INVESTIGATION OF DIGESTER F/M RATIO AS A PARAMETER TO AFFECT SLUDGE MINIMIZATION AND GAS PRODUCTION OF ULTRASONICALLY TREATED SLUDGE

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submitted by **GÖZDE TUĞBA KÖKSOY** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Göksel N. Demirer
Head of Department, **Environmental Engineering** _____

Prof. Dr. F. Dilek Sanin
Supervisor, **Environmental Engineering Dept., METU** _____

Examining Committee Members:

Prof. Dr. Celal F. Gökçay
Environmental Engineering Dept., METU _____

Prof. Dr. F. Dilek Sanin
Environmental Engineering Dept., METU _____

Assoc. Prof. Ayşegül Aksoy
Environmental Engineering Dept., METU _____

Assoc. Prof. İpek İmamoğlu
Environmental Engineering Dept., METU _____

Prof. Dr. Ayşenur Uğurlu
Environmental Engineering Dept., Hacettepe University _____

Date: 24.06.2009

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Gözde Tuğba Köksoy

Signature:

ABSTRACT

INVESTIGATION OF DIGESTER F/M RATIO AS A PARAMETER TO AFFECT SLUDGE MINIMIZATION AND GAS PRODUCTION OF ULTRASONICALLY TREATED SLUDGE

Köksoy, Gözde Tuğba

M.Sc., Department of Environmental Engineering

Supervisor: Prof. Dr. F. Dilek Sanin

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Ultrasonic sludge disintegration –the most commonly used mechanical pretreatment method- enables the occurrence of cavitation bubbles to extract intracellular material from the cell into aqueous phase. However, there is a lack of information on the volatile solids loading on the anaerobic digestion process performance of ultrasonically treated sludge.

In this thesis work, the effect of sonication on disintegration of waste activated sludge (WAS) and an important parameter digester F/M (food to microorganism) ratio on ultrasonically treated WAS were investigated.

First, preliminary studies were conducted. It was obtained that when the sonication power and time increased, soluble COD in the supernatant increased as well. Then, batch anaerobic digestion tests were conducted. Effect of F/M ratio in the digesters by using sonicated sludges at different powers was analyzed. For the sludge sonicated at high power, the methane content increased up to 55.1 % at F/M ratio of

10 compared to untreated sludge. On the other hand, methane generation rate slowed down with the increase in F/M ratio. Moreover, 10 % and 15 % increase in the destruction of MLVSS and total COD content was observed for sonicated sludges compared to the untreated sludges, respectively.

In summary, both the sonication as a pretreatment method and the increase in digester F/M ratio increased the biogas production and the solids reduction during anaerobic digestion prosess. These results may have important implications for the operation of full scale systems in terms of system efficiency and operation.

Key words: Activated sludge, anaerobic digestion, biogas, disintegration, F/M ratio, ultrasonication

ÖZ

ÖZÜMLEME TANKINDAKİ F/M ORANININ ULTRASONİKASYONLA ARITILMIŞ ÇAMUR ÜZERİNDE ÇAMUR MİKTARININ MİNİMİZASYONU VE GAZ ÜRETİMİNİ ETKİLEYEN BİR PARAMETRE OLARAK ARAŞTIRILMASI

Köksoy, Gözde Tuğba

Yüksek Lisans, Çevre Mühendisliği Bölümü

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Kullanılan en yaygın mekanik önarıtım metotu olan ultrasonik çamur parçalaması, hücre içi maddelerin hücreden sıvı faza çıkarılması amacıyla mikroorganizmaların dağılması için oyuk kabarcıklarının oluşmasını sağlar. Fakat, uçuçu katıların yüklemesinin ultrasonikasyonla arıtılmış çamurun özümleme işleminin performansı üzerindeki etkisi hakkında bilgi yetersizliği vardır.

Bu tez çalışmasında sonikasyonun çamur parçalanması ve özümleme tankındaki F/M (besin/mikroorganizma) oranının uygun koşullarda utrasonla arıtılmış çamur üzerindeki etkisi araştırılmıştır.

Öncelikle aktif çamur üzerinde özümleme öncesi ön çalışmalar yapılmıştır. Sonikasyon gücü ve zamanı arttıkça, çamur üst suyunun çözünmüş KOİ'sinin arttığı elde edilmiştir. Sonra, kesikli reaktörler kurulmuştur. F/M oranının özümleyecilerdeki etkisi değişik güçlerde sonikasyonlanmış çamurlar kullanılarak

araştırılmıştır. Yüksek güçte sonikasyonlanan çamur için F/M oranı 10 olduğu zaman, metan içeriği önarıtılmamış çamura oranla % 55.1'lere kadar artmıştır. Fakat, F/M oranı arttıkça metan gazı oluşum hızında yavaşlama görülmüştür. Ayrıca, toplam katı miktarı ve toplam KOİ içeriğinde % 10 ve % 15 azalma elde edilmiştir.

Sonuç olarak, bir ön arıtım metodu olan sonikasyon ve özümleme tankındaki F/M oranındaki artış anaerobik özümleme sonrası metan gazı içeriğini ve toplam katı miktarındaki azalma oranını arttırmaktadır. Bu sonuçların büyük ölçekli tesisler düşünüldüğünde sistem işletimi ve verimi açısından önemli etkileri olacağı düşünülmektedir.

Anahtar Kelimeler: Aktif çamur, anaerobik özümleme, biogaz, dezentegrasyon, F/M oranı, ultrasonikasyon

To my family…

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ABBREVIATIONS

ADS	: Anaerobic Digested Sludge
BM	: Basal Medium
BMP	: Biochemical Methane Potential
COD	: Chemical Oxygen Demand
CST	: Capillary Suction Time
DS	: Dissolved Solids
GC	: Gas Chromatograph
MLSS	: Mixed Liquor Suspended Solids
MLVSS	: Mixed Liquor Volatile Suspended Solids
NH_4-N	: Ammonia Nitrogen
PO_4-P	: Phosphate Phosphorus
sCOD	: Soluble Chemical Oxygen Demand
SOUR	: Specific Oxygen Uptake Rate
tCOD	: Total Chemical Oxygen Demand
TS	: Total Solids
TSS	: Total Suspended Solids
WAS	: Waste Activated Sludge
YTL	: New Turkish Liras

CHAPTER 1

INTRODUCTION

Wastewater sludge that typically contains 20 % solids by weight is a semisolid, nutrient-rich by-product of wastewater treatment. The quantity of sludge produced in a wastewater treatment plant is approximately 1 % of the quantity of treated wastewater (Turovskiy and Mathai, 2006). This produced sludge contains chemicals and microbes that can be a health hazard to people and it often smells bad. Therefore, high amounts of unstabilized sludge produced from activated sludge process get treated in anaerobic digestion systems for stabilization (Spinosa and Vesilind, 2001).

Anaerobic digestion is the most commonly applied sewage sludge stabilization technique resulting in the reduction of sludge volatile solids and the production of biogas. However, anaerobic stabilization is a slow process; because the first step in digestion, biological hydrolysis, has been identified as the rate-limiting step. Therefore, long residence times in the fermenters and large fermenter volumes are required for digestion (Eastman and Ferguson, 1981; Shimizu et al., 1993).

In order to improve the hydrolysis rate, various types of sludge pretreatment methods have been studied. These methods lead to rupture of the cell wall and membrane of bacteria in waste activated sludge resulting in release of organic substances to the outside of the cell (Wang et al., 1999). Therefore, sludge disintegration was introduced to solubilise and convert slowly biodegradable, particulate organic materials to low molecular weight, readily biodegradable compounds (Weemaes and Verstraete, 1998). Disintegration may be performed

biologically, chemically, thermally, mechanically or by a combination of these methods (Bougrier et al., 2005).

Ultrasonication is one of the effective mechanical methods that are used commonly as a pretreatment method. Its mechanism depends on the formation of cavitation bubbles by sound wave. There are several parameters that affect the efficiency of sonication as a pretreatment method on waste activated sludge. Therefore, before the full scale installation of ultrasound unit, lab scale and pilot scale experiments should be carried out to analyze these parameters such as sonication power and time (Khanal et al.,2007).

Food to microorganism (F/M) ratio is calculated by the amount of substrate (WAS) to inoculum (ADS) added to the anaerobic digester. It is an important digester parameter since volatile solids loading to the digester affects the performance of the process. There is lack of information in literature on the effect of F/M ratio on digester performance for sonicated sludge.

For this reasons, within the scope of this study, the effect of sonication on waste activated sludge was examined first during the disintegration studies. Then, batch anaerobic reactors were set for biochemical methane potential (BMP) tests in order to examine effect of the sonication and F/M ratio on anaerobic digestion process.

CHAPTER 2

LITERATURE REVIEW

2.1. Sludge

In fact, only about 35% of the organics are mineralized into carbon dioxide and water by microorganisms through aerobic biological treatment, such as the activated sludge process (Rittman and McCarty, 2001). The other part of the organic substances is converted into new bacterial cells known as biomass or biosolids (sewage sludge). This way, from biological wastewater treatment plants large quantities of sludge are produced. The sludge is highly putrescible (Nickel and Neis, 2007). It contains high quantities of organics, pathogens, nutrients and lots of water. Therefore, it has to be stabilized in order to enable an environmentally safe utilization and disposal (Neyens et al., 2003).

In wastewater treatment plants due to the growth in population and extended demands on higher effluent water quality lead to an increase in sludge production which makes sludge stabilization further and further important. For instance, in the United States the projected annual primary and secondary sludge production was about 7.6 million dry tons in 2005, which is about a 10% increase over data in 1998, and this is expected to increase to 8.3 million tons by 2010 (EPA U.S., 1999). The European Union (EU) had predicted that the sludge production within EU countries increased to 11 million dry tons in 2005 from about 6.6 million dry tons in 1998 (EC, 1991) - almost a 67% increase from 1998 to 2005. The quantity of sludge produced in a wastewater treatment plant is approximately 1 % of the quantity of treated wastewater. However, the handling, treatment and disposal of

this excess sludge account for 50-60% of the total operating costs (Saby et al., 2001; Canales et al., 1994). The reason is that while the treatment of wastewater takes several hours, processing and final destination (disposal or beneficial use) of the sludge generated take several days or even several weeks and require the use of more complex equipment (Turovskiy and Mathai, 2006).

The traditional disposal options such as landfill and incineration are becoming less acceptable for waste management. Sludge disposal by landfilling or incineration has formed environmental challenges due to the unavailability of landfill sites and incineration of solid wastes causing great difficulties in densely populated nations (Saby et al., 2001). In many countries the possibilities of sludge disposal suffer stringent national legislations, e.g. in Germany and in Switzerland disposal of sludge into landfills is completely forbidden (Strünkmann et. al., 2006). Thus, minimization of sludge production coupled with recovery of valuable byproducts and bioenergy is becoming increasingly critical for sustainable sludge management.

2.2 Anaerobic Digestion

Anaerobic digestion has become one of the most common methods for sludge stabilization in terms of producing biogas and reducing the volume of sludge. The following figure (Figure 2.1) explains the mechanism of anaerobic digestion schematically:

As illustrated in Figure 2.1, anaerobic digestion has four main stages. The detailed explanations of those steps are described below:

- **Hydrolysis:** Liquefaction of complex organic compounds to simpler forms by *hydrolytic bacteria;* principal end products include soluble sugars, amino acids, peptides, long-chain fatty acids (Scragg, 2005).

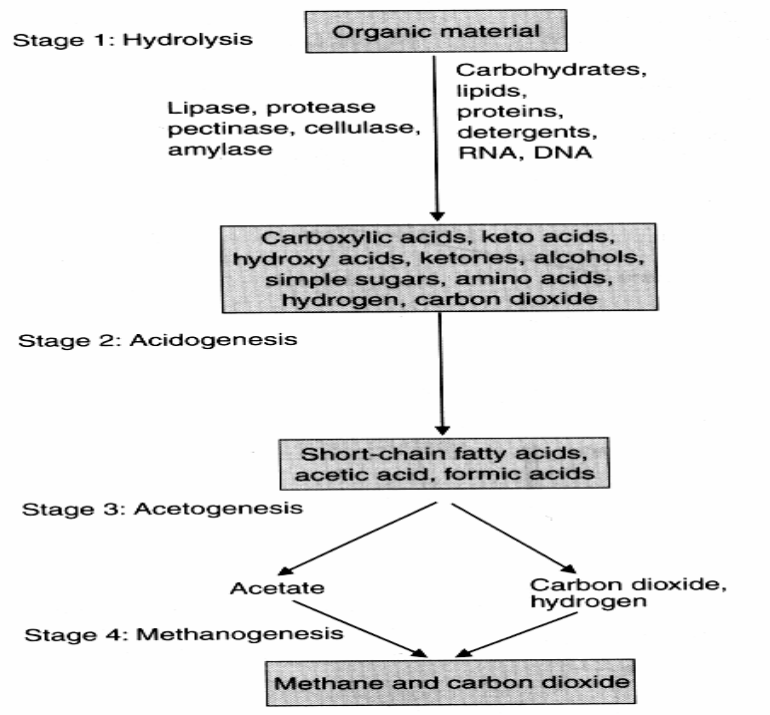


Figure 2.1. Schematic Representation of Anaerobic Digestion (Scragg, 2005)

- Acidogenesis: Metabolization of the products of hydrolysis by *Acidogenic bacteria;* principal end products include short-chain volatile organic acids (propionic, butyric, acetic and formic acids), CO_2, and H_2 (Scragg, 2005).

- Acetogenesis: Reduction of CO_2 and catabolization of short-chain fatty acids produced during acidogenesis by s*ynthrophic acetogenic and homoacetogenic bacteria;* principal end products include acetate, CO_2, H_2 (Scragg, 2005).

- Methanogenesis: Reduction of CO_2 and cleaved acetate by *Methanogens; e*nd products include CH_4, CO_2, trace gases (Scragg, 2005).

Although anaerobic digestion is the most widely applicable method in many treatment plants, the process is a very slow and may involve some problems such as difficulty in degrading facultative anaerobic microorganisms and foaming, which will affect the efficiency of sludge stabilization. In waste activated sludge most of the organics are located in the microbial cell. The cell wall of microorganisms is a stable semi-rigid structure that protects the cell from lysis. Therefore, there occurs a high resistance to biodegradation and this leads to long hydraulic retention time for the biological stabilization in digesters. Thus anaerobic digestion is a very slow process because of the rate-limiting step of hydrolysis (Show et al., 2006). Therefore, sludge pre-treatment by disintegration before anaerobic digestion has been found as a very beneficial technology in terms of improving the anaerobic degradation.

2.3. Sludge Pretreatment

Sludge pretreatment mechanically, chemically or thermally before anaerobic digestion has been applied to increase the digestability of excess sludge since pretreatment processes disrupt cell walls and release intracellular organic materials from the cells into the aqueous phase (Weemaes and Verstraete, 1998).

The cell destruction leads to the following advantages (Panter et al., 2002):

- Increase in performance of anaerobic digesters,
- Accelerated degradation of organic matter,
- Increase of biogas production,
- Disinfection of sludge,
- Disintegration of facultative anaerobic microorganisms,
- Reduction of specific sludge production,
- Reduction of bulking and foaming,
- Improvement of dewatering and settling capability.

Actually, improvement of dewatering and settling capability depend on the floc structure. In case of filamentous microorganisms, disintegration will destroy the voluminous floc structures and reduce the bulking and foaming problems and improve settling and dewatering. On the other hand, floc destruction may result in worse settling and dewatering character if the original sludge has a good floc structure (Müller et al., 2004).

Several sludge disintegration methods applied are (Spinosa and Vesilind, 2001):

- thermal energy treatment (Baier, 1997; Li and Noike, 1992; Stuckey and McCarty, 1984);

- chemical treatment using;

    ⇨ ozone (Scheminski et al., 1999; Yasui and Shibata, 1994; Yasui et al., 1996);

    ⇨ acids (Gaudy et al., 1971; Meunier et al., 1996; Woodard and Wukasch, 1994);

    ⇨ alkali (Haug et al., 1978; Lin et al., 1989; Mukherjee and Levine, 1992);

- mechanical energy (Müller, 1996);

    ⇨ high pressure (Lehne et al., 2001);

    ⇨ stirred ball mills (Baier and Schmidheiny, 1997; Kopp et al., 1997);

    ⇨ ultrasound (Tiehm et al., 1997);

- freezing and thawing (Chu et al., 1999);
- enzymes (Kayser and Nellenschulte, 1992; Knapp and Howell, 1978);
- irradiation (Etzel et al., 1969; Suess et al., 1982).

Sludge disintegration methods can be applied individually or one method can be combined with others such as thermo chemical methods to disintegrate sludge more efficiently.

2.3.1. Thermal Pretreatment

Thermal treatment was initially used in order to improve the dewaterability of sludge. But now it becomes a pretreatment method before anaerobic digestion (Weemaes and Verstraete, 1998). Thermal hydrolysis can be achieved at temperatures higher than 60^{o}C. At low temperatures very low solubilization rates are reached. As the temperature increases solubilization rate increases (Neyens et al. 2003). Brooks (1970) has found that solubilization of 20–60 % is achievable at 170^{o}C. When the temperature increases to 180^{o}C solubilization is more effective. Furthermore, as the temperature increases dewaterability increases. However, at these elevated temperatures, some refractory organics are formed. Therefore, operation temperature for hydrolysis can be kept at 175^{o}C where little or no refractory organics are formed and also dewaterability of the sludge is increased (Neyens et al., 2003).

The best conditions were determined to be as 170^{o}C, 30-60 min holding time and hydraulic retention time of 5-10 days based on gas production (Neyens et al. 2003). In addition, Li and Noike (1992) studied thermal pretreatment of waste activated sludge under conditions of 62 ºC to 175 ºC and 15 min. to 120 min. They observed a solubilization of 55 % at 75 ºC-30 min. It was found that after 60 min of

pretreatment there was no increase in methane gas production. The best condition was found as 170 ºC- 60 min.

Tanaka et al. (1997) examined the effect of thermal pretreatment on the combined WAS from domestic, commercial and industrial wastewaters. They found VSS solubilization rate as 15 % in between 115-150 ºC. It increased above 160 ºC and was 30 % at 180 ºC. Similar to trend of solubilization rate, the methane production was found around 40 % in between 115-150 ºC. It increased above 160 ºC and reached to 90 % at 180 ºC.

On the other hand, energy required for increasing the temperature to these elevated temperatures, huge amounts of heat must be applied. Thus, main operational cost is energy for thermal pretreatment (Müller, 2001).

2.3.2. Chemical and Thermo-Chemical Pretreatment

Chemical pretreatment techniques are applied by the addition of chemicals. These chemicals are chlorine, ozone, Fenton's reagent, acid and alkali. They convert hardly degradable compounds to easily ones (Neyens et al. 2003). These chemicals can also react with the hazardous compounds to make harmless compounds, such as, water and CO_2.

According to Neyens et al. (2004), acids and alkalis contribute to thermal hydrolysis of organic molecules. Smith et al. (1992) examined the thermo-chemical pretreatment of sludge at low and high pH values. Thermal acidic hydrolysis was studied with HCl and H_2SO_4. HCl showed a better performance than H_2SO_4 with solubilisation of about 30-50%. Thermal basic treatment was also performed by using $Ca(OH)_2$ and NaOH. NaOH had higher result with 40-60% solubilisation.

Woodard and Wukash (1994) showed that by the addition of 4 g sulphuric acid per g TSS, 61 % reduction in TSS was achieved. Tanaka et al. (1997) investigated chemical pretreatment of combined WAS from domestic, commercial and industrial wastewaters using alkali (NaOH). Increase in the alkali dose up to 0.5-0.6 g NaOH/g VSS, VSS solubilization rate increased to 15 %. Above 0.6, it was constant. On the other hand, there was a gradual increase in methane production which was about 50 % at the dose of 1.0.

Furthermore, in the study of Tanaka et al. (1997), the thermochemical pretreatment was also investigated using 0.3 g NaOH/g VSS at 130 ºC-5 min. Solubilization rate was 70-80 % for domestic WAS and 45 % for combined WAS. Pretreatment increased the methane production. It was 35 % to 50 % and 20 % to 35 % for domestic and combined WAS, respectively.

In the study of Saby et al. (2001), best possible dosage for chlorination was determined to be as 0.066 g Cl_2/g MLSS. By returning this chlorinated sludge to the activated sludge system, sludge production rate was reduced by 65% compared to the control system.

Bougrier et al. (2006) found that 20-25% solubilization was achievable by ozone dose of 0.1 and 0.16 gO_3/g TS. Organic amount decreased 5 % and gas production increased 11 % and 23 % for doses 0.1 and 0.16, respectively.

2.3.3. Freeze/Thawing Pretreatment

In freezing and thawing method, the sludge is frozen up to around –20 ºC, and then it is immersed into high temperature water bath for a several hours for thawing. Wang et al. (2003) compared the effect of freeze/thaw, acidic and sterilization pretreatments on sequential production of hydrogen and methane by anaerobic fermentation. The waste activated sludge was frozen at −17°C for 24 h in a freezer

and then thawed for 12 h in a water bath at 25°C. For acidic pretreatment perchloric acid (HClO4) was added into the sludge sample for 10 mins to adjust the pH to 3. Then, the sample was stored at 4°C for 6 h. In order to sterilize the sludge, the samples were pasteurized at 121°C and 1.2 kgf/cm^2 for 30 min. It was found that, sterilization produced two times more hydrogen than other pretreatments but freeze/thaw method showed the highest methane production in the methanogenic phase.

2.3.4. Mechanical Disintegration

Mechanical disintegration has grown rapidly in recent years due to the advances in technology (Panter et al. 2002). In order to rupture the cells by mechanical methods the energy is provided as pressure, translational or rotational energy. The cell of the microorganism resists the stress as long as the tension is lower than the strength of the cell wall. As the external pressure exceeds the cell internal pressure, cells are disrupted. All mechanical methods cause the lysis and disintegration of sludge cells. Therefore, intracellular matter is released and becomes more accessible for anaerobic microorganisms (Müller, 2001).

Most commonly used mechanical methods are as following:

- Ultrasonic homogenizer
- High pressure homogenizer
- Lysate Centrifuge
- Stirred ball mill

One of the most widely known methods for large scale operation is high pressure homogenizers. They consist of a multistep high-pressure-pump and a homogenizing valve. The pump compresses the suspension to pressures up to some thousand bars. (Weemaes and Verstraete, 1998). In order to disrupt the cells, pressures between

55 and 200 MPa was suggested by Geciova et al. (2001). The suspension then leaves the compressor through a valve at a high speed (300 m/s). The cells are then disintegrated because of turbulence, cavitation and shear stresses (Weemaes and Verstraete, 1998). Cell disintegrations up to 85% were achieved at relatively low energy levels (Harrison, 1991). Volatile solids reductions of 38-40% at retentions times of 2 days was found with high pressure homogenizer (Engelhart et al., 2000).

The Lysate-Centrifuge consists of decanter equipped with a disintegration device located at the discharge of the dewatered sludge. Tools on either the rotor or the stator stress the sludge by shear forces (Jomueller, 2008). Müller et al. (2004) studied the comparison of the energy consumption and efficiencies of different mechanical pre-treatment methods. Among them, the lysate centrifuge had a lower energy demand and its disintegration efficiency was lowest. The first full-scale experiment was carried out in Prague. The results showed that daily biogas production was increased about 7.5 % (Ødegaard, 2003).

Stirred ball mills consist of a cylindrical grinding chamber of up to 1 m^3 of volume which is almost completely filled with grinding beads (Jomueller, 2008). An agitator forces the beads into a rotational movement. The sludge is disintegrated in between the beads by the agitator making shear and pressure forces (Weemaes and Verstraete, 1998). Müller et al. (1998) found out that high degrees of disintegration was obtained when using the stirred ball mill for long grinding times, at high agitator speeds and small particle sizes of the grinding beads. The stirred ball mill was tested in Germany to produce an external carbon source for denitrification thereby decreasing the sludge production by 65% (Kunz, 1994).

Lehne et al. (2001) compared the energy requirements of ball mills, pressure homogenizers and ultrasonics and it was obtained from the results that ultrasonics used more energy than ball mills and homogenizers. On the other hand, Panter et al.

(2002) found that the ultrasonication is very simple method and the process can handle high viscosity materials.

2.4. Ultrasonication

Ultrasonic homogenizers consist of three major components: a generator with a frequency between 20 and 40 kHz, an electrical material transforms the electrical into mechanical impulses, and a sonotrode for the transmission of these impulses into fluid. Cavitation bubbles are produced by alternating overpressure and underpressure. In fact, there are applications of ultrasonic devices with thousand Watt performances in wastewater treatment plants (Jomueller, 2008).

Sonication at high powers provides 100 % disintegration of cells (Weemaes and Verstraete, 1998). Therefore, cell walls disrupt and intracellular materials pass through liquid phase. During anaerobic digestion the hydrolysis of sludge becomes easily and digestion time decreases.

2.5. Sonication Mechanism

Ultrasound is a sound wave at a frequency above the normal hearing range of humans that is 20 kHz (Figure 2.2). The ultrasound wave generates alternating compressions and rarefactions in the liquid. At higher intensities, ultrasound breaks up the aqueous medium during the rarefaction (Khanal et al., 2007).

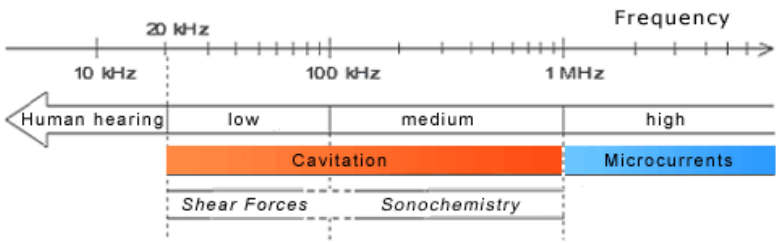


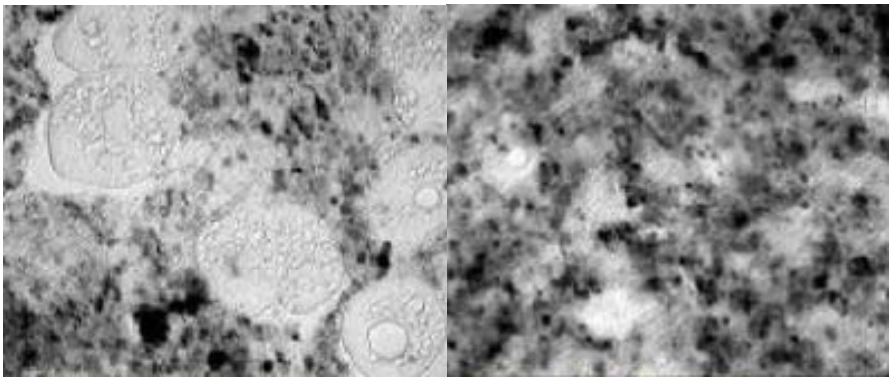
Figure 2.2. The sonication frequency interval (Ultrawaves, 2008)

These rarefactions are regions of excessively large negative pressure. This causes the breaking up of liquid and formation of microbubbles (cavitation bubbles) in the regions. These bubbles contain vaporized liquid and gas that was dissolved in the liquid earlier. As the wavefronts propagate, microbubbles oscillate under the influence of positive pressure, thus growing to an unstable size before violently collapsing at high velocity (Neppiras, 1980; Khanal et al., 2007).

The collapsing of the bubbles often results in localized temperatures up to 5000 K and pressures up to 180 MPa (Suslick, 1990; Flint and Suslick, 1991; Show et al., 2006). High temperature and pressure occured inside the collapsing bubbles could bring many physico-chemical effects (Monnier et al., 1999).

The researchers applied the ultrasonication because of these bubble explosions for counting the number of bacterial cells (Banks and Walker, 1977), extracting the exocellar polymer (Kiff and Thompson, 1979) and investigating the surface properties of microorganisms in sludge (Urban et al., 1993) (Chu et al., 2001).

