **39.3%** | **3.54%** |
| | 6 | 30 | 17.755 | 532.65 | 472.9 | | | **39.2%** | **3.53%** |
| | 9 | 30 | 19.17 | 575.1 | 515.8 | | | **42.7%** | **3.85%** |
| | 16 | 30 | 20.323 | 609.69 | 539.6 | | | **44.7%** | **4.03%** |
| | 58 | 30 | 25.877 | 776.31 | 700.6 | | | **58.1%** | **5.23%** |
| | | | | | | | | | |
| SRT=7d- 15 min. Ozonation | 0 | 30 | 3.732 | 114.94 | 19.5 | 13200 | 1183.7 | **1.7%** | **0.15%** |
| | 2 | 30 | 15.681 | 470.43 | 406.6 | | | **34.3%** | **3.08%** |
| | 4 | 30 | 18.357 | 550.71 | 493.7 | | | **41.7%** | **3.74%** |
| | 6 | 30 | 18.35 | 550.5 | 492.9 | | | **41.6%** | **3.73%** |
| | 9 | 30 | 20.65 | 619.5 | 565.5 | | | **47.8%** | **4.28%** |
| | 16 | 30 | 20.773 | 623.19 | 554.8 | | | **46.9%** | **4.20%** |
| | 58 | 30 | 26.088 | 782.64 | 707.7 | | | **59.8%** | **5.36%** |
| | | | | | | | | | |
| SRT=7d- 30 min. Ozonation | 0 | 30 | 3.577 | 119.07 | 24.2 | 13550 | 1249.3 | **1.9%** | **0.18%** |
| | 2 | 30 | 19.127 | 573.81 | 522.4 | | | **41.8%** | **3.86%** |
| | 4 | 30 | 21.274 | 638.22 | 591.8 | | | **47.4%** | **4.37%** |
| | 6 | 30 | 21.434 | 643.02 | 596.6 | | | **47.8%** | **4.40%** |
| | 9 | 30 | 22.842 | 685.26 | 639.1 | | | **51.2%** | **4.72%** |
| | 16 | 30 | 23.944 | 718.32 | 661.3 | | | **52.9%** | **4.88%** |
| | 58 | 30 | 28.413 | 852.39 | 785.8 | | | **62.9%** | **5.80%** |
| | | | | | | | | | |
| Seed SRT=7 d | 0 | 30 | 30.333 | 909.99 | | | | | |
| | 2 | 30 | 33.418 | 1002.54 | | | | | |
| | 4 | 30 | 34.181 | 1025.43 | | | | | |
| | 6 | 30 | 34.339 | 1030.17 | | | | | |
| | 9 | 30 | 35.652 | 1069.56 | | | | | |
| | 16 | 30 | 39.779 | 1193.37 | | | | | |
| | 58 | 30 | 46.916 | 1407.48 | | | | | |
| | | | | | | | | | |

