EFFECT OF DISINTEGRATION METHODS ON THE CHARACTERISTICS OF BIOLOGICAL SLUDGES AND THE BIOGAS PRODUCTION IN ANAEROBIC DIGESTION

by

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EFFECT OF DISINTEGRATION METHODS
ON THE CHARACTERISTICS OF BIOLOGICAL SLUDGES AND
THE BIOGAS PRODUCTION IN ANAEROBIC DIGESTION

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Dedicated to my family…

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EFFECT OF DISINTEGRATION METHODS ON THE CHARACTERISTICS OF BIOLOGICAL SLUDGES AND THE BIOGAS PRODUCTION IN ANAEROBIC DIGESTION

Pretreatment has become popular to enhance digestibility and minimization of the sludge by increasing its solubility.

This study investigated the effects of ultrasonic, microwave and alkaline pretreatments on the efficiencies of aerobic and anaerobic sludge digestion processes. The digestion efficiency was measured in terms of sludge minimization, pathogen reduction and biogas production. In the first part of the study, application conditions of pretreatment techniques were determined. In the second part, unpretreated (control) and pretreated sludge samples were digested aerobically at room temperature and anaerobically at 37°C for 30 d.

Sludge pretreatments improved the efficiencies of both aerobic and anaerobic digestion processes by increasing the solubility of sludge solids. In aerobic digestion, VS removal efficiencies improved by 23-68%, 24-33%, 28-78% for sludge pretreatment ratios (SPR) of 10%, 20%, and 25%, respectively. In anaerobic digestion, pretreatments led to 18-53% increase in VS removal efficiency and 41-77% increase in methane production efficiency. The highest biogas yield of 0.52 Lbiogas/gVS_{added} and methane yield of 0.37 LCH_4/gVS_{added} were obtained from microwave pretreated sludge samples. Sludge dewaterability was deteriorated with the application of pretreatments to 10% of the samples (SPR:10%), dewaterability of alkaline and microwave pretreated sludge samples improved when SPR increased to 20% and 25%.

DEZENTEGRASYON METOTLARININ BİYOLOJİK ÇAMUR KARAKTERİZASYONLARI VE HAVASIZ ÇÜRÜTMEDE BİYOGAZ ÜRETİMİ ÜZERİNE ETKİLERİ

Önarıtma, çamurun çözünürlüğünü arttırarak çamurun çürütülmesini ve azaltılmasını iyileştirdiği için yaygınlaşmaya başladı.

Bu çalışma ultrason, mikrodalga ve alkali önarıtmaların havalı ve havasız çamur çürütme proseslerinin verimleri üzerindeki etkilerini araştırdı. Çürütme verimliliği, çamur azaltımı patojen giderimi ve biyogaz üretimi açısından ölçüldü Çalışmanın başında, önarıtma tekniklerinin uygulama koşulları belirlendi. Çalışmanın ikinci bölümünde, ön arıtmasız (kontrol) ve önarıtılmış çamur numuneleri oda sıcaklığında havalı ve 37°C sıcaklıkta havasız olarak 30 gün çürütüldü.

Çamur önarıtmaları, çamur katılarının çözünmesini arttırarak hem havalı hem de havasız çürütme proseslerinin verimini arttırdı. Çamurun havalı çürütülmesinde, %10, %20 ve %25 çamur önarıtma oranları için organik madde giderim verimleri sırasıyla %23-68, %24-33, %28-78 arttı. Çamurun havasız çürütülmesinde, önarıtmalar organik madde giderim veriminde %18-53 ve metan üretim veriminde %41-77 artışa neden oldu 0.52 Lbiyogaz/gOrganikmadde$_{eklenen}$ olarak en yüksek biyogaz verimi ve 0.37 Lmetan/gOrganikmadde$_{eklenen}$ olarak en yüksek metan verimi, çamur numunelerinin mikrodalga önarıtımı ile arıtılmasından elde edildi. Numunelerin %10'na önarıtma uygulanmasıyla çamurun susuzlaştırılabilirliği kötüleştirildi, çamur arıtma oranı %20 ve %25'e yükseldiğinde alkali ve mikrodalga ile önarıtılmış çamur numunelerinin susuzlaştırılabilirliği iyileştirildi.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Units used
Alk.	Alkaline Pretreatment	
CER	Cation Exchange Resin	
C1,C2,C3	Aerobic Control Reactors	
COD	Chemical Oxygen Demand	(mg/L)
CST	Capillary Suction Time	(s)
CH_4	Methane	(%)
Cl^-	Chloride	(mg/L)
DD	Disintegration Degree	(%)
DO	Dissolved Oxygen	(%)
DOC	Dissolved Organic Carbon	(mg/L)
EC	Electrical Conductivity	(mS/cm)
EPS	Extracellular Polymeric Substances	
FC	Fecal Coliform	(CFU/100 mL)
FS	Fecal Streptococci	(CFU/100 mL)
MLSS	Mixed Liquor Suspended Solids	(mg/L)
MLVSS	Mixed Liquor Volatile Suspended Solids	(mg/L)
MW	Microwave Pretreatment	
MW+Alk.	Combined Microwave-Alkaline Pretreatment	
NH_4^+-N	Ammonium Nitrogen	(mg/L)
NO_2^--N	Nitrite Nitrogen	(mg/L)
NO_3^--N	Nitrate Nitrogen	(mg/L)
ORP	Oxidation Reduction Potential	(mV)
sCOD	Soluble Chemical Oxygen Demand	(mg/L)
SE	Specific Energy	(kJ/kg TS)
SO_4^{2-}	Sulfate	(mg/L)
SPR	Sludge Pretreatment Ratio	(%)
SRT	Solids Retention Time	(d)
SVI	Sludge Volume Index	(mL/g)

TCOD	Total Chemical Oxygen Demand	(mg/L)
TC	Total Coliform	(CFU/100 mL)
TKN	Total Kjeldahl Nitrogen	(mg/L)
TS	Total Solids	(mg/L)
U-15	Ultrasonic Pretreatment (SE of 12000 kJ/kg TS)	
U-30	Ultrasonic Pretreatment (SE of 24000 kJ/kg TS)	
VFA	Volatile Fatty Acid	(mmolar)
VS	Volatile Solids	(mg/L)
WAS	Waste Activated Sludge	
WWTP	Wastewater Treatment Plant	

1. INTRODUCTION

The rapid urbanization and the growth in human population has caused an increase in the number of the Wastewater Treatment Plants (WWTPs). Large quantities of sludge generated from biological WWTPs pose a great threat to the environment due to improper management of the sludges (Appels et al., 2013; Tyagi and Lo, 2013; Shao et al., 2012). The cost of excess sludge treatment has been estimated to be approximately 50-60% of the total expense of a WWTPs (Subramanian et al., 2010). There are high quantities of organic matter, pathogens and nutrients in the sludge produced from WWTPs. In order to alleviate a serious sludge disposal problem, the volume of sludge produced should be effectively reduced or even eliminated prior to final disposal (Shao et al., 2010).

In recent years, a wide range of sludge pretreatment techniques, such as thermal (microwave), mechanical (ultrasound, homogenizers, and mills), chemical (acids, alkali and other chemicals), oxidative (use of Ozone and Fenton process) and biological (pretreatments with or without enzyme addition) and combined methods (thermochemical) have been used to minimize the sludges. Pretreatment of waste activated sludge (WAS) causes disruption of cell walls and the release of extracellular and intracellular materials by speeding up the solubilization of organics and increasing the biodegradability of the sludges.

Digestion is a commonly performed biological process for the stabilization of sludge from WWTPs, and can be carried out either anaerobically or aerobically. Sludge stabilization results in the destruction of pathogens and the reduction in volatile solids and odors. Sludge pretreatment (disintegration) helps eliminate the hydrolysis step, and increases the biodegradability of organics and the degree of stabilization (Novak et al., 2003).

The purpose of this study is to investigate the effects of ultrasonic, microwave, alkaline and the combination of a microwave and alkaline pretreatment methods on the aerobic and anaerobic sludge digestion efficiency of the wastewater sludges.

2. THEORETICAL BACKGROUND

2.1. Sludge

Sludge is defined as the liquid, semisolid liquid or solid residuals of a wastewater treatment plant, depending on the operations and processes used. It usually contains pathogens, easily decomposable organics, hazardous heavy metals, harmful insect eggs and recalcitrant weed seeds. The total solid concentrations of sludge show great variations between 0.25 and 12% depending on the sources of sludge generation in the plants. According to the characteristics and composition of sludge, it may be classified as primary sludge, secondary sludge or chemical sludge (Shehu et al., 2012; Cho et al., 2012).

Primary sludge has a solids concentration of 4-8%, is characterized by a gray-brown color, bad odor, and is produced by removing the solids from the bottom of the primary clarifier. Secondary sludge is also referred to as biological sludge, and is generated throughout the biological process. It is separated from the liquid phase in the secondary clarifier, with the thickened solids, named waste activated sludge (WAS), which is also extracted from the system and directed to sludge treatment. WAS is characterized by a yellow-brown color, little odor and difficult dewaterability. It is very active biologically, and has a solids concentration of 0.5-1.5%. Chemical sludge is created by including chemical additives, such as metal salts into wastewater in order to settle the suspended solids (Vesilind, 1979).

2.2. Sludge Stabilization

The stabilization of sludge is a commonly applied technique for sludge disposal. After discharging the biomass (biodegradable materials) of the sludge to the environment, nuisance conditions are created. The application of stabilization provides degradation of the degradable portion of the sludge to a point that the sludge can be conveniently disposed of in the environment without the risk of creating odors and other nuisance conditions. Advantages of the stabilization process are pathogen destruction, organic matter reduction, and odor removal in raw sludge (Novak et al., 2003).

There exist different stabilization methods, which are anaerobic digestion, aerobic digestion, autothermal thermophilic digestion (ATAD), composting and thermophilic composting (Tchobanoglous et al., 2004). Anaerobic and aerobic digestion processes are employed worldwide in WWTPs in order to prevent the adverse effects of sludge on environment, to recover considerable amounts of methane, to increase biogas production, provide mass and odor reduction, and to improve dewaterability (Liu et al., 2012).

Aerobic digestion is often preferred in terms of its cost effectiveness and simplicity for small wastewater treatment plants. On the other hand, anaerobic digestion is complex process for the plants to operate (Moonkhum, 2007).

2.2.1. Anaerobic Digestion

Anaerobic digestion is the most commonly preferred sludge stabilization technique as a result of the generation of methane gas as by-product, reducing the volume of sludge, and the decomposition of organic and inorganic matter (Novak et al., 2003). With anaerobic digestion, the production of less amounts of sludge are an important benefit versus aerobic digestion (Tchobanoglous et al., 2004).

In the anaerobic digestion process, methane formers are very sensitive to the environmental conditions, which are characterized by solid retention time, hydraulic retention time, temperature, alkalinity, pH, the presence of inhibitory substances (toxic materials), and the presence of nutrients and trace elements. The first three factors effect the selection of process-in other words, the rapid growth of bacteria or the failure of the digestion process (Tchobanoglous et al., 2004).

Temperature in anaerobic digestion has an impact on the metabolic activities of the microbial population, and the rates of hydrolysis and methane formation. Anaerobic digestion can be operated in mesophilic (30-38°C) or in thermophilic (50-57°C) temperature range (Tchobanoglous et al., 2004). The difference between the mesophilic and thermophilic anaerobic digestion of sludge is that the latter has higher energy demand than the former. Heavy metals or other toxicants negatively influence the methane bacteria,

with the optimum pH range for anaerobic digestion being 6.4-7.5. The nutrients and trace elements are necessary for biological growth (Vesilind, 1979).

Anaerobic digestion of sludge is obtained through four main stages, involving hydrolysis, acidogenesis, acetogenesis and methanogenesis (Jang et al., 2012; Rani et al., 2012). To degrade the biodegradable organic matter and produce biogas, three kinds of bacteria work in conjunction during the phases of the anaerobic digestion process (Alibardi et al., 2010). The schematic mechanism of anaerobic digestion is shown in Figure 2.1. An explanation of main steps for anaerobic digestion is as follows:

2.2.1.1. Hydrolysis: In this first step, insoluble organic material and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids are degraded into soluble organic substances (e.g. amino acids and fatty acids) by extracellular enzymes (Zhang, 2010). Hydrolysis is the rate-limiting step of anaerobic digestion (Müller, 2001).

2.2.1.2. Acidogenesis: In the second step, the soluble organic substances are converted to volatile fatty acids (VFAs), hydrogen, carbon dioxide and other by-products by acidogenic bacteria (Shehu et al., 2012).

2.2.1.3. Acetogenesis: The third phase is where acetogenic bacteria digest the organic acids and alcohols in order to produce acetic acid (acetate), in addition to hydrogen and carbon dioxide (Vesilind, 1979).

2.2.1.4. Methanogenesis: This is the final phase, where acetate is transformed to methane and carbon dioxide by methane-forming bacteria. Hydrogen works as an electron donor and carbon dioxide is the acceptor produce methane (Vesilind, 1979).

Some of the advantages of anaerobic digestion include:

- the reduction of sludge volume,
- the stabilization of sludge,
- the disinfection of sludge,
- energy recovery in the form of methane (Park et al., 2012).

In anaerobic digestion, 30-35% Volatile Solids (VS) reduction can be achieved for secondary sludge, while VS reduction rates can reach up to 55-60% for primary sludge. Gas produced in anaerobic digestion, referred to as biogas, consists of trace amounts of water vapor, hydrogen, nitrogen, hydrogen sulphide, unsaturated hydrocarbons and other gases. Major components of biogas include methane and carbon dioxide, typically 65-70% and 30-35% by volume, respectively (Gray, 2005).

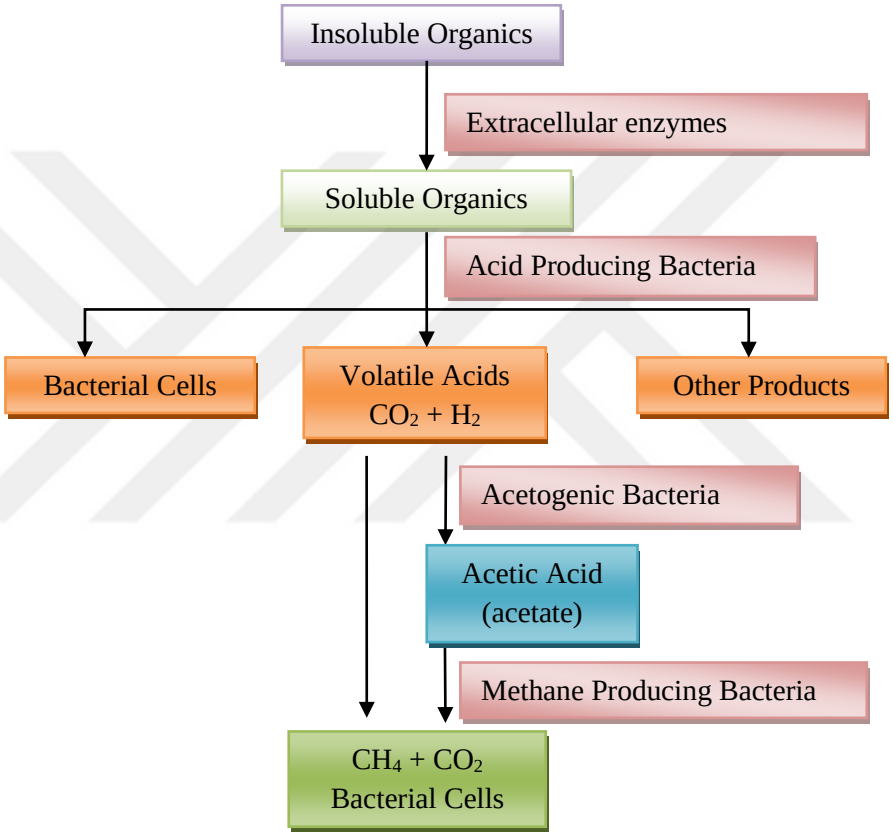

Figure 2.1. The steps of anaerobic digestion (Vesilind, 1979).