The localized high temperature and pressure induce sludge disintegration (Tiehm et al. 1997). The sudden and violent collapse of huge numbers of microbubbles generates powerful hydromechanical shear forces in the bulk liquid surrounding the bubbles (Kuttruff, 1991). The collapsing bubbles disrupt adjacent bacterial cells by extreme shear forces, rupturing the cell wall and membranes (Khanal et al., 2007). The structure of sludge flocs after sonication can be seen in Figure 2.3. 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 (Tatsuo et al., 1993; Jorand et al., 1994; Chiu et al., 1997a; Wang et al., 2005). Consequently, the amount of dissolved organic material is significantly increased (Chiu et al., 1997a; Tiehm et al., 2001). In the meantime, the excess power input could increase temperature after a long-duration operation. The heating effects are necessarily accompanied and cannot be disregarded (Chiu et al., 1997b).



Before sonication After sonication

Figure 2.3. The microscopic picture of sludge before and after sonication (VoR, 2008)

The average particle size decreases after sonication because of the dispersion of sludge flocs. This de-agglomeration process can be evaluated by particle size distribution. When flocs are de-agglomerated into single cells, the amount of bacteria counted increases. After longer sonication times the number of bacteria reduces because the cells are destroyed. The size of single bacteria may lie between 0.5 and 10 μm (Neis and Blume, 2002).

Sonication treatment of sludge is carried out with an ultrasound system that is equipped with a probe transducer. After adjusting the frequency and the sonication intensity, waves formed in the ultrasonic power supply go through the probe, which is placed in the middle of the sludge and mechanical vibrations form acoustic pressure in the system (Show et al., 2006). According to Chu et al. (2001), effects of sonication largely depend on the sample volume, geometry of container, and the probe position.

Sonication mechanism is affected by three factors: the energy provided, ultrasonic frequency and characteristics of sludge (Show et al., 2006; Khanal et al., 2007). The disintegration of cells is proportional to the enegy supplied. Bougrier et al. (2005)

found out that the minimum energy required to destruct the cells is about 1000kJ/kg TS, that is about 20 kj/L sludge which agrees with the Gonze et al. (2003) who have found a minimum energy of 30 kj/L sludge and Lehne et al. (2001) found a higher value which is 3000 kj/kg TS.

The disintegration of sludge is proportional to the provided energy (Müller and Pelletier, 1998, cited in Khanal, 2007; Lehne et al., 2001). The energy supplied is also proportional to sonication power and sonication time, inversely proportional to sludge volume and the total solids amount of sludge (Khanal et al., 2007). According to investigations of Tiehm et al. (1997) on sonication time and disintegration degree, it was obtained that as the sonication time increases, degree of disintegration also increases. It was also found that after 96 seconds of ultrasound treatment; more than 30% of the maximum disintegration was achieved compared to chemical disintegration method.

Although sonication duration is important parameter, sonication intensity affects the floc destruction. Show et al. (2006) found that that higher ultrasonic intensity with short sonication time provided better disintegration results than lower intensity with longer sonication time.

Furthermore, it was figured out that the disintegration of sludge is better at low ultrasonic frequencies (20-40 kHz) (Atchley and Crum, 1988; Tiehm et al., 2001). The reason is the formation of powerful hydrodynamic shear at low frequency. The bubble radius is inversely proportional to the ultrasound frequency. Therefore, the application of low frequencies creates larger cavitation bubbles.

2.6. Advantages and Disadvantages of Ultrasonication

Being a physical process, ultrasonic disintegration does not generate secondary toxic compounds and contribute additional chemical compounds. During sonication

highly oxidative reactive radicals-hydroxyl (OH•), hydrogen (H•), and hydroperoxyl (HO•2) and hydrogen peroxide (H2O2) are produced. Therefore, many toxic and recalcitrant organic pollutants, such as aromatic compounds, chlorinated aliphatic compounds, surfactants, organic dyes, etc., are broken down into simpler forms in addition to physical sludge disintegration (Adewuyi, 2001).

Some other advantages of ultrasound pretreatment are (Khanal et al., 2007):

- Compact design and easy retrofit within existing systems
- Low cost and efficient operation compared to several other pretreatments
- Production of an in situ carbon source for denitrification plants
- Complete process automation
- Potential to control filamentous bulking and foaming in the digester
- Better digester stability
- Improved VS destruction (5-25 %) and biogas production (30-45 %)

The increase in biogas production could produce as much as 240 million m^3 of gas or 480 GWh/yr of "green" electricity.

- Better sludge dewaterability
- Improved biosolids quality (i.e., biosolids with low residual biodegradable organics, low pathogen counts, etc.)

On the other hand, the ultrasound pretreatment also faces several challenges. One of the major issues is the high capital and operating costs of ultrasound units. The cost may go down when the technology becomes mature. Correspondingly, long-term performance data of full-scale ultrasound systems are still limited. This discourages design engineers to recommend ultrasound systems for full-scale application (Khanal et al., 2007).

The augmentation of cell break-up can be depicted by numerous chemical and biological parameters. The cell disruption drives to the release of intracellular organic compounds into the sludge water phase. Therefore, the increase of cell disintegration is specified by the increase of the Chemical Oxygen Demand (COD) in the sludge supernatant. If ultrasonic parameters are selected appropriately, the total sludge COD could be solubilised up to 90% (Clark and Nujjoo, 1998).

Investigations of Chu et al. (2001) explained that after 120 min sonication at 110 W., the sludge, which was originally 4ºC, increased to higher than 60ºC after the collapse of cavitation bubbles. In order to understand the temperature effect of the disintegration, beakers were immersed in an iced pool to maintain the bulk temperature approximately at 15ºC. It was obvious from the results that the sludge sonicated with a low density at 0.11W/ml with temperature control produced almost no COD release from solid state to the soluble state. However, the same sludge sonicated with same density of 0.11W/ml without temperature control showed approximately 2% of COD release from solid state. Consequently, bubble explosion and bulk solution temperature rise are important in sludge floc disintegration.

According to Bougrier et al. (2005) sonication not only reduces the amount of sludge after digestion, but also the increases the amount of biogas in digesters. Show et al. (2006) explains that the biogas yield is due to the help of sonication in accelerating the organics degradation and converting the biosolids into biogas. On the other hand as the COD loading increases, the biogas yield may decrease if sonication intensity is too high. According to Show et al. (2006), the reason was explained that because there is a large amount of organics degradation due to high COD loading, volatile fatty acid (VFA) formations will increase. The increase in VFA will adversely affect the biogas formation due to acidic conditions.

Although ultrasonic disintegration is a very effective method, studies of Chu et al. (2001) showed that as the sonication time and intensity increases in ultrasonic

homogenizer, particles disrupted causes to an increase in surface areas and water becomes more easily attachable on the surface of the flocs. As a result, dewaterability of sludge decreases because bound water content increases.

In the study of Chu et al. (2001) 20 % of the total COD turned to soluble state at 0.33 W/mL after 120 min sonication time. Therefore, the soluble COD in the supernatant increases 40 times. The results obtained from this study are similar to the study conducted by Tiehm et al. (1997). In addition, BOD/TCOD ratio increases from 66 % to 80 %. This shows the huge part of COD exerted can be disintegrated. Neis et al. (1997) investigated the effect of ultrasonication as pretreatment before anaerobic digestion. The sludge sonicated at frequency of 31 kHz and power of 3.6 kW. Soluble COD rises from 270 to 3500 mg/L. Furthermore, the detention time of solids in digesters decreases from 22 to 8 days.

In the other study conducted by Wang et al. (1999), it was seen that if sonication time increases up to 30 min, the methane amount increases as well. If the pretreatment time extends to 40 min, the increase in methane amount is same with that at 30 min. when compared to control sludge, at day 11, methane amount increases with 12 %, 31 %, 64 % and 69 % were found at sonication times of 10, 20, 30, and 40 minutes, respectively. On the other hand, all organic disintegration efficiency values for ultrasonic pretreatment were determined above 30 %. At day 11, the increases in the efficiencies with respect to control were obtained 11 %, 20 %, 38 % and 46 % for sonication times of 10, 20, 30 and 40 minutes, respectively.

Lyons (1951) investigated the settleability of WAS sonicated at 30 W for different time periods. According to this study, it was found out that the settleability of sludge improves at all times and the 15 min was the optimum time with a overall sonication of 450 W-min. In another study (Hall, 1981), when the sonication time and the supplied power per volume are low, capillary suction time (CST) values increases extremely thereby, the dewaterability of sludge deteriorates. Moreover, if

the sonicated sludge is led to stand for little time, CST values decreases because of reflocculation of the particles at the sludge. However, it was found that at all time CST value of sonicated sludge is higher than that of untreated sludge (King and Forster, 1990).

According to the study conducted by Shimizu et al. (1993) on ultrasound treatment of WAS and the effect on subsequent anaerobic digestion, solubilization ratio increased with pretreatment time with a maximum value of 75 to 80% obtained for a pretreatment time of 90 minutes at 20 kHz of oscillation frequency and power supply of 200 W at 25-30ºC.

Tiehm et al. (1997) carried out both batch and semi-continuous experiments to find the effect of ultrasonic pretreatment on the anaerobic digestion of primary and WAS (53/47 dry weight) sludge. The ultrasonic pretreatment was done using a high performance ultrasound reactor (3.6 kW) operated at 21 kHz and a pretreatment time of 64 seconds. Ultrasonic pretreatment appeared to result in only a slight improvement (5% at 22 day SRT) in VS reduction.

The enhancement of anaerobic digestion of waste activated sludge using ultrasonic pretreatment was examined by Neis et al. (2000). The sludge was sonicated by a 3.6 kW ultrasound reactor at frequency of 31 kHz and an acoustic intensity ranged between 5 and 18 W/cm^2. The degree of disintegration was related to the sludge solids concentration, the type of sludge treated and the intensity applied. It was determined during the anaerobic digestion for a sonicated reactor operated at 4, 8 and 16 days SRT were 32.0, 38.1 and 42.4 %, respectively. Disintegration degree of 27.0 and 32.2% were found for untreated reactors operated at 8 and 14 days SRT.

In the study of Bougrier et al. (2004) the effects of ultrasonic, ozonation and thermal pretreatment of WAS were examined. First, the optimal conditions of each pretreatment method were determined. For ultrasonic treatment two energies 7200

and 11500 kJ/kg TS were supplied. Moreover, two dosages 0.13 and 0.19 g O_3/g TS for ozonation and two temperatures 190 and 170ºC with 30 min treatment time for thermal pretreatment were applied on the same sludge in order to compare the methods. The best result (COD solubilization of 60% and 18% increase in biogas formation compared to untreated sludge) was found for thermal pretreatment at 190ºC.

Wang et al. (1999a) compared ultrasonic pretreatment with thermal methods on their effect on anaerobic digestion of WAS. Ultrasonic treatment at 9 kHz and 200 W, thermal treatment using an autoclave at 120ºC, thermal treatment in a hot bath at 60ºC and freezing at –10ºC were examined. The anaerobic test was 7 days but the differences in methane production were minor after the first three days. In the first three day of digestion the methane production was higher in all reactors with pretreated sludge compared to untreated reactors. On the second day the pretreated reactors produced maximum methane with values of 766, 737, 616, and 560 mL/L per day for ultrasonic, autoclave, hot bath, and freezing pretreatments, respectively.

2.7. Food to Microorganism (F/M) Ratio in Anaerobic Digestion

Food to microorganism (F/M) ratio is the ratio of substrate amount as waste activated sludge to the inoculum amount as anaerobic digested sludge added to the reactor. It is an important digester parameter in terms of volatile solids loading performance of the system (Pranshanth et al., 2006). According to Tanaka et al. (1997), F/M ratio should be in the range of 0.45-0.50 in order to solubilize cells efficiently. Pranshanth et al. (2006) found the best value of F/M ratio between 0.57-0.68 for anaerobic digestion. In the study of Braguglia et al. (2006) they mentioned that the optimum range for F/M ratio was found between 0.5 and 2 for untreated excess sludge by Engelhart (2002) (Braguglia et al., 2006). Furthermore, for the untreated excess sludge batch digestion tests at different F/M ratios (0.15 and 8.37), the initial lag-phase in biogas production becomes longer with the increase in F/M

ratio. Nevertheless, the specific biogas production was approximately constant for all ratios. In the study of Braguglia et al. (2006), it was found that the ultrasound pretreatment was effective on hydrolysis kinetics and biogas production for all investigated F/M ratios. The VS degradation data for untreated and sonicated sludge during continuous anaerobic digestion were well correlated by a first order kinetic equation. The hydrolysis rate values (0.06-0.17 d^{-1}) increased with decreasing F/M for untreated sludges. The hydrolysis rate of sonicated sludges (0.13-0.23 d^{-1}) was found higher than untreated ones. Moreover, it was obtained that at all F/M ratios, biogas production was higher for sonicated sludges and the most convenient gain (25 %) in biogas production was at F/M ratio of 0.5 compared to F/M ratios of 1 and 2. Initially for the F/M ratio of 2, the biogas production occured slowly due to accumulation of soluble organic substances caused a kinetic discoupling between hydrolysis and methanogenesis. However, after a while the biogas production increased and overtook the others.

2.8. Full Scale Ultrasound Pretreatment and Cost of Ultrasound Process

There are many full scale plants using sonication especially in Germany and Switzerland in order to enhance the anaerobic digestion and aerobic sludge stabilization and to prevent foaming and bulking of sludge. Table 2.1 shows a few full scale ultrasound plants and the results of sonication.

Capital and operation and maintanence (O&M) costs for the ultrasound pretreatment differ by the type and size of facility. Capital costs for the ultrasound process are generally $30,000/kW and 1 kW of the ultrasound process treats approximately 10,000 population equivalents). O&M costs are minimum and generally involve the replacement of the probes once every 1.5 to 2 years. The payback times for plants range from 8 months to 3 years. This saves approximately €2.5($3.97)/population equivalent/year (approximately €1.50($2.38)/population equivalent/yr) (PA, 2008).

Table 2.1. Full scale examples of ultrasound treatment (PA, 2008; Ultrawaves, 2008)

Treatment plant	Country	Flow rate (MGD)	Results of sonication
Bad Bramstedt	Germany	4.49	reduction in digestion time from 20 to 4 days increase in biogas production by a factor of 4 25 % reduction of digested sludge mass (PA, 2008)
Bamberg	Germany	12.15	30 % increase in VS destruction and biogas production. avoided the construction of a new anaerobic digester (3,000 m^3) (PA, 2008)
Ergolz	Switzerland	3.43	15 % increase in VS destruction 25 % increase in biogas production (Ultrawaves, 2008)
Mannheim	Germany	31.5	70 % and 45 % increase in VS destruction and biogas production respectively 3 % reduction in polymer consumption (Ultrawaves, 2008)

CHAPTER 3

MATERIALS AND METHODS

3.1. Sludge

Waste activated sludge (WAS) and anaerobic digested sludge were obtained from Ankara Central Wastewater Treatment Plant.The plant is conventional biological treatment plant that includes screening, primary clarification, secondary treatment by activated sludge and sludge treatment by anaerobic digestion. WAS was taken from the recycle stream before secondary clarifier. Anaerobically digested sludge (ADS) was sampled from the full scale digester of the plant fed with primary and secondary sludge.

3.2. Sludge Disintegration Studies

The disintegration by ultrasound was performed with an ultrasonic homogenizer Labsonic P (Sartorius AG, Germany) operating at maximum power 400 W and 24 kHz (Figure 3.1). The power has amplitude of 20-100%. The probes used in the experiments are suitable for sample volumes 100- 2000 mL and have diameters of 14 mm (average power output of 100 W; 0.29 W/mL) and 22 mm (average power output of 255 W; 0.73 W/mL).

3.2.1. Effect of Sonication Power

The ultrasonic probe with a diameter of 14 mm was used to disintegrate 350 mL of WAS. The probe has an average power of 100 W. By controlling the amplitude of

homogenizer (20% to 100 %), different ultrasonic powers were applied to the samples. The time was set to 15 minutes to see only the effect of power.

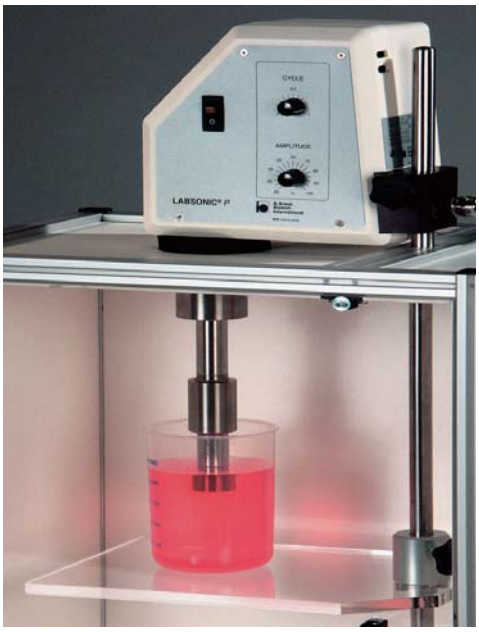


Figure 3.1. Ultrasonic homogenizer

3.2.2. Effect of Sonication Time

The ultrasonic probe with a diameter of 14 mm was used to disintegrate 350 mL of WAS. The power was set to 100 W. Different ultrasonic energies were applied to the samples with changing the sonication time.

3.2.3. Effect of Temperature

In order to see the effect of temperature on disintegration, 350 mL of WAS samples were sonicated at different sonication powers without any temperature control. On the other hand, at all powers applied, another set of WAS samples was sonicated

with temperature control. These samples were put in beakers filled with ice to keep the temperature between 20-25 ºC. After this experiment, the sonication pretreatments were always done with controlled temperature.

3.2.4. Effect of Solid Concentration

Sludge samples with difeerent solids concentration were disintegrated with 14 mm ultrasonic probe at the same parameters (100 W and 15min).

3.2.5. Effect of Probe Size

The ultrasonic probe which has a diameter of 22 mm (average power of 255 W) was used with changing amplitudes and times applied for the 14 mm probe in order to see the effect of probe size.

3.3. Anaerobic Digestion Tests

3.3.1. 250 mL Batch Anaerobic Reactors

In the experiments, 250 mL serum bottles of batch anaerobic reactors were operated. The reactors were fed with WAS and ADS either untreated or sonicated at required F/M ratios calculated as below:

$$\frac{\mathrm{F}}{\mathrm{M}}\left(\frac{\mathrm{mg}}{\mathrm{mg}}\right)=\frac{MLVSS_{WAS}\left(mg/L\right)\times V_{WAS}\left(mL\right)}{MLVSS_{ADS}\left(mg/L\right)\times V_{ADS}\left(mL\right)} \qquad \text{(equation 3.1)}$$

The working volume in the reactors of 250 mL was 120 mL. Of this total volume, 100 mL was WAS and ADS (their one-to-one amounts depend on F/M ratio); 20 mL was basal medium. The aim of using basal medium was to supply nutrients to

the system and help to adjust pH. Basal medium components are given in the Table 3.1. The remaining volume of the reactor was left empty for gas collection.

All reactors were purged with N_2 gas for 10 minutes to prevent oxidation of readily oxidizable organics and remove oxygen in the system. The tops of the bottles were closed with rubber stoppers, and shaken well. Finally, reactors were incubated in 37ºC by shaking them manually once a day.

Table 3.1. Basal medium components

Component	Unit	Concentration
KH_2PO_4	g/L	0.43
$Na_2HPO_4.7H_2O$	g/L	0.80
$NaHCO_3$	g/L	0.10
NH_4CL	g/L	0.30
$MgSO_4.7\ H_2O$	g/L	0.47
$CaCl_2.2\ H_2O$	g/L	0.12
$FeSO_4.7\ H_2O$	g/L	2.80
H_3BO_3	mg/L	0.05
$Al_2(SO_4)_3.\ 18\ H_2O$	mg/L	119.77
$MnCl_2.4H_2O$	mg/L	0.05
$CuSO_4.5H_2O$	mg/L	92.80
EDTA	mg/L	641.22
$ZnSO_4.7H_2O$	mg/L	142.52
$NH_4Mo7O_{22}.H_2O$	mg/L	79.97
$CaCl_2$	mg/L	49.99
$NiCl_2.6H_2O$	mg/L	91.60
HCl	mL	1.00

3.3.2. 3 L Batch Anaerobic Reactors

In order to follow the effect of studied parameters in a larger scale well-mixed reactor, large batch anaerobic reactors were also constructed. Glass reactors with 3 L total volume were used. The working volume in these reactors was 2 L. Totally 2 L of WAS and ADS in a certain proportion to satisfy the desired F/M ratio of the reactors. This time, no basal medium was added to the reactors. The remaining 1 L volume of the reactors was left empty. Reactors are purged with N_2 gas for 10 minutes to prevent oxidation of readily oxidizable organics and remove oxygen in the system. Each reactor was connected to a 4 L glass gas collection system by silicone tube. Gas collectors were placed in brine solution to prevent the solubility of gases. All connections were secured in order to prevent the leakage of gas into and out of the reactor system. Finally, the reactors were put on magnetic stirrers and incubated in hot room at 37ºC. 3 L reactor with gas collector system is shown in Figure 3.2.

Figure 3.2. 3 L anaerobic reactor with 4L gas collector

3.4. Analytical Procedures

MLSS and MLVSS

MLSS and MLVSS were measured in preliminary studies to specify sludge content, before and after anaerobic digestion tests, to determine initially F/M ratio, and to analyze solids destruction. The sludge samples were analyzed using Method 2540D and 2540E (APHA, 2005) for MLSS and MLVSS measurement, respectively.

sCOD

To analyze organic content in the soluble phase, after 2 h of sedimentation, the supernatant was centrifuged in Hettich Rotofix 32A centrifuge at 3500 rpm for 10 min. Then, it was filtered through 0.45 μm pore size membrane filters by Millipore Vacuum Pump and filtration kit. Soluble COD of supernatants measured in duplicates, by closed reflux colorimetric method using Hach kits and Hach DR2000 spectrophotometer.

tCOD

In order to analyze the organic content in the sludge, total COD was measured in duplicates by closed reflux colorimetric method using Hach kits and Hach DR2000 spectrophotometer.

Total Gas Analysis

Total gas production of reactors was measured by open-tube manometer. In the system, since there is a graduated pipette and the two tips of the manometer are opened to the atmosphere, the liquid in the manometer is in equilibrium. After the syringe is inserted to the septa of the reactor, the gas formed in the reactor is

emptied to the graduated pipette by the help of syringe and drops the level of the liquid in the pipette. Since total liquid amount dropped in the pipette is equal to the gas volume in the reactor emptied to the manometer, liquid volume difference in the pipette is taken as total gas production of the reactor.

Gas Composition

The gas composition in the reactors was analyzed by gas chromatography (GC) with TCD detector (Agilent Technologies 6890N). The carrier gas in the system is helium. Gas composition analysis is made with 30.0 m x 530 µm x 40.0 µm HP-Plot Q capillary column. Column temperature stays at 45 ºC for 1 min, and then increases to 65 ºC by 10 ºC at a minute. The average velocity of helium gas is 29 cm/sec. The equipment splits the sample at a ratio of 1:20 for analysis. In the calibrations done according to method used, the calibration gas with known content was injected to the equipment and from results, it was concluded that nitrogen, methane and carbondioxide gases were eluting at 2.1, 2.3 and 2.9 min, respectively. 0.3 mL of gas from the top of serum bottles is drawn by a syringe and 0.1 mL of it is given to air to clean the tip of syringe. Thus, 0.2 mL of gas is injected to GC for composition analysis. The gas composition of all reactors was measured in duplicates. The calculation of methane amount of a reactor during anaerobic digestion is given in Appendix B.

Turbidity

Turbidity is caused by suspended matter, organic and inorganic matter, soluble colored organic compounds and other microorganisms. In order to measure turbidity, the supernatant of the sludge after 2 hour settlement was put into turbidity measurement cell and analyzed by using Hach Turbidimeter 2100N with two times. The unit of the turbidity was expressed as NTU.

pH

pH was measured by CyberScan PC 510 pH meter/conductivity meter in order to specify the acidity or basicity of samples.

Specific Oxygen Uptake Rate (SOUR)

SOUR is as an indicator of the microbial activity in terms of oxygen consumption rate of sludge. It was calculated by using a dissolved oxygen (DO) meter according to Method 2710B (APHA, 2005) with the given formula in equation 3.2. Oxygen uptake rate has the unit of mg O_2 per time per g VSS. DO was measured by Hach Sension 378 pH/conductivity/DO meter.

$$SOUR = \frac{slope(mg / L.time)}{MLVSS(g / L)} \qquad \text{(equation 3.2)}$$

CST

The dewatering property was measured with capillary suction time (CST) applying Method 2710G (APHA, 2005). Type 304 M Triton Electronics Capillary Suction Timer consisting a test block and sludge reservoir was used for the CST measurement as seen in Figure 3.2. 7x9 cm Whatman 17 chromotographic paper was placed into the test block on which two electrical contact points were present. A small cylinder called as a sludge reservoir was insert into the block and the sludge sample was added in the sludge reservoir. The time is started when the filtrate of sludge flowing in the paper reaches inner electrical contact and the time ends when the filtrate reaches the outer contact. The CST measured as seconds was read from digital display. For each samples, it was measured in duplicates.

Ammonium-Nitrogen and Phosphorus Analysis

The soluble phase of the sludge in the reactors was analyzed for ammonium-nitrogen and phosphorus after removing suspended particles by filtering through 0.45 μm pore size membrane filters. Test kits by HACH-Lange LCK 303 and LCK 350 were used for ammonium-nitrogen and phosphorus by using Hach DR5000 spectrophotometer, respectively.

Carbohydrate Analysis

After removing suspended particles, carbohydrate content of the soluble phase of sludge from the reactors obtained after anaerobic digestion was measured using phenol-sulphuric acid method developed by Dubois *et al.* (1956) with alginate used as the standard. For measurement of carbohydrate concentration, 2 mL samples were taken and put into test tubes set in triplicate for each reactor. 50 μL phenol which was prepared as 80% (w/w) and 5 mL sulphuric acid were added into each tube. The samples were allowed to stand for 10 minutes at room conditions. Then, the tubes were vortexed and placed into an incubator at 30°C for 15 minutes. The formation of yellow-orange color is the characteristic for this method and then the absorbance of each sample was measured at 480 nm using Pharmacia LKB Novaspec II Spectrophotometer. Each carbohydrate concentration was calculated by using a standard calibration curve which was prepared before experiment by using alginate. The calibration curve is given in Appendix A.

Protein Analysis

The protein content of the sludge in the reactors was measured after removing suspended matter by using folin-ciocalteu phenol reagent method which is developed by Lowry *et al.* (1951). Bovine serum albumin was used as a standard. Four reagents named as A, B, C and D was applied in the analysis. Reagent A was

composed of 2% w/v sodium carbonate in 0.1 N NaOH. Reagent B was prepared by dissolving 1% w/v sodium potassium tartarate in 0.5 % w/v cupric sulphate. Reagent C consisted of 1mL of Reagent B and 49 mL of Reagent A. Lastly, Reagent D contained the Folin-Ciocalteu's phenol reagent and it was diluted with deionized water by the ratio of 10:10. 600 µL samples were taken from the supernatants of each reactor sample. It was supplemented into triplicate ordered test tubes. Each tube including sample was mixed with 3 mL Reagent C and then they were allowed to stand for 10 minutes at room temperature. Then, 300 µL Reagent D was put and the tubes were vortexed. After that, the samples were kept at room temperature for half an hour. Blue color occurrence let to measure the absorbance with spectrophotometer. The absorbance based on the intensity of the blue color was measured at 750 nm by using Pharmacia LKB Novaspec II Spectrophotometer. The calculation of the protein concentrations was carried out by using a standard calibration curve by using Bovine Serum Albumin as a standard. For each analysis a separate calibration curve was prepared; one of which is given in Appendix A.