Table D.11. Anaerobic digestion batch test, 15 day SRT WAS, Ammonia generation

| Sample | Day | ×Dilution | Conc. | Conc.×Dilution | Corrected Ammonia conc. (280/250 × (Bottles - (3/28)*Seed)) | TCOD mg/l | TKN mg/l | Ammonia Conc. / Total TKN (mg ammon/l)/(mg-N/l) | Ammonia Con / TCOD % (mg ammon/l)/(mgCOD/l) |
|---|---|---|---|---|---|---|---|---|---|
| SRT=15d-Raw | 0 | 30 | 3.483 | 104.475 | 7.8 | 11525 | 1211.7 | **0.6%** | **0.07%** |
| | 2 | 30 | 13.669 | 410.07 | 339.0 | | | **28.0%** | **2.94%** |
| | 4 | 30 | 15.06 | 451.8 | 383.0 | | | **31.6%** | **3.32%** |
| | 6 | 30 | 15.436 | 463.08 | 395.0 | | | **32.6%** | **3.43%** |
| | 9 | 30 | 18.087 | 542.61 | 479.4 | | | **39.6%** | **4.16%** |
| | 16 | 30 | 19.619 | 588.57 | 516.0 | | | **42.6%** | **4.48%** |
| | 58 | 30 | 24.201 | 726.03 | 644.3 | | | **53.2%** | **5.59%** |
| | | | | | | | | | |
| SRT=15d- 15 min. Ozonation | 0 | 30 | 3.702 | 114.94 | 19.5 | 12200 | 1210 | **1.6%** | **0.16%** |
| | 2 | 30 | 14.305 | 429.15 | 360.3 | | | **29.8%** | **2.95%** |
| | 4 | 30 | 15.881 | 476.43 | 410.6 | | | **33.9%** | **3.37%** |
| | 6 | 30 | 17.246 | 517.38 | 455.8 | | | **37.7%** | **3.74%** |
| | 9 | 30 | 17.967 | 539.01 | 475.3 | | | **39.3%** | **3.90%** |
| | 16 | 30 | 19.405 | 582.15 | 508.8 | | | **42.0%** | **4.17%** |
| | 58 | 30 | 23.748 | 712.44 | 629.0 | | | **52.0%** | **5.16%** |
| | | | | | | | | | |
| SRT=15d- 30 min. Ozonation | 0 | 30 | 3.603 | 119.07 | 24.2 | 11475 | 1240 | **1.9%** | **0.21%** |
| | 2 | 30 | 17.86 | 535.8 | 479.8 | | | **38.7%** | **4.18%** |
| | 4 | 30 | 19.54 | 586.2 | 533.5 | | | **43.0%** | **4.65%** |
| | 6 | 30 | 20.586 | 617.58 | 568.1 | | | **45.8%** | **4.95%** |
| | 9 | 30 | 21.776 | 653.28 | 603.3 | | | **48.7%** | **5.26%** |
| | 16 | 30 | 23.421 | 702.63 | 643.7 | | | **51.9%** | **5.61%** |
| | 58 | 30 | 27.904 | 837.12 | 768.7 | | | **62.0%** | **6.70%** |
| | | | | | | | | | |
| Seed SRT=15 d | 0 | 30 | 30.333 | 909.99 | | | | | |
| | 2 | 30 | 33.418 | 1002.54 | | | | | |
| | 4 | 30 | 34.181 | 1025.43 | | | | | |
| | 6 | 30 | 34.339 | 1030.17 | | | | | |
| | 9 | 30 | 35.652 | 1069.56 | | | | | |
| | 16 | 30 | 39.779 | 1193.37 | | | | | |
| | 58 | 30 | 46.916 | 1407.48 | | | | | |
| | | | | | | | | | |