2.2.2. Aerobic Digestion

Aerobic digestion is widely used stabilization process in WWTPs in order to oxidize and decompose the organic part of the sludge by microorganisms in the presence of oxygen. Under aerobic conditions, the carbonaceous fraction of the sludge is oxidized to carbon dioxide and water, with the nitrogenous fraction oxidized to ammonium and nitrate. Because of the almost complete depletion of the food supply, microorganisms consume their own protoplasm and cellular matter in order to obtain the energy required for the

metabolism. This condition is referred to as endogenous respiration, which is the predominant reaction in the aerobic system (Khalili et al., 2000; Moonkhum, 2007; Gregor et al., 2008). Aerobic digestion is more suitable for small and medium-sized WWTPs due to the simple operation and maintenance control, and less investment cost (Liu et al., 2012). The schematic appearance of the aerobic digestion is shown in the Figure 2.2.

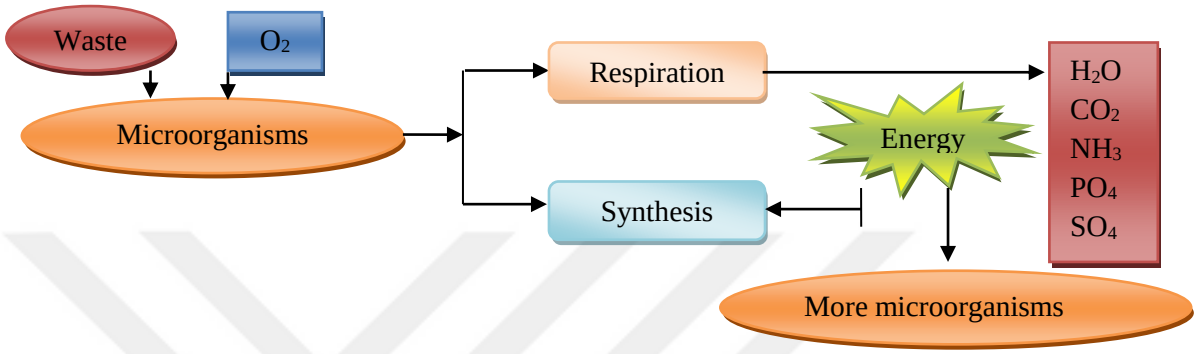

Figure 2.2. The path of aerobic digestion (Vesilind, 1979; Whiteley and Lee, 2006).

There are two steps of the aerobic digestion process, which can be illustrated as the direct oxidation of biodegradable matter to cellular material in Eqaution 2.1. Endogenous respiration, where cellular material is oxidized to digested sludge, as seen in Equation 2.2 (Moonkhum, 2007; Gregor et al., 2008). These processes are demonstrated in the following equations:

$$Organic\ matter + O_2 + NH_4 \rightarrow CO_2 + H_2O + Cellular\ material \quad (2.1)$$

$$Cellular\ material + O_2 \xrightarrow{bacteria} CO_2 + H_2O + NO_3 + digested\ sludge \quad (2.2)$$

$C_5H_7NO_2$ is a typical formula used for representative of the microorganisms (Moonkhum, 2007; Gregor et al., 2008). The aerobic process is identified as stoichiometry in the following equations:

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 + energy \quad (2.3)$$

$$C_5H_7NO_2 + 7O_2 \rightarrow 5CO_2 + 3H_2O + NO_3^- + H^+ + energy \quad (2.4)$$

In the aerobic digestion process, nitrogen appears in the form of ammonia as indicated in Equation 2.3, which is a system inhibiting nitrification. The Equation 2.4 represents the event of nitrification. Theoretically, 1.42 g of oxygen are required per gram of active cell mass in the non-nitrifying system, whereas 1.98 g of oxygen are required per gram of active cell mass when nitrification occur (Tchobanoglous et al., 2004).

The performance of aerobic digestion process are affected by certain parameters, such as the rate of sludge oxidation, sludge temperature, system oxygen requirements, sludge loading rate, sludge age, and sludge solids characteristics (Tchobanoglous et al., 2004). The design criteria of the aerobic digestion system are given in Table 2.1.

Table 2.1. The parameters and criteria affecting the aerobic digestion process (Tchobanoglous et al., 2004).

Parameters	**Range**
SRT for waste activated sludge (d)	10-15
Solid loading rate (kg VS/m^3d)	1.6-4.8
Oxygen requirement (kg/kg)	2-4
Energy requirements	
Mechanical aerator (W/m^3)	20-40
Blower (m^3/m^3 min)	0.02-0.04
DO residual in liquid (mg/L)	1-2
Sludge temperature (^{o}C)	Room temperature
Reduction in VSS (%)	40-50

The process performance of an aerobic digestion system is influenced significantly by the operating temperature, which is very significant for the competition of microorganisms. Temperatures between 25-30°C are proper for nitrification, with maximum nitrification rate occuring at 30°C (Genç et al., 2002). Higher rates of microbial metabolic activity are maintained in higher temperatures, which leads to a shorter retention time needed to obtain the inactivation of pathogenic organisms and the removal of solids to a desired level (Bernard and Gray., 2000). The pH of the aerobic digestion should be controlled to improve the metabolic activity and enhance the digester efficiency. The optimum pH interval is between 6 and 8 for nitrification steps (nitrite and nitrate formation).

Nitrosomonas and Nitrobacter are the most important and common nitrifiers present in all activated sludge systems. The growth of these two nitrifiers occurs in at least 2 d, with Nitrobacter growing slower than Nitrosomonas. After the beginning of nitrite formation, the nitrate formation starts between 0 and 11 d, because nitrate formation rate is always lower than the nitrite formation rate. In addition, low organic loadings are needed to activate nitrifiers in the aerobic systems (Genç et al., 2002).

The benefits of the aerobic stabilization can be clarified as below:

- the reduction of sludge volume, which is equal to those achieved by anaerobic digestion,
- the application of land reclamation,
- the uses as fertilizer in agricultural purposes,
- the production of an odorless, humus like biologically stable sludge for disposal in the environment,
- the stabilization of sludge,
- the easy operation and lower capital cost,
- the generation of good dewatering characteristics (Tchobanoglous et al., 2004).

2.3. Sludge Pretreatment

Sludge pretreatment processes that disintegrate before aerobic and anaerobic digestion is a very beneficial technology that leads to the release of extracellular and intracellular organic materials from cells into an aqueous phase, breaking the cells walls and membranes, solubilization of the sludge by reducing floc particle size, disintegration of sludge cells and increasing the digestibility of excess sludge (Show et al., 2007; Park et al., 2012).

In order to accelerate the rate limiting stage of hydrolysis in anaerobic digestion (Müller et al., 2004), there are many kinds of pretreatment methods, which can be classified in these ways: physical (freezing and thawing), chemical (use of ozone, acids, alkali and other chemicals), mechanical (ultrasound, homogenizers, mills, and others),

thermal (microwave), biological hydrolysis with or without enzyme addition, and combination of these methods (such as thermochemical) (Müller, 2001; Bougrier et al., 2005; Rani et al., 2012; Cho et al., 2012; Dhar et al., 2012; Athanasoulia et al., 2012**)**.

The advantages of employing the disintegration methods can be summarized in the following:

- enhancing of aerobic and anaerobic stabilization processes,
- reduction of pathogens and disinfection of sludge,
- improvement of settling properties,
- acceleration of organic matter degradation,
- developing of conditioning and dewatering,
- increase of biogas production in anaerobic digestion systems (Müller, 2003).

2.3.1. Thermal (Microwave) Pretreatment

Microwave (MW) pretreatment is an alternative method which makes use ofan electromagnetic radiation with an oscillation frequency of 0.3-300 GHz. In the mechanism of the microwave application, polar molecules such as water, proteins, some lipids or other organic complexes in the sludge are irradiated. These molecules begin to rotate with microwave electrical field and the resulting molecular rotation causes frictional heat which will result in the dissipation of energy to the sludge in the system. (Dogan and Sanin, 2009; Eskicioglu et al., 2009; Yu et al., 2010; Appels et al., 2013). MW technology includes rapid heating, less energy than conventional heating, pathogen destruction, ease of control, system compactness, and low overall cost (Kennedy et al., 2007; Park et al., 2010).

In several microwave pretreatment studies, the most common temperatures used were between 60 and 180°C (Appels et al., 2013). The microwave pretreatments can be carried out at temperatures higher than 60°C because very low solubilization rates are obtained at low temperatures. At temperatures higher than 180°C, biodegradability of sludge decreases due to the formation of toxic compounds, refractory organics and recalcitrant inhibitory intermediates (Wilson and Novak, 2009; Shehu et al., 2012). The time range of microwave

pretreatment has changes to between 15 and 60 min (Dhar et al., 2012). In several review papers published recently, optimal temperatures in microwave pretreatment was achieved at 160 to 180°C and treatment times ranged from 30 to 60 min due to the increase in the efficiency and the rate of degradation of WAS, the improvement of sludge dewaterability, the achievement of sludge sanitation and the generation of biogas. At optimal conditions, better digestion results are provided at a rate of a 40-60% and 40-100%, increasing volatile solid (VS) destruction and biogas produced, respectively (Bougrier et al., 2008; Liu et al., 2012; Shahriari et al., 2012).

2.3.2. Alkaline Pretreatment

Alkaline pretreatment, compared with other methods, is a widely performed method for sludge pretreatment due to its simple procedure, ease of operation and high solubilization efficiency (Shao et al., 2012). In the alkaline pretreatment, NaOH is the preferred reagent over chemicals like KOH and $Ca(OH)_2$ as a result of its greater (40-60%) solubilization efficiency than these chemicals (Park et al., 2012; Li et al., 2012). The order of efficiency of the chemicals mentioned above is given such as NaOH > KOH > $Mg(OH)_2$ and $Ca(OH)_2$ (Carrère et al., 2010). The mechanism of alkaline pretreatment is carried out by splitting complex materials into smaller molecules at a high pH (Kim et al., 2010). The benefits can be described as the disruption of floc structure and cell walls, the enhancement in the biodegradation of complex materials (Yunqin et al., 2009), the increase in levels of solubilization, the improvement of aerobic and anaerobic digestion performances (Shao et al., 2012), the releasing of inner organic matters and the acceleration of sludge hydrolysis (Li et al., 2008), and lastly, the enhancement in sludge dewatering ability (Erdincler and Vesilind, 2000).

A higher NaOH dosage generally leads to a higher sludge pH, which could result in extra costs to neutralize the sludge for sludge digestion. At a high pH, some refractory components in the liquid phase are formed, which leads to low biodegradability. In addition, the alkaline treatment affects positively the sewage sludge disintegration. Until a 0.05 mol/L of NaOH dosage, the disintegration degree (DD) showed a rapid increase as the NaOH dosage was increased. Furthermore, when the dosage of NaOH exceeded 0.05 mol/L value, the DD had no significant development. It was observed that after applying a

0.06 mol/L NaOH dosage for 2 h, the DD was obtained about 24% (Zhang et al., 2012). Additionally, Li et al. (2008) investigated the effects of different doses (0-0.5 mol/L) of NaOH and $Ca(OH)_2$ at 0-40^oC. For sludge disintegration, it has been observed that NaOH was more convenient reagent compared to $Ca(OH)_2$. Most efficient dose for NaOH was about 0.05 mol/L (0.16 g/g DS) and 60-71% solubilization of organic matters was achieved in the first 30 min during its 24 h treatment. Besides of this, low dose of NaOH (<0.2 mol/L) negatively influenced the ability of sludge dewatering, while the ability was recovered gradually with the increase of NaOH dose.

2.3.3. Ozonation Pretreatment

Ozone (O_3) is a very strong oxidizing agent that has the potential to treat and reduce the quantity of sludge generated from WWTPs. It destroys the structure of natural organic matter and improves the conversion of high molecular weight compounds (slowly biodegradable particulate organics) into low molecular weight (readily biodegradable) products, such as carboxylic acids, hydrophilic acids, carbohydrates, amino acids, etc.. After the implementation of sludge disintegration by ozone, the floc disintegration, solubilisation and mineralization (oxidation of the soluble organic matters into carbon dioxide) processes are accomplished. Ozonation is considered an attractive pretreatment method for solid hydrolysis prior to aerobic and anaerobic digestion (Salsabil et al., 2010; Braguglia et al., 2012; Cesaro et al., 2012).

The disintegration efficiency is influenced by ozone dose and dosing method (Erden et al., 2010). After investigations by several studies, an optimal ozone dose for the improvement of anaerobic biodegradability has been determined to be 0.1 g O_3/g COD, 0.2 g O_3/g TSS, 0.15 g O_3/g TS (Carrère et al., 2010). Extra ozone doses or long treatment times are not desired because of the mineralization of dissolved organics (Chu et al., 2009). Salsabil et al. (2010) investigated the effect of ozone oxidation on aerobic digester performance in terms of sludge solubilization and reported that the application of 0.1 g O_3/g TS ozone dose before aerobic digestion leads to 71% TSS reduction in sludge at the end of the 55 days of operation. When the ozone dosage was higher than 0.02 g O_3/g TSS, sludge cells were disintegrated in the study of Yan et al. (2009). At a dose of 0.5 g O_3/g TS, compared to the control, WAS mass was reduced as 70%, along with an 85% reduction

in sludge volume (Park et al., 2003). Furthermore, Bougrier et al. (2006) achieved 20-25% solubilization by using ozone dose of 0.1 and 0.16 g O_3/g TS. In addition, the organic amount was decreased by 5% and gas production was increased by 11% and 23% for doses of 0.1 and 0.16, respectively. Moussavi et al. (2008) reported that TS reduction was achieved between 15.4% and 80.7% at 0.125 to 2 g O_3/g TS ozone doses. It can be stated that reduced TSS and VS were obtained 8.3% to 47.9% and 5.8% to 45.9% at the same ozone doses, respectively.

2.3.4. Advanced Oxidation by Fenton's process

One of the commonly used advanced oxidation techniques is Fenton's process. H_2O_2 and ferrous iron are utilized as a reagent in Fenton's reaction, which leads to the disintegration of H_2O_2 and the formation of highly reactive hydroxyl (OH) radicals. The OH radicals having strong oxidation ability can completely decompose organic substances like CO_2 and H_2O. The ferrous iron and H_2O_2 concentration and solution pH are significant system parameters for determining the Fenton process rate. The process is applied in two ways: the mineralization of some parts of activated sludge into the CO_2 and H_2O, and the solubilization of the other part of sludge to biodegradable organics (Erden and Filibeli et al., 2011). The basic equation of Fenton process is shown in the Equation 2.5:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \qquad (2.5)$$

Fenton's process leads to the destruction of bacteria cell membranes, and the conversion of biomass particulates into soluble compounds such as proteins, lipids and polysaccharides (Tokumura et al., 2009). In addition, the H_2O_2 used in the process reduces odors and destroys pathogenic organisms (Kim et al., 2009).

Kim et al. (2009) investigated the effects of hydrogen peroxide oxidation on an excess activated sludge reduction. Four different concentrations of H_2O_2 (0.4, 0.8, 1.2 and 1.6 M) were performed on the sludge through 2 h of reaction testing, with the initial pH of the sludge samples set up to 3., 5, 7., 9 and 11 to determine the concentrations of H_2O_2 and pH effect on a sludge reduction. From the results, it was reported that a 49% solid reduction was obtained at pH 11 and 1.6 M H_2O_2 after 2 h of reaction at ambient temperature and

pressure. sCOD/TCOD ratio improved to 57.4% indicating high amount of release in the intracellular materials. The authors stated that a 66.7% of settleability was enhanced after H_2O_2 treatment. A 61% decrease (from 34.5 μm to 10.8 μm) was observed in the median size of sludge particles.

Neyens et al. (2003a) established the optimum values of 25 g H_2O_2/kg DS, and 1.67 g Fe(II)/kg DS, pH 3, at ambient temperature and pressure. In the study of Dewil et al. (2007), the Fenton treatment was performed in a batch reactor, containing 2 L of sludge at ambient temperature and pressure. A 26.6% reduction in VS was recorded and the methane content was found to be between 65 and 70% using 0.07 g Fe(II)/g H_2O_2 and 50 g H_2O_2/kg DS at pH 3 through 60 min reaction. Kaynak et al. (2008) investigated the effects of Fenton process on anaerobic sludge digestion. The optimum dose using the Box-Wilson Experimental Design Method was found as 0.067 g Fe (II)/g H_2O_2 and 60 g H_2O_2/kg DS at pH 3. The results of this study showed a 25.4% reduction in VS was achieved at mesophilic conditions after 5 days operation period. Pham et al. (2011) also carried out the Fenton oxidation under following conditions: 0.01 mL H_2O_2/g SS, 150 $[H_2O_2]_o/[Fe^{2+}]_o$, 25 g/L TS, at pH 3, 25°C, and 150 rpm with 60 min duration.