Metal Concentration Measurement in Sludge

Ion concentration was measured in each sludge sample after anaerobic digestion with microwave assisted digestion process. The sludge samples were analyzed by the procedure of the digestion process described by Özsoy et al. (2006). Sludge taken from each reactor centrifuged for 10 minutes at 3500 rpm to separate water part from the sludge. After removing of the supernatant part, pellet containing sludge was dried at 103ºC for 24 hours. A 0.25 g dried and powdered sludge was weighed from each reactor and then added into teflon vessels of Berghof speedwave MWS-2 microwave digester. In order to take average and find out the standard deviation, the vessels were triplicated for each reactor. 5 mL nitric acid (65% w/v) and 5 mL hydrofluoric acid (40% w/v) was put into teflon vessels containing dried sludge in order to accomplish microwave digestion of sludge samples. A blank (no sludge) was used to get contribution coming from the used

HNO_3 and HF. After inserting the vessels into digester table, microwave-digestion program was started. The program was comprised of three stages with different time, temperature and power and it was given in Table 3.2. After digestion, the sludge samples and supernatants were put into teflon beakers and then they were boiled till 3-5 mL sample was remained in the beakers. Distilled water was added to the samples to dilute them to 25 mL. Then, these samples were filtered through Millipore filter. The necessary dilutions for the samples were made for measurement. The concentration of ions in diluted samples was determined by using Analyst 400 model atomic absorption spectrometer and Jenway PFP7 Model Flame Photometer.

Table 3.2. Stages in microwave digestion process program

Program Stage	Time (min)	Temperature (°C)	Power (W)
Stage 1	40	200	800
Stage 2	25	100	400
Stage 3	1	20	400

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Disintegration Studies 1

Before performing batch anaerobic tests, preliminary studies were conducted to see the effects of sonication and its parameters on WAS characteristics. In this part, 14 mm probe was used for sonication. Thereby, the average power output at 100 % amplitude was 100 W.

4.1.1. Effect of Sonication Power on WAS without Controling Temperature

The first part of the preliminary studies was to inveatigate the effect of ultrasonic temperature on sludge disintegration. 350 ml of WAS (8085 mg/L MLSS; 6418 mg/L MLVSS; 11308 mg/L tCOD) was sonicated at different power outputs without temperature control. Sonication time was fixed at 15 minutes. Thereby, the effect of sonication power and temperature on WAS were examined. As the sonication power increases, the temperature of the sonicated WAS increases as shown in Table 4.1.

Table 4.1. The variation in the temperature of WAS according to sonication power

Sonication Power (W)	0	20	30	40	50	60	70	80	90	100
Temperature (°C)	16	34.5	37.5	41	45	50	54	58	63.5	67

In disintegrated sludge, intracellular materials pass into the liquid phase and increase the soluble COD values. It is evident from the soluble COD values in Figure 4.1 that when the sonication power increases soluble COD values increase. This illustrates that at high powers the sludge disintegration is better since high ultrasonic power generates high mechanical shear forces during cavitation bubble implosion (Grönroos et al., 2005).

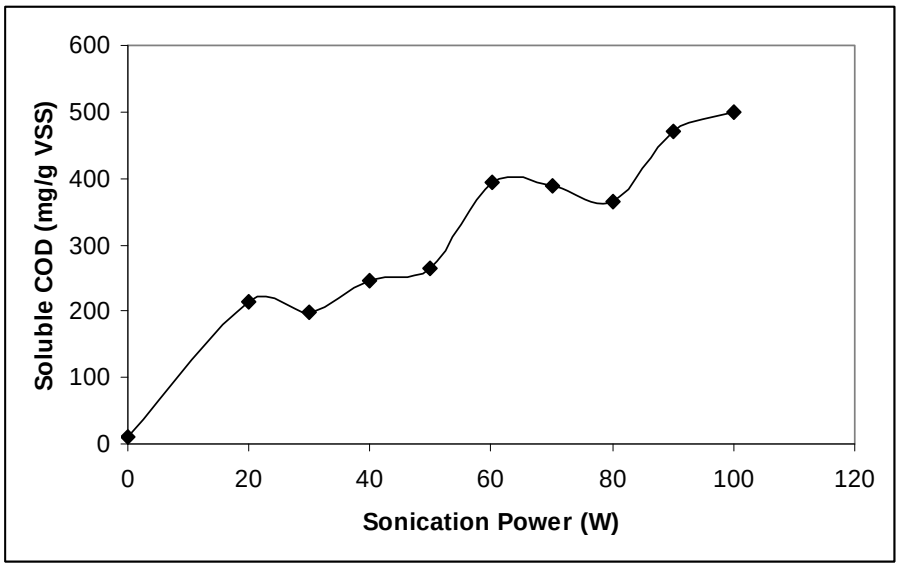


Figure 4.1. The variation in soluble COD according to sonication power (without temperature control)

The filterability was measured using CST. The CST helps to estimate the sludge ability to dewater. Sonication pretreatment disintegrates the sludge flocs and particle size decreases (Chu et al., 2001). Therefore, the bound water linked to the particles surface increases. This makes deterioration in filterability. According to literature, the CST value of the sludge disintegrated at lower powers increase (Hall, 1981). Similar to findings in the study of Chu et al. (2001), in Figure 4.2 when sonication power increases, CST values increase as an indirect indication of disintegration. However, at high sonication powers CST values decrease even CST value of the disintegrated sludge at 100 W is same with that of control sludge in

Figure 4.2. The reason of this decrease in CST values after 70 W could be the effect of high temperature. The temperature may have effect on hydrogen bonds and gave a structure to sludge. Consequently, a part of the initial boundwater could be released (Haug et al., 1978).

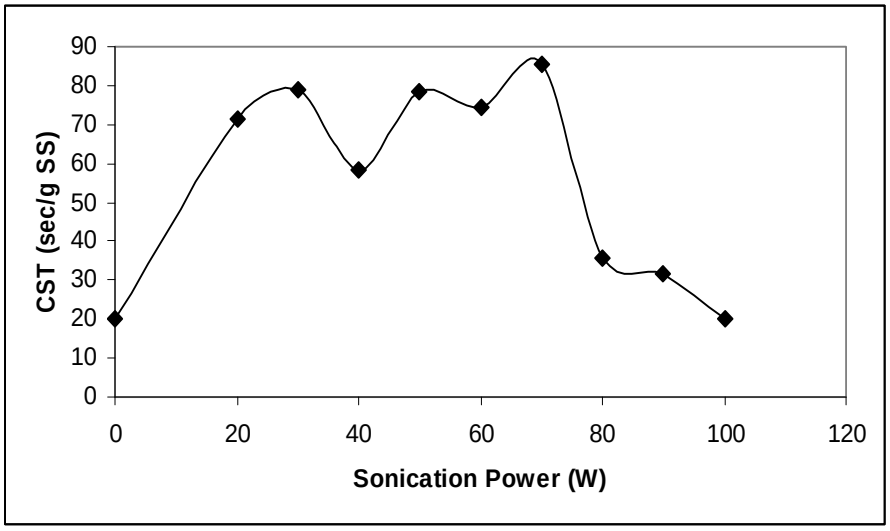


Figure 4.2. The variation in CST according to sonication power (without temperature control)

According to Figure 4.3, even at sonication at 20 W the turbidity of the sludge deteriorates. When the sonication power is increased, turbidity values increase by fluctuating. This can be accepted as the proof of disintegration. It is expected that when the sludge is disintegrated, the particle size decreases, floc structure is broken and the turbidity of the soluble phase increases.

4.1.2. Effect of Sonication Power on WAS with Controled Temperature

Samples of 350 ml of WAS (8835 mg/L MLSS; 6200 mg/L MLVSS; 9757 mg/L tCOD) was sonicated at different power outputs. Sonication time was fixed at 15 minutes. Temperature was controlled between 20 C º ± 5 with ice bath. Thus, the effect of sonication power on WAS was investigated by temperature cooling.

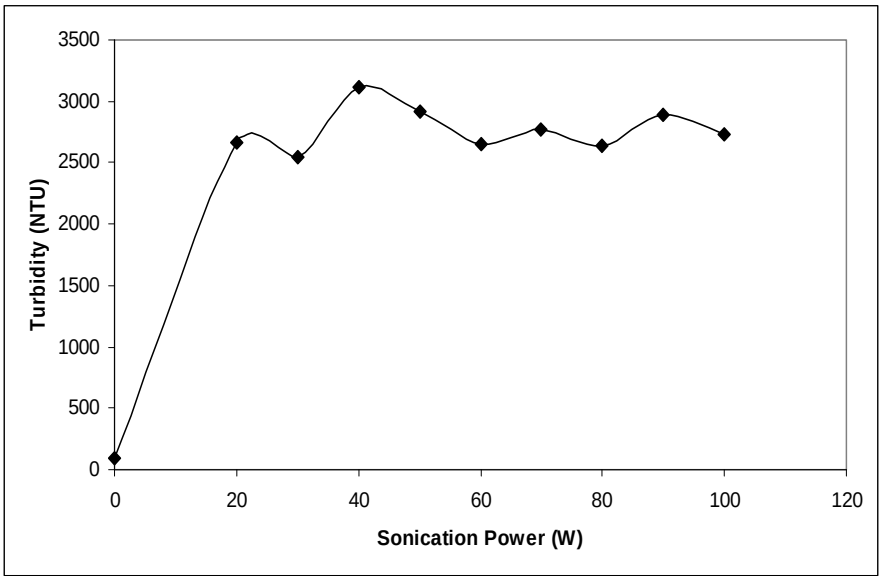


Figure 4.3. The variation in turbidity according to sonication power (without temperature control)

In order to examine the effect of sonication pretreatment on microbial viability, the oxygen consumption by microorganisms in the untreated and sonicated sludge samples were analyzed. It is clear from Figure 4.4 that when the sonication power increases, bacterial activity decreases. In control sludge, there is rapid decrease in oxygen concentration. This shows there is an intense microbial activity. After 60 W pretreatment, this slope slows down and the oxygen consumption in the sonicated sludge at 100 W is slow compared to other ones. That is, the microbial activity slows down. This is an indirect measurement of disintegration. According to Hua and Thomson (2000), higher intensities will enhance inactivation rates of E.coli in the wastewater media.

If the sonication power increase, soluble COD amount increase (Figure 4.5). The higher soluble COD obtained is found at 100 W. Therefore, 100 W was chosen as effective sonication power when the time was fixed at 15 min. When this soluble COD trend is compared with the trend of the experiment without temperature controling (Figure 4.1), there is an important effect of temperature in COD increase.

For example, at the sonication power of 100W, the sCOD is approximately 500 mg/g VSS when the temperature is controlled. On the other hand, with temperature control the sCOD value is nearly 200 mg/g VSS. Similar findings were found by Chu et al. (2001). When the sonication density was 0.33 W/mL and time was 60 min, the sCOD/tCOD ratio was increased from 0.07 to 0.17 without temperature control. According to Chu et al. (2001), both heterotrophic bacteria and total coliform are disinfected at higher temperatures and this causes an increase in the COD values.

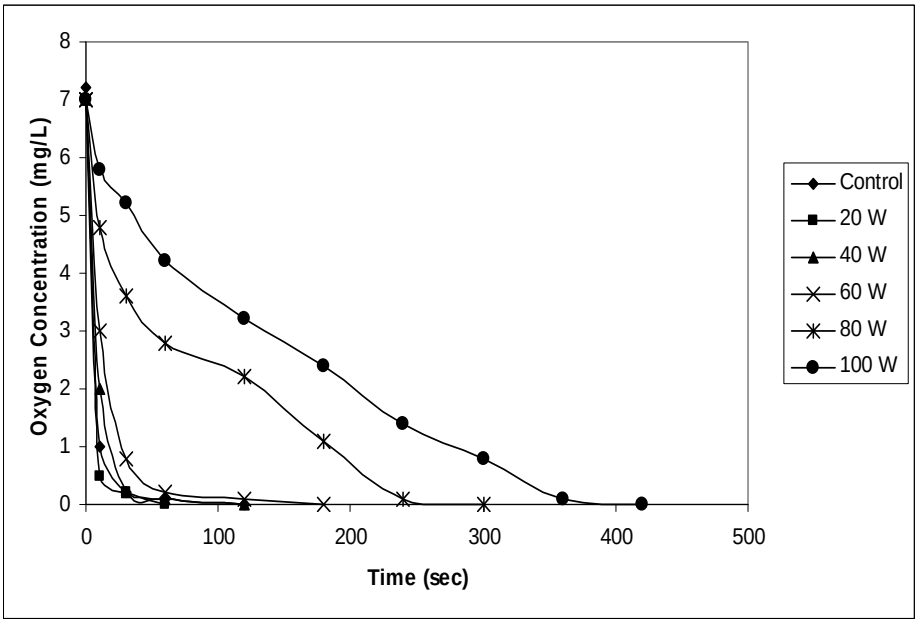


Figure 4.4. The variation in oxygen concentration according to sonication power

According to Figure 4.6, when the sonication power was increases, CST values increase up to a point. After 60 W, CST values decrease. However, CST value of sonicated sludge at 100 W is above that of control sludge. When this result is compared with Figure 4.2, if temperature is not controlled CST values of disintegrated sludges at high powers are low. If the temperature is controlled, CST value of sonicated sludge at 100 W is higher than the CST of control sludge. This fact could originate from the lacking influence of temperature on sludge structure.

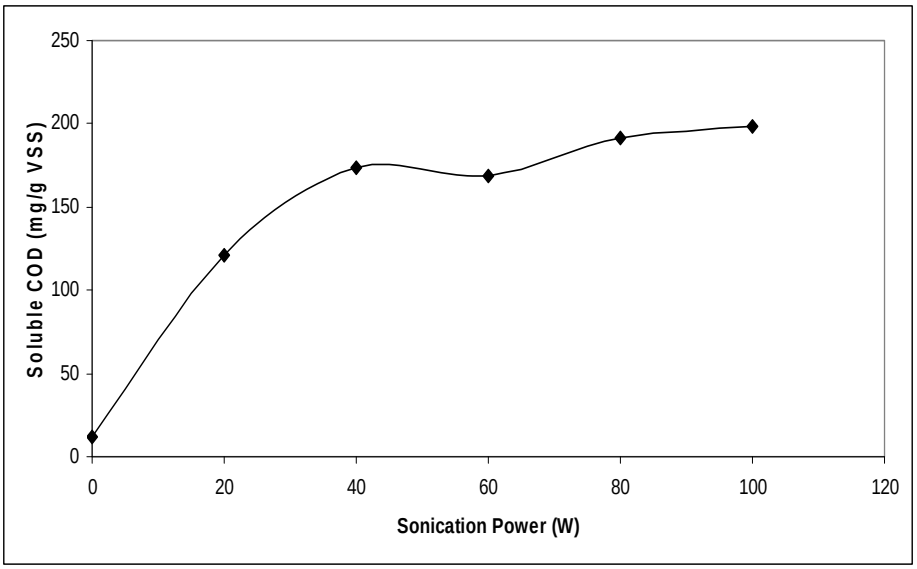


Figure 4.5. The variation in soluble COD according to sonication power (using 14 mm probe)

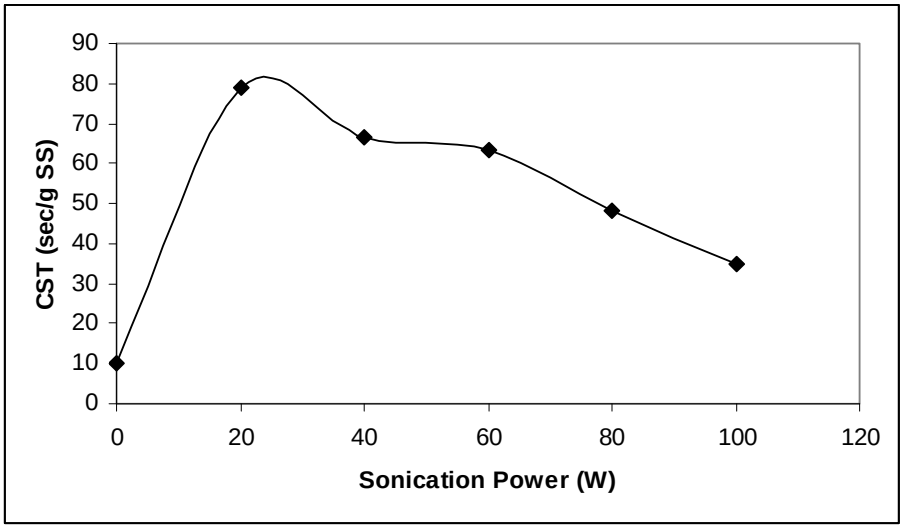


Figure 4.6. The variation in CST according to sonication power (using 14 mm probe)

With the pretreatment at 20 W turbidity increases excessively compared to control and then it increased slowly when the sonication power is further increased. (Figure 4.7).

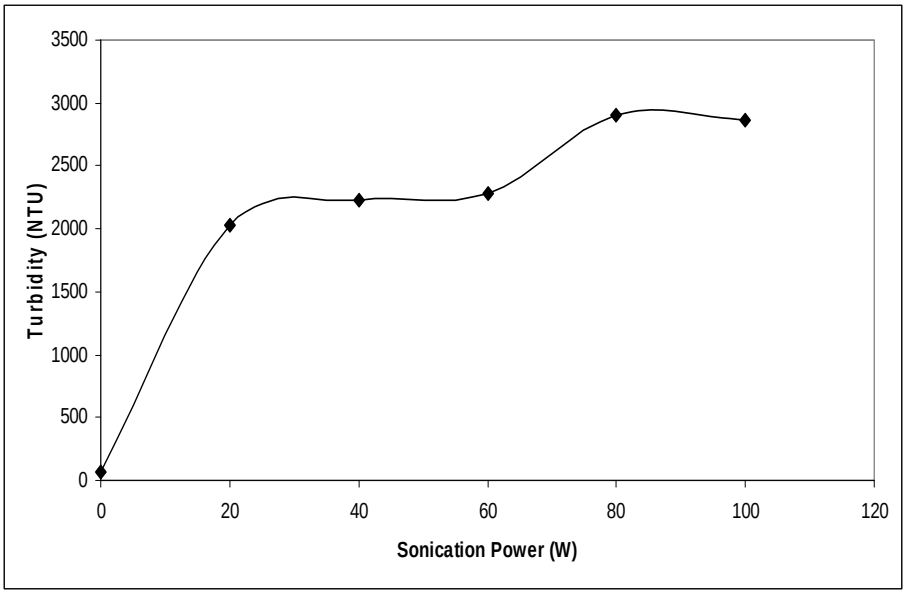


Figure 4.7. The variation in turbidity according to sonication power (using 14 mm probe)

4.1.3. Effect of Sonication Time on WAS

During the investigation of sonication time effect on WAS, the power was fixed at 100 W and 5, 10, 15 ve 20 minutes of sonication were applied. According to Figure 4.8, when sonication time increases, soluble COD increases as well. Moreover, it is seen that there is no more increase in soluble COD values between 15 and 20 minutes sonication time. After this point, the soluble COD values become constant and no more organic material can be released into aqueous phase. For this reason, the better sonication time was taken as 15 min.

Figure 4.9 demonstrates the variation in CST according to change in the sonication time. If the sonication time increases, CST values of the sludge increases up to 10 min of sonication time and then decreases. That is, after 15 minutes of pretreatment, the dewaterability of sludge improves. However, according to the values in literature, these findings are above the CST value of control.

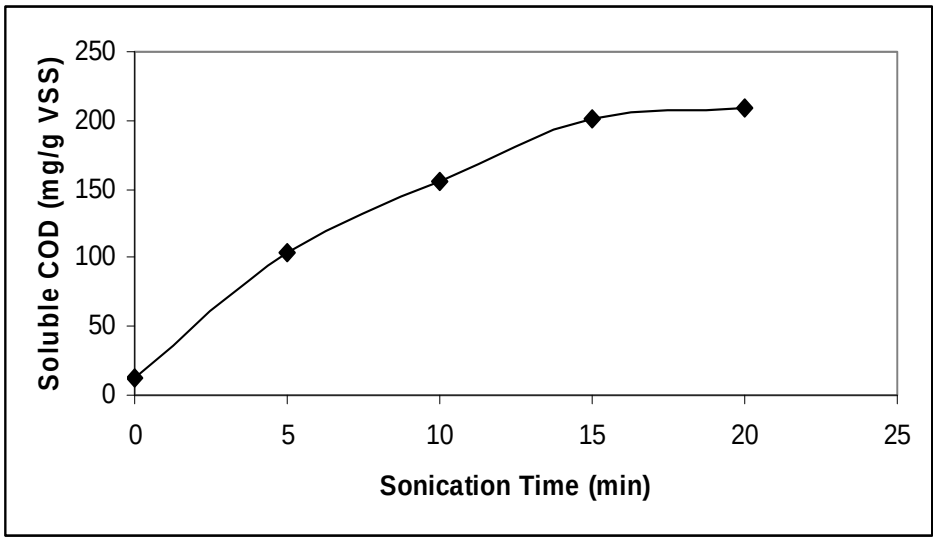


Figure 4.8. The variation in soluble COD according to sonication time (using 14 mm probe)

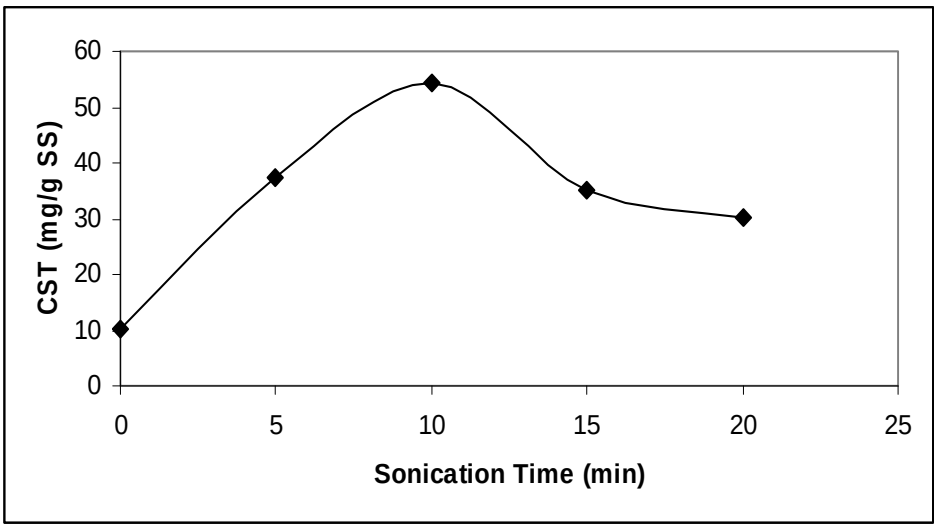


Figure 4.9. The variation in CST according to sonication time (using 14 mm probe)

Turbidity of the supernatants increases when the sonication time increases. After 10 minutes, it starts to decrease and stay around 2750 NTU (Figure 4.10). That is, disintegrated floc particles in the soluble phase do not cause any more turbidity after 15 min sonication time.

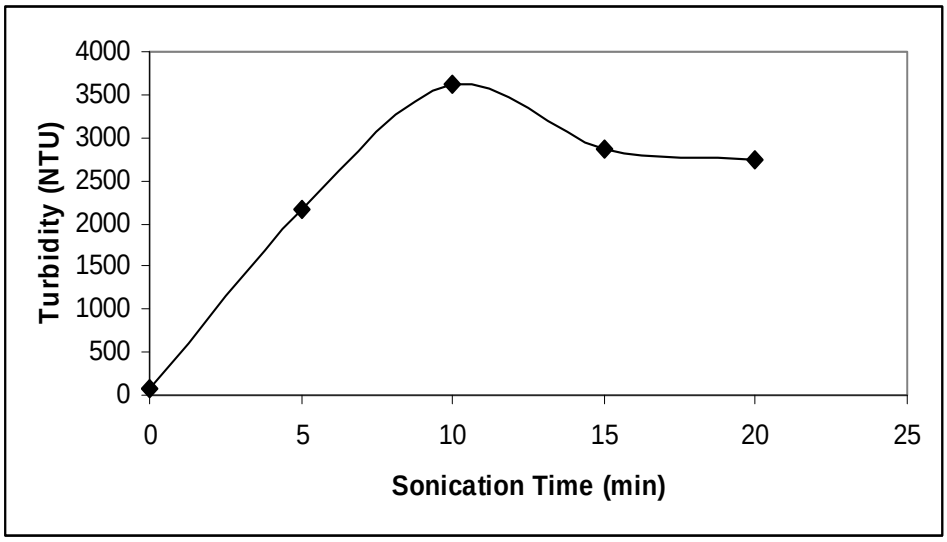


Figure 4.10. The variation in turbidity according to sonication time (using 14 mm probe)

4.1.4. Effect of Total Solids Concentration on Sonication of WAS

In this part, waste activated sludges with different MLSS values were sonicated in order to see the effect of total solids concentration on sonication. Table 4.2 gives the MLSS, MLVSS and total COD values of waste activated sludges. Temperature was kept around 25 ºC ± 5. Sonication power and time were fixed at 100 W and 15 minutes, respectively.

In Figure 4.11, oxygen consumption rates that microbial activity of control and sonicated sludges having different MLSS concentrations can be seen. Sample 1 refers to sludge number 1, sample 2 refers to sludge number 2, etc in Table 4.2. The specific oxygen uptake rate of sludge samples was calculated and given in Table 4.3. It is observed that in the sludge that has high MLSS concentration microbial activity is high due to rapid oxygen consumption rate. On the other hand, slope of oxygen consumption rate of sludge with low MLSS concentration is slow that is, microbial activity is not dense. Furthermore, in the pretreated sludge at

most of the MLSS concentration, oxygen consumption rate is approximately 10 to 60 % slower than related control sludges.

Table 4.2. MLSS, MLVSS and Total COD values of waste activated sludges

	MLSS (mg/L)	MLVSS (mg/L)	tCOD(mg/L)
Sludge no.1	4350	3160	4310
Sludge no.2	5605	4070	5550
Sludge no.3	6160	4430	5930
Sludge no.4	7365	5265	8030
Sludge no.5	8835	6200	9757

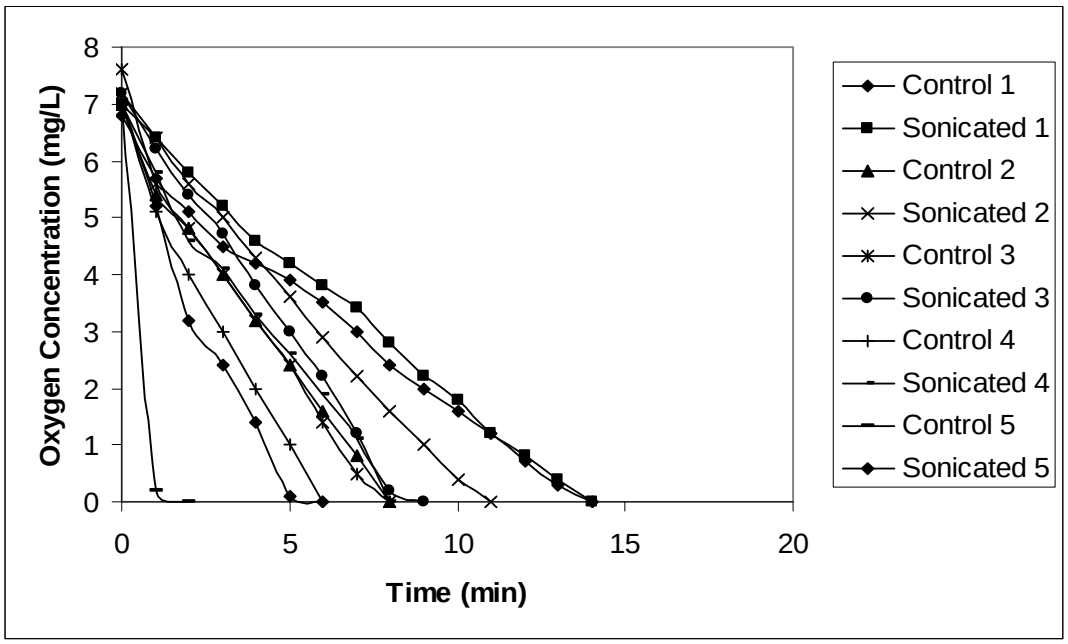


Figure 4.11. Oxygen concentration in sludges at different MLSS concentrations with respect to time

Table 4.3. Specific oxygen uptake rate (SOUR) of sludges at different MLSS concentrations

	SOUR (mg/g.min)
Control 1	0.145
Sonicated 1	0.158
Control 2	0.204
Sonicated 2	0.163
Control 3	0.204
Sonicated 3	0.186
Control 4	0.213
Sonicated 4	0.156
Control 5	0.565
Sonicated 5	0.190

From Figure 4.12, it can be seen that if MLSS concentration of sludge increases, soluble COD amount increases. This increase is almost exponential with respect to MLSS content. The reason for this increase is disintegration of sludge with high solids concentration results in the increase in SCOD amount. Then, it is decided to normalize the sCOD values with MLSS concentrations.