Table D.12. Anaerobic digestion batch test, 1.95 day SRT WAS, VFA generation

| Sample | Day | ×Dilution | Acetate | Propionate | Butyrate | Iso Butyrate | Valerate | Corrected values | | | | TCOD(mgCOD/L) | TVFA (mg COD/l | TVFA/TCOD (mg COD/l) / (mgCOD/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Acetate | Propionate | Butyrate+ Iso Butyrate | Valerate | | | |
| SRT=1.95d-Raw | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7025 | **0** | **0%** |
| | 2 | 5 | 117 | 47.8 | 6.89 | 13.1 | 2.19 | 652.1 | 265.4 | 111.9 | 12.3 | | **1326** | **19%** |
| | 4 | 5 | 147 | 72.9 | 11.4 | 17.6 | 3.9 | 803.8 | 402.4 | 162.4 | 21.8 | | **1806** | **26%** |
| | 6 | 5 | 165 | 85.4 | 12.3 | 18.4 | 4.71 | 895.3 | 472.9 | 171.9 | 26.4 | | **2037** | **29%** |
| | 8 | 5 | 174 | 85.7 | 13.9 | 19.8 | 5.95 | 922.3 | 473.1 | 188.7 | 33.3 | | **2111** | **30%** |
| | 10 | 5 | 189 | 86.3 | 15.8 | 19.8 | 5.71 | 988.8 | 477.6 | 199.4 | 32.0 | | **2205** | **31%** |
| | Correction e.g. for acetate: $(Act\text{-}Act_{seed} \times V_{seed}/V_T) \times V_T / (V_T - V_{seed}) \times Dil$ | | | | | | | | | | | | | |
| SRT=1.95d-15 min. Ozonation | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7000 | **0** | **0%** |
| | 2 | 5 | 146 | 52.7 | 13.7 | 17 | 0 | 814.5 | 292.8 | 171.9 | 0.0 | | **1625** | **23%** |
| | 4 | 5 | 183 | 93.3 | 16.5 | 22 | 0 | 1005.4 | 516.7 | 215.6 | 0.0 | | **2246** | **32%** |
| | 6 | 5 | 194 | 94.7 | 16.4 | 23.7 | 9.62 | 1057.7 | 525.0 | 224.6 | 53.9 | | **2441** | **35%** |
| | 8 | 5 | 202 | 101 | 17.2 | 25.1 | 4.28 | 1079.1 | 558.8 | 236.9 | 24.0 | | **2476** | **35%** |
| | 10 | 5 | 211 | 101 | 17.5 | 26.8 | 8.81 | 1112.0 | 559.9 | 248.1 | 49.3 | | **2585** | **37%** |
| SRT=1.95d-30 min. Ozonation | 0 | | | | | | | | | | | | | |
| | 2 | | | | | | | | | | | | | |
| | 4 | | | | | | | | | | | | | |
| | 6 | | | | | | | | | | | | | |
| | 8 | | | | | | | | | | | | | |
| | 10 | | | | | | | | | | | | | |
| Seed SRT=1.95d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| | 2 | 5 | 5.21 | 3.87 | 0 | 0 | 0 | | | | | | | |
| | 4 | 5 | 32.3 | 9.7 | 0 | 0 | 0 | | | | | | | |
| | 6 | 5 | 47.9 | 8.95 | 0 | 0 | 0 | | | | | | | |
| | 8 | 5 | 86.9 | 11.3 | 0 | 0 | 0 | | | | | | | |
| | 10 | 5 | 116 | 9.55 | 0 | 0 | 0 | | | | | | | |