2.3.5. Ultrasonic Pretreatment

Ultrasonic pretreatment is considered one of the most effective mechanical methods. The principle of ultrasonic pretreatment depends on two significant mechanisms; cavitation, which is favored at low frequencies, and chemical reactions as a result of the formation of $OH^{\cdot}$, $HO_2^{\cdot}$, $H^{\cdot}$ radicals at high frequencies (Carrère et al., 2010). Although ultrasound frequencies range from 20 kHz to 10 MHz, better sludge disintegration is generally carried out at a lower frequency range of 20 to 40 kHz (Tiehm et al., 2001; Khanal et al., 2007; Carrère et al., 2010; Braguglia et al., 2012). This is because low-frequency ultrasound forms larger cavitation bubbles and produces more powerful hydrodynamic shear forces, therefore providing efficient sludge disintegration. In sewage sludge treatment, particularly, 20 kHz is commonly performed in sludge disintegration before digestion (Gallipoli and Braguglia, 2012)

The process of this pretreatment can be described as the propagation of an ultrasound pressure wave through the sludge medium with a huge amount of energy dissipation, compressions and rarefactions generated. Low pressure happens in the rarefactions region (excessively large negative pressure). As result of reduced pressure, microbubbles (cavitation bubbles) which contain vaporized liquid and gas that had dissolved in the liquid are formed in this region. The positive pressure cavitation bubbles oscillate and grow to an unstable size before violently collapsing. Large numbers of cavitation bubbles grow and then violently collapse, producing local heating and high pressure on liquid-gas interface and high shearing forces in the surrounding liquid phase and formation of radicals (Braguglia et al., 2012). Due to the local extreme conditions, the highly oxidative radicals hydroxyl ($OH^{\bullet}$), hydrogen ($H^{\bullet}$), and hydroperoxyl ($HO_2^{\bullet}$) and hydrogen peroxide (H_2O_2) can be formed (Rani et al., 2012). The collapsing bubbles disrupt adjacent bacterial cells with extreme shearing force, rupturing the cell walls and membranes. The high temperature and pressure developed inside the collapsing bubbles is also seen to be beneficial to sludge disintegration (Moonkhum, 2007). In addition, the chemistry of ultrasonic pretreatment consists of a combination of shearing and chemical reactions with radicals, and pyrolysis and combustion (Fernández-Cegrí et al., 2012). During ultrasound pretreatment, the radicals generated during the cavitation phenomena induces improved sludge destruction rates, disintegrating the biological sludge flocs and microorganisms lyses, enhancing hydrolysis rates, breaking down complex compounds into simpler forms, reducing the sludge treated and oxidizing of toxic chemical compounds (Moonkhum, 2007; Carrère et al., 2010; Gallipoli and Braguglia, 2012).

The efficiency of ultrasonic process is affected by some factors; supplied energy, ultrasonic frequency, power and treatment time, nature of the influent (Khanal et al., 2007). In the study of Khanal et al. (2007), biogas production increased up to 40% using ultrasounds of specific energies ranging from 1000 to 10000 kJ/kg TS on WAS pretreatment.

Akin, (2008) observed the effect of the different total solid (TS) concentrations (2, 4 and 6%) in WAS during ultrasonic performance. For each of these TS concentrations, low ultrasonic frequency (20 kHz) and high ultrasonic densities (2.07-3.05 W m/L) were used. Ultrasonic times were arranged in the following values; 0, 10, 30, 60, 120, 240 s. After the

application of ultrasonic pretreatment, it was stated that sCOD release was improved with the increase in specific energy input and ultrasonic density at all TS contents. At 2% TS, the highest sCOD release of 329 mg/g TS was obtained at an energy input of 5 kWs/g TS. Otherwise, the sCOD release decreased to 248.5 and 124.2 mg sCOD/g TS at TS contents of 4 and 6%, respectively. In addition, at a TS content of 2% and specific energy input of 10 kWs/g, the highest protein release was found to be 1449 mg/L (80.7 mg/g TS), acquired in the first 60 s.

2.3.6. Biological Pretreatment of Sludge

The biological sludge disintegration process is catalyzed by the addition of external enzymes into the sludge (Müller, 2001). The cracking of cell wall compounds is achieved by the enzyme catalyzed reactions and autolytic processes (Perez-Elvira et al., 2006).

Barjenbruch and Kopplow (2003) studied enzymatic pretreatment by applying enzyme carbohydrates. The authors stated that this pretreatment had no effect on reducing the foaming problem but in terms of dewaterability of sludge (decrease in CST value), the pretreatment is more effective than thermal pretreatments at 80, 90 and 121°C and ultrasonic pretreatment. A 15% improvement of DD was achieved after enzymatic pretreatment.

Roman et al. (2006) observed the effects of two enzymes such as cellulase and pronase E added alone and in combination. After an addition of 0.03% of enzyme solution, the sludge samples were incubated on a rotary shaker (200 rpm) at 40ºC for 120 h. It was found that an 80% reduction (decreased from 25 g/L to 5 g/L) in total solids (20% in the control), whereas the sludge samples treated with cellulase and pronase E were reduced by 29% and 36%, respectively. Optimal results were attained with the mixture of these enzymes, achieving 93% removal of particulate COD (59% in the control) and 97% TCOD removal (63% in the control). With the application of single enzymes, there was little or no impact on sludge solubilisation, final COD and total suspended solid (TSS), but a decrease in the production of volatile fatty acids (VFAs) was achieved by all of the enzyme additions.

2.3.7. Thermochemical Pretreatment

Thermochemical pretreatment is a process that combines chemical pretreatment with thermal pretreatment. In order to obtain a higher degree of disintegration, alkaline pretreatment has often been combined with other treatment methods, such as microwave treatments (Kim et al., 2010; Dogan and Sanin, 2009).

Carrère et al. (2010) studied the combination of alkali treatment (pH of 12, NaOH) with microwave treatment (160°C) and found 10% methane production in the thermochemical pretreatment higher than microwave alone. In another study, Dogan and Sanin (2009) stated that dewaterability improved about 22% after anaerobic digestion with thermochemical pretreatment (pH of 12, 160°C microwave), compared to anaerobic digestion of WAS.

2.4. Literature Review of Pretreatment Methods

2.4.1. Ultrasonic Pretreatment

Bougrier et al. (2006) observed that the ratio of sCOD/TCOD in WAS increased from 4% to 32% when increasing specific energy input from 0 to 10000 kJ/kg TSS. After ultrasonic pretreatment, volatile suspended solid (VSS) decreased by 23%, 28%, and 30% for the 1000, 5000, and 10000 kJ/kg TSS specific energy inputs, respectively. It can be stated that there was no significant improvement in the VSS reduction as the specific energy increased from 5000 to 10000 kJ/kg TSS. In addition, soluble carbohydrates increased by 162%, 282%, and 730% for the specific energy inputs of 1000, 5000, and 10000 kJ/kg TSS, respectively.

Salsabil et al. (2009) compared the effects of ultrasonic pretreatment on aerobic and anaerobic digestions in reactors. With a sonication treatment of 108000 kJ/kg TS, aerobic and anaerobic biodegradability showed an improvement due to the increase of specific sCOD uptake rate. This ultrasonic disintegration led to an increase of biogas production as a result of the sCOD increase. Sonication time and density (W/mL) play a significant role in COD dissolution efficiency. Yan et al. (2010) have also reported that the sCOD increased from 200 to 10000 mg/L after 15 min of ultrasonic vibration applied at 1000 W of power, 20 kHz of frequency and 2 W/mL of density in the raw sludge. In the research of Li et al. (2007), the sCOD concentration increased from 810 to 6499 mg/L by utilizing 25 min of ultrasonic vibration time set at 200 W and 20 kHz.

Chang et al. (2011) investigated the effects of ultrasonic pretreatment processes occurring at a frequency of 20 kHz, with different energy levels (low - 240 W and high - 480 W) and vibration times (2, 5, 10, 20, 30, 40, 50, 60 min) on mixed sludge, which had an average 50:50 volume ratio of primary-to-secondary sludge in aerobic digestion. The ultrasonic densities at corresponding energy levels (240 W and 480 W) were 1.2 and 2.4 W/mL, respectively. Based on the results, better cell wall destruction efficiency and a 44.4% increase in dissolution rate of COD was achieved at 30 min of ultrasonic pre-treatment applied at an energy output of 480 W, i.e. SE of 397 kJ/g SS, in comparison to non-pretreated sludge. The initial sCOD and TCOD were found to be 128 mg/L and 15200

mg/L, respectively. The sCOD dissolution rate increases when the sonication time increases. Furthermore, the ultrasonic disintegration leads to decrease in suspended solid (SS) content of the sludge and release of extracellular polymeric substances (EPS) content present within the sludge into the solution. It was observed that the sCOD did not increase significantly at both energy outputs when the sonication time was below 5 min. On the other hand, the sCOD increased linearly and rapidly from 640 mg/L to 4480 mg/L for sonication time between 5 and 20 min and from 1000 mg/L to 6880 mg/L for sonication time between 5 and 30 min for 240 W and 480 W respectively. Until 20 min of the sonication time, the sCOD increment rate was almost similar for both 240 W and 480 W energy output. However, higher sCOD values were achieved with 480 W energy output in comparison to 240 W energy output. It can be stated that the increase in sCOD was a function of sonication time at different energy output. With the low (240 W) and high (480 W) energy output, the maximum of approximately 40% and 49.7% dissolution rate of sCOD was observed respectively. Additionally, SS concentration decreased by 1160 mg/L after 30 min, and by 2600 mg/L after 60 min at 240 W energy output, whereas it decreased by 2360 mg/L after 30 min and by 3660 mg/L after 60 min at 480 W energy output. According to these results, it can be seen that SS concentration after 30 min of sonication at 480 W energy output did not change. Considering the above-mentioned results, the optimal ultrasonic energy output was selected as 480 W and 30 min. In the optimum conditions, SS concentration was 10880 mg/L and its optimal specific energy was about 397 kJ/g SS.

Zhang et al. (2007) reported the influence of the ultrasound pretreatment (0.5 W/mL of ultrasonic density and 10-30 min of ultrasonic time) on biological activity, sCOD, proteins and nucleic acid concentrations in sludge. The increase in ultrasonic time and power density results in an increase of the sCOD, supernatant proteins and nucleic acids concentrations, DD, SS and VS reduction increased. It can be stated that higher energy ultrasound was more efficient than lower energy ultrasound for the sludge treatment. After ultrasonic treatment at 0.5 W/mL for 30 min, the sludge disintegration degree was 30.1%, the solid mass was reduced by 23.9%, the sludge viability was decreased by 95.5%, with biological cells almost completely inactivated. In addition, at these pretreatment conditions, the sCOD, supernatant proteins and nucleic acids concentrations increased by

690%, 560% and 1640%, respectively, indicating very effective sludge floc disintegration and cells lysis.

Erden et al. (2010) investigated that the effect of the ultrasonic treatment with an operating frequency of 20 kHz and a supplied power of 200 W. Specific energy of 9690 kJ/kg TS was applied to sludge samples. At the end of the 30th operation day, TS values decreased to 15.2% and 26.5% with reference to the raw sludge and ultrasonically pretreated sludge, respectively. In addition, ultrasonic treatment resulted in 36% higher VS reduction rates, compared to the control reactor (22.75%). VSS/SS ratio decreased from 0.75 and 0.89 to 0.63 and 0.57 at the end of the operation for raw sludge and ultrasonically pretreated sludge, respectively. In the first 15 days of operation, VSS/SS ratio decreased especially then kept relative constant value for each digester. Total nitrogen in sludge's supernatant also increased from 107 to 138 mg/L for raw sludge, and from 13 to 62 mg/L for ultrasonically pretreated sludge. At the end of operation period, particle size reduced the ratio of 13.6% and 57.1% for raw sludge and ultrasonically pretreated sludge respectively. Protein concentration showed the highest decrease in the digester fed with sonicated sludge. At the end of the 30 days of operation, 66.1% and 11.4% decrease was obtained in CST of raw sludge and ultrasonically pretreated sludge

Benabdallah et al. (2007) utilized the ultrasonic apparatus with an operating frequency of 20 kHz and supplied power of 70 W. In order to determine the optimal conditions, specific energy arranged from 0 to 15000 kJ/kg TS (0, 5000, 8000, 11000, 15000). Approximately 40% improvement in organic matter (expressed as VS) solubilization was achieved for SE greater than 11000 kJ/kg TS. Based on the results, it can be said that when the SE increased, matter solubilization improved. Since little difference in VS solubilization improvement was detected between 11000 (42.2%) and 15000 kJ/kg TS (43.4%), optimum SE value of 11000 kJ/kg TS was selected. Moreover, DD was obtained in the range of 27.5-31.5% at higher SE applied. SE of 11000 kJ/kg TS gave optimum results in terms of solid distribution and organic matter solubilization. The other disintegration values were found to be 75% at 20000 kJ/kg TS, Müller and Pelletier (1998) and 60% at 15000 kJ/kg TS, Bougrier et al. (2005) when compared to the study of Benabdallah et al. (2007). The average particle size decreased to 10.12-13.26 mm, while the average particle size of untreated samples ranged from 0.4 to 150 mm. When the

applied SE was changed from 11000 to 15000 kJ/kg TS, the biogas yield was enhanced only 2.5% and at low SE, and the total methane production increased significantly between 11.0 and 19.7% under mesophilic conditions. It can be maintained that the ultrasound pretreatment has proved to be an effective technique to disintegrate the particulated matter.

Dewil et al. (2006) stated that dewaterability decreased with increasing SE. The reason was explained that EPS and the cell walls of microorganisms in the sludge were damaged from the shear forces of ultrasonic energy. Smaller flocs offer an extended surface area; therefore, huge amounts of water were not removed from more surface area. On the other hand, the EPS were highly hydrated polymeric substances; thus, large amounts of water were bound onto these structures due to its high absorption capacity.

Yan et al. (2010) investigated the effect of ultrasonic specific energy on WAS solubilization. The ultrasonic treatment was used with an operating frequency of 20 kHz, supplied a power of 100 W and SE ranged from 0-90000 kJ/kg DS (0, 3000, 6000, 9000, 15000, 30000, 60000, and 90000 kJ/kg DS). Results showed that particle size reduction and the solubilization of organic matter in WAS were obtained with an increase in ultrasonic specific energy. When the SE increased from 0 to 90000 kJ/kg DS, DD improved from 0 to 60.8%, on the other hand, DD of 38.2% was reached with the specific energy of 30000 kJ/kg DS. According to results, it can be determined that the mean particle size of WAS decreased with the SE. At SE of 15000 kJ/kg DS, the mean particle size was 13.1 μm, which was only one half of that of original WAS. With the ultrasonic treatment of WAS, the mean particle size of WAS deceased from 25.0 to 3.2 μm in the SE range of 0-90000 kJ/kg DS. It was clearly observed that both the concentrations of soluble protein and carbohydrate increased with the specific energy. At the specific of 90000 kJ/kg DS, the soluble protein of WAS increased from 44.1 mg COD/L (raw sludge) to 3104.5 mg COD/L. Similarly, the concentration of soluble carbohydrate increased from 23.8 to 274.8 mg COD/L in the specific energy range of 0-90000 kJ/kg DS. The reason as to why concentrations of soluble protein at different specific energies were much higher than those of soluble carbohydrate can be explained with the fact that protein is the predominant component of WAS. The initial concentrations of ammonia nitrogen and soluble phosphorus in raw WAS were 6.5 and 28.0 mg/L, respectively. The concentrations of ammonia nitrogen were 33.3, 50.9, 61.7, 72.8 and 78.4 mg/L, respectively, and the

corresponding soluble phosphorus amounts were 39.3, 46.2, 57.3, 61.3 and 67.8 mg/L, respectively, when WAS was treated with ultrasonic at the specific energies of 3000, 15000, 30000, 60000 and 90000 kJ/kg DS. It can be clearly noted from the results that the release of ammonia nitrogen and soluble phosphorus both increased with SE during the ultrasonic treatment of WAS.