When soluble COD values are normalized according to MLSS values, as it is clear from Figure 4.13 soluble COD values fluctuate in a small range of 5 % and remain same. For this reason, it is meaningful to pretreat high concentration of MLSS values since soluble COD amount increases significantly with the increase in MLSS amount of sludge as expected.

As the MLSS concentration in the sonicated sludge increases, CST and the turbidity of supernatant increases directly, as expected due to solids destruction. The results

are given in Figures 4.14 and 4.15. In Figure 4.14, CST of soinicated sludge with MLSS of 8835 mg/L is lower than the previous one in which the reason could be the reflocculation.

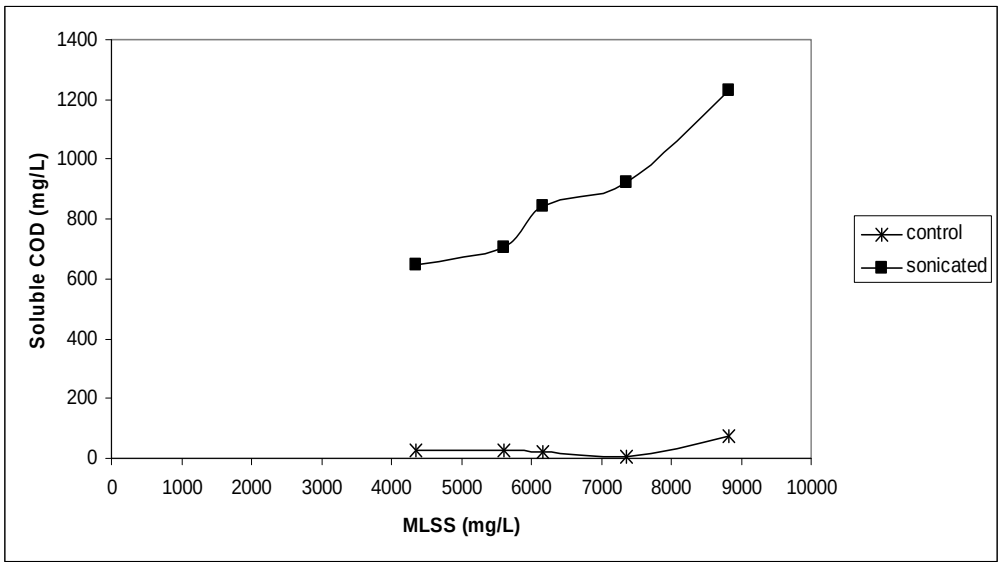


Figure 4.12. Change in soluble COD with respect to MLSS concentration of sludge

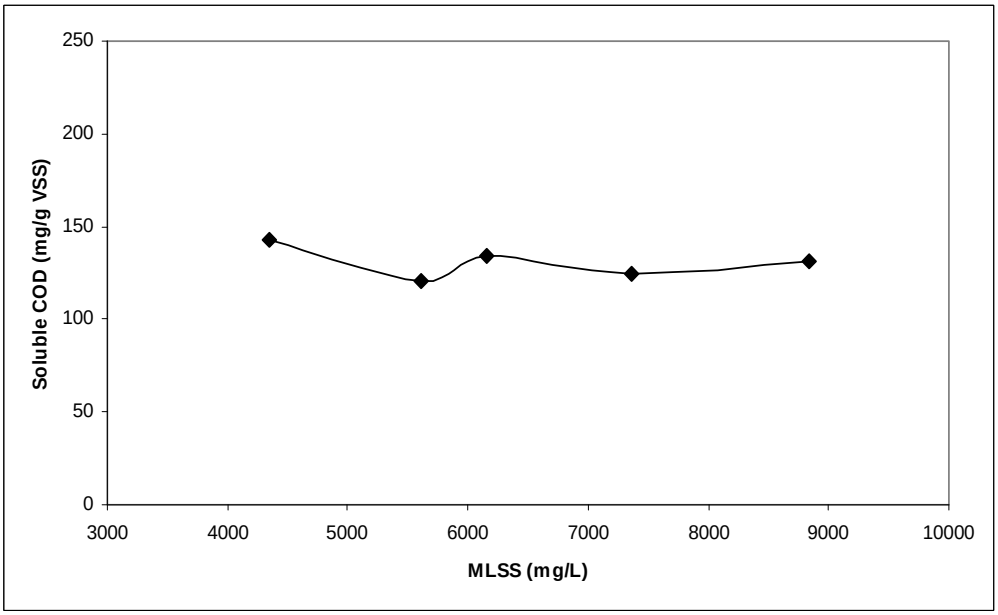


Figure 4.13. Change in normalized soluble COD with respect to MLSS concentration of sludge

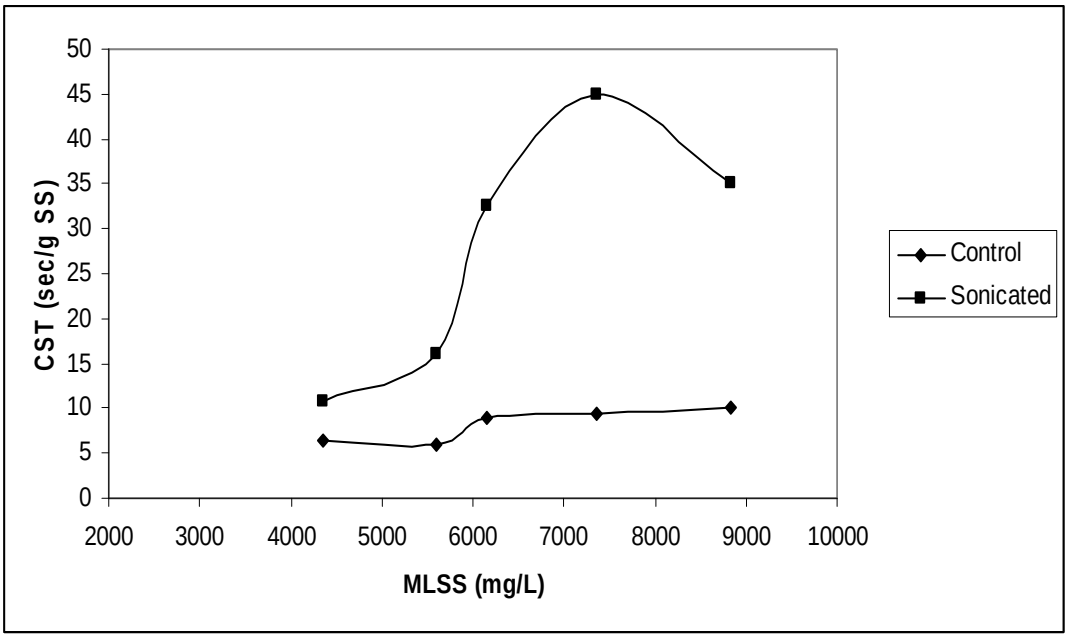


Figure 4.14. Change in CST with respect to MLSS concentration of sludge

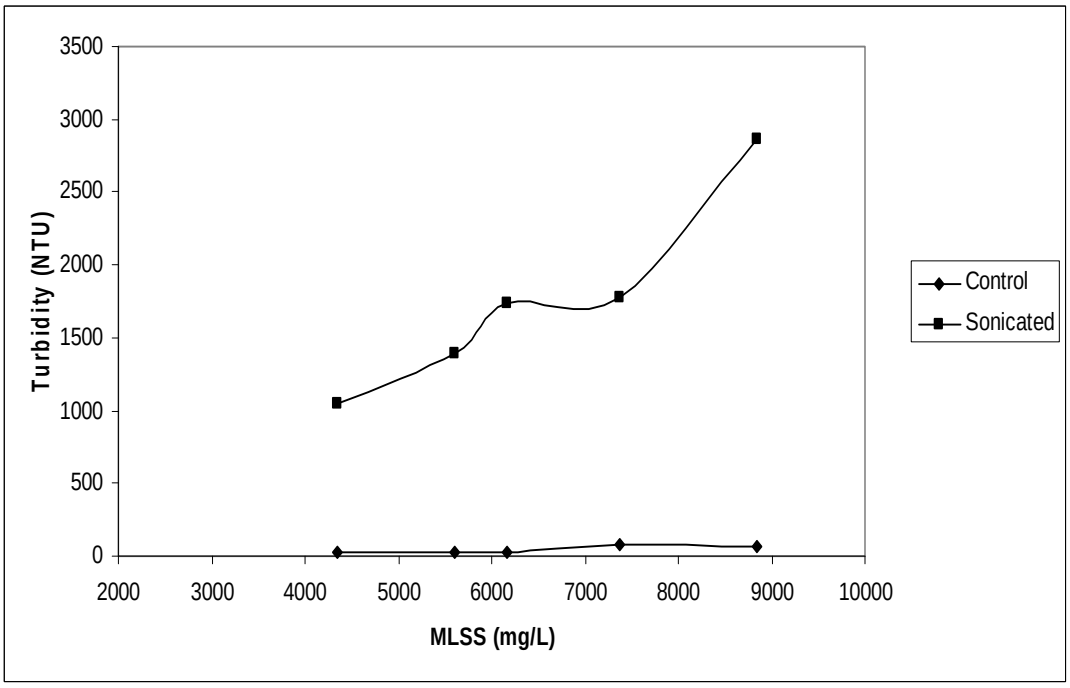


Figure 4.15. Change in turbidity with respect to MLSS concentration of sludge

4.2. Disintegration Studies 2

In this part, 22 mm probe was used for sonication under temperature control. Thereby, the average power output at 100 % amplitude was 255 W.

4.3.1. Effect of Sonication Power on WAS

Samples of 350 ml of WAS (5185 mg/L MLSS; 4035 mg/L MLVSS; 8800 mg/L tCOD) was sonicated for 4 minutes at different power outputs. In this part in order to control the energy consumption and minimize the costs, shorter time periods were tested since higher powers were used.

It is clear from Figure 4.16 that soluble COD amount increases when the sonication power increases. If these findings are compared with the results from Figure 4.5, when the sonication time decreases from 15 min to 4 min and sonication power increases from 100 W to 255 W, the soluble COD in the supernatant of the sonicated sludge increases from about 200 to 250 mg /g VSS.

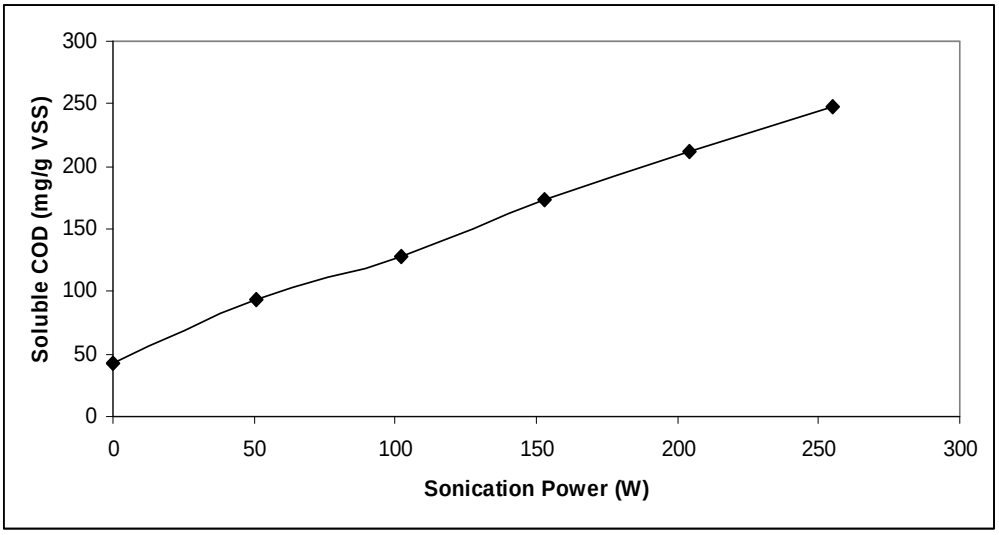


Figure 4.16. The variation in soluble COD according to sonication power (using 22 mm probe)

According to Figure 4.17, when the sonication power increases, CST values increase as well. However, after 200 W, CST value of sonicated sludge decreases mildly. The cause of this decrease may be the reflocculation of sludge. Furthermore, turbidity of the supernatant of sonicated sludge increases excessively by increasing sonication power compared to control. (Figure 4.18).

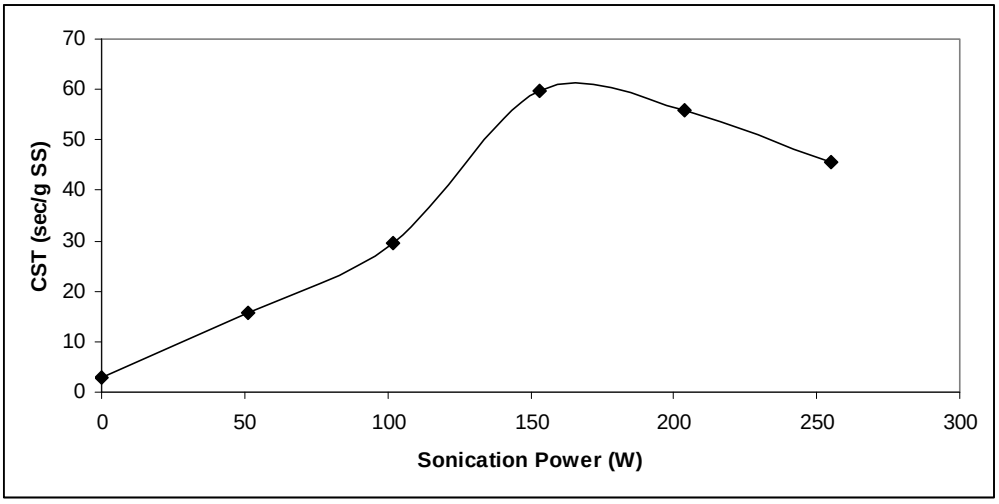


Figure 4.17. The variation in CST according to sonication power (using 22 mm probe)

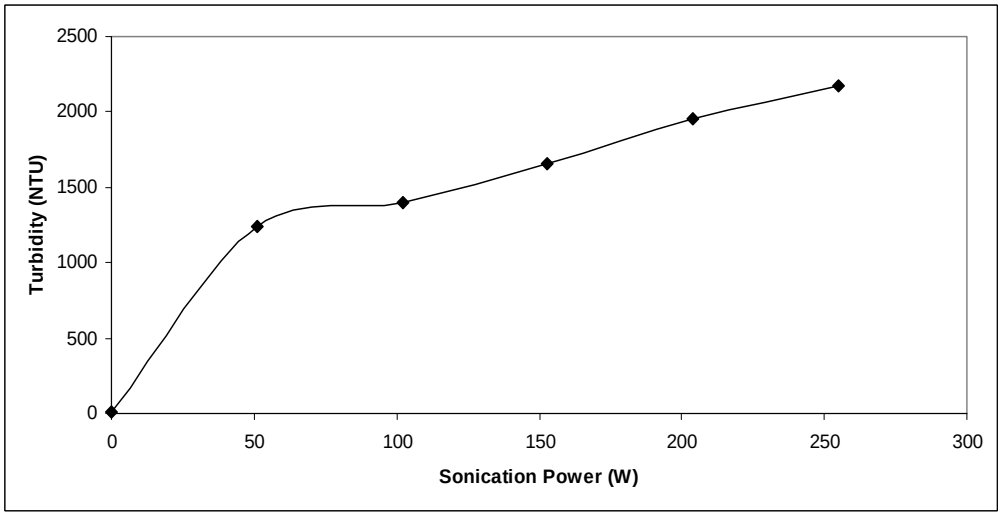


Figure 4.18. The variation in turbidity according to sonication power (using 22 mm probe)

4.3.2. Effect of Sonication Time on WAS

WAS samples of 350 ml (5495 mg/L MLSS; 4330 mg/L MLVSS; 8990 mg/L tCOD) was sonicated at 255 W with different sonicaton times.

As shown in Figure 4.19, if sonication time increases, soluble COD increases as well. After 8 min this increase slows down.

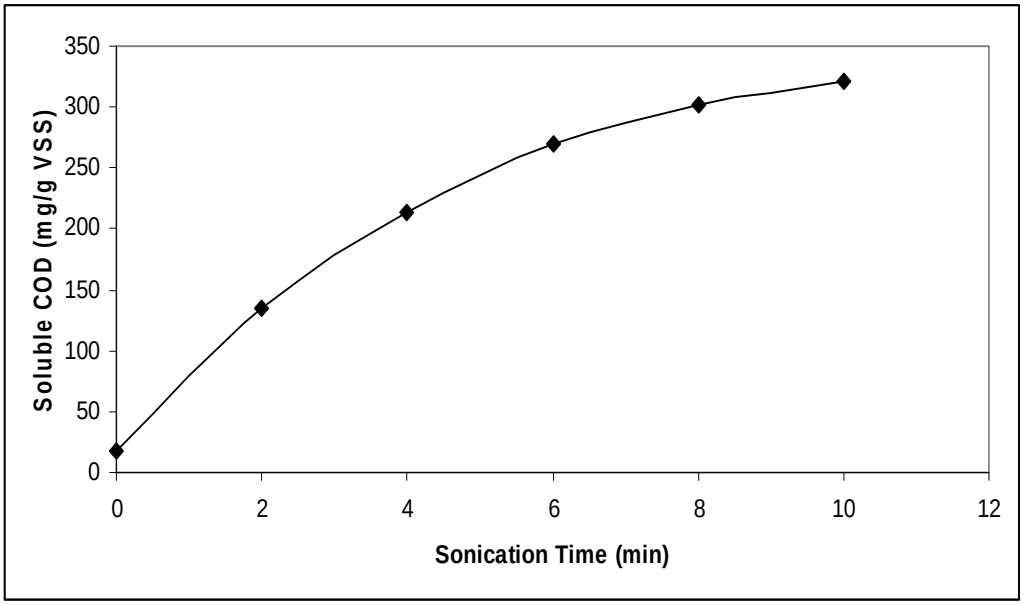


Figure 4.19. The variation in soluble COD according to sonication time (using 22 mm probe)

Figure 4.20 represents the variation in CST according to change in the sonication time. When the sonication time increases, CST values of the sludge increase from 3 to 50 sec/g SS and then decrease to 30 sec g/SS. The reflocculation may be the cause of this decline.

With increase in sonication time, turbidity of the supernatant increases and this increase reaches to 2500 NTU at 10 min sonication. After 6 min, the increase slows down (Figure 4.21).

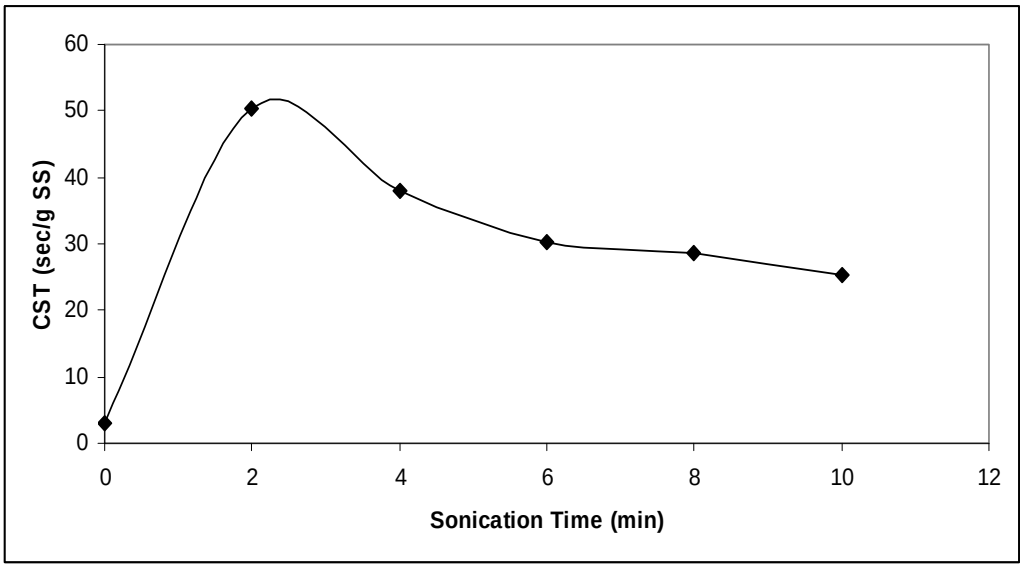


Figure 4.20. The variation in CST according to sonication time (using 22 mm probe)

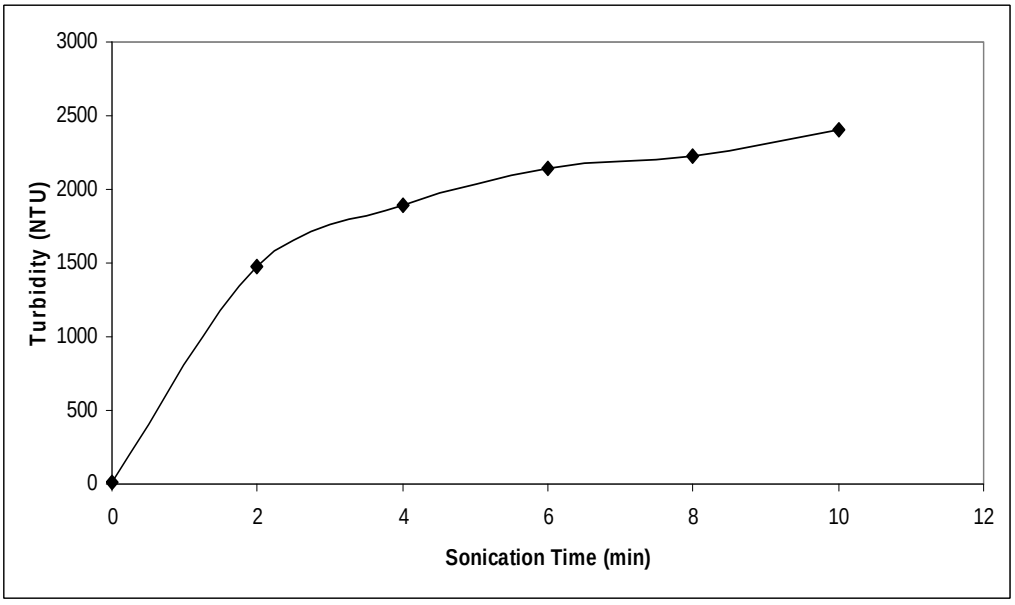


Figure 4.21. The variation in turbidity according to sonication time (using 22 mm probe)

When the different size sonication probes (14 and 22 mm) were compared as soluble COD contributed to the supernatant of sonicated sludge according to sonication power and time, it is found in Figure 4.22 that even at lower sonication time and sonication power more soluble COD was measured in the liquid phase after sonication with 22 mm probe.

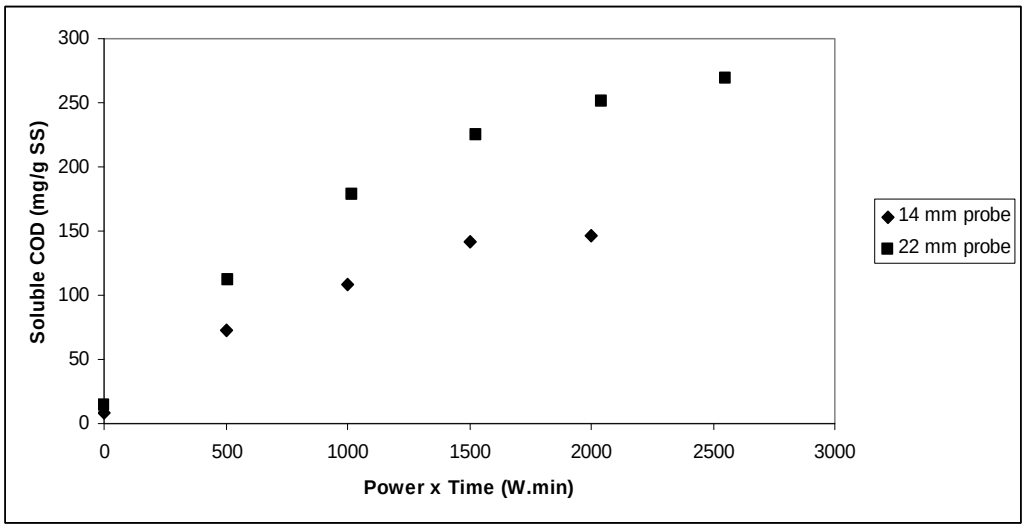


Figure 4.22. The comparison of probes as soluble COD according to sonication power x time

4.3. Anaerobic Batch Tests 1-Using low power ultrasound probe

4.3.1. Effect of Sonication Time in Anaerobic Digestion

Regarding results of first set of disintegration experiments, 100 W was chosen as best point of sonication power. On the other hand, effect of sonication time on soluble COD was found very similar for different times. For this reason, in order to observe the effect of sonication time on biomethane potential, tests were applied on 10, 15 and 20 minutes disintegrated sludges. Thus, after this test, not only the best sonication time was determined but also, the effect of sonication on total gas amount and methane gas composition was understood.

For anaerobic digestion, WAS was used with MLSS , MLVSS and total COD values of 5225 mg/L , 4325 mg/L and 6120 mg/L, respectively. The MLSS and MLVSS values of ADS were 13065 mg/L ve 7265 mg/l, respectively. To set the F/M ratio at 0.5, 46 mL WAS, 54 mL ADS and 20 mL basal medium were added to the reactors. 12 reactors were set: triplicate reactor for control sludge and triplicate reactors for all different sonication time at fixed sonication power (100 W) according to results of disintegration studies.

Reactors were named as control, 10 min, 15 min and 20 min. During the 56 days anaerobic digestion period, total gas production and gas composition were measured in definite time intervals.

Before setting reactors, the soluble COD of the sonicated sludges at different times were analyzed one more time. According to Figure 4.23, when the sonication time increases soluble COD amount increases as well and also there has not been much difference between the soluble COD of 15 and 20 min sonicated sludge.