Table D.13. Anaerobic digestion batch test, 7 day SRT WAS, VFA generation

| Sample | Day | ×Dilution | Acetate | Propionate | Butyrate | Iso Butyrate | Valerate | Corrected values: Acetate | Propionate | Butyrate+ Iso Butyrate | Valerate | TCOD(mgCOD/L) | TVFA (mg COD/l | TVFA/TCOD (mg COD/l) / (mgCOD/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SRT=7d- Raw | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | **0** | **0%** |
| | 2 | 5 | 165 | 68.6 | 15.7 | 31.9 | 10.1 | 920.9 | 381.8 | 266.6 | 56.6 | | **2160** | **16%** |
| | 4 | 5 | 196 | 94.5 | 18.7 | 35.2 | 12.8 | 1078.2 | 523.4 | 301.8 | 71.7 | | **2637** | **20%** |
| | 6 | 5 | 211 | 103 | 19.4 | 35.8 | 17.6 | 1152.9 | 571.4 | 309.1 | 98.4 | | **2857** | **22%** |
| | 8 | 5 | 223 | 104 | 21 | 37.4 | 10.7 | 1196.7 | 575.6 | 327.0 | 59.9 | | **2864** | **22%** |
| | 10 | 5 | 246 | 115 | 23.3 | 36.4 | 11.4 | 1308.0 | 638.3 | 334.3 | 63.8 | | **3099** | **24%** |
| | Correction e.g. for acetate: $(Act\text{-}Act_{seed} \times V_{seed}/V_T) \times V_T / (V_T - V_{seed}) \times Dil$ | | | | | | | | | | | | | |
| SRT=7d- 15 min. Ozonation | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | **0** | **0%** |
| | 2 | 5 | 176 | 71.3 | 17.8 | 31 | 14.9 | 982.5 | 397.0 | 273.3 | 83.3 | | **2316** | **18%** |
| | 4 | 5 | 211 | 96.8 | 20.5 | 34.7 | 13.2 | 1162.2 | 536.3 | 309.1 | 74.0 | | **2764** | **21%** |
| | 6 | 5 | 219 | 103 | 21.2 | 36 | 20.7 | 1197.7 | 571.4 | 320.3 | 116.1 | | **2962** | **22%** |
| | 8 | 5 | 238 | 110 | 23.9 | 38.9 | 0 | 1280.7 | 609.2 | 351.7 | 0.0 | | **2928** | **22%** |
| | 10 | 5 | 261 | 118 | 26 | 39.1 | 9.87 | 1392.0 | 655.1 | 364.6 | 55.3 | | **3252** | **25%** |
| | | | | | | | | | | | | | | |
| SRT=7d- 30 min. Ozonation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | **0** | **0%** |
| | 2 | 5 | 198 | 53.5 | 26.4 | 28.3 | 7.16 | 1105.7 | 297.3 | 306.3 | 40.1 | | **2268** | **17%** |
| | 4 | 5 | 240 | 75.9 | 29.7 | 37 | 16.2 | 1324.6 | 419.2 | 373.5 | 90.8 | | **2912** | **21%** |
| | 6 | 5 | 277 | 103 | 28.5 | 39.6 | 22 | 1522.5 | 571.4 | 381.4 | 123.2 | | **3433** | **25%** |
| | 8 | 5 | 297 | 112 | 30.5 | 47 | 0 | 1611.1 | 620.4 | 434.0 | 0.0 | | **3447** | **25%** |
| | 10 | 5 | 312 | 117 | 30.7 | 54.2 | 22.4 | 1677.6 | 649.5 | 475.4 | 125.4 | | **3893** | **29%** |
| | | | | | | | | | | | | | | |
| Seed SRT=7d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| | 2 | 5 | 5.21 | 3.87 | 0 | 0 | 0 | | | | | | | |
| | 4 | 5 | 32.3 | 9.7 | 0 | 0 | 0 | | | | | | | |
| | 6 | 5 | 47.9 | 8.95 | 0 | 0 | 0 | | | | | | | |
| | 8 | 5 | 86.9 | 11.3 | 0 | 0 | 0 | | | | | | | |
| | 10 | 5 | 116 | 9.55 | 0 | 0 | 0 | | | | | | | |
| | | | | | | | | | | | | | | |