Cho et al. (2012) examined the effect of WAS hydrolysis during ultrasonication for one to 60 min at specific energy of 0.1-50 kJ/g TS. After 60 min of ultrasonication, 37% solubilization rate enhancement was obtained at specific energy of 50 kJ/g TS. Similarly, Liu et al. (2008) achieved 37.9% solubilization rate improvement after 60 min of ultrasonication (40 kHz, 0.1 W/mL), and in another study, Zhang et al. (2007) reported a 30% disintegration rate enhancement through 30 min of ultrasonication (25 kHz, 0.5 W/mL). Regarding the different increasing pattern of the solubilization rate, this issue can be explained through observation of thedisintegration of the loose part (sludge floc) and subsequent disintegration of the rigid part (microbial cells). In the research of Cho et al. (2012) it was observed that the concentrations of bound EPS-protein and carbohydrate decreased during ultrasonication, indicating the destruction of sludge floc. Until 10 min of ultrasonication, an initial steep decrease in bound EPS-carbohydrate was observed from 213 mg/L to 121 mg/L, after the rate of decrease then stabilized.

Yan et al. (2010) performed the ultrasonic pretreatment of WAS at a frequency of 20 kHz and time of 10 min. It observed that the concentrations of soluble protein and carbohydrate both increased with ultrasonic energy density. With fermentation time of 72 h, the soluble protein at ultrasonic density of 0, 0.25, 0.50, 1.0, 2.0 and 4.0 kW/L were, respectively, 1756.8, 1853.4, 1959.3, 2030.9, 2087.0 and 2157.0 mg/L and the corresponding soluble carbohydrate were 326.8, 356.7, 376.7, 383.0, 410.1 and 440.7 mg/L. It was stated that VFA production increased with ultrasonic energy density between 0.25 and 1.0 kW/L and decreased in the range of 1.0 and 4.0 kW/L. The decrease of VFA accumulation at higher than 1.0 kW/L ultrasonic density was due to the fact that most microbial cells were destroyed and sludge activity decreased drastically. The acetic acid percentage accounting for the total VFA decreased from 52.3 to 44.9%. The iso-valeric and propionic percentages both increased from 14.9 to 21.5 and 11.4 % to 15.2%, respectively, increasing from zero to 4.0 kW/L ultrasonic energy density. The average NH_4^+-N were,

increased respectively 327.4, 344.3, 357.0, 368.6, 388.9 and 418.2 mg/L and the corresponding PO_4^3-P concentration were 97.5, 101.1, 105.4, 109.3, 113.4, and 120.6 mg/L, respectively, as ultrasonic energy density increased from 0, 0.25, 0.5, 1.0, 2.0 and 4.0 kW/L. The result of the release of NH_4^+-N was mainly from the hydrolysis of WAS protein. It can be clearly said that the average NH_4^+-N release was greater than that of PO_4^3-P at ultrasonic energy density of 1.0 kW/L.

2.4.2. Microwave Pretreatment

In the most studies, an optimal temperature was reported in the range of 160-180ºC and treatment times from 30 to 60 min. Pressure may vary from 600 to 2500 kPa. Sludge biodegradability caused a decrease in spite of achieving high solubilization efficiencies at temperatures higher than 170-190ºC.

Several researchers have stated that MW pretreatment is an efficient method in the enhancement of aerobic and anaerobic digestion and disintegration of WAS. Eskicioglu et al. (2009) investigated the effect of MW pretreatment temperature on sCOD/TCOD ratios in the anaerobic digestion since sCOD to TCOD ratios gives a general indication of the extent of hydrolysis, A wide range of pretreatment temperatures such as 50, 75, 96, 120, 150, 175ºC were used in the study. Based on the results of the analysis, a linear relation was observed between these MW temperatures and level of hydrolysis. sCOD/TCOD ratios increased from 9±1 (control) to 12±1, 21±0, 24±1, 24±3, 28 ±1 and 35±1% at 50, 75, 96, 120, 150 and 175ºC, respectively. In addition, the highest cumulative biogas production of 31+6% was acquired at 175ºC after 18 d of digestion when compared to control.

Park et al. (2010) examined the influence of the MW pretreatment on the degree of solubilization of WAS, determining the combination of output power (400-1600 W), target temperature (60-120ºC) and TS concentration (1-3% TS). In this study, the sCOD/TCOD ratios showed an increase from 6.9% (control) to 8.0-17.5% after MW pretreatment to 60-120ºC. The highest sCOD/TCOD ratio was found as 17.5% at 400W of output power, 120ºC temperature, and 2% TS concentration. It was reported that higher VS reduction (25.7%) was seen in in the anaerobic reactors after MW pretreated sludge as compared to

the control reactors (23.2%). Moreover, in total COD removal and methane production, 64% and 79% improvements were observed, respectively. Similarly, Hong et al. (2006) reported that the sCOD/TCOD ratios of WAS increased from 8% (control) to 18% after microwave pretreatment to 72.5ºC. A 68% more biogas production was achieved at 5 d SRT for the reactor digesting MW pretreated sludge (72.5ºC) in comparison of the control reactors operating at 11 d SRT. In another study, Ivo and Jing (2009) stated that there was no difference between the microwave pretreatment temperatures of 50-70ºC and the increase of sCOD/TCOD ratio was reported as from 2% to 21%.

Pino-Jelcic et al. (2006) observed a 53.9% and 49% VS reduction for MW pretreated anaerobically digested sludge and control, respectively. In addition, MW-digested sludge showed higher biogas production by 16.4 and 6.3%, as compared to control. As another research with MW pretreatment, Liu et al. (2012) reported that thermal pretreatment (at 175ºC/60 min) improved the solubilization of VSS (27.5%), increased methane potential of WAS (34.8%), decreased viscosity (from 13500 to 1625 mPa.s), enhanced the dewatering performance, as well as increased sCOD, soluble protein and carbohydrate. In addition, the maximum methane production rate of WAS was found as 1.78 mL CH_4/g VS at this pretreatment conditions.

Grübel and Machnicka (2011) stated that MW disintegration (2.45 GHz of frequency and 900 W of power) resulted in transfer of organic matter from solid phase to the liquid phase. Disintegration was carried out over periods of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 s. The most effective operation time was found to be 180 s of exposition for sludge. In the MW disintegration process, protein concentration increasedwith the conduction time, similar to the COD value change. Based on sCOD and protein concentration, the value increased from 58.5 to 673 mg O_2/dm^3 and from 26 to 366 mg/dm^3, respectively. The MW pretreatment positively influences sludge volume index (SVI), a decrease of it from 195.5 to 54.1 cm^3/g was observed. A high dewatering ability of sludge was determined by a definite improvement on sedimentation properties of sludge due to the low values of SVI.

Dhar et al. (2012) evaluated the influence of MW disintegration on WAS using three thermal pretreatment temperatures (50, 70 and 90°C) at the set temperature for 30 min. VSS was reduced as 25, 26, and 27% at 50, 70 and 90°C pretreatment temperatures, respectively. In this way, it can be said that VSS reductions were nearly the same for the different thermal pretreatments although the sCOD/TCOD ratio increased with temperature. Total volatile fatty acids (TVFA) solubilization increased from 156 to 181, 196, and 178 mg/L among the three thermal pretreatments, respectively. Additionally, soluble carbohydrates increased by 350%, 264%, and 434% at 50, 70 and 90°C pretreatment temperatures, respectively. The increases in methane production during anaerobic digestion were nearly the same among the three thermal pretreatments. After a detailed investigation of the literature about MW disintegration, COD solubilization was improved up to 20% and 26% after pretreatment of WAS at boiling (96°C) and higher treatment temperatures (up to 175°C), respectively (Tyagi et al., 2013).

Ahn et al. (2009) reported the influence of microwave treatments carried out 0, 3, 5, 7, 9, 11, and 15 min in a microwave oven (2450 MHz, 700 W) on the disintegration of municipal secondary sludge. As microwave irradiation time increased from 0 to 15 min, sCOD concentration increased from 380 to 5520 mg/L, and the solubilization degree of sludge was increased from 2% at 0 min to 22% at 15 min. Due to sludge disintegration by microwave pretreatment, the concentrations of soluble protein and carbohydrate also increased. Maximum TVFA concentration was less than 5000 mg COD/L, which was lower than the inhibitory concentration.

2.4.3. Alkaline Pretreatment

Many researchers have investigated the effect of alkaline pretreatment on sludge disintegration and their results are given in the following studies:

Kim et al. (2003) investigated the effect of alkaline pretreatments at pH 12 using alkaline agents NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$. For sCOD/TCOD ratio, 8.1% (control) to 39.8% (NaOH), 36.6% (KOH), 10.8% ($Mg(OH)_2$) and 15.3% ($Ca(OH)_2$) increase was acquired. Different NaOH doses were applied to investigate the effect on COD solubilization on the anaerobic digestion. Since no significant increase in the rate of

COD solubilization was found for doses higher than 7g/L NaOH, this dose was studied in the anaerobic batch reactors. With a dose of 7g/L NaOH, 20.5% and 29.8% of VS destruction for control and alkaline pretreatment, respectively, was found.

Hwang et al. (1997) assessed different pretreatment methods, including alkaline, ultrasonic treatment and the different combinations of these two methods. In terms of COD solubilization, the combination pretreatment (40% COD solubilization) showed the best results when compared to single treatments. In the aerobic digestion, the performance of digester fed with alkaline pretreatment, was better than that of raw sludge in terms of COD and SS removal. Based on the results, it was obvious that at short treatment time, alkaline pretreatment was more effective than ultrasonic treatment. sCOD concentrations decreased to from 1200 to 220 for raw sludge and 2600 to 230 mg/L for alkaline pretreatment after 40 d, resulting in 82-91% removal. These observations supported the conclusion that soluble organic matters are degrade easily in the aerobic system. Pretreatment shortened the retention time of aerobic digestion, as it was observed that at 5 d, 47% of TSS removal rate was obtained in the alkaline pretreatment and 71% of final TSS removal was achieved.

Shao et al. (2012) examined that the influences of alkaline pretreatment on anaerobic digestion and sludge dewaterability. In the study of Shao et al. (2012), WAS was disintegrated using different pH values (8, 9, 10, 11, 12) with the addition of 4 mol/L NaOH at ambient temperature for 24 h. After anaerobic digestion, a 50.6% and 57.1% of VSS reduction was provided for control and alkaline pretreatment at pH 11, respectively. TSS reduction reached 41.2% for the control and 46.6% for pH 11. As the alkaline dose increased, biogas production increased to 476 mL/g VSS at pH 10. At pH 12, 18.1% decrease in the biogas production was acquired as compared to the control, while biogas production improved by 7.2%-15.4% at pH 9-11, respectively. Sludge dewaterability improved at pH 8 and 9, while it deteriorated at pH 10, 11 and 12. The distribution of 97.9% of proteins and 97.2% of carbohydrates in the inner fractions (TB-EPS and pellet) was carried out for the control, whereas 73.3% of proteins and 91.6% of carbohydrates in these fractions were distributed for the pH 12.

Jin et al. (2009) studied the effects of alkaline sludge disintegration using NaOH in the range of 0.04 to 0.5 mol/L (100-1250 g/kg DS) and $Ca(OH)_2$ in the variations of 0.02 to

0.3 mol/L (92.5-1387.5 g/kg DS). Regarding the results of the solubilization of organic substances, NaOH was more efficient than $Ca(OH)_2$. For aerobic digesters, dissolved oxygen (DO) was arranged as 5 mg/L and air flux in the aerobic digesters was 40 L/h. Without any pretreatment, reactors ran normally from 1 d to 5 d, with ultrasonic pretreatment (7500 kJ/kg DS) being introduced from 6 d to 13 d. Only NaOH pretreatment (0.04 mol/L, 30 min) was used from 14 d to 22 d and with the pH of the pretreated sludge then being adjusted to 7.0. After NaOH (0.5 mol/L) treatment, d10 of sludge particles decreased from 15.77 to 11.87 μm, d90 of that increased from 115.69 to 135.51 μm during 30 min of NaOH treatment, sCOD increased rapidly from 60% to71% and slow SCOD increased in subsequent periods. NaOH dosage of 0.04mol/L in a 30 min NaOH treatment was selected sufficient pretreatment dosage because no further increase of sCOD was observed when the NaOH dosage exceeded 0.04 mol/L. DD was about 30% at 0.04 mol/L NaOH dosage and 30 min treatment duration time. With ultrasonic, alkaline, and combining pretreatments, VSS removal increased from 38.0% to 42.5%, 43.5% and 50.7%, respectively. sCOD, TN, and TP concentrations in the supernatant of the digested sludge increased slightly.

2.4.4. Thermochemical Pretreatment

Dogan and Sanin (2009) assessed the pretreatment methods of alkaline solubilization using NaOH, MW irradiation at 160°C and a combination of pretreatments for WAS. The effects of these methods on COD solubilization, turbidity, and CST were investigated. The sCOD/TCOD ratio of WAS increased from 0.005 (control) to 0.18, 0.27, 0.34, and 0.37 for combined methods of MW and pH 10, 11, 12, and 12.5, respectively. It was observed that 22% improvement of dewaterability was acquired after combined pretreatments (MW+pH 12) compared to the non-pretreated digested sludge. Additionally, the highest total gas and methane production achieved were MW+pH 12 pretreatment with 16.3% and 18.9% improvements over control reactor, respectively.

Stuckey et al. (1978) reported that WAS solubilization was improved by thermochemical pretreatment. A 55% COD solubilization was observed when pretreating at 175°C for 30 min and at pH 12 instead of 48% COD solubilization at 175°C for 30 min without any pH adjustment.

Tanaka et al. (1997) carried out the thermochemical pretreatment on domestic WAS using 0.3 g NaOH/g VSS at 130ºC and for 5 min. For domestic WAS, 70-80% of solubilization rate was found and 45% for combined WAS was observed. In the study, the methane production increased from 35% to 50% for domestic WAS and from 20% to 35% for thermochemical pretreatment of WAS.

Vlyssides et al. (2004) studied the solubilization of WAS at moderate range temperature (50, 60, 70, 80 and 90ºC) and the pH in the alkaline region (at 8, 9, 10, and 11) in anaerobic digestion. Before hydrolysis, TOC, TVS, and TN were obtained as 27.9±0.25%, 68.2±0.35%, and 4.92±0.2%, respectively. At pH>10 and T>80ºC, the COD solubilization increased significantly until the 8th hour of hydrolysis, where about 80% of the solubilization had been achieved. At pH 11 and T 90ºC and after 10 h of hydrolysis, the concentration of the VSS was 6.82%, the VSS reduction reached 45%, at the same time the soluble COD was 70000 mg/L and the total efficiency for methane production 0.28 $LCH_4/g\ VSS_{added}$ when compared with 50ºC temperature and pH 8, the total efficiency (for methane production) was only 0.07 L CH_4/g VSS.

Kim et al. (2003) carried out thermal (121ºC), chemical (7 g/L NaOH), ultrasonic (42 kHz, 120 min) and thermochemical (121ºC, 7 g/L NaOH) pretreatment prior to batch anaerobic digestion. The best results for methane production obtained were with the thermal (3390 LCH_4/m^3WAS) and thermochemical (3367 LCH_4/m^3WAS) pretreatments, followed by the ultrasounds (3007 LCH_4/m^3WAS) and chemical (2827 LCH_4/m^3WAS) pretreatments, the production from raw sludge being equal to (2507 L CH_4/m^3WAS).

Penaud et al. (1999) investigated the influence of NaOH addition during the thermochemical pretreatment of a microbial biomass in terms of COD solubilization and anaerobic biodegradability. In the study, NaOH was added to 600 mL of WAS to reach concentrations ranging from 0 to 26.1 g NaOH/L. The optimal conditions for COD solubilization were 140°C and pH 12 for 30 min. When no NaOH was added, 19.6% COD solubilization was achieved at ambient temperature and 37% at 140°C. When 26.1 g of NaOH/L was added, 75.4% COD solubilization were obtained at ambient temperature and 85.1% at 140°C. Higher NaOH additions (26.1 g/L) at 140°C for 30 min led to 85% COD solubilization instead of 53.2%, which was at ambient temperature. Since the dose of alkali

increased COD solubilization and reduced TS, the COD solubilization and TS elimination reached 63% and 33% when 4.6 g of NaOH/L were added. When no NaOH was added, soluble proteins were equivalent to 4.1 g of COD/L and 33.4 g of COD/L when 26.1 g of NaOH/L were used. A 95.2 mg of NH_4^+-N/L was obtained without NaOH addition and 268.8 mg NH_4^+-N/L with 26.1 g of NaOH/L because NH_4^+-N was released as a consequence of protein solubilization. When 4 to 10 g of NaOH/L was added, biodegradability rates reached 50%. However, biodegradability performances decreased when NaOH concentrations exceeded 10 g of NaOH/L. The highest biodegradability rates were obtained when added sodium concentration was around 4-5 g of NaOH/L.