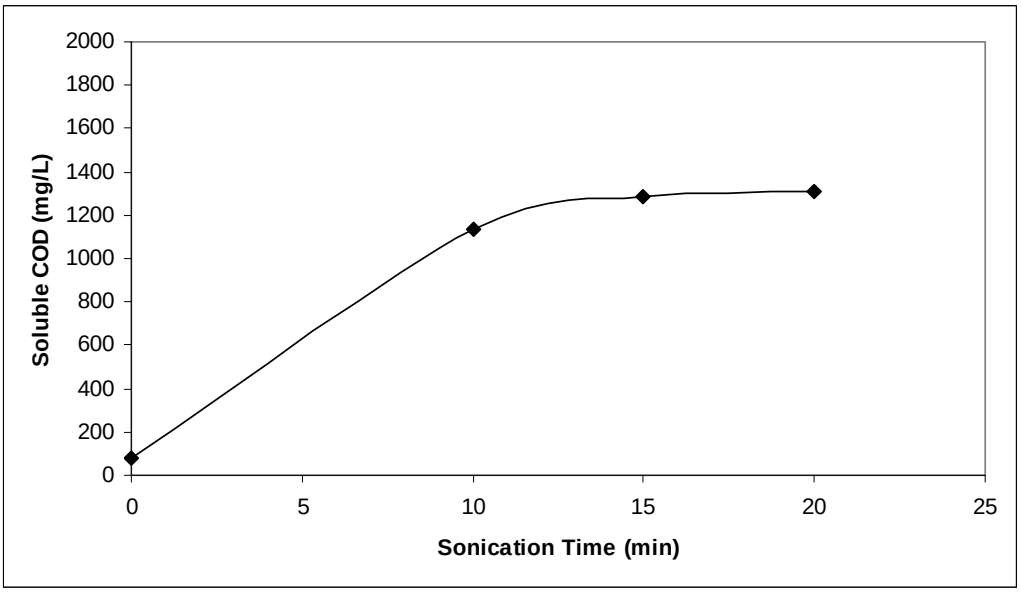


Figure 4.23. Soluble COD concentration in sludges sonicated at different times before digestion

Figure 4.24 illustrates the difference in total gas production between control and sonicated sludges. It is obvious that ultrasonic pretreatment method increases the hydrolysis rate of sludge and the hydrolysis rate of control sludge is lower than sonicated sludges beginning from second day in anaerobic digestion. The difference that shows the hydrolysis rate decreases to the end of digestion period. This confirms that the sonicated sludges produced total gas that they could at the beginning and the disintegration rate of control sludge is slow. At the end of 56 days of digestion period, total gas production value of 10 minutes, 15 minutes and 20 minutes sonicated sludge is 6 % and 12 % higher compared to control sludge, respectively. No difference is seen in total gas amounts between 15 and 20 minutes pretreatment time.

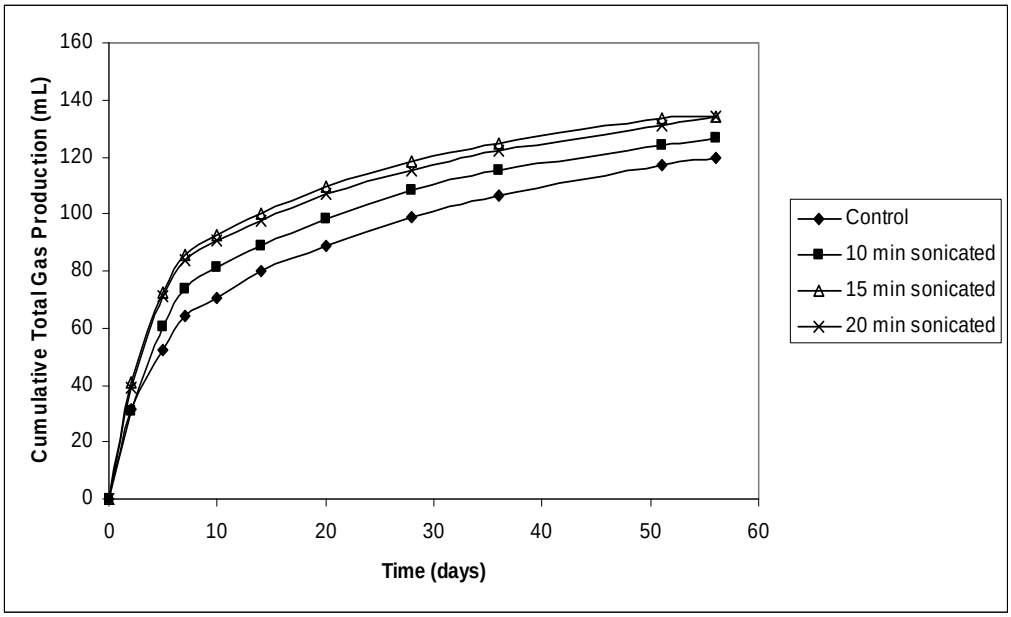


Figure 4.24. Cumulative total gas production of sludges sonicated at different times during anaerobic digestion

According to Figure 4.25, at the end of 56 days, parallel to effects of sonication times on soluble COD, sludges sonicated at 10, 15 and 20 minutes produce higher methane gas at the same ratio compared to control sludge. There is no difference as

the cumulative methane production in all sonicated sludges. But one thing obvious is the initial faster rate in sonicated reactors compared to control for the first 5 to 10 days.

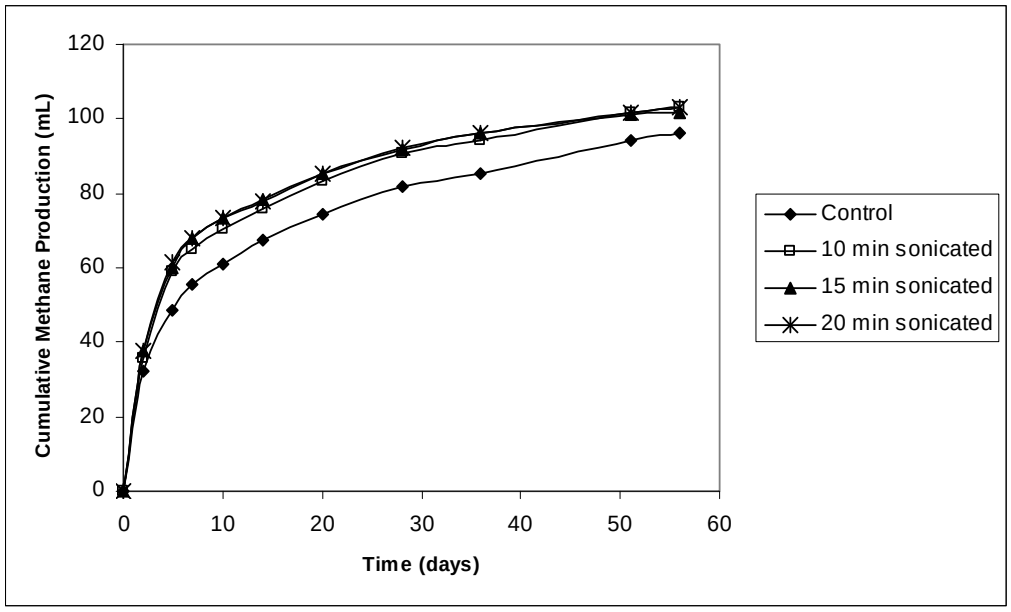


Figure 4.25. Cumulative methane gas production of sludges sonicated at different times during anaerobic digestion

After 56 days of anaerobic digestion period, reactors were opened and analyzed. The initial and final MLSS, MLVSS, total COD and final soluble COD are given in Table 4.4. According to these results, there 5 % - 7 % and 3 % - 5 % difference in MLSS and MLVSS values to compare the control and sonicated reactors, respectively. On the other hand, the total COD destruction of sonicated reactors is higher than control reactors as seen in Table 4.5. This shows that organic matter as total COD turns into the gas.

Considering the loss of some portion in MLSS/MLVSS because of sonication of sludge before anaerobic digestion, the initial data of control sludge should be taken to calculate decrease in the MLSS/MLVSS after sonication and digestion process.

Thus, the initial MLSS and MLVSS values were used to mention reduction after all anaerobic digestion tests in this study.

Table 4.4. MLSS and MLVSS values of 250 mL reactors before and after anaerobic digestion

	Initial MLSS (mg/L)	Final MLSS (mg/L)	Reduction in MLSS (%)	Initial MLVSS (mg/L)	Final MLVSS (mg/L)	Reduction in MLVSS (%)
Control	8550	5455	36	5190	2755	47
10 min son.	7310	4890	43	4570	2475	52
15 min son.	7880	4870	43	4650	2475	52
20 min son.	7270	5095	41	4230	2595	50

Table 4.5. Soluble COD and total COD values of 250 mL reactors before and after anaerobic digestion

	Initial tCOD (mg/L)	Final tCOD (mg/L)	Reduction in tCOD (%)	Final sCOD (mg/L)
Control	6998 ± 130	5988 ± 301	14	233
10 min son.	7078 ± 10	5732 ± 151	19	434
15 min son.	7103 ± 39	5710 ±155	20	371
20 min son.	7653 ± 95	5897 ± 112	23	331

After anaerobic digestion, the pH and CST of the sludge in the reactors was analyzed in order to examine sludge characteristics. Moreover, the turbidity of supernatants of sludges was investigated (Table 4.6). The pH and CST values of

sonicated reactors are similar to control reactor, but turbidity of sonicated ones is 13 to 30 % higher compared to control reactor.

Table 4.6. pH, CST and turbidity after anaerobic digestion

	pH	CST (sec)	Turbidity (NTU)
Control	7.38	123.7 ± 7.68	416.6 ± 21.59
10 min son.	7.33	120.7 ± 6.42	542.2 ± 32.90
15 min son.	7.35	123.8 ± 6.59	467.3 ± 8.15
20 min son.	7.38	127.6 ± 5.91	523.9 ± 44.76

4.2.2. Effect of F/M Ratio in Anaerobic Digestion

In this part of the study, in order to see the effect of different F/M values, batch anaerobic reactors with total volume of 250 mL were set. WAS sonicated at 100 W and 15 min and anaerobic digested seed were added to the reactors with F/M ratios with 0.5, 2, 5 and 10. In order to satisfy these ratios, WAS and ADS were added to the reactors after determining MLSS values. Table 4.7 shows the amounts of WAS and ADS added to the control and sonicated reactors. The working volume in the reactors is 120 mL with 20 mL of basal medium. The other part is left empty for gas production. For all system, reactors were set three times to increase the replicability.

All reactors are purged with N_2 gas for 10 minutes to prevent oxidation of readily oxidizable organics and remove oxygen in the system. Followed by, the tops of the bottles were closed with rubber stoppers, and shaken well. Finally, conventional digesters were incubated in 37ºC. Even if the system is conventional, reactors were shaken once in a day.

Table 4.8 indicates the MLSS and MLVSS values of control and sonicated reactors before anaerobic digestion.

Table 4.7. WAS and ADS amounts added to 250 mL control and sonicated reactors regarding F/M ratios

	WAS (mL)		ADS (mL)	
F/M ratio	control	sonicated	control	sonicated
0.5	46	48	54	52
2	77	79	23	21
5	89	90	11	10
10	94	95	6	5

Table 4.8. MLSS and MLVSS values of 250 mL control and sonicated reactors before anaerobic digestion

	Control Reactor		Sonicated Reactor	
F/M ratio	MLSS (mg/L)	MLVSS (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
0.5	7208	4319	6743	3955
2	5441	3696	4663	3086
5	4757	3454	3857	2749
10	4472	3354	3522	2609

Figure 4.26 demonstrates the total gas produced by reactors with different F/M ratios during anaerobic digestion. For first 8 days, control and sonicated reactors with F/M ratios of 2, 5 and 10 produced lower amount of gas compared to reactors

with F/M ratio of 0.5. The other days, they catched and passed the reactors with F/M ratio of 0.5. That is, when the F/M ratio of reactors increases, the period for gas production in the reactors lags behind.

Figure 4.27 demonstrates the methane gas composition of gases produced by reactors with different F/M ratios during anaerobic digestion. Regarding the produced total gas amounts, at the beginning of the digestion control and sonicated reactors with F/M ratios of 2, 5 and 10 produced lower amount of methane gas compared to reactors with F/M ratio of 0.5. Next, their methane amounts pass the methane gas amount of reactors with F/M ratio of 0.5.

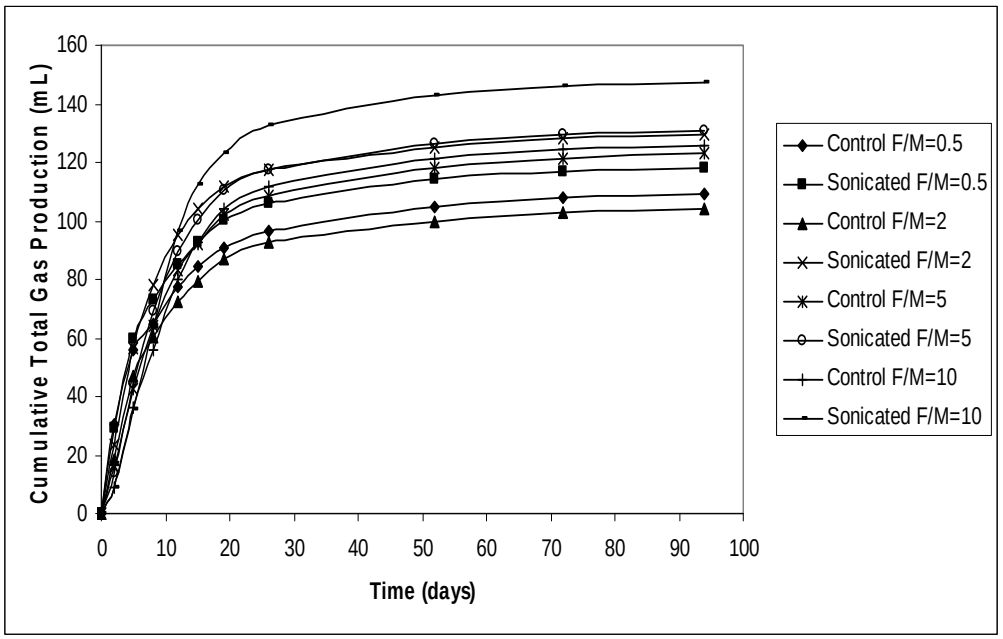


Figure 4.26. The daily change in cumulative total gas production of 250 mL reactors at different F/M ratios during anaerobic digestion

Looking at the total gas amount of reactors produced per gram volatile solids after digestion period in Figure 4.28, sonicated reactors with F/M ratio of 0.5, 2, 5 and 10 produced 18.5 %, 49.2 %, 33.4 % and 51 % higher amounts of total gas compared to their own control reactors, respectively.

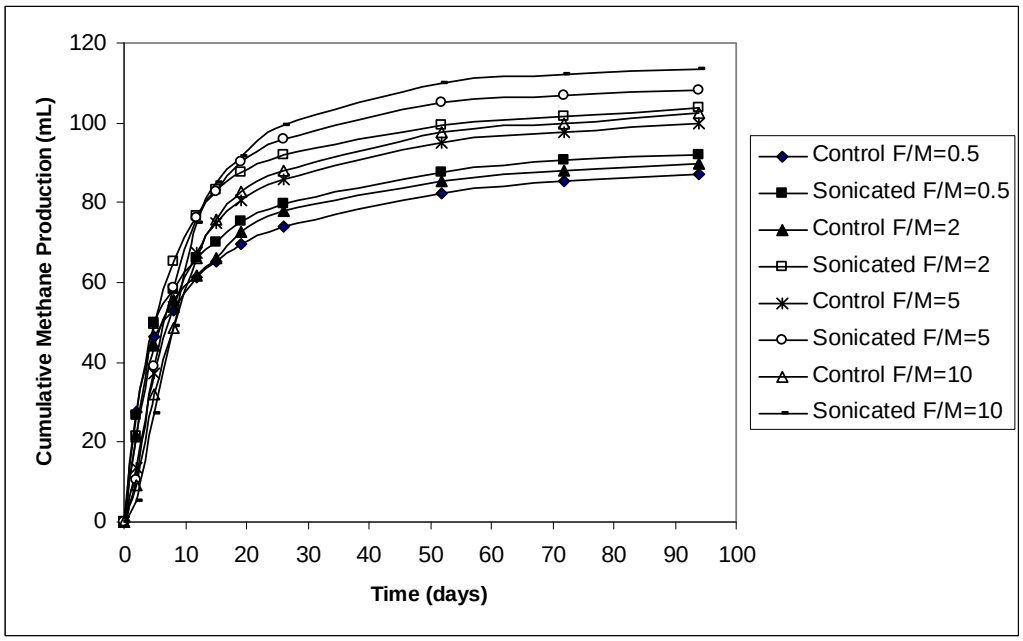


Figure 4.27. The daily change in cumulative methane gas production of 250 mL reactors at different F/M ratios during anaerobic digestion

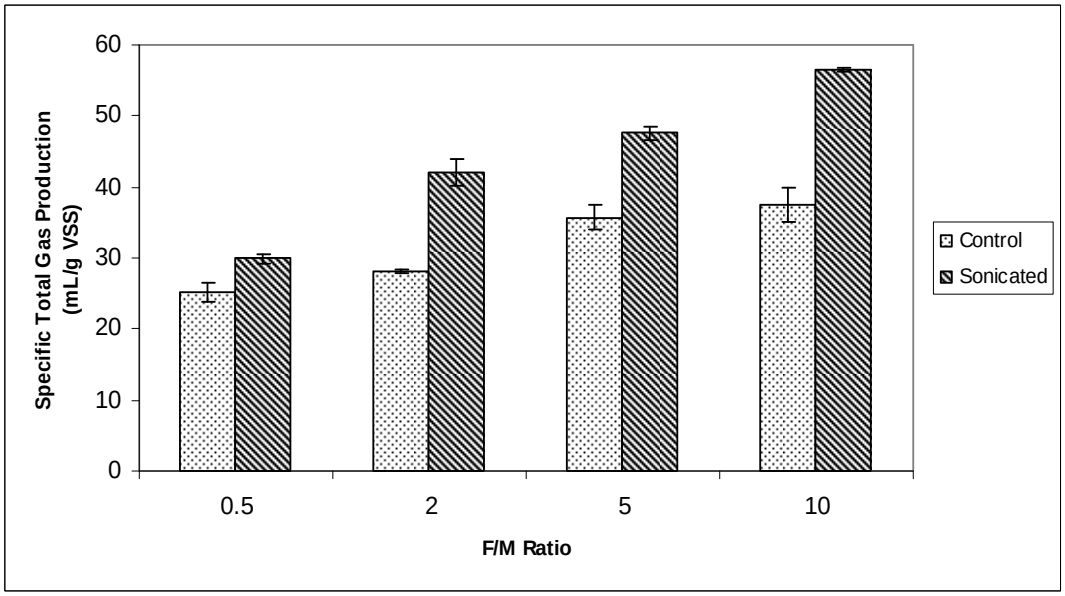


Figure 4.28. Total gas amount of 250 mL reactors at different F/M ratios as per gram VSS after anaerobic digestion

Looking at the methane gas amount of reactors produced per gram volatile solids after digestion period in Figure 4.29, sonicated reactors with F/M ratio of 0.5, 2, 5 and 10 produced 15.3 %, 38.1 %, 36 % and 42.3 % higher amounts of methane gas compared to their own control reactors, as respectively.

So with these results, the lower difference between control and its sonicated reactor in terms of total gas and methane production is found in the reactors with F/M ratio of 0.5. This difference is high when the F/M ratio is 2 and 10. Thereby, F/M ratio of 10 can be recommended considering high methane production.

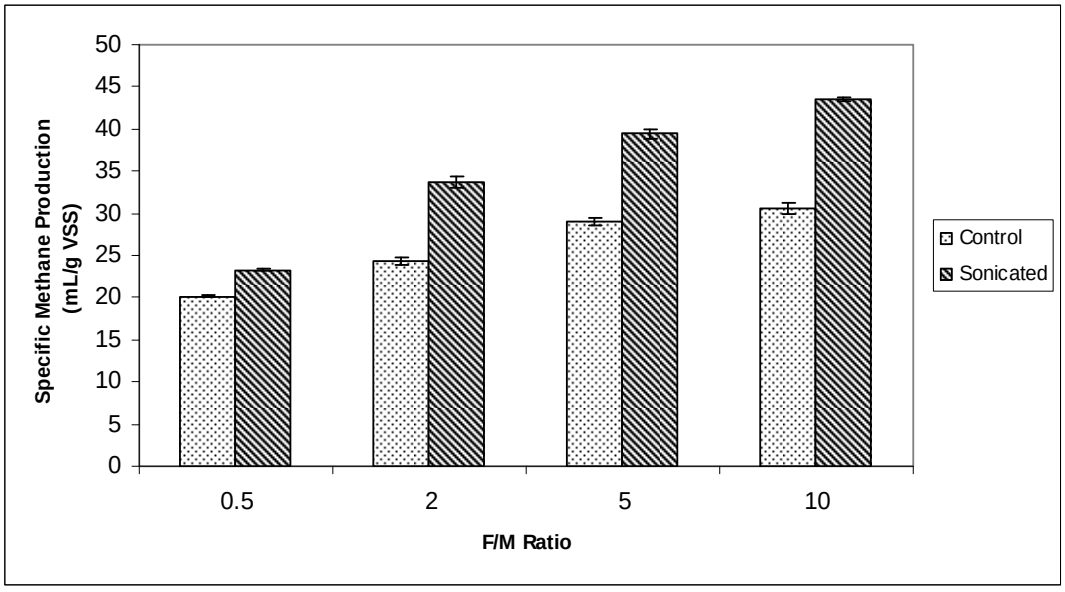


Figure 4.29. Methane gas amount of 250 mL reactors at different F/M ratios as per gram VSS after anaerobic digestion

Total MLSS and MLVSS of reactors at the first day of setting up and at the end of 94 days of anaerobic digestion are given in Table 4.9. It is found that the initial MLSS values of control reactors measured in the reactor setting day are higher than the MLSS values of sonicated reactors at all F/M ratios. That is, during pretreatment by sonication some portion of MLVSS was converted into dissolve solids.

In total COD values, an important difference before and after anaerobic digestion was not seen, as expected. Despite observing an increase in total COD destruction (%1-4.9) in sonicated sludges relative to control reactors, these values are in the range of experimental variations and not considered as big difference.

Table 4.9. MLSS and MLVSS values of 250 mL reactors with different F/M ratios before and after anaerobic digestion

Reactor	Initial MLSS (mg/L)	Final MLSS (mg/L)	Reduction in MLSS* (%)	Initial MLVSS (mg/L)	Final MLVSS (mg/L)	Reduction in MLVSS* (%)
Control F/M=0.5	7208	4235	41.2	4319	2085	51.7
Sonicated F/M=0.5	6743	4270	40.8	3955	2005	53.6
Control F/M=2	5441	3115	42.8	3796	1650	56.5
Sonicated F/M=2	4663	2885	47.0	3086	1530	60.0
Control F/M=5	4757	2430	48.9	3454	1460	57.7
Sonicated F/M=5	3857	2150	54.8	2749	1185	65.7
Control F/M=10	4472	2060	53.9	3354	1185	64.7
Sonicated F/M=10	3522	2045	54.3	2609	1140	66.0

* Reduction values of sonicated reactors are calculated with respect to control reactors at t=0

According to Table 4.10, initially, sonicated sludges have slightly more total COD value compared to control sludges. As expected, during digestion, some portion of total COD was converted into biogas. After digestion, it was found that total COD values of sonicated and control reactors decreased and they were close to each other.

As shown in Figure 4.30, dewaterability of sludge deteriorates for sonicated reactors compared to control reactors. This deterioration increases when F/M ratio increases. However, CST value of reactor with F/M ratio of 0.5 is better than the other reactors and CST decreases by increasing F/M ratio.

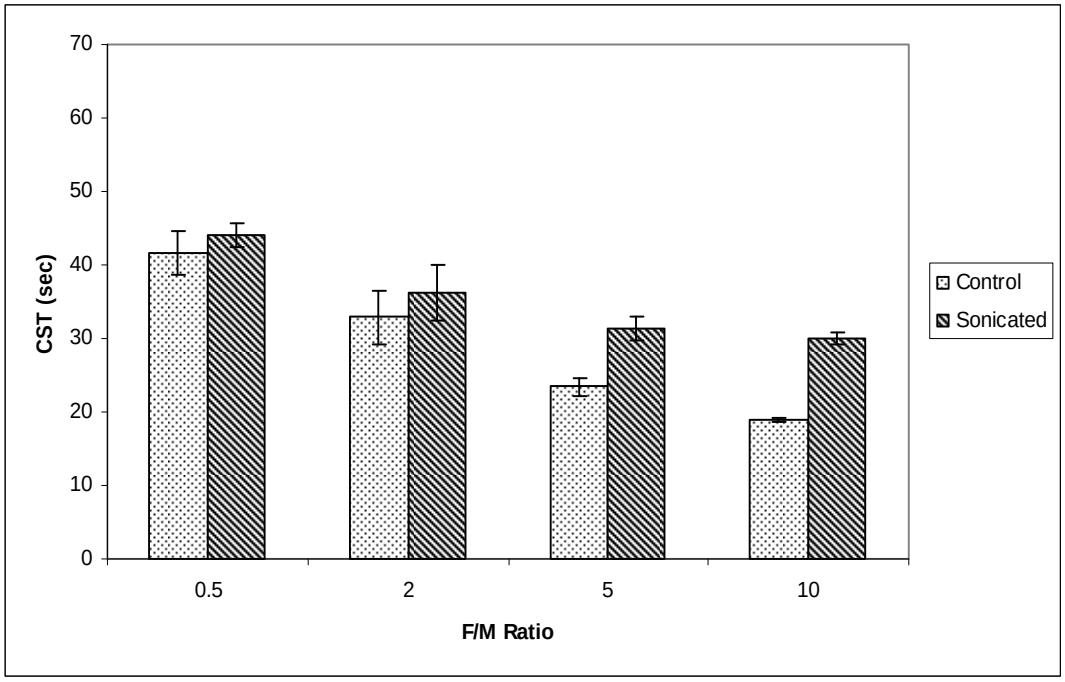


Figure 4.30. CST values of 250 mL reactors at different F/M ratios after digestion

Turbidity of the supernatants of the sonicated reactors at different F/M decreases compared to control reactors as shown in Figure 4.31. This decline is higher at the reactor with F/M ratio of 0.5. When F/M ratio increases, the turbidity of sonicated

reactors approaches to that of control reactors. However, generally turbidity trend decreases by increasing F/M ratio as similarly observed in CST findings.

Table 4.10. Total COD values of 250 mL reactors with different F/M ratios before and after anaerobic digestion

Reactor	Initial tCOD (mg/L)	Final tCOD (mg/L)	Reduction in tCOD (%)
Control F/M=0.5	6350 ± 185	4872 ± 243	23.3
Sonicated F/M=0.5	6500 ± 170	4919 ± 218	24.3
Control F/M=2	5300 ± 195	3314 ± 311	37.5
Sonicated F/M=2	5560 ± 80	3452 ± 57	37.9
Control F/M=5	5010 ± 75	3098 ± 114	38.2
Sonicated F/M=5	5100 ± 155	2943 ± 188	42.3
Control F/M=10	4800 ± 150	2595 ± 367	45.9
Sonicated F/M=10	5000 ± 90	2666 ± 111	46.7

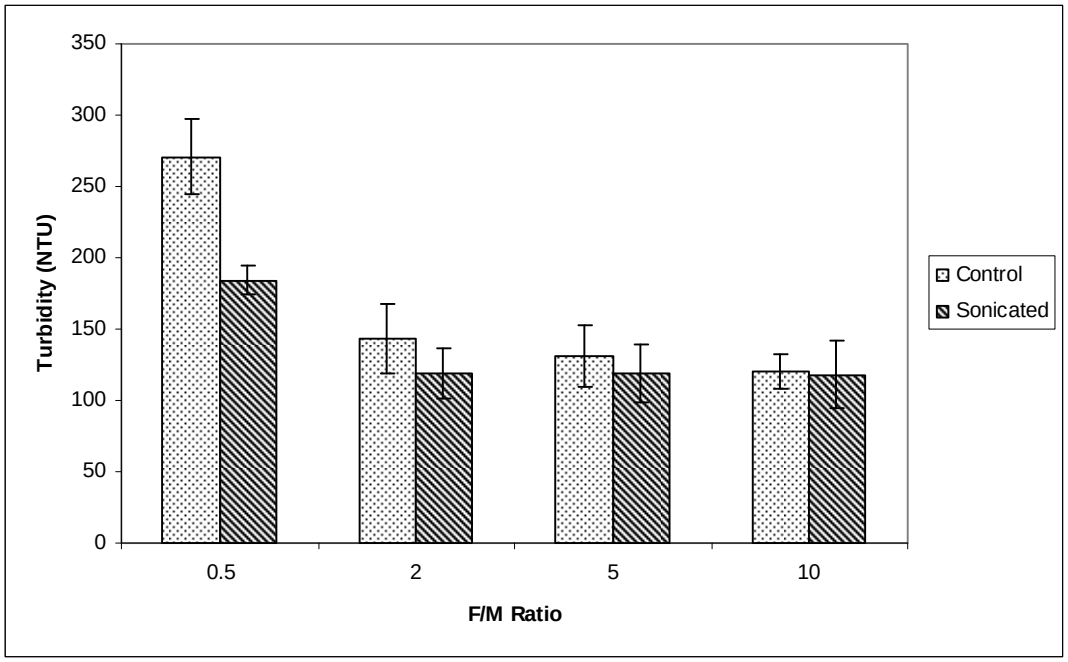


Figure 4.31. Turbidity values of 250 mL reactors at different F/M ratios after digestion

4.4. Anaerobic Batch Tests 2 – Using high power ultrasound probe

4.4.1. Effect of F/M ratio in Anaerobic Digestion

4.4.1.1. Batch Reactors with Volume of 250 mL

In this part, to replicate the results of anaerobic digestion at different F/M values and to observe the effect of using high power probe, batch anaerobic reactors with total volume of 250 mL were set again. WAS sonicated at 255 W and 10 min and anaerobic digested seed were added to the reactors with F/M ratios with 0.5, 2, 5 and 10. In order to satisfy these ratios, WAS and ADS were added to the reactors after determining MLSS values. Table 4.11 shows the amounts of WAS and ADS

added to the control and sonicated reactors. The working volume in the reactors is 120 mL with 20 mL of basal medium.