Table D.14. Anaerobic digestion batch test, 15 day SRT WAS, VFA generation

| Sample | Day | ×Dilution | Acetate | Propionate | Butyrate | Iso Butyrate | Valerate | Corrected values: Acetate | Corrected values: Propionate | Corrected values: Butyrate+ Iso Butyrate | Corrected values: Valerate | TCOD(mgCOD/L) | TVFA (mg COD/l | TVFA/TCOD (mg COD/l) / (mgCOD/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SRT=15d-Raw | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | **0** | **0%** |
| | 2 | 5 | 141 | 67 | 11.3 | 20.4 | 4.6 | 786.5 | 372.9 | 177.5 | 26.0 | | **1779** | **15%** |
| | 4 | 5 | 173 | 85.8 | 16 | 24.1 | 10.98 | 949.4 | 474.7 | 224.6 | 60.9 | | **2264** | **19%** |
| | 6 | 5 | 188 | 77.1 | 16.9 | 25.4 | 11.9 | 1024.1 | 426.4 | 236.9 | 66.8 | | **2305** | **20%** |
| | 8 | 5 | 210 | 98.3 | 19.5 | 26.7 | 10.7 | 1123.9 | 543.7 | 258.7 | 59.9 | | **2614** | **22%** |
| | 10 | 5 | 234 | 113 | 22.5 | 26 | 0 | 1240.8 | 627.1 | 271.6 | 0.0 | | **2766** | **24%** |
| | Correction e.g. for acetate: $(Act\text{-}Act_{seed} \times V_{seed}/V_T) \times V_T / (V_T - V_{seed}) \times Dil$ | | | | | | | | | | | | | |
| SRT=15d- 15 min. Ozonation | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | **0** | **0%** |
| | 2 | 5 | 174 | 68.6 | 16 | 0 | 0 | 971.3 | 381.8 | 89.6 | 0.0 | | **1777** | **15%** |
| | 4 | 5 | 203 | 88 | 19.5 | 32.1 | 12.9 | 1117.4 | 487.0 | 289.0 | 72.1 | | **2601** | **23%** |
| | 6 | 5 | 209 | 91.1 | 18.9 | 31.7 | 12.3 | 1141.7 | 504.8 | 283.4 | 68.9 | | **2638** | **23%** |
| | 8 | 5 | 229 | 97.9 | 20.9 | 33.2 | 12.9 | 1230.3 | 541.5 | 303.0 | 72.6 | | **2831** | **25%** |
| | 10 | 5 | 243 | 109 | 23.2 | 32.9 | 0 | 1291.2 | 604.7 | 314.2 | 0.0 | | **2864** | **25%** |
| SRT=15d- 30 min. Ozonation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | **0** | **0%** |
| | 2 | 5 | 187 | 57.3 | 24.1 | 28.5 | 4.85 | 1044.1 | 318.6 | 294.6 | 27.2 | | **2187** | **19%** |
| | 4 | 5 | 226 | 79 | 27.8 | 38 | 14.1 | 1246.2 | 436.6 | 368.5 | 79.1 | | **2821** | **25%** |
| | 6 | 5 | 247 | 95.9 | 26 | 39.4 | 14.1 | 1354.5 | 531.7 | 366.2 | 78.9 | | **3076** | **27%** |
| | 8 | 5 | 272 | 110 | 28.8 | 47.9 | 19.7 | 1471.1 | 609.2 | 429.5 | 110.6 | | **3498** | **30%** |
| | 10 | 5 | 303 | 123 | 30.2 | 53.4 | 20.3 | 1627.2 | 683.1 | 468.2 | 113.8 | | **3853** | **34%** |
| Seed SRT=15d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| | 2 | 5 | 5.21 | 3.87 | 0 | 0 | 0 | | | | | | | |
| | 4 | 5 | 32.3 | 9.7 | 0 | 0 | 0 | | | | | | | |
| | 6 | 5 | 47.9 | 8.95 | 0 | 0 | 0 | | | | | | | |
| | 8 | 5 | 86.9 | 11.3 | 0 | 0 | 0 | | | | | | | |
| | 10 | 5 | 116 | 9.55 | 0 | 0 | 0 | | | | | | | |

# Appendix E

# Calculations:

Table E.1. List of files in compact disc showing calculations*

| File name | Format | Subject |
|---|---|---|
| **COD Calculation** | **Excel** | **Methodology for calculation of TCOD, FCOD and FFCOD** |
| **TKN Calculation** | **Excel** | **Methodology for calculation of TKN and FTKN** |
| **Solid Calculation** | **Excel** | **Methodology for calculation of TS, VS and VDS** |
| **Calculation of $K_{VFA}$** | **Excel** | **Methodology for calculation of $K_{VFA}$ in anaerobic batch tests** |
| **Calculation of $K_{Ammonia}$** | **Excel** | **Methodology for calculation of $K_{Ammonia}$ in anaerobic batch tests** |
| **Modeling - COD Fractionation** | **Excel** | **Methodology for calculation of COD fraction of WAS** |

*The calculations on the compact disc are only available in the printed version of the thesis.