Qiao et al. (2008) studied the effects of combined microwave and alkaline pretreatment on WAS in terms of both the solubilization of organic matters and the settleability of WAS. The VSS dissolution ratio and SS dissolution ratio increased above 20% and 10%, respectively, with addition of NaOH. The settleability was improved after 1 min microwave treatment (170°C) with alkali (0.2 g NaOH/g TS) pretreatment. Approximately 72% VSS of the mixture of primary and secondary sludge dissolved into aqueous phase at 170°C with 0.2 g NaOH/g SS and 30 min holding time. It can be stated that combined microwave and alkaline pretreatment had a strong effect of on sludge solubilization.

3. MATERIALS AND METHODS

This chapter gives the information on the materials and methods that were used in this experimental analyses.

3.1. Materials

3.1.1. Waste Activated Sludge (WAS) and Inoculum

In this study, WAS sample was periodically supplied from the recirculation line of Kullar Municipal Wastewater Treatment Plant, located in Kocaeli, Turkey. The plant serves a population of 300000 population equivalent (PE) and has a wastewater treatment capacity of 63000 m^3/d. Inoculum sludge was collected from a full scale mesophically operated anaerobic digester of a yeast factory (Pakmaya Manufacturing Plant) in Kocaeli. The collected sludge samples were immediately transferred to the laboratory and stored in a plastic container at 4°C prior to use. WAS and inoculum characteristics were analyzed immediately. Before starting the experimental analysis, all sludge samples were brought to the room temperature and the WAS was concentrated to 1.0-1.5% by gravity settling. The sludge samples to be analyzed were shown in Figure 3.1.



Figure 3.1. Inoculum sludge (left) and WAS (right) taken from the plants.

3.1.2. Chemicals and Instrumentation

All chemicals and instruments used in experimental analyses were given in Table 3.1, Table 3.2, and Table 3.3.

Table 3.1. Liquid chemicals used in the experiments.

Liquid Chemicals	**Formula**	**Source**	**Experiment**
Sulfuric Acid (95-97%)	H_2SO_4	Merck	COD Analysis TKN Digestion TP Digestion Soluble Carbohydrate
Sodium Hydroxide	NaOH	Sigma-Aldrich	Alkaline Pretreatment pH Arrangement
Hydrochloric Acid (37% Puriss)	HCl	Sigma-Aldrich	pH Arrangement
Phosphoric Acid (85.0-88.0%)	H_3PO_4	Sigma-Aldrich	The protection of VFAs samples
Hydrogen Peroxide (30-31%)	H_2O_2	Sigma-Aldrich	TKN and TP Digestion
Liquid Phenol (90% w/w)	C_6H_5OH in H_2O	Sigma-Aldrich	Soluble Carbohydrate

Table 3.2. Solid chemicals used in the experiments.

Solid Chemicals	**Formula**	**Source**	**Experiment**
Total Protein Kit, Micro Lowry, Peterson's Modification	-	Sigma-Aldrich	Soluble Protein
Phenolphthalein Indicator	-	Sigma-Aldrich	NH_4^+-N, TP
Phosphate Buffered Saline	-	Sigma-Aldrich	EPS Extraction
D(+) Glucose Monohdyrate	-	Sigma-Aldrich	Carbohydrate Calibration
Bovine serum albumin (BSA)	-	Sigma-Aldrich	Protein Calibration
Potassium Dichromate	$K_2Cr_2O_7$	Riedel-de Haën	COD Analysis
Potassium Hydrogen Phthalate	KHP	Merck	COD Calibration
Mercury(II) Sulfate	$HgSO_4$	Merck	COD Analysis
Silver Sulfate	Ag_2SO_4	Merck	COD Analysis
Sodium Chloride	NaCl	Sigma-Aldrich	Chloride Analysis
Potassium Chromate	K_2CrO_4	Sigma-Aldrich	Chloride Analysis
Sodium Carbonate	Na_2CO_3	Merck	pH Arrangement in Aerobic Reactors
m-FC Agar Endo-Type Agar m-Enterococcus Agar	-	Acumedia	Microbiology Analysis

Table 3.3. Models and applications of the instrumental equipments used in this study.

Instrumental Equipment	**Model**	**Experimental Analysis**
pH Meter	WTW 3110 Set 2	pH, Temperature
EC meter	WTW LF 320	EC and Salinity
DO Meter	WTW Oxi 330	DO
ORP Probe	WTW 3110 Set 2	ORP
Analytical Balance	Precisa (XB 220A)	TS, VS, MLSS, MLVSS
Drying Oven	Nüve (FN 500)	TS, VS, MLSS, MLVSS,
Muffle Furnace	Protherm	TS, VS, MLSS, MLVSS,
Filtration Apparatus	Schott Duran	MLSS, MLVSS, DOC
Shaker	Nüve (SL 350)	DOC
Centrifuge	Hettich Zentrifugen	sCOD, NH_3-N, NO_2^--N, NO_3^--N, Soluble Protein and Carbohydrate
COD Reactor	Velp Scientifica	TCOD
Spectrophotometer	Hach (DR/2010)	TCOD, sCOD, TKN, TP, NH_3-N, NO_2^--N, NO_3^--N, SO_4^{-2}, Cl^-, Soluble Protein, Soluble Carbohydrate
Digesdahl Digestion	Hach	TKN, TP Digestion
CST Instrument	Type 304M	CST
Viscometer	Brookfield DV-I Prime	Viscosity
Particle Size Analyzer	Malvern Matersizer 2000	Particle Size Distribution
Gas Chromatograph	Agilent HP 5890	Gas Composition
Miligascounter	Ritter MGC 1	Gas Production
Elemental Combustion	Costech	Elemental Analysis (C/N/H_2)
DOC Instrument	Shimadzu TOC-V CSH	DOC
Gas Chromatograph	Perkin Elmer Clarus 600	VFA Analysis
Ultrasonic Homogenizer	Bandelin Sonopuls	Ultrasonic Digestion
Microwave	Berghof MWS-3+	Thermal Digestion
Jar Test Apparatus	F.615	Alkaline Disintegration
Water Bath	Nüve BM402	Provide Constant Temperature of Anaerobic Reactors

3.2. Methods

3.2.1. Sludge Pretreatments

In this study, sludge samples were disintegrated by ultrasonic, microwave and alkaline pretreatments before and after the aerobic and anaerobic digestions.

The disintegration degree (DD) is an important parameter to describe the grade of solubilization of organic matter. The disintegration degrees of the sludge samples were calculated according to the following Equation 3.1 defined by Müller and Pelletier (1998). The maximum sCOD of raw sludge was obtained by adding NaOH concentration (Bougrier et al., 2005).

$$DD_{COD} = \left[\frac{sCOD - sCOD_0}{sCOD_{NaOH} - sCOD_0} \right] x100 \quad (3.1)$$

Where,

DD_{COD} : disintegration degree (%)
sCOD : the COD in the supernatant of the pretreated sludge (mg/L)
$sCOD_0$: the COD in the supernatant of the untreated sludge (mg/L)
$sCOD_{NaOH}$: the COD in the supernatant of the alkaline disintegrated sludge (in the ratio of 1 mol/L NaOH for 24 h at room temperature as described by Bougrier et al., 2005). In this study, $sCOD_{NaOH}$ value was found as 3918 mg/L.

3.2.1.1. Ultrasonic Disintegration. Ultrasonic pretreatment was applied to sludge samples by using a Bandelin-Sonopuls HD 3400 shown in Figure 3.2, having a probe with tip diameter of 25 mm (VS 200 T). The ultrasonicator was operated at a frequency of 20 kHz with a supplied power of about 50-60 Hz. The homogenizator was equipped with a generator (GM 3400), an ultrasonic converter (UW 3400), which converts electrical energy into ultrasound energy, and a booster horn (SH 3425) that contributes the ultrasonic energy to the sludge bulk.

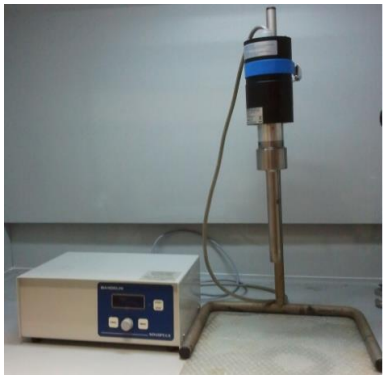

Figure 3.2. The ultrasonic homogenizer used in this study (Bandelin-Sonopuls HD 3400).

Ultrasonic Calibration. To determine the acoustical power entering the ultrasonic system, several methods are available such as calorimetric dosimetry (Toe et al., 2001), acoustical dosimetry (Mason and Peters, 2002), chemical dosimetry (Koda et al., 2003), and electrical power measurement (Boucaud et al., 1999). In this study, the calorimetry-which is also the most common and easiest method-was applied to find the all of the power dissipated into the ultrasonic medium (Teo et al., 2001). The actual power applied to sludge samples were calculated by using Equation 3.3. In literature, the heat capacity of sludge, Cp_{sludge}, is 4.18 J/g/^{o}C (Xu and Lancaster, 2009). In this study, heat capacity of sludge was calculated to be 4.15 J/g/^{o}C by using heat capacity of water (Cp_{water} = 4.186 J/g/^{o}C) and solids in sludge (Cp_{solids}=1.95 J/g/^{o}C) (Equation 3.2). The calculated heat capacity (4.15 J/g/^{o}C) was similar to the value in literature. Therefore, Cp_{sludge} was accepted to be 4.18 J/g/^{o}C.

$$Cp_{sludge} = \text{Water content of sludge} \times Cp_{water} + \text{Water content of solids} \times Cp_{solids} \quad (3.2)$$

$$Cp_{sludge} = 0.988 \times 4.186 \text{ J/g/}^{o}\text{C} + 0.012 \times 1.95 \text{ J/g/}^{o}\text{C} = 4.15 \text{ J/g/}^{o}\text{C}$$

The calorimetric method involves the measurement of the temperature (T) rise versus time (t) with 30 s intervals using a thermocouple situated in 400 mL of deionized water with temperature controlling. The value of dT/dt was estimated from the slope of the curves fitting the best polynomial/straight line or the tangent at time zero. The ultrasonic power dispersed into the system was computed by substituting the value of (dT/dt) at time zero into Equation 3.3. Results were given in the Chapter 4.

$$\text{Power} = \left(\frac{dT}{dt}\right) \text{x } C_p \text{ x } M \tag{3.3}$$

where,

P : the power dissipated in the system (W)

$\left(\frac{dT}{dt}\right)$: the change in temperature at certain time interval (^oC/sec)

C_p : the heat capacity of sludge (4.18 J/g)

M : the mass of water (g)

Specific Energy. The power/energy input for ultrasonic disintegration of sludge can be stated as specific energy. The specific energy (SE) is defined as the energy supplied per unit of mass of sludge solid (as TS) to evaluate of the solubilization and disintegration performance of the sludge (Moonkhum, 2007; Fernández-Cegrí et al., 2012). SE is a function the product of the ultrasonic power, initial TS concentration, ultrasonic time and volume of sonicated sludge, which are crucial parameters affecting the ultrasonic disintegration (Akin, 2008). It is calculated using by Equation 3.4 (Bougrier et al., 2005; Salsabil et al., 2010; Park et al., 2012; Braguglia et al., 2012)**:**

$$\text{SE (kJ/kg TS)} = \frac{\text{P x T}}{\text{TS}_0 \text{ x V}} \tag{3.4}$$

where,

SE : the specific energy input (kJ/kg TS)

P : the ultrasonic power (kW)

T : the ultrasonic time input (s)

V : the volume of sonicated sludge (L)

TS_0 : the solids concentration (kg/L)

In the optimization studies of ultrasonic applications, 400 mL of WAS at ambient temperature was placed in a 500 mL glass beaker and submerged in an ice bath and mixed

well prior to ultrasonication. The sludge temperatures were maintained below 30°C during the experiments in order to minimize the increment of sludge temperature due to the thermal effect of the cavitation phenomenon. The ultrasonic probe was always situated in such a way that it was immersed into the sludge at a depth 2 cm during tests in order to prevent the mixing of air into the sludge samples. The optimization studies of ultrasonic applications were explained in the Chapter 4 in detail.

3.2.1.2. Microwave (MW) Disintegration. MW disintegration was performed by using Berghof, MWS-3+ Digestion System (Figure 3.3) having 10 teflon vessels with a capacity of 60 mL. The system works with a maximum power supply of 50-60 Hz, the power output of 1450 W, the maximum temperature of 300°C, the maximum pressure of 150 bars and the frequency of 2460 MHz. Five staged temperature profiles of MW program were adjusted by using a digital screen of the MW digestion system. 30 mL of WAS was irradiated in each vessel with a total 400 mL of WAS.



Figure 3.3. Berghof, MWS-3+ digestion system.

3.2.1.3. Alkaline Disintegration. Alkaline pretreatment was performed at pH 10, 11, 12. The pH of the samples were adjusted to 10, 11 and 12 by with adding the alkaline agents, 2 N NaOH, into 400 mL of WAS samples in 500 mL Low Form Griffin Beakers. The sludge samples were mixed homogenously by using a magnetic stirrer. NaOH treatment is more efficient than treatments using other chemicals such as KOH and $Ca(OH)_2$ in solubilization of WAS (Park et al., 2012; Li et al., 2008). The alkaline pretreatment was applied to the sludge samples for 2, 6, 12 and 24 h at 150 rpm. NaOH addition into the WAS samples was continued throughout alkaline pretreatment in order to keep the pH of the sludge constant at the desired pH value. The pH of the WAS drops slightly with time during the

alkaline pretreatment. The fluctuations of the desired pH values were held within ±0.1. The pH values of the pretreated sludge samples were neutralized to pH 7 adding 2N HCl in order to provide suitable conditions for anaerobic digestion. The adjusted NaOH doses and the pH of the sludge samples before and after the alkaline pretreatment were shown in Table 3.4.

Table 3.4. Initial and final pH values of WAS and adjusted NaOH concentrations.

Pretreatment Time (h)	Initial pH	Final pH	The Dose of NaOH adjusted (mL/400 mL WAS)
2	6.62	10	2.5
	6.74	11	3.4
	6.62	12	5.9
6	6.62	10	2.6
	6.61	11	3.9
	6.65	12	6.3
12	6.62	10	2.4
	6.63	11	3.3
	6.64	12	6.2
24	6.63	10	2.5
	6.60	11	3.5
	6.59	12	6.3

3.2.1.4. Combined Microwave - Alkaline Disintegration. In the combination microwave and alkaline pretreatments (MW+Alk.), 400 mL of microwave pretreated WAS in the optimum conditions (175°C, 30 bar, and 30 min) was exposed to alkaline pretreatment in the optimum alkaline condition of pH 12 for 2 h at 150 rpm.

3.2.2. Anaerobic Reactors

The ten borosilicate (amber colored) glass bottles having volume of 2.5 L sealed with two-hole rigid plastic covers were used as anaerobic batch reactors. One port in the rigid plastic cover was connected to Miligascounter® (MGC) by a PVC hose with 7 mm inlet and 10 mm outlet diameter to measure the volume of biogas produced and the other port capped by a small rubber septa used as a biogas sampling port. At predetermined time intervals, biogas samples were collected manually using a syringe from this port to

determine the gas composition. The anaerobic reactors used in this study were shown in Figure 3.4.



Figure 3.4. Anaerobic reactors used in this study.

The working volume in the reactors was 1.6 L and the remaining volume of 0.9 L was left empty for gas collection. Prior to anaerobic digestion, TS of the sludge samples were adjusted to 6.5% by centrifugation. The inoculum (seed sludge) to substrate ratios (ISR) was adjusted to be 1:1 on weight basis. The control reactor contained just inoculum and untreated WAS sample. The other reactors contained inoculum, pretreated and thickened WAS. The contents of the reactors were given in Table 3.5.