Table 4.11. WAS and ADS amounts added to 250 mL control and sonicated reactors regarding F/M ratios

	WAS (mL)		ADS (mL)	
F/M ratio	control	sonicated	control	sonicated
0.5	33	36	67	64
2	67	70	33	30
5	83.5	85	16.5	15
10	91	92	9	8

Table 4.12 indicates the MLSS and MLVSS values of control and sonicated reactors before anaerobic digestion.

Table 4.12. MLSS and MLVSS values of 250 mL control and sonicated reactors before anaerobic digestion

	Control Reactor		Sonicated Reactor	
F/M ratio	MLSS (mg/L)	MLVSS (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
0.5	8874	4436	8498	4251
2	7746	4432	7107	4073
5	7198	4430	6494	3994
10	6949	4429	6207	3957

Figure 4.32 illustrates the total gas production amounts of reactors at different F/M ratios during 48 days of anaerobic digestion. For the first days, when the F/M ratio increases the gas amount produced gets lower. Then, the gas produced from reactors at high F/M ratio passes that at lower F/M ratio. This gas formation trend is similar to the trend at Figure 4.24 but the gas values for sonicated reactors in Figure 4.32 are high compared to that in Figure 4.24 because of using high power sonication in this case. On the other hand, the gas values are similar to each other for control reactors as seen in two figures.

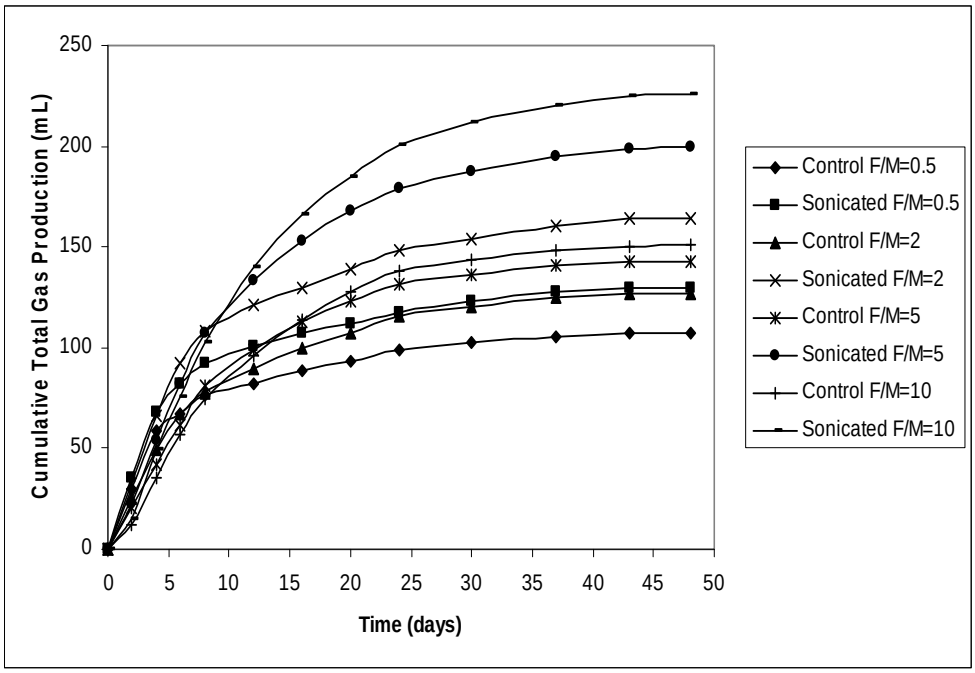


Figure 4.32. The daily change in cumulative total gas production of 250 mL reactors at different F/M ratios during anaerobic digestion

Figure 4.33 demonstrates the methane gas composition of gases produced by reactors with different F/M ratios during anaerobic digestion. Similar to the produced total gas amounts, control and sonicated reactors with F/M ratios of 2, 5 and 10 produced lower amount of methane gas compared to reactors with F/M ratio of 0.5 at the beginning of the digestion. Next, their methane amounts surpass the

methane gas amount of reactors with F/M ratio of 0.5. This produced methane gas trend is parallel to the trend at Figure 4.25 but the methane gas values for sonicated reactors in Figure 4.33 are high compared to that in Figure 4.25 because of using high power sonication in this case.

Total gas amount of reactors produced per gram volatile solids after digestion period in Figure 4.34 points that, sonicated reactors with F/M ratio of 0.5, 2, 5 and 10 produced 17.5 %, 40.4 %, 54.7 % and 67.5 % higher amounts of total gas compared to their own control reactors, as respectively. If these increments are related with the increments obtained in Figure 4.21, for F/M ratio of 0.5 and 2 there is not much difference. However, for F/M ratio of 5 and 10 there is a significant difference in the increments due to excess amount of sludge sonicated with high power.

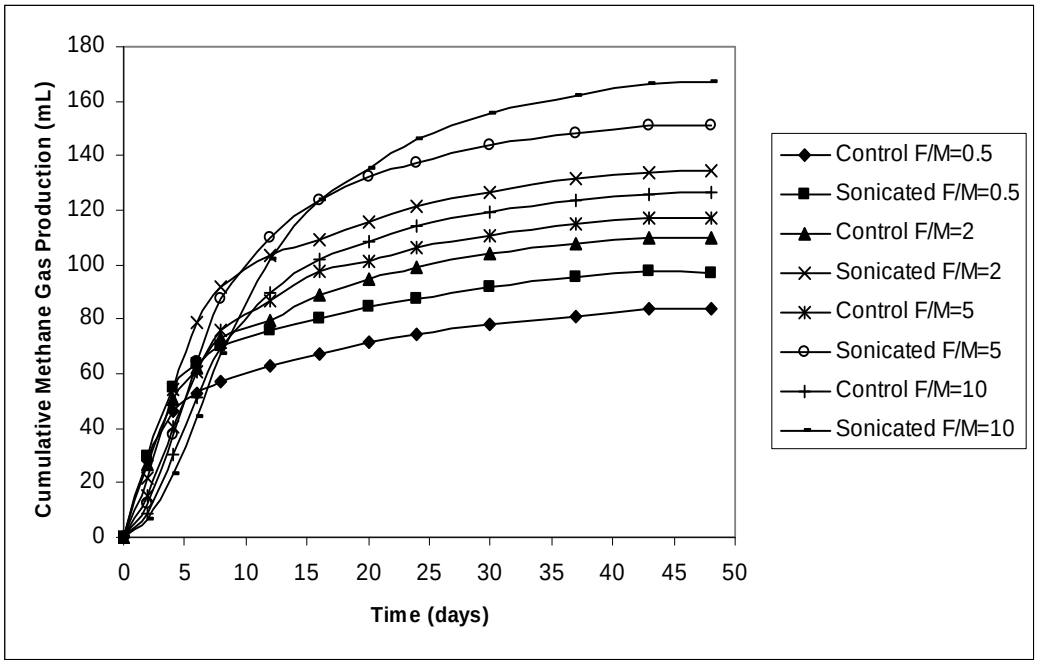


Figure 4.33. The daily change in cumulative methane gas production of 250 mL reactors at different F/M ratios during anaerobic digestion

When produced methane gas amount of reactors per gram volatile solids after digestion period in Figure 4.35 is examined, sonicated reactors with F/M ratio of 0.5, 2, 5 and 10 produced 12.8 %, 32.6 %, 43.5 % and 48.1 % higher amounts of methane gas compared to their own control reactors, as respectively. Comparing these increments with the increments found in Figure 4.22, there is difference in methane increment at F/M 5 and 10 since high power sonicated sludge amount is abundant in higher F/M ratios.

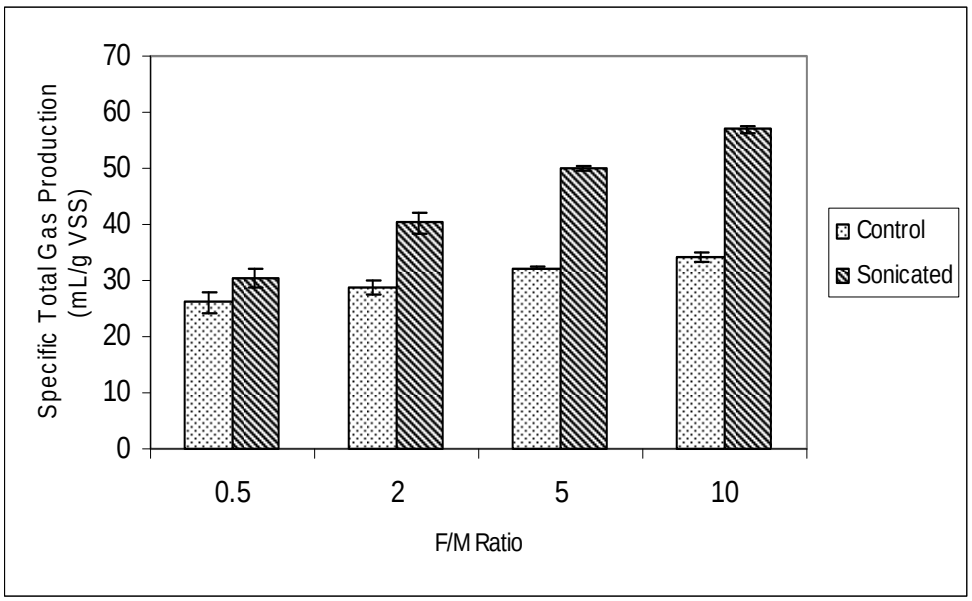


Figure 4.34. Total gas amount of 250 mL reactors at different F/M ratios as per gram VSS after anaerobic digestion

Total MLSS and MLVSS of reactors at the first day of set up and at the end of anaerobic digestion are given in Table 4.13. It is found again that the higher MLSS value measured in the first day is the MLSS of control reactor. Moreover, the first day MLSS value of the reactors set by sonicated sludge were measured lower. That is because during pretreatment by sonication some portion of MLVSS was converted into dissolved solids.

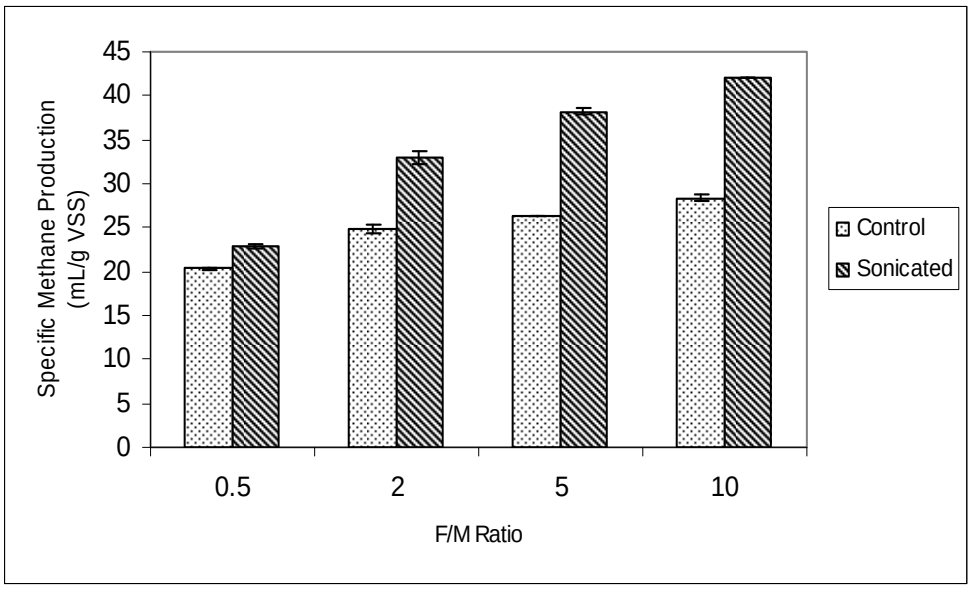


Figure 4.35. Methane gas amount of 250 mL reactors at different F/M ratios as per gram VSS after anaerobic digestion

Unlike total COD results obtained from anaerobic digestion of reactors sonicated at 100 W, there is a significant increase in total COD destruction in sonicated reactors at all F/M ratio compared to their control reactors as shown in Table 4.14. This shows that sonication pretreatment make organic matter in the cell pass into the liquid phase and this matter as total COD is converted into gas during digestion.

As shown in Figure 4.36, dewaterability of sludge improves for sonicated reactors compared to control reactors. This improvement decreases when F/M ratio increases. However, CST itself decreases by increasing F/M ratio.

Turbidity of the supernatants of the sonicated reactors at different F/M decreases compared to control reactors as shown in Figure 4.37. This decline is higher for the reactor with F/M ratio of 0.5. When F/M ratio increases, the turbidity of sonicated reactors approaches to that of control reactors. However, generally turbidity trend decreases by increasing F/M ratio as similarly observed in CST findings.

Table 4.13. MLSS and MLVSS values of 250 mL reactors with different F/M ratios before and after anaerobic digestion

Reactor	Initial MLSS (mg/L)	Final MLSS (mg/L)	Reduction in MLSS* (%)	Initial MLVSS (mg/L)	Final MLVSS (mg/L)	Reduction in MLVSS* (%)
Control F/M=0.5	8874	6590	25.8	4436	2650	40.3
Sonicated F/M=0.5	8498	6495	26.8	4251	2430	45.2
Control F/M=2	7746	4900	36.7	4432	2005	54.8
Sonicated F/M=2	7107	4340	44.0	4073	1745	60.6
Control F/M=5	7198	4515	37.3	4430	1935	56.3
Sonicated F/M=5	6494	4015	44.2	3994	1645	62.9
Control F/M=10	6949	3985	42.7	4429	1720	61.2
Sonicated F/M=10	6207	3880	44.2	3957	1680	62.1

* Reduction values of sonicated reactors are calculated with respect to control reactors at t=0

Table 4.14. Total COD values of 250 mL reactors with different F/M ratios before and after anaerobic digestion

Reactor	Initial tCOD (mg/L)	Final tCOD (mg/L)	Reduction in tCOD (%)
Control F/M=0.5	6940 ± 110	4972 ± 156	28.4
Sonicated F/M=0.5	7080 ± 85	4620 ± 87	34.8
Control F/M=2	6550 ± 90	3931 ± 97	40.0
Sonicated F/M=2	6640 ± 60	3538 ± 41	46.7
Control F/M=5	6630 ± 55	3495 ± 65	47.3
Sonicated F/M=5	6730 ± 70	3067 ± 30	54.4
Control F/M=10	6800 ± 60	2936 ± 199	56.8
Sonicated F/M=10	7020 ± 45	2553 ± 98	63.6

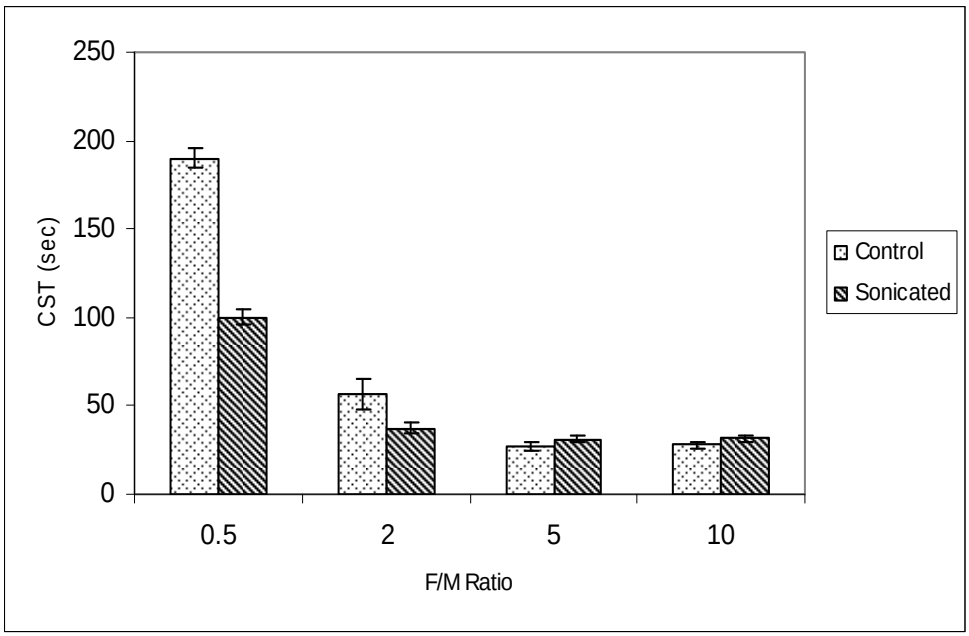


Figure 4.36. CST values of 250 mL reactors at different F/M ratios after digestion

4.4.1.2. Batch Reactors with Volume of 3 L

It is obvious that in the batch anaerobic digestion tests by using sonication at 100 W-15 min and 255 W-10 min, at all F/M ratios, the similar gas production profiles are found. However, for both cases significantly higher destruction in MLSS values for sonicated reactors compared to control reactors could not be observed. To overcome the disadvantage of working in small scale reactors and to observe whether reactor volume has effect on MLSS reduction or not, 3 L batch reactors were set by using sonication at 255 W- 10 min and at minimum and maximum F/M ratios of the anaerobic tests conducted before. Since, the lowest but the fastest gas production was observed at the minimum F/M ratio studied (0.5) in small anaerobic batch reactors. On the other hand, at the maximum F/M ratio (10), the highest but the slowest gas production was found.

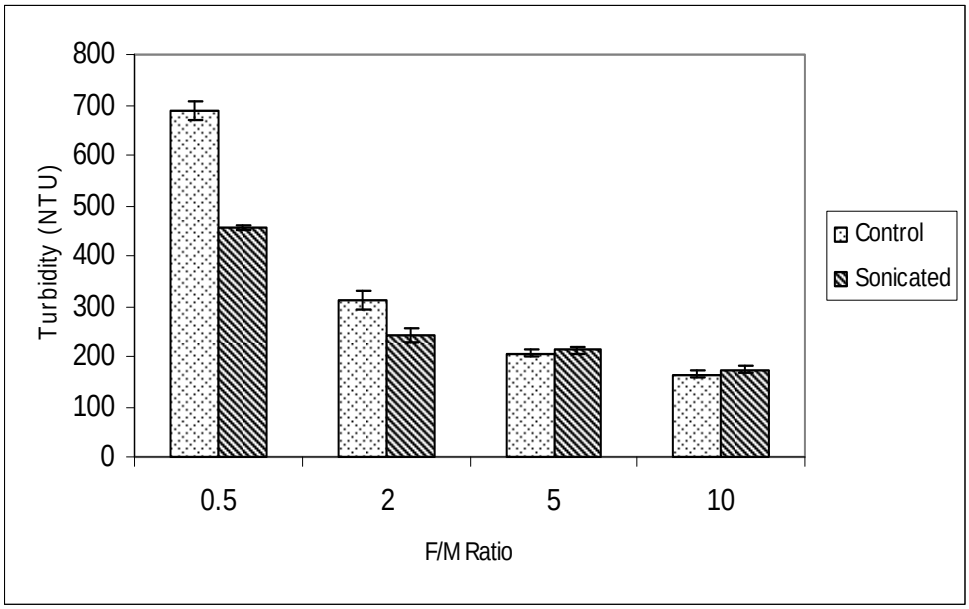


Figure 4.37. Turbidity values of 250 mL reactors at different F/M ratios after digestion

WAS sonicated at 255 W and 10 min and anaerobic digested seed were added to the reactors with F/M ratios with 0.5, 2, 5 and 10. In order to satisfy these ratios, WAS and ADS were added to the reactors after determining MLSS values. Table 4.15 shows the amounts of WAS and ADS added to the control and sonicated reactors. The working volume in the reactors was 2 L. No basal medium was added to the reactors.

Reactors were set as duplicate reactors. All reactors were put on magnetic stirrers and incubated in hot room at 37ºC.

4 L glass gas collectors that were connected to the reactors from the top were put in saline brine solution to prevent the solubility of gases. At the same time, the decrease in alkalinity caused by the solubility of carbondioxide gas can be avoided.

Table 4.15. WAS and ADS amounts added to 3 L control and sonicated reactors regarding F/M ratios

	WAS (L)		ADS (L)	
F/M ratio	control	sonicated	control	sonicated
0.5	1.06	1.08	0.94	0.92
10	1.91	1.93	0.09	0.07

Table 4.16 indicates the MLSS and MLVSS values of control and sonicated reactors before anaerobic digestion.

Table 4.16. MLSS and MLVSS values of 3 L control and sonicated reactors before anaerobic digestion

	Control Reactor		Sonicated Reactor	
F/M ratio	MLSS (mg/L)	MLVSS (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
0.5	11698	5768	10635	5598
10	5555	3923	5115	3656

Figure 4.38 illustrates the total gas production amounts of reactors at 0.5 and 10 F/M ratios during 45 days of anaerobic digestion. For the first days, for reactors with high F/M ratio the gas amount produced is lower compared to the ones with low F/M ratio. The reason is the low seed concentration for anaerobic process to consume the food in the reactor. After day 16, the gas produced from reactors at high F/M ratio passes that of the reactors at lower F/M ratio. The gas produced

from sonicated reactors at all F/M ratios is higher at the end of digestion period. This gas formation trend is similar to the trend at Figure 4.19 and 4.32 but the gas values for reactors in Figure 4.38 are high because of larger volume and high amount of sludge used.

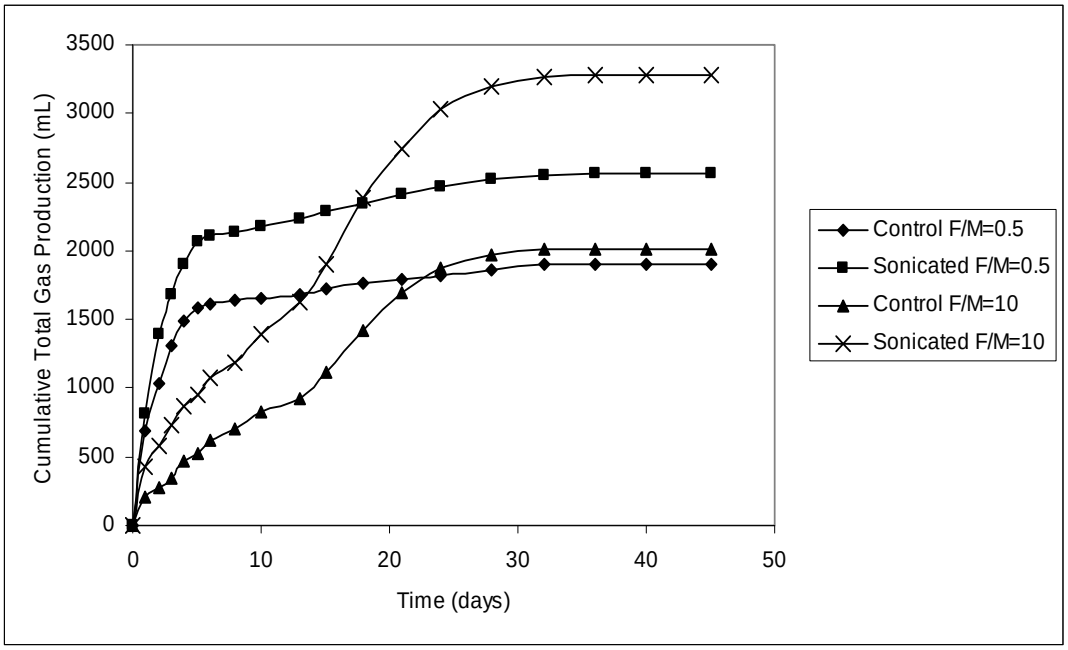


Figure 4.38. The daily change in cumulative total gas production of 3 L reactors at different F/M ratios during anaerobic digestion

Figure 4.39 demonstrates the methane content of gases produced by reactors with F/M ratios of 0.5 and 10 during 45 days of anaerobic digestion. Similar to the total gas amounts produced, control and sonicated reactors with F/M ratio of 10 produced lower amount of methane gas compared to reactors with F/M ratio of 0.5 at the beginning of the digestion. Next, their methane amounts pass the methane gas amount of reactors with F/M ratio of 0.5. This produced methane gas trend is parallel to the trend in Figure 4.20 and 4.33.

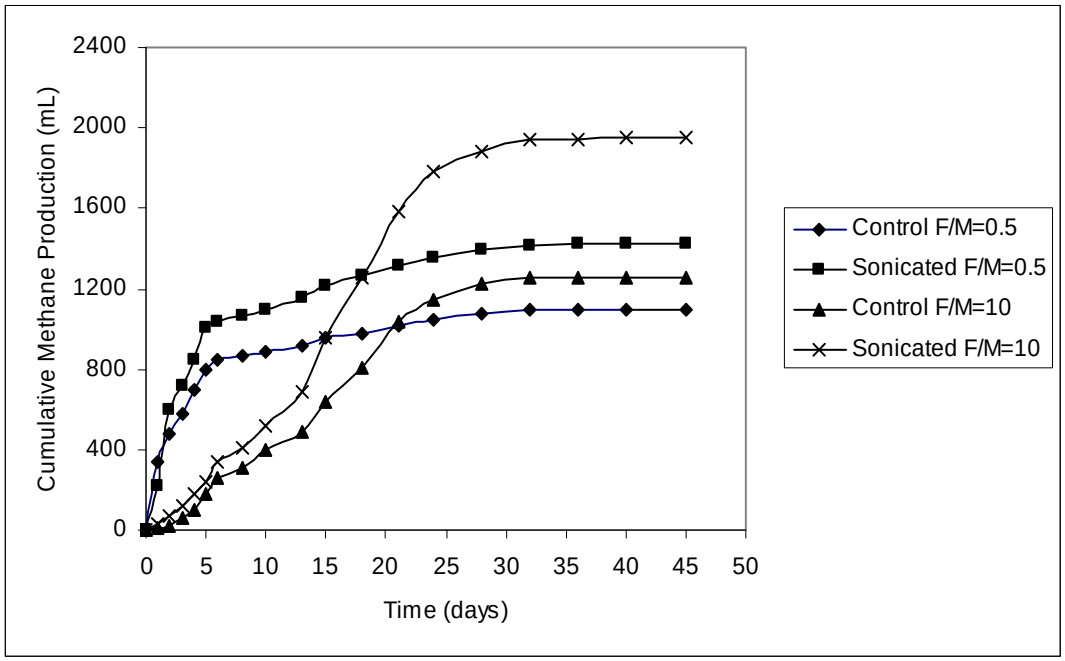


Figure 4.39. The daily change in cumulative methane gas production of 3 L reactors at different F/M ratios during anaerobic digestion

The percentage increase in total gas and methane gas of sonicated reactors compared to control reactors at F/M ratios of 0.5 and 10 at the end of digestion period are given in Table 4.17. These results are comparable with the increments found from anaerobic reactors set at a volume of 120 mL. There is not much increase in the methane content with the increase in F/M ratio like in the study of Bunrith (2008).