The pH of reactors was adjusted to 7.0-7.5 by the addition of 1N KOH and H_2SO_4 to make suitable conditions for methanogens. Then, the lids and tubing connections completely sealed with silicon to provide anaerobic conditions in the reactors. The headspaces in the reactors were purged with pure nitrogen gas for 5 min in order to eliminate oxygen from the system. Then, the port was immediately sealed with rubber stoppers. The reactors were incubated at 37 ± 1°C in a water bath (Nüve BM402) under mesophilic conditions for approximately 30 d in duplicate. The reactors were shaken daily. To avoid sunlight, reactors were covered with aluminum foil. Anaerobic batch reactors were opened at the end of a 30 days of digestion period since the biogas productions were ceased.

Table 3.5. The contents of the anaerobic reactors.

Anaerobic Reactor	Pretreatments	Application Conditions	Inoculum (g)	WAS (g)	Thickened WAS (g)
Inoculum	-	-	800	-	-
Control	Unpretreated	-	800	400	400
U-15	Ultrasonic (12000 kJ/kg TS)	200 W 70% Amplitude 15 minutes	800	400	400
U-30	Ultrasonic (24000 kJ/kg TS)	200 W 70% Amplitude 30 minutes	800	400	400
MW	Microwave	175 ºC 30 minutes	800	400	400
Alk.	Alkaline	pH=12 2 h	800	400	400
MW+Alk	Combined Microwave - Alkaline	175 ºC 30 minutes and pH=12, 2 h	800	400	400

3.2.3. Aerobic Reactors

A total of 12 plexiglass aerobic semi-continuous reactors with 10 L of working and 15 L of total volume were operated. Extra head space was provided to avoid overflow of foamed. 10 L of WAS were fed to the reactors. The reactors were covered with removable plexiglass lids to minimize the evaporation. Three reactors were operated as control reactors containing unpretreated sludge samples, while the other nine were fed with ultrasonic, microwave, and alkali pretreated sludge samples. The digesters were aerated by compressed air in order to maintain adequate mixing and dissolved oxygen level. DO level was maintained above 2 mg/L. The reactors were operated at ambient air temperature of 25±2ºC for 30 d. When the pH of the reactors dropped below 6.8, the pH was stabilized at 7+0.2 with the addition of 1N NaOH or sodium bicarbonate ($NaHCO_3$) (as solid forms). When the pH increased above 7, 1N HCl solution was added to decrease the pH back to a level of 7+0.2. Evaporation losses, which were generally small, were not made up in order to improve the sludge volume reduction. Solids adhering to the freeboard of each reactor were carefully returned to the mixed liquor every day. Sludge samples were collected and analyzed periodically for the analyses to evaluate the effect of pretreatments.

Ultrasonication, microwave, and alkaline pretreatments were applied to the 10%, 20%, and 25% of the total volume of WAS samples.



Figure 3.5. Aerobic reactors used in this study.

3.2.4. Sludge Characterization Analyses

The analyses of sludge characteristics were performed according to Standard Methods (American Public Health Association [APHA], 2005) for the Examination of Water and Wastewaters. In this study, most of the analyses in this study were done in triplicates.

3.2.4.1. pH, Oxidation Reduction Potential (ORP) and Temperature. The pH, ORP and temperature measurements were done by using a pH meter (WTW model 3110 Set 2 analyzer).

3.2.4.2. Dissolved Oxygen (DO). DO measurements were carried out by using a WTW Oxi 330 Portable Oximeter.

3.2.4.3. Electrical Conductivity (EC) and Salinity. The measurement of EC and Salinity were performed by using a WTW LF 320 conductivity meter.

3.2.4.4. Alkalinity. Alkalinity of the samples were measured by the titration method (Method 2320) described in Standard Methods (APHA, 2005).

3.2.4.5. Total Solids (TS) and Volatile Solids (VS). TS concentrations of the samples were measured and evaluated according to Standard Methods by using the Method 2540B (APHA, 2005). The VS concentrations of the samples were determined according to Method 2540E (APHA, 2005).

3.2.4.6. Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS). MLSS and MLVSS concentrations were determined in accordance with the Standard Methods by using the Method 2540D and 2540E, respectively (APHA, 2005).

3.2.4.7. Total Chemical Oxygen Demand (TCOD) and Soluble Chemical Oxygen Demand (sCOD). COD analyses were made by using the closed reflux, colorimetric method (Method 5220D) in the Standard Methods (APHA, 2005). For sCOD analysis, the supernatant of sludge was obtained after centrifugation of the sample for 20 minutes at 9000 rpm. The supernatant portion of the sludge samples were digested for 2 h at 150°C after adding sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$) and then the absorbance of the digested samples was determined at 600 nm. Using the potassium hydrogen phthalate (KHP) calibration curve, TCOD and sCOD concentrations are determined. All analyses were performed in three parallel samples. The calibration curve for COD analysis was given in Appendix A., Figure A.1.

3.2.4.8. Dissolved Organic Carbon (DOC). After centrifugation of the sludge samples at 9000 rpm for 20 minutes, the supernatants of the sludge samples were filtered from a Glasfibre Pre-filter paper having 47 mm diameter and first then, a Cellulose Acetate Filter paper having 0.45 µm pore size by using a Filtration Apparatus. After that, samples were diluted not to exceed the detection limits of the Shimadzu TOC-V CSH analyzer. The diluted samples of 25 µL were injected into the TOC analyzer. The combustions of the samples were provided at 680ºC. After a couple of minutes, DOC concentrations of the samples were automatically measured by the analyzer.

3.2.4.9. Total Kjeldahl Nitrogen (TKN). TKN can be described as the sum of the organic nitrogen and ammonia nitrogen (Tchobanoglous et al., 2004). This parameter was analyzed by using Nessler Method (Method 8075), which defined in HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997), subsequent to the digestion of the sample.

Sludge sample was digested at 440°C using a Digesdahl Digestion Apparatus. After digestion of the sample, TKN indicator, KOH solution, Mineral Stabilizer, Polyvinyl Alcohol Dispersing Agent, and Nessler Reagent were used as chemicals. The TKN of the samples was read as mg/L at 460 nm and TKN concentrations were calculated using the formula in Equation 3.5 (Hach, 1997):

$$\mathrm{TKN(mg/L)} = \frac{75 \times A}{B \times C} \qquad (3.5)$$

where,

A : mg/L read from the spectrophotometer
B : mL of sample taken for digestion
C : mL analysis volume of digested sample

3.2.4.10. Total Phosphorous (TP). The same sample that was digested in Digesdahl Digestion Apparatus for TKN measurement was used in the TP experiment. For this analysis, Phosver 3 Ascorbic Acid Method (Method 8048) was performed according to HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997). PhosVer 3 Phosphate Powder Pillow kits were poured into the digested sample in the 10 mL of sample cell (Hach) and TP content of the sample was measured at 880 nm. TP concentrations were calculated using the formula in Equation 3.6 (Hach, 1997):

$$\mathrm{TP(mg/L)} = \frac{A \times 2500}{B \times C} \qquad (3.6)$$

where,

A : mg/L read from the spectrophotometer
B : mL of sample taken for digestion
C : mL analysis volume of digested sample

3.2.4.11. Ammonium Nitrogen (NH_4^+-N). The NH_4^+-N concentrations of the samples were monitored using the Nessler Method (Method 8038) in accordance with HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997). In the analysis, Mineral Stabilizer, Polyvinyl Alcohol Dispersing Agent, and Nessler Reagent were added to the samples and the concentrations were obtained at 425 nm by using HACH DR/2010 Spectrophotometer.

3.2.4.12. Nitrite-Nitrogen (NO_2^--N). This parameter was analyzed according to the Ferrous Sulfate Method (Method 8153) as described in the HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997). Standard NitriVer 3 Nitrite Reagent Powder Pillow kits and 25 mL of sample cells provided by Hach were used in the measurement.

3.2.4.13. Nitrate-Nitrogen (NO_3^--N). According to Cadmium Reduction Method (Method 8039) described in HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997), this analysis was measured by using standard NitraVer 5 Nitrate Reagent Powder Pillow kits and 10 mL of sample cells provided by Hach.

3.2.4.14. Sulfate (SO_4^{2-}). SO_4^{2-} analysis was done according to Method 8051 mentioned in HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997) by using SulfaVer 4 Sulfate Reagent Powder Pillow kits taken from Hach.

3.2.4.15. Chloride (Cl^-). The chloride concentration was analyzed by using TS 4164 ISO 9297- $AgNO_3$ Titration Method.

3.2.4.16. Particle Size Distribution. Particle size analysis was performed by using Malvern Mastersizer 2000 (with the wet dispersion unit of Hydro2000MU). The refractive index of water (used as dispersant liquid) and sludge samples were adjusted to be 1.33 and 1.55, respectively. Obscuration was adjusted to value between 10 - 20% after preliminary studies. The speed of the stirrer and pump was kept at 600 rpm in order to minimize the damage to the sludge particles. The sample was introduced into the water using a Pasteur pipette with as wide an end-opening as possible (Gonze et al., 2003). In order to measure the particle size, sludge sample was slowly added to 800 mL of deionized water, which applied as dispersion tank, in 1000 mL low form beaker. All of the samples were analyzed in triplicate, the average values were reported and the plots of volume (%) vs particle size

(μm) were maintained. The size distribution of particles was measured in terms of cut-off diameters dp10, dp50, and dp90.

3.2.4.17. Viscosity. Viscosity is known as the basic rheological parameter and plays a major role in the increase of biodegradability of sludge (Pham et al., 2010). A plot of shear stress (τ, Pa) as a function of shear rate (γ, s^{-1}) describes the behaviour of sludge flow during wastewater treatment to be characterized. The viscosity (η, Pa s) is calculated by the ratio between the shear stress and the shear rate: [η (Pa s) = τ/γ] (Yang et al., 2009).

In this study, viscosity measurements were performed by using a Brookfield RVDV-I Prime digital viscometer. In order to measure the viscosity of the samples, firstly, the level of the viscometer was adjusted turning the three feet on the bottom of the base by controlling the bubble on the top of head. The viscometer was turned on, pressed a button and autozeroed. The level was again controlled prior to each measurement. The viscosity was obtained using RV Guardleg and four different Spindles #1, 2, 3, or 4 (namely S01, S02, S03, or S04) of the RV spindle set for 400 mL of sludge samples in 600 mL Low Form Griffin Beaker. In the experiments, 100 rpm was used as the operating speed. All measurements were done at ambient temperature of 25±1°C. All samples were measured in triplicate.

3.2.4.18. Capillary Suction Time (CST). CST test was conducted to examine and compare dewaterabilities of sludges by using Type 304 M Triton Electronics Capillary Suction Time Apparatus. The CST apparatus (Figure 3.6) consists of a cylindrical sludge column, which is centered in the middle of two concentric electrodes at diameter DI (inner electrode), and D2 (outer electrode), resting on CST filter paper. The water released from the sludge and spreads out onto the filter paper by the capillary suction pressure of the paper underneath. When the waterfront reaches D1, CST gives an alarm, then, time measurement starts. When the wet front reaches to D2, the time count stops. The time required for water to travel between two concentric electrodes on the filter paper is called as CST (Bougrier et al., 2007; Moonkhum, 2007) Lower time, means a shorter CST and better dewaterability of sludge (Vesilind, 1988). Filterability constant (X) was calculated by using Equation 3.7 developed by Vesilind (1988).

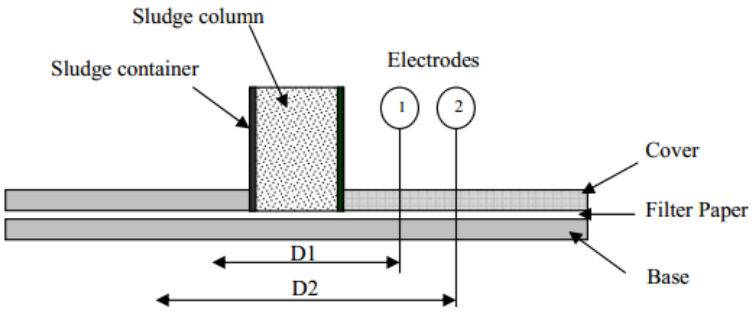

Figure 3.6. Schematic diagram of CST device (Moonkhum, 2007).

$$X = \left[\left(D_2^{\,2} - D_1^{\,2}\right)\left(\frac{\pi.d}{A.P}\right)\right]\left[\left(\frac{\mu.C}{t}\right)\right] \quad \Longrightarrow \quad X = \phi\left[\left(\frac{\mu.C}{t}\right)\right] \tag{3.7}$$

where,

X : filterability constant ($kg^2/s^2.m^4$)
D1, D2 : diameters of sensor locations 1 and 2 (m)
d : filter paper depth (m)
A : area of the bottom of the collar (m^2)
P : capillary pressure of filter paper (m)
μ : viscosity of filtrate (N/m^2)
C : solids concentration of sludge (kg/m^3)
t : capillary suction time, CST (s)
ø : a dimensionless instrument constant (0.118)

3.2.4.19. Sludge Volume Index (SVI). In order to determine the SVI of sludge, the sludge mixed liquor was allowed to settle for 30 minutes in a 1 L graduated cylinder described in the Standard Methods (APHA, 2005) according to the Method 2710D and then, settled sludge volume was measured from this graduated cylinder. SVI was calculated by using Equation 3.8:

$$SVI = \frac{\text{Settled sludge volume (ml/L)}}{\text{Suspended solids (mg/L)}} \text{x} 1000 \tag{3.8}$$

3.2.4.20. Microbiology Analysis. For the microbiologic observation, total coliform (9222B), fecal coliform (9222D) and fecal streptococci (9230C) bacteria in the sludge samples were investigated with Membrane Filtration Method described in the Standard Methods (APHA, 2005). Total coliforms were calculated by Equation 3.9. Fecal coliforms were calculated by Equation 3.10. The amounts of fecal streptococci were computed as above equation used in the calculation of total coliforms.

$$\text{Total coliforms}/100\,\text{mL} = \frac{\text{coliform colonies counted x}\,100}{\text{mL sample filtered}} \tag{3.9}$$

$$\text{Fecal coliforms /g dry weight} = \frac{\text{colonies counted}}{(\text{dilution chosen})\,\text{x}\,(\text{dry solid \%})} \tag{3.10}$$

3.2.4.21. Volatile Fatty Acids (VFAs). A Perkin Elmer Clarus 600 Gas Chromatograph with a flame ionization detector (FID) and an ID DF column (30 m x 0.32 mm x 0.25 µm) was used to determine the concentrations of VFAs as mmolar. Maximum temperature of column was 260°C and the amount of sample injected was 2 µL. The set point and maximum temperature of the oven was 100°C and 240°C, respectively. Helium (0.8 mL/min) was applied as a carrier gas in the gas chromatograph.

Prior to measurement, samples were centrifuged at 9000 rpm for 30 minutes in order to get the supernatant parts of sludge samples. Then, soluble forms of samples were filtered using 0.2 µm syringe filter (hydrophilic) provided by Sartorius Stedim Biotech. If necessary, the samples were protected for 2 months by adding 0.2 mL of 10 N Phosphoric Acid (H_3PO_4) into 1.8 mL of sample. After analysis, the concentrations of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and, heptanoic acids were obtained as mmolar.

3.2.4.22. Elemental Analysis. The elemental characterization ($C/N/H_2$) of sludge samples were performed by using the Elemental Analyzer. The C/N ratio is based on total carbon and total nitrogen as a percentage of the total dry mass. It was calculated according to Equation 3.11, which is the most common method for determining the C/N ratio (Rughoonundun et al., 2012).

$$\text{C/N ratio} = \frac{\text{total carbon mass}}{\text{total nitrogen mass}} \tag{3.11}$$

3.2.4.23. Extracellular Polymeric Substances (EPS). The method which was used for extraction of EPS using a Cation Exchange Resin (CER) is similar to the description of Frølund et al. (1996). DOWEX 50*8, 20-50 mesh in the sodium form, provided from Fluka, was used as a strongly acidic CER. Before the extraction process, a washing was performed to remove any EPS from bulk water of sludge. 50 mL of sludge samples was centrifuged at 3000 g for 15 min using centrifuge instrument (Hettich Universal 16 A). By using phosphate buffer solution, supernatants were discarded and sludge pellets were resuspended to their original volume (50 mL). Phosphate buffer solution consisted of 2 mM Na_3PO_4, 4mM NaH_2PO_4, 9 mM NaCl, and 1 mM KCl at pH 7. These samples were transferred to extraction beakers and CER was added to each sample at a dosage of 75 g/g VSS. Then, samples were stirred at 150 rpm for 7 h. In this extraction method, tightly bound EPSs were shifted from sludges to the liquid phase. After the extraction operation, sludge samples were first settled by gravity and then the supernatant was centrifuged at 10000 g for 20 min in order to separate EPS/sludge suspension from CER. The supernatants were again centrifuged twice at the same speed for 10 minutes to remove remaining flocs. These samples were used to determine the soluble EPS of the sludge.