Total MLSS and MLVSS of reactors at the first day of setting up and at the end of anaerobic digestion are given in Table 4.18. It was observed that when the F/M ratio increases the destruction in MLSS amount increases as well. If the sonicated reactors are compared with control ones the MLSS destruction is higher. That is, during digestion organics are turned into biogas and new cell formation is lower in sonicated reactors.

Table 4.17. Increase in produced gas with respect to controls and methane content of 3 L sonicated reactors

F/M Ratio	Increase in total gas (%)	Increase in methane (%)	Methane Content (%)
0.5	35.1	29.4	55.6
10	62.9	55.1	59.5

Table 4.18. MLVSS values of 3 L reactors with different F/M ratios before and after anaerobic digestion

Reactor	Initial MLVSS (mg/L)	Final MLVSS (mg/L)	Reduction in MLVSS with respect to controls at t=0 (%)
Control F/M=0.5	5768	3890	32.6
Sonicated F/M=0.5	5598	3230	42.3
Control F/M=10	3923	1770	54.9
Sonicated F/M=10	3656	1270	65.3

Similar to total COD results obtained from anaerobic digestion of small size batch reactors sonicated at 255 W, total COD destruction is enhanced considerably in sonicated reactors at all F/M ratio compared to their control reactors as shown in Table 4.19.

Table 4.19. Total COD values of 3 L reactors with different F/M ratios before and after anaerobic digestion

Reactor	Initial tCOD (mg/L)	Final tCOD (mg/L)	Reduction in tCOD (%)
Control F/M=0.5	11625 ± 92	7480 ± 396	35.7
Sonicated F/M=0.5	12135 ± 7	6308 ± 223	48.0
Control F/M=10	7680 ± 127	3400 ± 100	55.4
Sonicated F/M=10	8565 ± 205	2608 ± 83	70.0

Figure 4.40 indicates that dewaterability of sludge improves for sonicated reactors compared to control reactors. Furthermore, CST decreases via increasing F/M ratio. Turbidity of the supernatants of the sonicated reactors at different F/M decreases compared to control reactors as shown in Figure 4.40.

One disadvantage of sonication has been identified as the enrichment of supernatant in terms of organics, nitrogen and phosphorus content. For this reason after digestion the supernatants of reactors were analyzed for soluble COD. The findings, as mg/L, are presented in Figure 4.41. As F/M increases, soluble COD of control reactors does not change much more. However, soluble COD of sonicated reactors decrease with the increase in F/M ratio. If these results are normalized with the MLVSS in the reactors (Figure 4.42), the trend for soluble COD values of sonicated reactors is same but the soluble COD value of control reactor at F/M ratio of 10 increases. Therefore, one can conclude that sonication does not excessively increase

the soluble organics in the supernatant at the end of digestion as compared to the unsonicated samples.

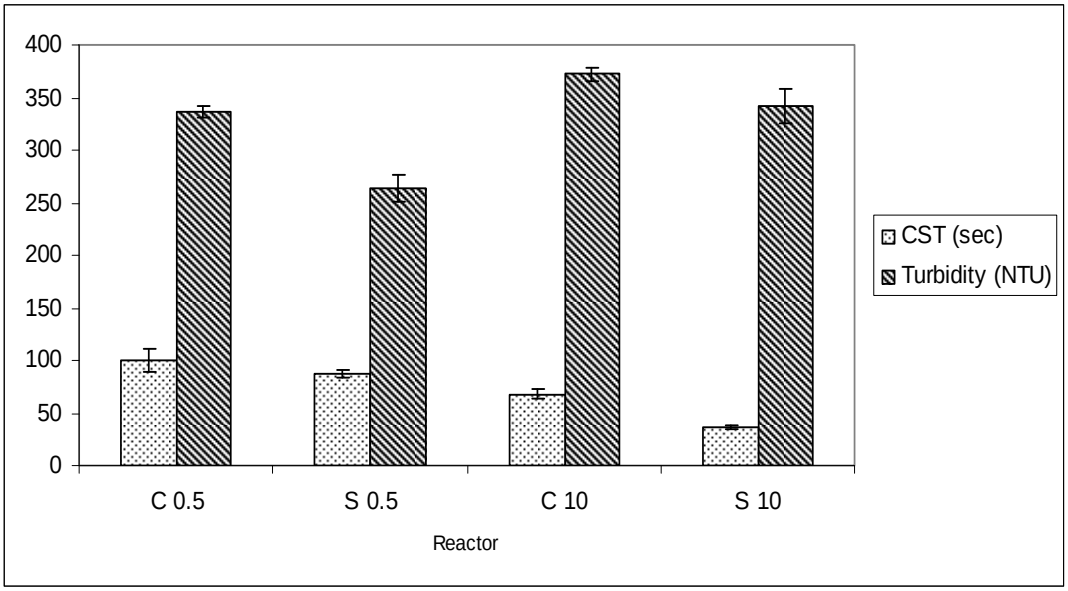


Figure 4.40. CST and turbidity values of 3 L reactors at different F/M ratios after digestion

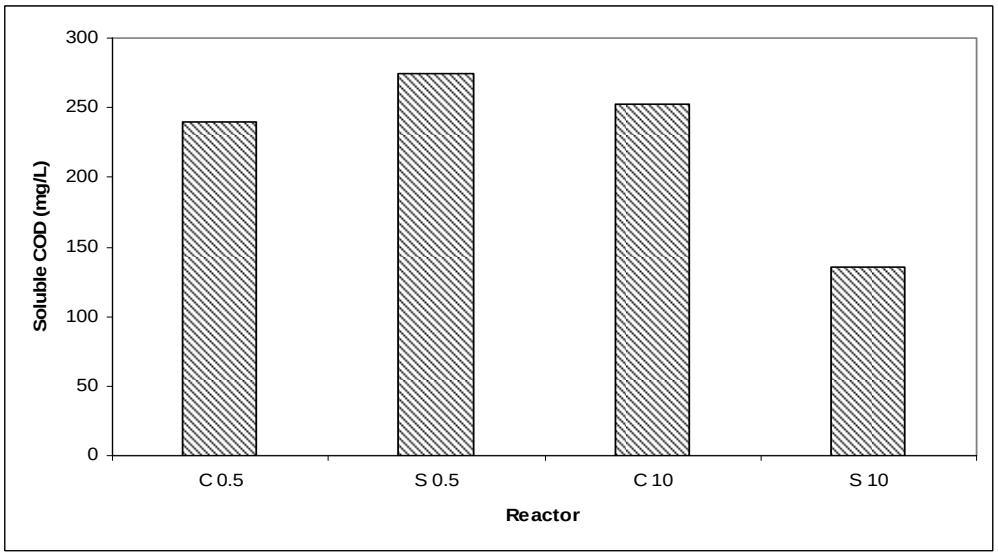


Figure 4.41. Soluble COD of 3 L reactors at different F/M ratios after digestion

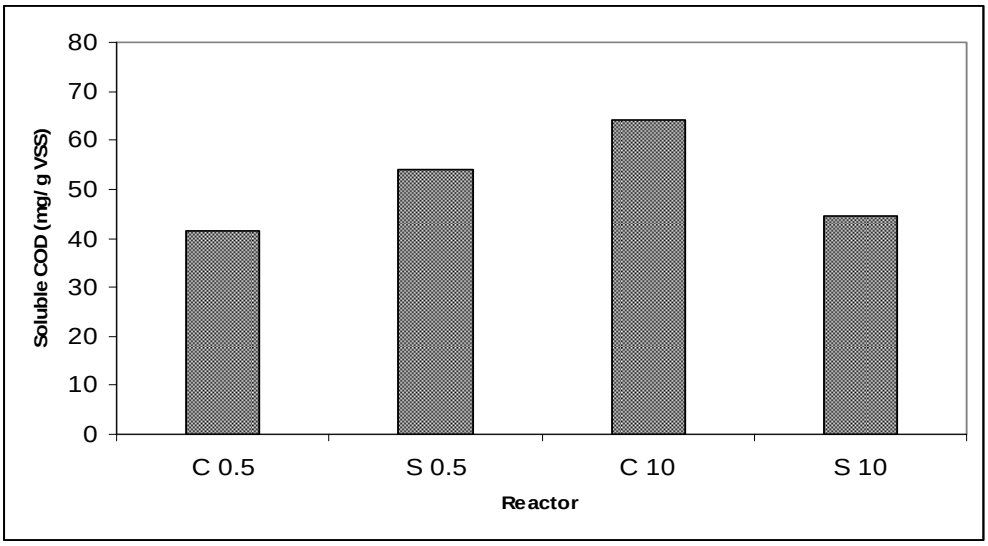


Figure 4.42. Normalized soluble COD of 3 L reactors at different F/M ratios after digestion

In order to see the soluble organic fraction, the carbohydrate and protein content of supernatants of reactors were examined and the results are presented in Figures 4.43 and 4.44. The carbohydrate in the soluble phase in reactors was found as nearly the same for all the reactors. The protein content of the sonicated reactor at the F/M ratio of 0.5 was higher than its control reactor. When the F/M ratio was 10, the protein in the sonicated reactor was lower than that in the control reactor.

The supernatants of reactors were also analyzed for phosphate phosphorus (PO_4-P) and ammonia nitrogen (NH_4-N) content. The findings as mg/L are represented in Figure 4.45. As F/M increases, PO_4-P amount do not change for sonicated and control reactors. However, sonicated reactors have approximately 120 % higher PO_4-P content compared to control ones for F/M ratios of 0.5 and 10. When these results were normalized with the MLVSS in the reactors (Figure 4.46), the trend obtained was the same. For NH_4-N, the increase in the F/M ratio causes the decrease in NH_4-N content and there is an increase in NH_4-N content between control and sonicated reactors. Sonicated reactors with F/M ratios of 0.5 and 10 have 3 % and 15 % higher NH_4-N content in mg/g VSS compared to control ones,

respectively. The findings for the reactor with F/M ratio of 0.5 are close with that in the study of Tiehm et al. (2001). Tiehm et al. (2001) measured the nitrogen and phosphorus content of untreated and sonicated WAS after anaerobic digestion. The concentration of ammonium and phosphorus of the sludge supernatants was higher as compared to the control due to the biodegradation of disintegrated WAS.

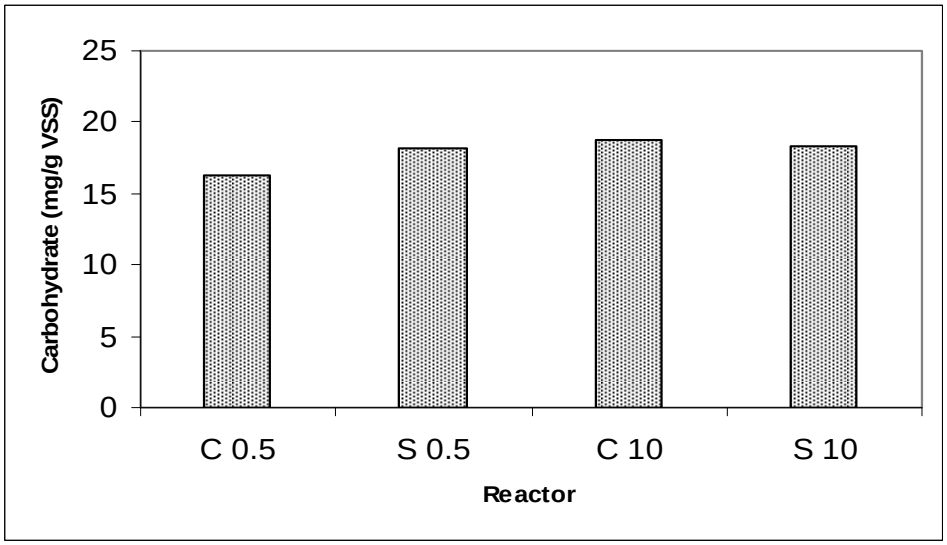


Figure 4.43. Carbohydrate content of 3 L reactors at different F/M ratios after digestion

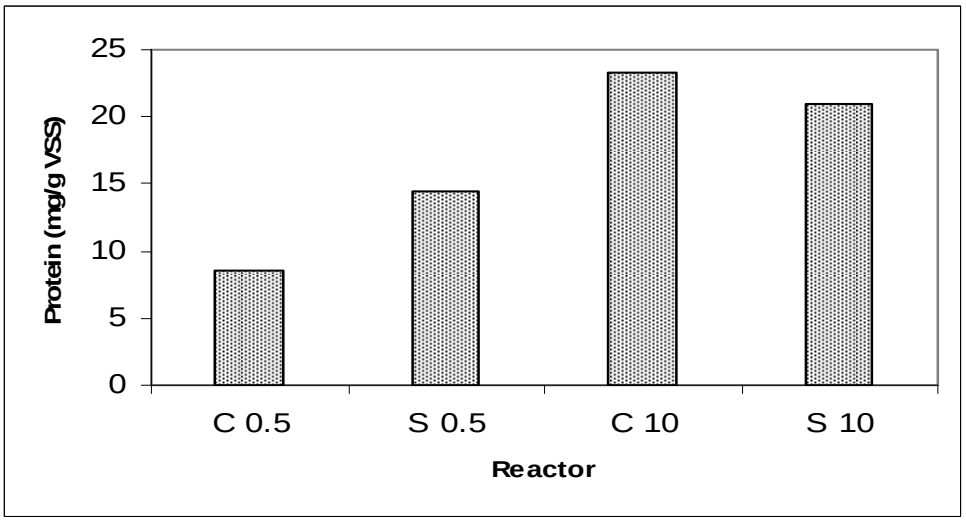


Figure 4.44. Protein content of 3 L reactors at different F/M ratios after digestion

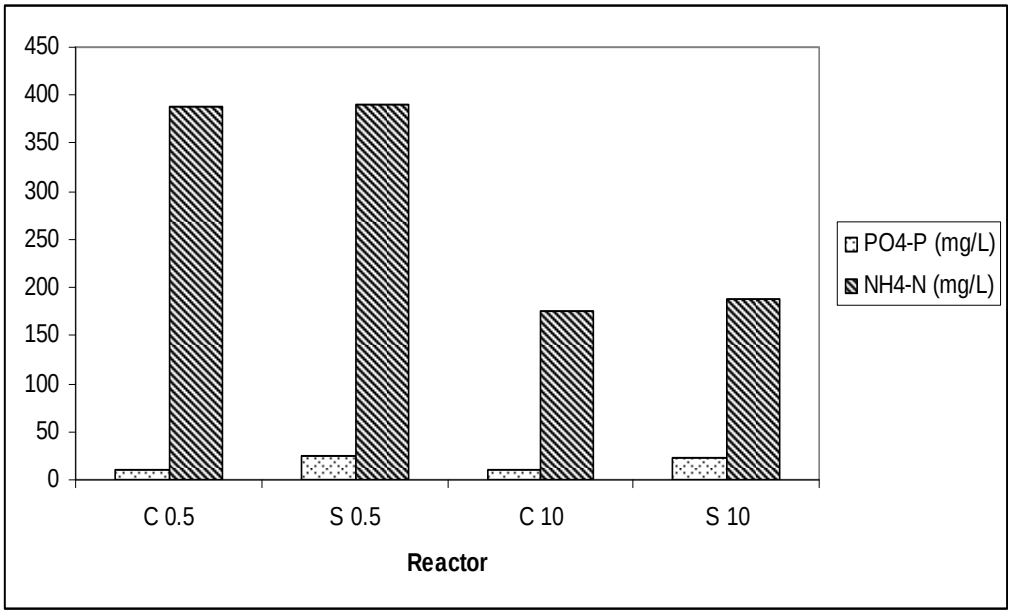


Figure 4.45. Phosphorus and nitrogen content of 3 L reactors at different F/M ratios after digestion

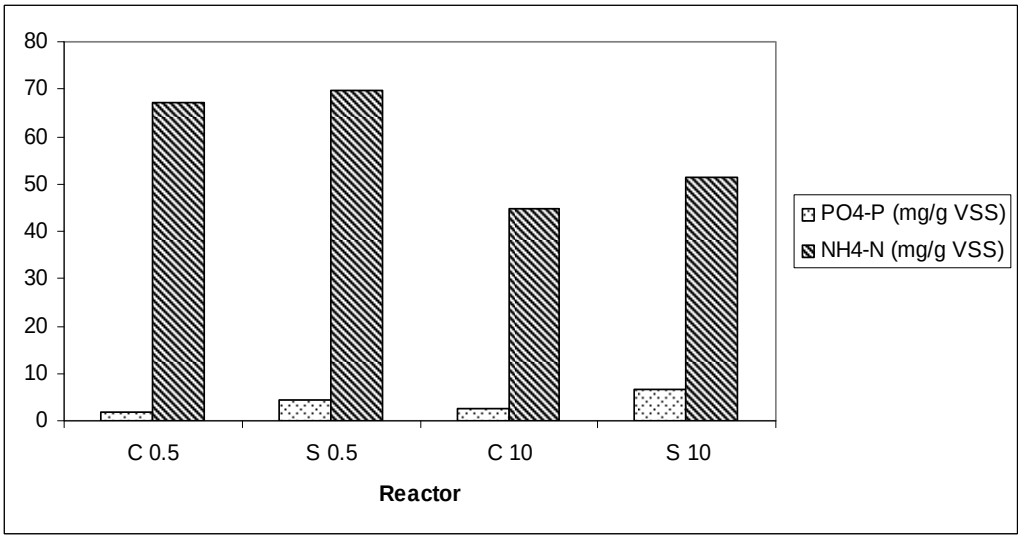


Figure 4.46. Normalized phosphorus and nitrogen content of 3 L reactors at different F/M ratios after digestion

These results altogether indicates that sonication of sludge does not necessarily worsen the supernatant quality for anaerobically digested sludge. The organic, nitrogen and phosphorus contents of supernatants from sonicated reactors were almost the same for those from unsonicated reactors.

Since there is generally a concern about heavy metal concentration in sludge due to organic matter deduction and sludge mass reduction after sonication, heavy metal contents of sonicated and control sludge samples were measured and checked whether they were below the limits in soil pollution regulation or not for sludge disposal. According to Table 4.20, there is no cadmium and low amount of lead, copper, nickel, zinc, mercury, and chromium in all reactors. That is, the heavy metal concentration of the sludges is below the limits in the regulation (Table 4.21). Furthermore, calcium and magnesium amounts are determined in reactors (Figure 4.47). If the F/M ratio increases, the concentrations of these ions decrease and the sonicated reactors have higher ion concentrations compared to their controls.

Table 4.20. Metal concentration in 3 L reactors at different F/M ratios after digestion

Heavy Metal (mg/kg oven dried material)	Control F/M=0.5	Sonicated F/M=0.5	Control F/M=10	Sonicated F/M=10
Lead (Pb)	22.3	65.8	35.8	83.0
Cadmium (Cd)	0.0	0.0	0.0	0.0
Chromium (Cr)	47.8	59.7	71.4	56.8
Copper (Cu)	52.2	65.7	60.1	67.3
Nickel (Ni)	17.5	23.5	19.5	21.5
Zinc (Zn)	617.7	747.7	567.7	577.7
Mercury (Hg)	0.69	0.71	0.54	0.59

Table 4.21. The maximum allowable limits in Soil Pollution Regulation for heavy metal concentration in stabilized treatment sludges

Heavy Metal (Total)	Limits (mg/kg oven dried material)
Lead (Pb)	1200
Cadmium (Cd)	40
Chromium (Cr)	1200
Copper (Cu)	1750
Nickel (Ni)	400
Zinc (Zn)	4000
Mercury (Hg)	25

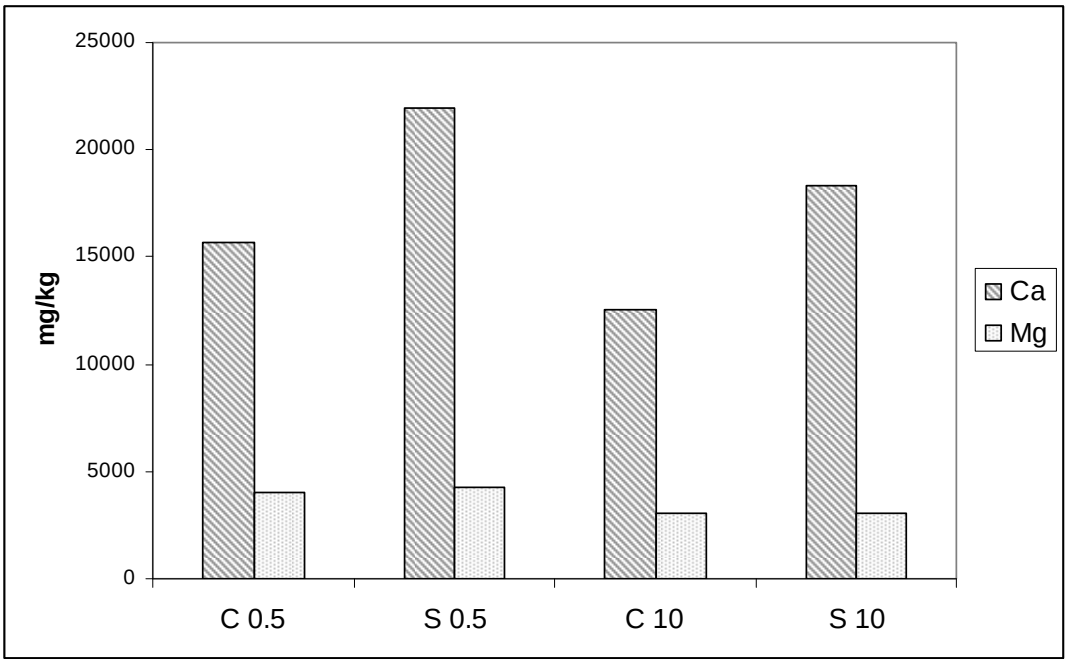


Figure 4.47. Calcium and magnesium concentration in 3 L reactors at different F/M ratios after digestion

4.4.2. Methane Generation Rates for Sonicated and Unsonicated Sludge Samples

Death, lysis and hydrolysis are important mechanisms in anaerobic digestion (Figure 4.48). Pavlostathis and Giraldo-Gomez (1991) found that hydrolysis of activated sludge (particulate, degradable and dead) is a much slower step among them. Gossett and Belser (1982) also examined anaerobic digestion of waste activated sludge and found that the rate limiting step was hydrolysis. In anaerobic digestion, the waste stabilization is accomplished by the methane generation (Speece, 1996). For that reason, the overall methane generation rate is also a sign of the waste stabilization rate.

In this part of the study attempts are made to express the rates of methane production with the use of two different methods. The calculated rates are not necessarily expressing solely the hydrolysis rate but rather an overall rate is calculated and a discussion is made on these. A comparison of sonicated vs. unsonicated samples as well as the values obtained at different F/M ratios are conducted after these calculations.

In the studies of Eastman and Ferguson (1981), Gossett and Belser (1982) and Pavlostathis and Gossett (1986), the hydrolysis model of the anaerobic digestion of waste activated sludges was fitted to first order reaction kinetics. Therefore, the cumulative methane generation data obtained in this study is assumed to follow first order kinetics like other studies (Lin et al., 1999; Pranshanth et al., 2006) by the given equation:

$$G_t = G_f\left(1 - e^{-kt}\right) \qquad \text{(equation 4.1)}$$

where;

G_t :Cumulative methane generation at time t (mL)

G_f *:* Ultimate methane generation (mL)

k : First-order rate constant (day^{-1})

t : Time (days).

The overall methane generation rates of batch anaerobic tests in 250 mL and 3 L reactors calculated by the given equation in order to see the methane generation rates.

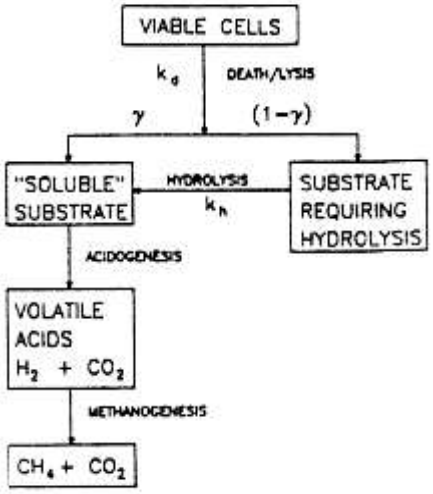


Figure 4.48. Anaerobic digestion model for biological solids (Pavlostathis and Gossett, 1986)

In solving for the two unknowns of the above equation two approaches were used. First, the experimentally recorded cumulative net methane productions (Gt) were modelled to fit first-order rate functions by using SigmaPlot version 10.0, Systat Software, Inc. Representation of non-linear regression analysis of methane gas productions are given in Appendix C. G_f and k values are obtained from this program. Also, R^2 values obtained from regression results are examined to evaluate the fit.

Second, Thomas method (Thomas, 1950) was used for modelling the experimental data. Using the same equation, G_f, k and R^2 values were calculated by this method (Lin et al., 1999) following the linearization of the data. One of the regression graphs is given in Appendix C.

Moreover, statistical analyses were performed on data sets by using software GraphPrism 5, Graphpad, San Diego, Calif. The value R^2 (coefficient of determination) obtained from Sigmaplot modeling and Thomas method gives the goodness of fit. It is the fraction of the total variance of y that is explained by the model or equation. Its value is between 0.0 and 1.0, has no units. Higher values of R^2 specify that the curve moves closer to data. However, it does not show that the fit is sensible. The best-fit values of the parameters may have values that make no sense or the confidence intervals may be very wide (Motulsky and Christopoulos, 2003). For this reason, statistical comparisons are used to compare the two models with the experimental data that make scientific sense such as runs test.

The runs test asks whether the curve deviates systematically from the data. A run is a series of consecutive points that are either all above or all below the regression curve. That is, a run is a consecutive series of points whose residuals are either all positive or all negative. After fitting a curve, the actual number of runs is counted and calculates the predicted number of runs (based on number of data points) is calculated by the program. The runs test compares these two values (Motulsky and Christopoulos, 2003).

If the model fits the data poorly, clusters of points on the same side of the curve are seen. This means there are fewer runs than predicted from sample size, and the runs test will produce a low P value. The P value responds if the data are randomly scattered above and below the curve and what is the probability of observing as few runs (or even fewer) than actually observed in this analysis. If the runs test reports a low P value, conclude that the curve does not describe the data very well (Motulsky and Christopoulos, 2003).

250 mL anaerobic batch reactors

Table 4.22 and 4.23 show the Gf, k and R^2 values - obtained from the 2 methods mentioned for the data of 250 mL the anaerobic reactors using low and high power sonication, respectively.

When Tables 4.22 and 4.23 are examined, it can be seen that at majority of the F/M ratios studied (especially low power probe), the rate of methane production was higher in the case of sonicated reactors compared to the control reactors. The deviations for this are observed either at lowest or highest F/M ratios. This brings the concern, that at high F/M ratios such as 10, there is plenty of substrate, but not enough microorganisms to degrade the substrate especially in the early phases of the reactor operation. This ends up with a lag period and when an overall rate is calculated the rate is low. Moreover, in some cases, the k value of control sludges is somewhat higher than that of sonicated sludges. This can be explained by different biodegradability characteristics of the COD fractions in the sonicated sludges and these may follow different biochemical fermentation pathways (Tiehm et al., 2001).

Also, it is seen from the tables that when F/M ratio is increased, the methane generation rate is decreased. The reason is that the hydrolyzing bacteria are poor scavengers and they need high concentrations of feed material for the production of hydrolytic exoenzymes in efficient operation (Pavlostathis and Gossett 1986). This condition seems to be better satisfied at lower F/M ratios.

When the ultimate methane generations are examined, the obvious difference between the sonicated and unsonicated sludges and the effect of different F/M ratios are easily observed. Independent of the F/M ratio and the level of sonication, the methane generated are predicted much higher in the case of sonicated sludge

compared to the unsonicated sludge. As the level of power increases, the difference between the sonicated and unsonicated reactors increases.

Table 4.22. Computed and calculated G_f and k values for 250 mL anaerobic batch reactors-low power probe

	SigmaPlot			Thomas Method		
Reactor	k	G_f	R^2 P	k	G_f	R^2 P
Control F/M=0.5	0.132	81.4	0.9633 0.0476	0.077	110.5	0.9476 0.0476
Sonicated F/M=0.5	0.137	86.6	0.9710 0.0476	0.078	117.5	0.9551 0.0476
Control F/M=2	0.119	85.4	0.9827 0.0476	0.073	113.8	0.9643 0.0476
Sonicated F/M=2	0.124	99.8	0.9947 0.1905	0.073	132.8	0.9753 0.0476
Control F/M=5	0.095	97.3	0.9967 0.1905	0.062	122.0	0.9848 0.1667
Sonicated F/M=5	0.096	107.2	0.9940 0.1667	0.060	131.5	0.9687 0.1905
Control F/M=10	0.084	110.0	0.9938 0.1905	0.055	120.5	0.9668 0.1905
Sonicated F/M=10	0.078	114	0.9820 0.0476	0.045	134.2	0.8128 0.0476

Table 4.23. Computed and calculated G_f and k values for 250 mL anaerobic batch reactors-high power probe

	SigmaPlot			Thomas Method		
Reactor	k	G_f	R^2 P	k	G_f	R^2 P
Control F/M=0.5	0.170	102.5	0.9788 0.0152	0.119	96.3	0.9540 0.0152
Sonicated F/M=0.5	0.169	123.8	0.9813 0.0152	0.120	113.0	0.9639 0.0152
Control F/M=2	0.115	124.8	0.9935 0.0152	0.105	125.1	0.9763 0.0130
Sonicated F/M=2	0.128	158.9	0.9895 0.0758	0.095	152.2	0.9734 0.0671
Control F/M=5	0.094	145.4	0.9980 0.0758	0.084	131.6	0.9651 0.0758
Sonicated F/M=5	0.086	204.1	0.9971 0.0242	0.063	176.1	0.8532 0.0130
Control F/M=10	0.077	158.3	0.9941 0.0152	0.056	150.2	0.7559 0.0152
Sonicated F/M=10	0.070	239.0	0.9933 0.0152	0.025	280.4	0.2885 0.0152

Comparing the rate calculation methods, k values from Thomas method are lower than that from Sigmaplot. This was also found in the study of Pranshanth et al., 2006. The reason is that, under high microorganism population the initial methane production is fast, that is the process is limited by substrate availability. On the other hand, initial methane production is slow due to more food and less bacteria population.

The comparison between the two approaches can also be made based on the regression fit coefficients (R^2). In that case, it can be argued that Sigmaplot model makes better predictions compared to the Thomas Method due to the higher correlation coefficients obtained in modeling. Moreover, P values obtained from two approaches show that the difference between the experimental data and them is statistically significant for control and sonicated reactors with 22 mm probe at F/M ratio of 0.5 and 10 ($P< 0.05$).

3 L anaerobic batch reactors

Table 4.24 show the G_f, k and R^2 values - obtained from 2 methods - of the anaerobic reactors of 3 L. Again, it is found that when F/M ratio is increased, the methane generation rate is decreased because of the low concentration of feed material to produce hydrolytic exoenzymes. Moreover, the k value of sonicated sludges is lower than that of control sludges due to different biochemical fermentation pathways. On the other hand, the ultimate methane generated is obtained much higher in the case of sonicated sludge compared to the unsonicated sludge at two F/M ratios. Different from the rate data of 250 mL anaerobic reactors, the rate values of reactors with F/M ratio of 0.5 are higher since the reactors were stirred continuously during digestion.

When the methods are compared, k values from Thomas method are lower than that from Sigmaplot and G_f values were highly estimated in the Thomas method

compared to Sigmaplot fitting which is similar to the study of Pranshanth et al., 2006. The regression values are lower in the reactors with F/M ratio of 10, especially for Thomas method because of the lag period. Because of the limitations in the feed, Thomas method can not predict the rate value well. Also, P values indicate that deviation from data is statistically significant for both approach ($P<0.05$) and Thomas method doesn't describe the data well compared to Sigmaplot modeling since P values from Thomas method were very lower.

Table 4.24. Computed and calculated G_f and k values for 3 L anaerobic batch reactors

	SigmaPlot			Thomas Method		
Reactor	k	G_f	R^2 P	k	G_f	R^2 P
Control F/M=0.5	0.274	1038.7	0.9666 0.0175	0.157	1345.3	0.9516 0.0004
Sonicated F/M=0.5	0.230	1351.2	0.9685 0.0043	0.146	1685.8	0.9621 0.0033
Control F/M=10	0.029	1926.9	0.9595 0.0010	0.018	2775.0	0.3760 0.0006
Sonicated F/M=10	0.026	3218.1	0.9531 0.0010	0.010	6709.5	0.0840 0.0006

4.4.3. Cost Calculation

Wastewater treatment including sludge disposal is a high cost process. The renewable energy coming from anaerobic digestion of wastewater sludge decreases

this cost. However, there is still huge load due to disposal of sludge. With the application of ultrasound, these costs decrease because of benefits of high biogas formation and sludge amount reduction for disposal. For this reason in this part, a simple cost calculation is made.

Economic analysis of ultrasonication of waste activated sludge was evaluated considering capital cost of ultrasound, operational cost, bioenergy recovery cost due to pretreatment and cost from reducing sludge to landfill. The operational cost includes only energy consumption cost for ultrasonic unit, whereas the energy cost for heating sludge, mixer, heat loss, consumables of equipment and chemical cost were not taken into account for this evaluation. The sludge transportation and disposal cost and electricity cost used in this study was based on local data in Turkey.

Assumptions on sludge production

As an example, a municipal waste water treatment plant (10,000 P.E.) with a daily WAS production of 5 tons (DS = 25 %, VS = 75%) is considered. Then, for the treatment of 5 tons/day sewage sludge a 2 kW ultrasonic reactor before anaerobic digestion is designed.

Assumptions on capital cost

The capital cost of ultrasonic reactor for 1 ton of sludge was approximately 4200 € (Nickel, 2002). 1 € was taken as 1.9598 YTL (ISO, 2008). That is, for the investment cost, 41156 YTL is needed in this case. The life-cycle of the system was assumed as 10 years (Nickel, 2002).

Assumptions on operating costs

The operating costs are dominated by the electrical power consumption. The average price in Turkey is approximately 0.15 YTL/kWh (TEDAS, 2008). The annual operating costs then are 2600 YTL.

Assumptions on transportation and disposal costs

Assuming the sludge solid reduction as 10 % after anaerobic digestion of the sonicated sludge (F/M=10) with respect to that of the untreated sludge, the transportation and disposal costs will decrease. For the transport, a truck with a capacity of 5 ton was assumed and transport cost of 1.3 YTL/km considering a 10 km far disposal site was taken. Disposal cost of 1 ton of sludge was taken as 45 YTL (BURSA, 2008). Therefore, annual transportation and disposal costs decrease approximately 6,500 YTL.

Table 4.25. Summary table for cost analysis

	Without Ultrasonication	With Ultrasonication
Capital Cost (YTL)	-	41,156
Operational Cost (YTL/year)	-	2,860
Transportation Cost (YTL/year)	2,790	2,429
Disposal Cost (YTL/year)	48,290	42,048
Total Cost (YTL/year)	51,080	88,493
Methane Revenue(YTL/year)	98,550	164,250

Assumptions on revenue from methane generation

Assuming the increase in the methane production per VS from 150 L/kg to 250 L/kg after anaerobic digestion of the sludge sonicated at F/M ratio of 10 with respect to that of untreated sludge, the gain as renewable energy was calculated. When the calorific value of methane was 10 kWh/m^3 with an efficiency of 0.32 (Nickel, 2002), the annual methane production was 98,550 YTL. However, this value increases to 164,250 YTL when the sludge is sonicated before digestion.

The payback period of ultrasound treatment can be found by the dividing the capital cost of it to the difference in profit obtained from two situations. Then, it was found 1.5 years to pay off the investment. Considering all costs of ultrasonication, besides being environment-friendly; its application is more profitable.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1. Conclusions

The effect of sonication and F/M ratio on waste activated sludge as preliminary studies and on anaerobic digestion process as anaerobic batch tests was investigated. The conclusions obtained from the experimental results are depicted below.

- If the temperature of the experimental sonication set was not controlled, the soluble COD values were found higher than that in controlled temperature. At 100 W- 15 min temperature reached 67 °C and soluble COD value was found around 500 mg/ gVSS. On the other hand, soluble COD was determined as 200 mg/g VSS in the temperature controlled case. Moreover, CST values were lower for uncontrolled case because of the effect of temperature on dewaterability. Turbidity values are near 3000 NTU at 100 W-15 min sonication.

- When sonication power increased, soluble COD values also increased directly. This was also true for the increase in sonication time. However, power was more critical parameter than time as mentioned in literature since this was found by using different probes with different power inputs in this study. The soluble COD exerted was 75 and 200 mg/g VSS at 100 W- 5 min and 255 W -5 min, respectively.

- Waste activated sludges with different solids concentration were sonicated at the same sonication power and time. Soluble COD amount increases

with the increase in MLSS concentration. However, when soluble COD values are normalized according to MLSS values, the results fluctuate in a small range and remain the same. For this reason, it is meaningful to pretreat high concentration of MLSS values since soluble COD amount increases linearly with the increase in MLSS amount of sludge as expected.

- The sludge was sonicated at 100 W and different sonication times of 5, 10, 15 and 20 minutes. The soluble COD values were found close to each other for 10, 15 and 20 min sonication times. For this reason, the batch anaerobic reactors of 250 mL were carried out for biogas production. Related to exerted soluble COD values before digestion, the biogas amount after digestion was similar for 10, 15 and 20 min. sonicated reactors.

- The untreated and sonicated reactors at different F/M ratio (0.5, 2, 5 and 10) were conducted for anaerobic digestion in 250 mL. This was done twice: one by using ultrasound at 100 W and 15 min and the other at 255 W and 10 min. For two experimental sets, the total gas and biogas production trends were similar. Total gas and biogas production were getting lower at the beginning with the raise in the F/M ratio. However, the gas amounts obtained at high F/M ratio overtake the others after a while.

- In order to see this trend obtained from 250 mL reactors in large volume batch reactors, batch anaerobic reactors of 2 L were conducted for untreated and sonicated reactors at F/M ratios of 0.5 and 10 (the min and max ratio taken from the sets carried out before). Again, the same gas production trend is observed. As a result, for F/M ratio of 10 total gas and methane is increased by 62.9 % and 55.1 % compared to control reactors, as respectively. Approximately 10 % VS and 15 % total COD reduction with respect to control was obtained at F/M ratio of 10.

- The CST values after digestion decrease with the increase in F/M ratio due to lower solids concentration. The sonicated reactors have a lower CST value compared to untreated ones. On the other hand, turbidity values increase when the F/M ratio increases and the sonicated reactors are lower turbid than untreated ones.

- After digestion, carbohydrate content of all the reactors is nearly same. The protein content increases with the increase in F/M ratio and there is an increase in sonicated reactor at F/M ratio of 0.5 compared to its control. On the other hand, the protein amount of control is higher than that of sonicated reactor at F/M ratio of 10.

- When F/M increases, phosphorus amount do not differ for sonicated and control reactors much more. However, sonicated reactors with F/M ratios of 0.5 and 10 have approximately 120 % higher phosphorus content compared to control ones. On the other hand, the increase in the F/M ratio causes the decrease in nitrogen content and there is an increase in nitrogen contents between control and sonicated reactors same as the phosphorus content. Sonicated reactors with F/M ratios of 0.5 and 10 have 3 % and 15 % higher nitrogen content compared to control ones, respectively.

- Heavy metal concentrations in all reactors after digestion were determined and found below the limits in solid waste regulation. Moreover, calcium and magnesium amounts were analyzed in reactors. If the F/M ratio increases, the concentrations of these ions increase and the sonicated reactors have higher ion concentrations compared to their controls.

- Although, the methane production increases with the increase in F/M ratio, the methane generation rate gets slower with the increase in F/M ratio at

the beginning of the digestion. The curve fitting of experimental data is better with Sigmaplot modeling rather than that with Thomas method.

- Taking into account the decrease in sludge transportation and disposal costs and the increase in renewable energy, capital and operational cost of ultrasound process can be compensated within 1.5 years.

5.2. Recommendations for Future Work

Following the investigations described in this thesis, a number of projects could be taken up:

- The effect of sonication and F/M on sludge disintegration should be examined in the continuously fed reactors.

- It would be of interest to measure the daily COD values of the continuous reactors during anaerobic digestion together with gas production data. Therefore, the kinetics of hydrolysis of anaerobic digestion of sonicated sludge can be calculated on COD basis.

- For the reliable cost benefit analysis of ultrasonic application, pilot scale experiment should be conducted.

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APPENDIX A

CALIBRATION CURVES

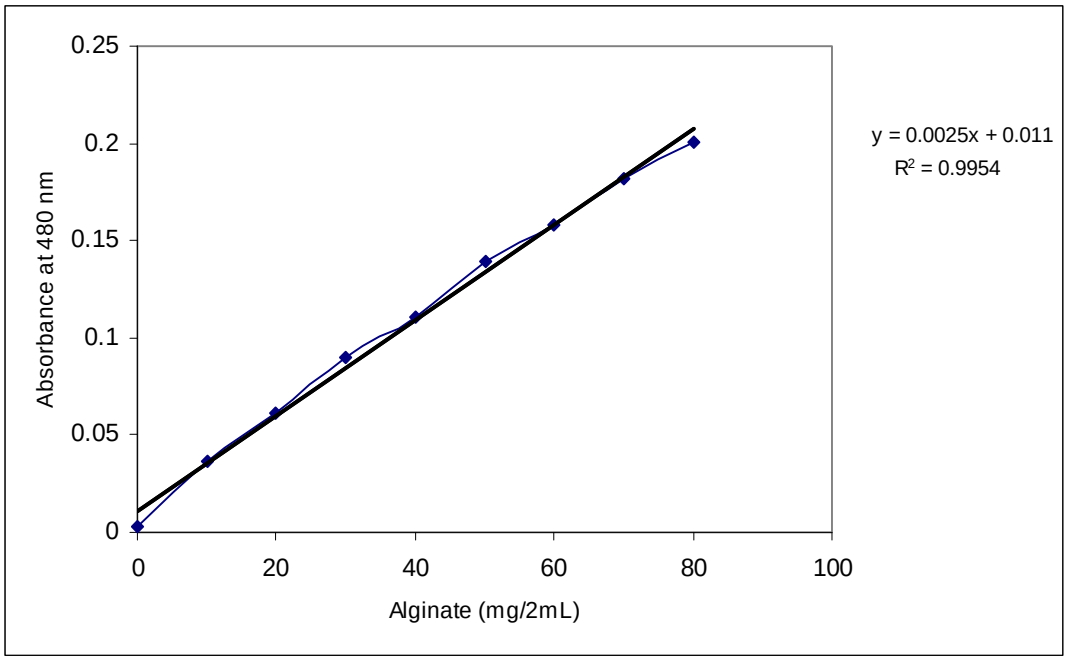

Figure A.1. The calibration curve obtained for the carbohydrate analysis

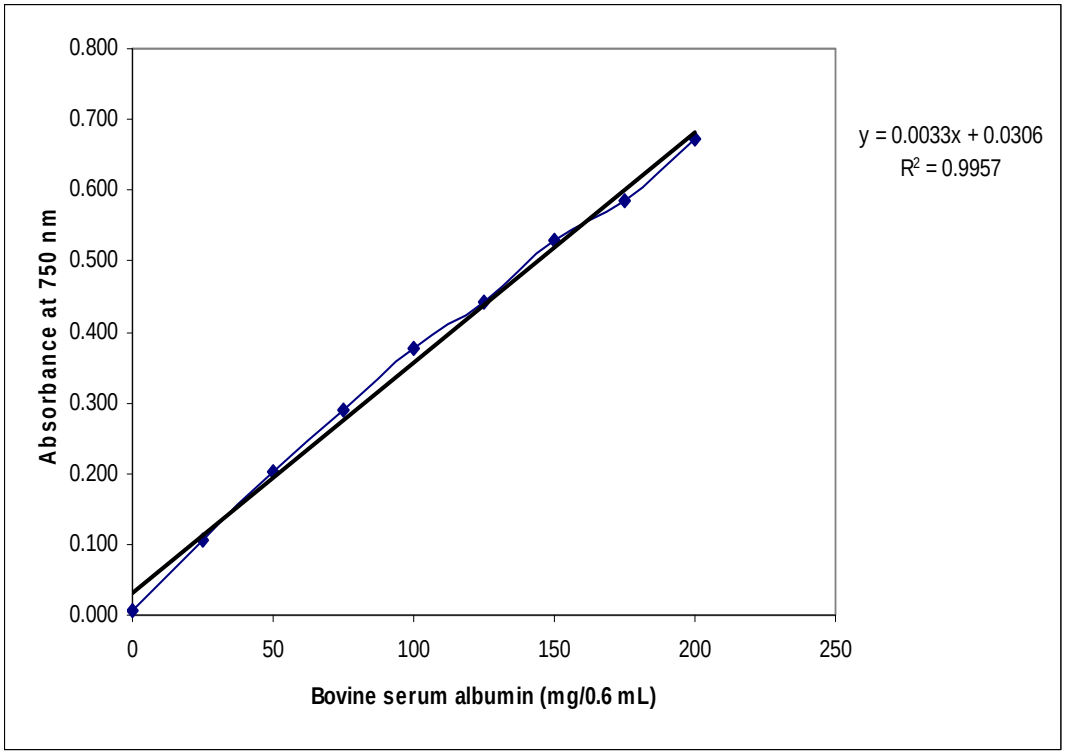

Figure A.2. The calibration curve obtained for the protein analysis

APPENDIX B

METHANE GENERATION CALCULATIONS FOR BATCH SYSTEMS

In order to calculate methane generation of the reactors during anaerobic digestion, mass balance evaluations were carried out by the equations given below. Calculations were performed periodically using two consecutive analyses of biogas compositions (P_1 and P_2) and total biogas production data (V_b) obtained between these analyses.

Generation = Accumulation in headspace of the reactor + Output

$$G = \left[(M_2 - M_1) \times V_h\right] + \left[\frac{(M_1 + M_2)}{2} + V_t\right]$$

where; G : Methane generation (mL),
G_r : Methane generation in reactor (mL),
M_1 : Initial methane content in biogas (%),
M_2 : Final methane content in biogas (%),
V_h : Volume of headspace (mL),
V_t : Volume of total biogas produced (mL),

APPENDIX C

REGRESSION ANALYSIS GRAPHS

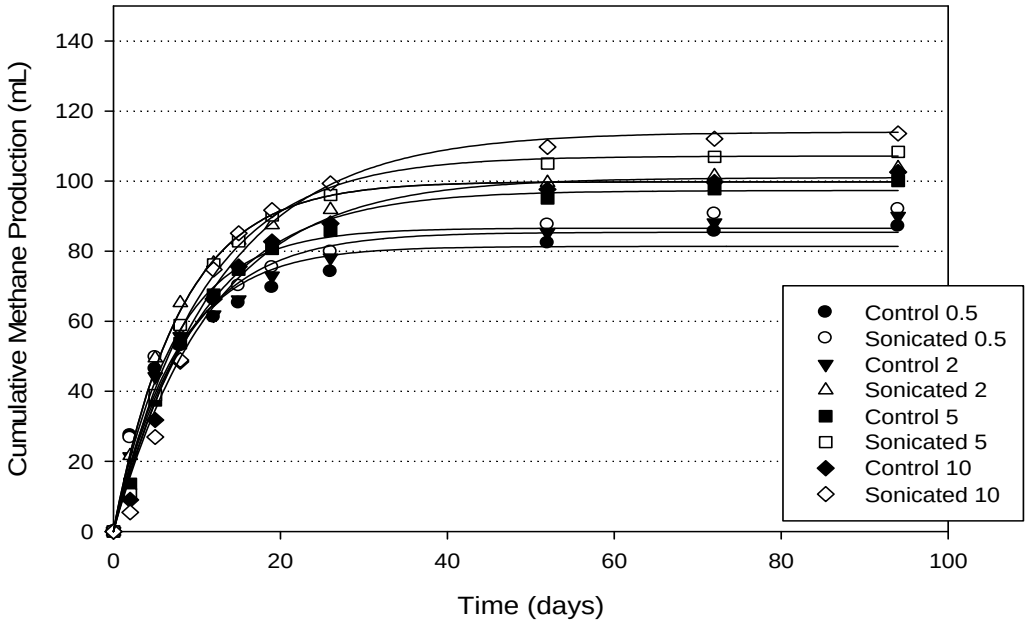

Figure C.1. Representation of non-linear regression analysis of methane gas productions of 250 mL unsonicated and low power-sonicated reactors

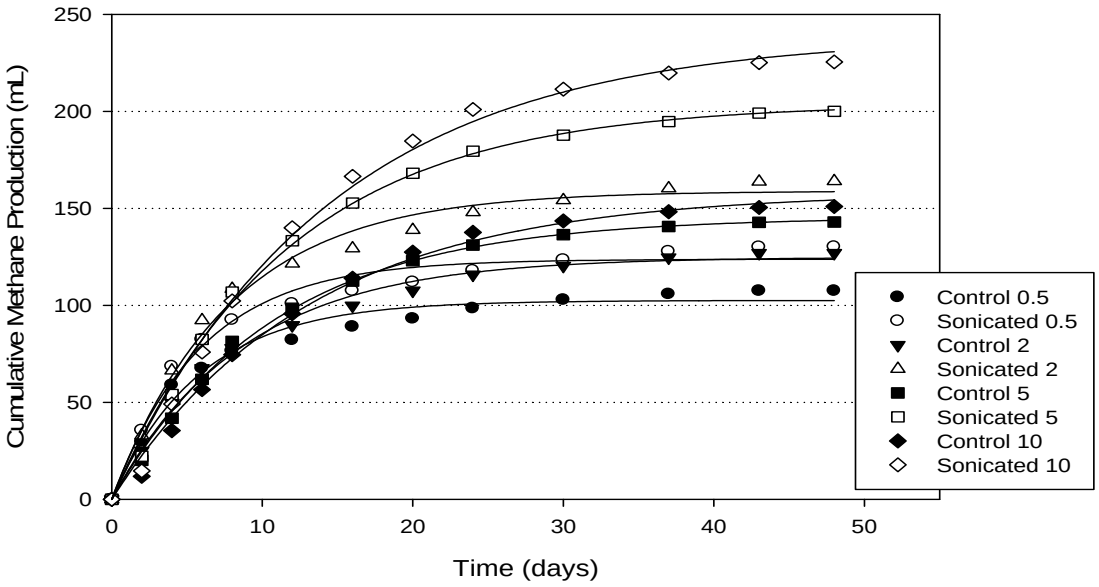

Figure C.2. Representation of non-linear regression analysis of methane gas productions of 250 mL unsonicated and high power-sonicated reactors

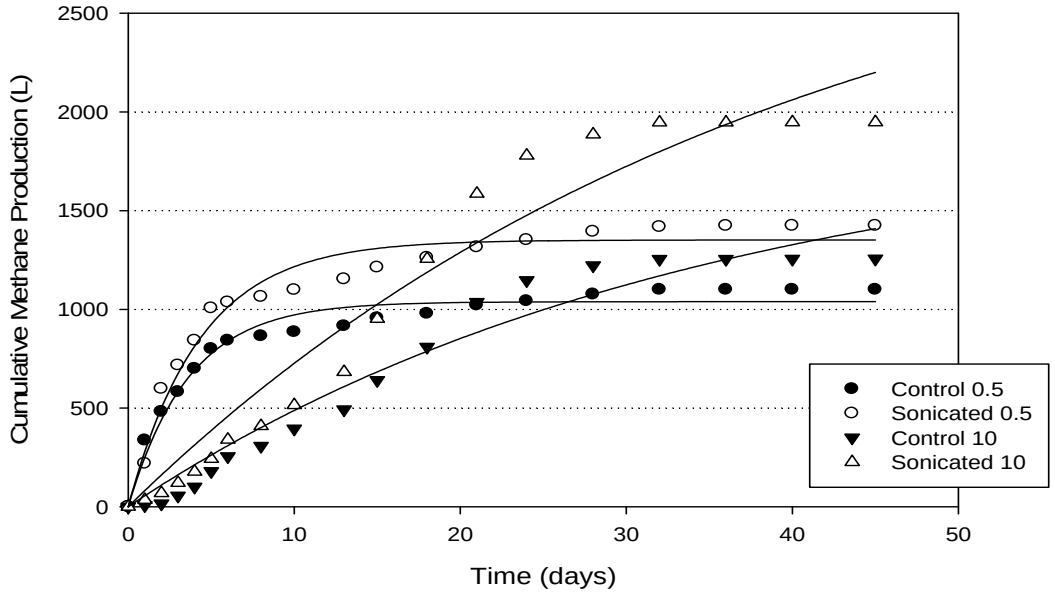

Figure C.3. Representation of non-linear regression analysis of methane gas productions of 3 L unsonicated and high power-sonicated reactors

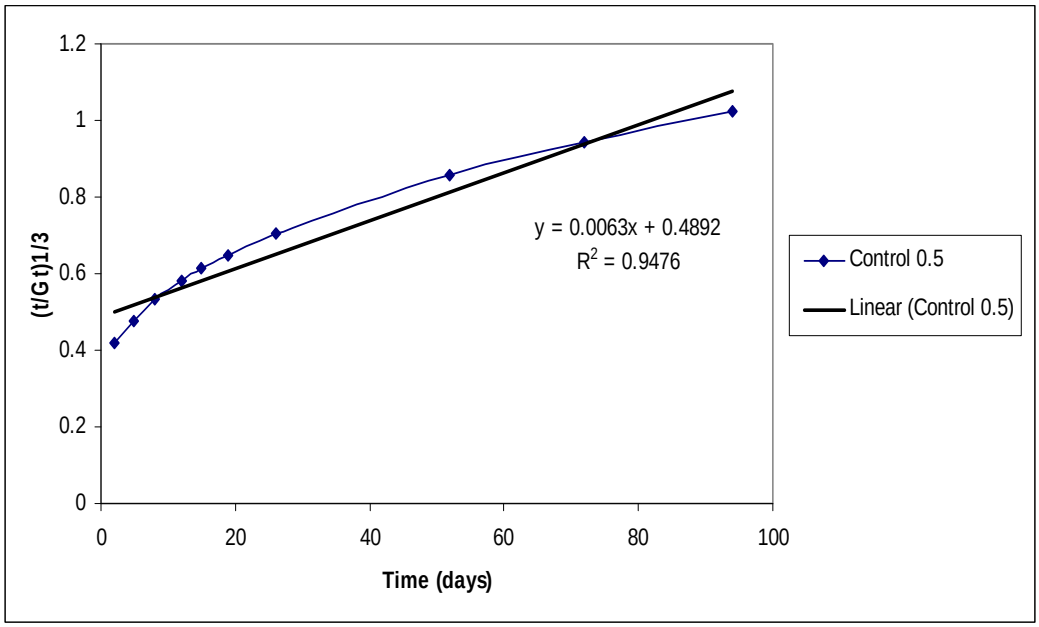

Figure C.4. Representation of regression analysis of methane gas production of control reactor at F/M ratio of 0.5 using Thomas Method