3.2.4.24. Soluble Protein. Soluble protein analysis was performed by using Total Protein Kit, Micro Lowry, Peterson's Modification (Product No: TP 0300-1KT) and Bovine serum albumin (BSA) solution, used as a standard for preparation of the calibration curve. In the procedure, 1 mL of Modified Lowry Reagent Solution was put into 1 mL of supernatant of sludge sample in a glass test tube. After that, solutions were allowed to stand at room temperature for 20 min. With rapid and immediate mixing, 0.5 mL of prepared Folin & Ciocalteu's Phenol Reagent Working Solution was added to the tubes. Samples were allowed to stand for 30 min in order to develop color. The mixture was transferred to micro cuvets and the absorbance readings were obtained at 750 nm. The protein concentrations of the samples were determined from the calibration curve. The calibration curve applied in this study is seen in Appendix A, Figure A.3.

3.2.4.25. Soluble Carbohydrate. Phenol-Sulfuric Acid Method described by Rao and Pattabiraman (1989) was followed to determine soluble carbohydrate concentrations of the samples after extraction. The calibration curve was prepared using D-Glucose as a standard solution.

For the experiment, 2 mL of supernatant of the sludge samples was taken into the glass test tubes. After that, 6 mL of concentrated sulphuric acid were added into the test tubes. They were allowed to cool, then, 0.1 mL of 90% liquid phenol prepared was added into the tubes. Then, all tubes were vortexed and waited for 30 min at room temperature for the formation of yellow-orange color. The procedure was applied for both all samples and the glucose standard as triplicate. Absorbance values of the prepared samples were measured at 480 nm and the concentrations were calculated using the calibration curve. The calibration curve that was used throughout this study is given in Appendix A, Figure A.4.

3.2.4.26. Total Gas Production. Daily gas productions in the anaerobic reactors were measured by using Miligascounters® (Ritter MGC 1, Germany) connected to the reactors.

3.2.4.27. Gas Composition. The composition of biogas produced (O_2%, N_2%, CO%, CO_2%, and CH_4%) in the anaerobic reactors was analyzed three times a week by using Gas Chromatograph (Agilent Technologies 6890N). The calibration of the instrument was implemented using 99.99 % Supelco CH_4 and CO_2 standards and 5% gas mixture. Helium gas was used as the carrier gas (2 mL/min). For the measurement, 5 µL of gas is drawn via a locked syringe (Hamilton) from the port capped by small rubber septa and 2.5 µL of the collected gas is given to air and the latter is immediately injected to GC for gas composition analysis. The theoretical methane yield, the amounts of molecular methane were determined from Equation 3.12 and 3.13, respectively (Nielfa et. al., 2015). The biodegradability was calculated by using Equation 3.14 (Kim et.al., 2015).

$$\text{Theoretical Methane Yield} = \frac{n_{CH_4} R T}{p VS_{added}} \tag{3.12}$$

$$n_{CH_4} = \frac{COD}{64\ g/mole} \tag{3.13}$$

$$\text{Biodegradability}\ (\%) = \frac{\text{Methane Yield}}{\text{Theoretical Methane Yield}} \text{x} 100 \tag{3.14}$$

where,

R : the gas constant (0.082 atm L/mol.K)

T : temperature of the glass bottle (310 K)

p : the atmospheric pressure (1 atm)

VS_{added}: the volatile solids of the substrate (g)

n_{CH4} : the amount of molecular methane (mole)

4. RESULTS AND DISCUSSION

In this chapter, results and evaluations of the experimental studies were given to determine the effects of the ultrasonication, microwave, and alkaline pretreatments on aerobic and anaerobic digestion.

4.1. Preliminary Studies for Sludge Pretreatment Methods

4.1.1. Optimization of Ultrasonic Pretreatment

In order to determine the optimum power output and amplitude in the ultrasonication system, 200 W, 225 W, 250 W and 300 W power output and 40%, 50%, 60%, 70%, 80%, and 90% amplitude values were applied to 400 mL of WAS samples. The actual energy imparted to the sludge among the different pretreatment conditions were given in Table 4.1. The highest actual power (65 W) was obtained in the 200 W power output and 70% amplitude. The optimum amplitude was determined to be 70% for 400 mL of WAS.

Disintegration degree (DD%) is directly proportional to sCOD as can be seen in Table 4.2. Changes in sCOD, CST, viscosity and supernatant turbidity values of the sludge samples with different ultrasonic power output at the same sonication time were given in Table 4.2. DD(%) values increased for ultrasonic power outputs between 60 W and 250 W as can be seen in Table 4.2. The results indicated that the highest DD (20%) was obtained in the 250 W ultrasonic power output. However, 200 W was selected as the best power output to be applied before ultrasonic pretreatment in order to avoid unnecessary loss of energy. As it noted in the study of Grönroos et al. (2005) high ultrasonic power was required due to the fact that disintegration of sludge presupposes high mechanical shear forces caused by jet streams during cavitation bubble implosion.

Table 4.1. Result of ultrasonic calibration for 400 mL sludge volume.

Amplitude (%)	Power Output (W)	Actual Power (W)
40	200	48
	250	50
	300	29
50	200	40
	225	56
	250	32
	300	37
60	200	51
	225	58
	250	62
	300	38
70	**200**	**65**
	225	47
	250	60
	300	60
80	200	47
	225	58
	250	61
	300	60
90	200	55
	225	50
	250	59
	300	42

Table 4.2. sCOD, DD, CST, viscosity, and supernatant turbidity values of ultrasonicated WAS with different power outputs at the same sonication time.

Ultrasonic Power (W)	Application time (min)	sCOD (mg/L)	DD (%)	CST (s)	Viscosity (mPa.s)	Supernatant Turbidity (NTU)
-	-	37.1	-	26	27.7	6
60	15	154	2	43	13.2	650
100	15	543	4	1099	18.5	800
140	15	1187	16	1090	20.8	1330
200	**15**	**1328**	**18**	**1638**	**12.4**	**2068**
225	15	1002	14	1761	14.4	1468
250	15	1410	20	1602	13.2	1805

In order to determine the optimum sonication time for the ultrasonic pretreatment, the sludge samples were sonicated for 10, 15, 20, 30 and 60 min at the 200 W power and 70% amplitude as given in Table 4.3. The results showed that DD% increased with increasing

specific energy (SE). However, the high SE applications are energy intensive. In this study, ultrasonic pretreatment application conditions were selected to be 200 W power output, 70% amplitude and 15 min of application time, giving SE of 12000 kJ/kg TS, considering the energy consumption of the system. For SE value of 12000 kJ/kg TS, DD was found to be 18%. The sludge disintegration degree increased strongly for SE values higher than 12000 kJ/kg TS as shown in Table 4.3. In the anaerobic digestion studies, ultrasonic pretreatment was applied to sludge samples in two different specific energy values of 12000 kJ/kg TS and 24000 kJ/kg to see the effect of the high degree of sludge disintegration on the methane production.

Table 4.3. sCOD, DD, CST, viscosity, and supernatant turbidity values of ultrasonicated WAS with different specific energies.

SE (kJ/kg TS)	Application time (min)	sCOD (mg/L)	DD (%)	CST (s)	Viscosity (mPa.s)	Supernatant Turbidity (NTU)
8000	10	1055	14.2	1136	15.6	1000
12000	**15**	**1328**	**18.0**	**1638**	**12.4**	**2068**
16000	20	2875	39.5	1947	14.0	2370
24000	30	3031	41.6	1046	13.2	3175
48000	60	3843	52.9	1857	11.6	4350

As can be seen in Table 4.3, supernatant turbidity increased strongly with the increasing SE from 8000 to 48000 kJ/kg TS. This increasing supernatant turbidity can be explained with the disintegration of the sludge flocs. The CST values increased with increasing of SE applied to the sludge but, longer sonication times lead to fluctuations in terms of CST. The decrease in the CST after 20 min of sonication was most probably due to the higher SE values. Dewaterability of sludge was seriously deteriorated after the ultrasonic pretreatment. The operational conditions of ultrasonic pretreatment for the anaerobic digestion studies were given in Table 4.4.

Table 4.4. Operational conditions of ultrasonic pretreatment before anaerobic digestion.

Ultrasonic Power and Amplitude	Application time (min)	Initial Temperature (°C)	Final Temperature (°C)	Specific Energy (kJ/ kg TS)
200 W Power	15	20.3	23.6	12000
70% Amplitude	30	22.1	25.1	24000

Table 4.5 showed the changes in the particle size distributions of unpretreated and ultrasonically disintegrated sludge samples. The size of the sludge particles decreased with increasing SE applied to the sludge samples. Applied energy disintegrated the sludge particles and caused to release of extracellular and intracellular materials.

Table 4.5. Particle size of ultrasonicated WAS with different specific energies.

SE (kJ/kg TS)	Surface Weighted Mean D[3.2] µm	Volume Weighted Mean D[4.3] µm	d(0.1) µm	d(0.5) µm	d(0.9) µm
0	39.953	85.607	21.856	64.518	161.709
8000	23.222	73.045	12.946	43.484	138.339
12000	10.139	53.383	5.367	33.951	122.394
16000	8.246	47.094	4.010	25.017	120.080
24000	7.292	44.537	3.733	24.561	115.465
48000	6.636	40.599	2.812	14.079	94.697

4.1.2. Optimization of Microwave (MW) Pretreatment

In the microwave pretreatment, total 400 mL of WAS (30 mL of WAS was irradiated in each vessel with a total 400 mL of WAS) was disintegrated with different temperatures (100, 150, 175, 190°C) and pretreatment times (10, 20 and 30 min) in order to determine the application conditions. The results of sCOD concentration, DD, CST, viscosity, and particle size analysis of the WAS were given in Table 4.6 and Table 4.7.

In table 4.7., the rise of the median diameter, d(0.5), indicated the reflocculation of intracellular and extracellular materials (Bougrier et al., 2005).

Table 4.6. sCOD, DD, CST, and viscosity values of microwave pretreated WAS with different temperatures and pretreatment times.

MW Pretreatment Temperature (°C)	Application time (min)	sCOD (mg/L)	DD (%)	CST (s)	Viscosity (mPa.s)
100	10	1033	16.8	477	31.8
	20	1308	21.4	528	29.6
	30	1625	26.7	492	28.9
150	10	1034	16.9	316	31.7
	20	1325	21.7	286	29.4
	30	1531	25.1	284	29.1
175	10	1588	26.1	334	29.2
	20	1687	27.7	306	28.6
	30	**1801**	**30.1**	**317**	**27.8**
190	10	1417	23.2	406	29.6
	20	1326	21.7	525	28.6
	30	1390	22.8	410	29.6

Table 4.7. Particle size of microwave pretreated WAS with the temperature of 175°C and different pretreatment times.

MW Application time (min)	Surface Weighted Mean D[3.2] µm	Volume Weighted Mean D[4.3] µm	d(0.1) µm	d(0.5) µm	d(0.9) µm
0	32.507	69.099	18.603	53.612	133.748
10	86.094	173.504	50.102	147.851	331.816
30	67.027	145.140	38.547	112.010	261.114

Considering the sludge solubilization and energy consumption, the pretreatment temperature and holding time for the MW irradiation were found to be 30 min and 175°C, respectively, based on the results of MW optimization study. According to the study of Kepp et al. (2000), the highest hydrolysis rates in MW pretreatment were achieved with the temperature range of 165 and 180°C.

4.1.3. Optimization of Alkaline Pretreatment

For the alkaline pretreatment, different pH values (10, 11, and 12) were examined to determine the application conditions. sCOD concentration increased with increasing pH. because the sludge became basic with increasing pH and so hydroxyl anions destroyed floc structures and cell walls with alkaline pretreatment (Rani et al., 2012). pH 12 and 2 h of

pretreatment time at 150 rpm were chosen to be the application conditions of alkaline pretreatment in terms of chemical consumption (Table 4.8 and Table 4.9).

Table 4.8. sCOD, DD, CST, and viscosity values of alkaline pretreated WAS with different pretreatment times.

Alkaline Application time (h)	pH	sCOD (mg/L)	DD (%)	CST (s)	Viscosity (mPa.s)
0	6.62	390.0	-	17.6	8
2	10	792.2	4.25	162.4	32
	11	1618	12.9	461.5	30
	12	**3256**	**30.0**	**992.5**	**36**
6	10	817	4.5	181.4	40
	11	1852	15.4	661.1	32
	12	3990	38.0	1200.4	34
12	10	921	5.6	151.4	36
	11	1905	16.00	590.3	32
	12	4088	39.0	1402.2	30
24	10	678	3.0	142.6	38
	11	2192	19.0	617.1	34
	12	4117	39.3	1670	32

Table 4.9. Particle size of alkaline pretreated WAS with different times.

Alkaline Pretreatment Time (h)	pH	Surface Weighted Mean D[3.2] µm	Volume Weighted Mean D[4.3] µm	d(0.1) µm	d(0.5) µm	d(0.9) µm
0	6.62	34.794	73.044	18.295	57.781	141.734
2	10	32.972	70.213	17.351	56.192	132.949
	11	33.200	73.921	17.578	57.204	135.624
	12	**29.586**	**75.342**	**16.386**	**52.786**	**142.217**
6	10	29.691	66.627	15.597	49.190	122.350
	11	28.211	60.383	16.677	48.840	115.664
	12	28.210	67.134	15.956	51.071	130.765
12	10	29.167	67.134	15.625	50.115	125.714
	11	30.125	67.134	16.712	49.088	121.548
	12	29.687	67.134	16.121	51.116	131.116
24	10	32.139	68.754	16.917	52.877	130.162
	11	28.020	74.939	17.354	54.489	165.577
	12	34.902	63.652	15.985	50.47	128.901

4.2. Sludge Characteristics of WAS and Inoculum

The initial characteristics of the WAS and inoculum were presented in Table 4.10.

4.3. Anaerobic Digestion Experiments

WAS was taken from the recirculation line of the Kullar Municipal wastewater treatment plant in Kocaeli. Inoculum sludge was obtained from a full-scale mesophically operated anaerobic digester of a yeast factory (Pakmaya) in Kocaeli. Ten 2.5 L anaerobic reactors, each having 1.6 L of working volume, were operated. The contents of the anaerobic reactors were given in the previous chapter (Table 3.5). The initial characteristics of the sludge samples used in anaerobic digestion were given in Table 4.10.

Prior to anaerobic digestion, ultrasonic, microwave and alkaline and combined of microwave-alkaline pretreatments were applied to sludge samples. In the anaerobic digestion studies, ultrasonic pretreatment was applied to sludge samples in two different specific energy values of 12000 kJ/kg TS and 24000 kJ/kg TS. The microwave pretreatment was applied to the sludge samples for 30 min at 175 ^{o}C by using Berghoff MWS-3+ digestion system. For the alkaline pretreatment, pH value of the samples increased to 12 for 2 h by the addition of 2 N NaOH. In the combination of microwave and alkaline pretreatments, 400 mL of microwave pretreated sludge at 175 ^{o}C for 30 min were exposed to the alkaline pretreatment. Disintegration degrees of the pretreated sludge samples were given in Table 4.11.

To evaluate the performances of the anaerobic reactors, all parameters were analyzed at the beginning (before setting up the anaerobic reactors) and at the end of 30 days of operation period, except GC analyses, which were conducted three times a week.

Table 4.10. The characteristics of the WAS and inoculum used in the reactors.

Parameter	Unit	WAS	Inoculum
pH	-	7.26	7.51
Temperature	^{o}C	24.6	24.2
EC	mS/cm	2.78	33.8
Salinity	$‰_{o}$	1.3	20
Alkalinity	mg $CaCO_3$/L	1250	2500
ORP	mV	77.6	-390.9
TS	%	1.52	2.5
VS	%	0.99	1.41
VS	%*TS	64.90	56.28
MLSS	mg/L	7700	14220
MLVSS	mg/L	6570	9360
TCOD	mg/L	14360	10420
sCOD	mg/L	62.5	2583
DOC	mg/L	54	1057
DO	mg/L	2.75	-
TKN	mg/L	1013	900
NH_4^+-N	mg/L	375	550
NO_2^--N	mg/L	0	0
NO_3^--N	mg/L	25	250
TP	mg/L	500	200
PO_4^{3-}	mg/L	1600	700
SO_4^{-2}	mg/L	150	350
Cl^-	mg/L	203	418
CST	s	32.3	113.6
Viscosity	mPa.s	34.0	23.2
Total Coliform	cells/mL	$12x10^6$	$6x10^6$
Fecal Coliform	cells/mL	$2.3x10^5$	$2x10^5$
Fecal Streptococci	cells/mL	$1x10^5$	$5.1x10^4$

Table 4.11. Disintegration degrees of the pretreated sludge samples.

Pretreatment	DD (%)
Ultrasonic (15 min, 12000 kJ/kg TS)	18.0
Ultrasonic (30 min, 24000 kJ/kg TS)	41.6
Microwave	30.1
Alkali	30.0

4.3.1. pH and Temperature

pH measures the hydrogen ion concentration of the sludge and indicates whether the sludge is acid or alkaline. The anaerobic digester performance was influenced by pH since methanogenesis only occur at a high pH rate. Anaerobic system is very sensitive to pH changes and it is very important to maintain a pH of 6.5 to 8.2 for a healthy system and promote methane gas formation (Rani et al., 2012). The pH of the anaerobic reactors in this study was measured in the range of 7 to 7.8 during the anaerobic digestion. In all reactors, the pH values were in adequate values for methanogenic microorganisms and a potentially well-balanced anaerobic digestion process was provided. The results of the initial and final pH of the all reactors were shown in Figure 4.1.

CO_2 concentration in the gas phase and HCO_3^- alkalinity of the liquid phase controls the pH of the system. If the CO_2 concentration in the gas phase remains constant, the possible addition of HCO_3^- alkalinity cause to increase the pH values in the anaerobic reactors (Rani et al., 2012). After anaerobic stabilization, the increase in the pH of the sludge samples could be related with bicarbonate buffering system supporting the growth of methanogenic bacteria.

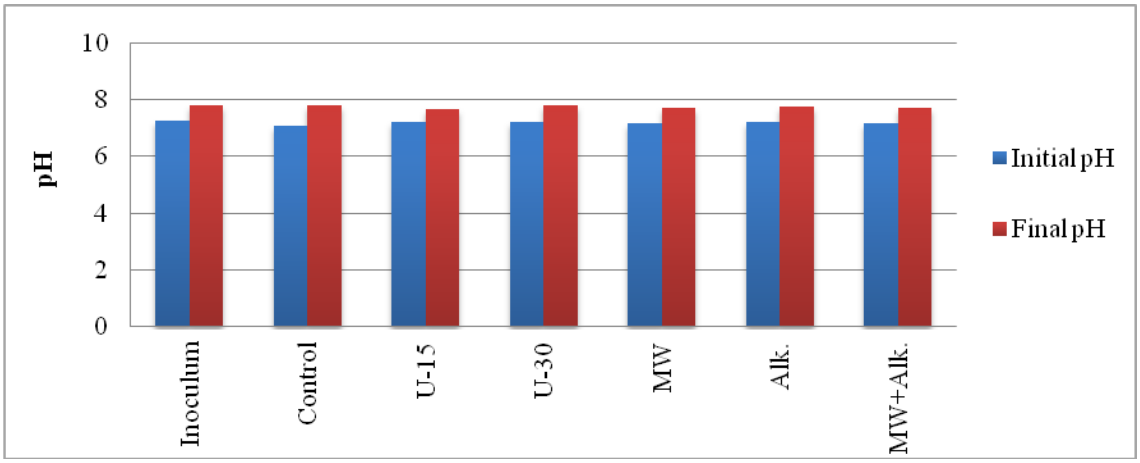

Figure 4.1. pH change in the anaerobic reactors.

The temperature was kept at 37ºC during the anaerobic digestion. After opening the anaerobic reactors, the final temperatures in the reactors were measured to be 37±2ºC. The temperature has a significant effect on the growth rate and metabolism of microorganisms

in the anaerobic reactors because methanogens are one of the most sensitive groups to fluctuations in temperature (Appel et al., 2008).

4.3.2. Oxidation Reduction Potential

Oxidation reduction potential (ORP) could be used to measure very low oxygen concentrations. ORP has been used extensively to monitor and control anaerobic proces in terms of methane production (Ishizaki et. al., 1974). ORP values of the sludge samples in the reactors at the beginning and ending of anaerobic digestion were shown in Figure 4.2. Methane production was found to be optimized at ORP levels between -500 and -520 mV (Koch and Oldham, 1985). In this study, the ORP values higher than -350 mV were obtained for the anaerobic reactors.

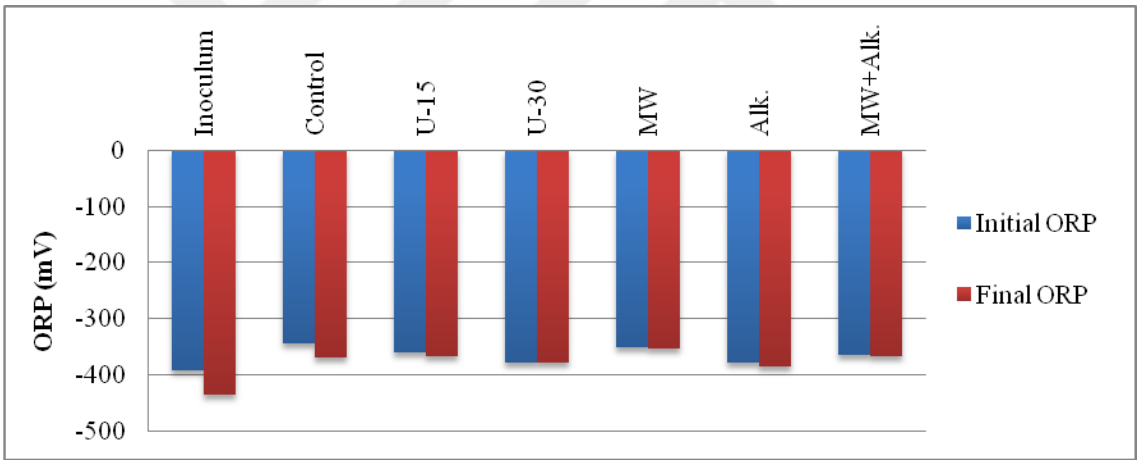

Figure 4.2. ORP change in the anaerobic reactors.

4.3.3. Electrical Conductivity and Salinity

Electrical conductivity **(**EC) is a numerical expression of the ability of an aqueous solution to carry an electrical current (Reid et al., 2006). EC and salinity data of the sludge samples in the anaerobic reactors at the beginning and end of the digestion were presented in Figures 4.3. and 4.4. The trend of EC and salinity in the anaerobic reactors were similar as expected. At the beginning of the anaerobic digestion, EC and salinity values in the reactors containing pretreated sludge samples were same or a bit higher/lower than control reactor. Initially, no important changes were seen in the EC and salinity values with the

application of pretreatments. At the end of the anaerobic digestion, the EC and salinity data of the sludge samples in the reactors increased, almost doubled, from 19 to 50 mS/cm and 11 to 28 ‰, respectively, which might be related to sludge samples after anaerobic digestion contains higher ion concentration (conduct electrical current) than before digestion.



Figure 4.3. EC change in the anaerobic reactors.



Figure 4.4. Salinity change in the anaerobic reactors.

4.3.4. Alkalinity

In an anaerobic digester, alkalinity is always required to avoid a drop in the pH and to maintain a stable and a safe process (Nah et al., 2000). In order to maintain an optimum methanogenesis, an alkalinity of at least 2000 mg $CaCO_3$/L would be needed for an anaerobic system (Farquhar and Rovers, 1973). The initial alkalinity concentrations of the

sludge samples in the reactors were varied between 2500 and 12500 mg $CaCO_3$/L as shown in Figure 4.5. These results were in accordance with the data stated by Farquhar and Rovers, (1973). The increase of alkalinity concentration was normally due to the activity of the methanogenic bacteria, which could produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate.

Anaerobic digester stability was enhanced by a high alkalinity concentration (Yunqin et al., 2009). The highest increase in the alkalinity (64%) was observed in the reactor containing sludge sample pretreated with the combined microwave and alkaline pretreatment. Increase in alkalinity concentrations were measured to be 8%, 44% and 48% in the control, microwave pretreated and alkaline pretreated reactors, respectively.

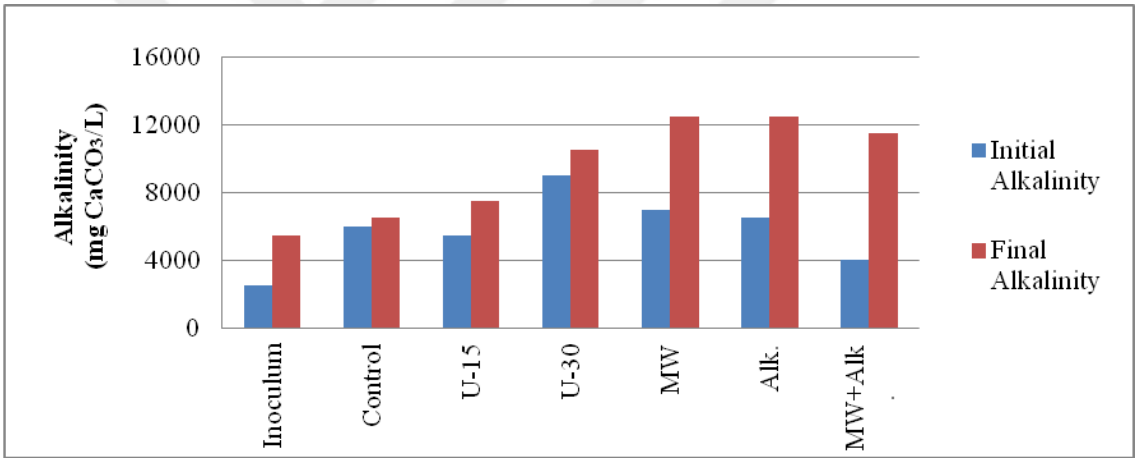

Figure 4.5. Alkalinity concentration change in the anaerobic reactors.

4.3.5. Total Solids and Volatile Solids

Total solids (TS) and volatile solids (VS) concentrations were analyzed for the anaerobic reactors in order to determine the organic matter of the reactors prior to anaerobic digestion and calculate the removal efficiencies as a result of the biological degradation. Initial TS concentrations of the anaerobic reactors changed between 6 - 7 %. The initial VS/TS ratio of the sludge samples were about 60%, little bit lower than the typical VS/TS ratio of 75-80%. TS and VS concentrations of all anaerobic reactors decreased at the end of the anaerobic digestion as a consequence of biodegradability of the

sludge. The results of TS and VS concentrations and the removal efficiencies were given in Figures 4.6 and 4.7.

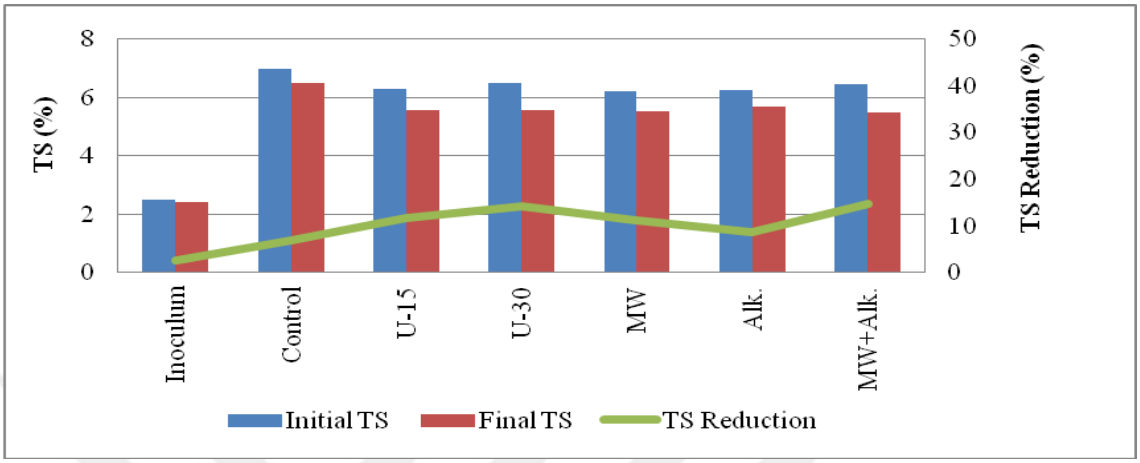

Figure 4.6. TS concentrations and TS removal efficiencies in the anaerobic reactors.

The highest TS removal efficiency of 15% was achieved in the combined microwave-alkaline pretreatment. The value of TS reduction for the combined microwave-alkaline pretreatment was similar to ultrasonic pretreatment at specific energy of 24000 kJ/kg TS (14%). Among the pretreatment methods, the lowest TS removal efficiency of 9% was obtained in the alkaline pretreatment. The similar TS reduction of 11% and 12% was observed in the microwave and ultrasonically pretreated sludge (at specific energy of 12000 kJ/kg TS), respectively. The TS removal efficiency of the reactors were little bit lower than expected. Relatively low VS/TS ratio of the sludge explains these low TS removal percentages.

VS degradation is one of the most important parameters in order to calculate biogas yield and methane yield and evaluate the performance of an anaerobic digestion system (Chi et al., 2011). VS reductions of the anaerobic reactors containing pretreated and unpretreated sludges were obtained in the range of 17-26%. The highest VS removal efficiency of 26% was observed in the combined microwave-alkaline pretreatment. Besides, ultrasonic pretreatment at specific energy of 24000 kJ/kg TS (25% VS reduction) indicated better performance on VS removal efficiency when compared to the microwave pretreatment (22% VS reduction), ultrasonic pretreatment at specific energy of 12000 kJ/kg TS (21% VS reduction) and alkaline pretreatment (20% VS reduction). The results

are compatible with the literature. In the study of Dogan and Sanin (2009), VS reduction in the control reactors was observed as 23.2±1.3%, while it was 25.7±0.8% for the reactors containing microwave pretreated sludge. Park et al. (2004) observed 25.7% higher VS reduction in the anaerobic reactors containing microwave pretreated sludge as compared to the control reactor (23.2%).

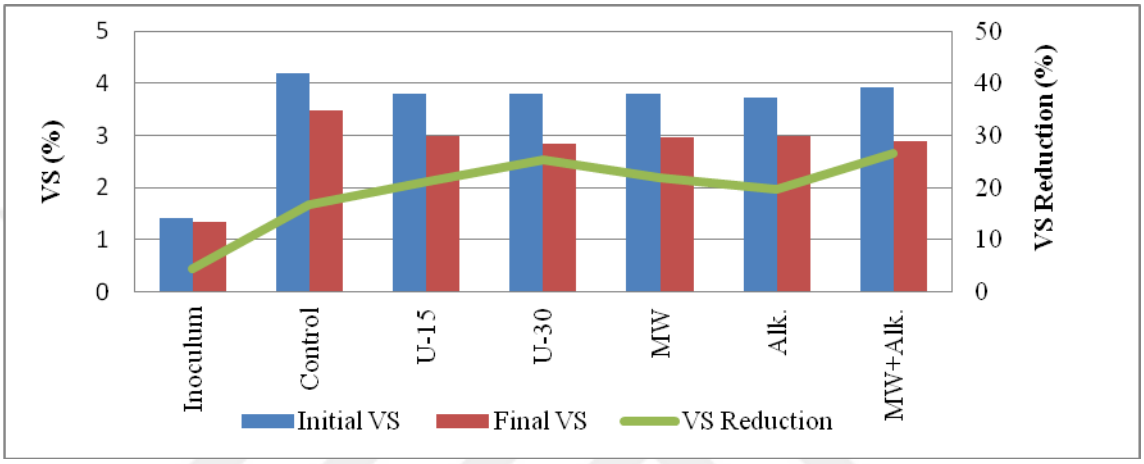

Figure 4.7. VS concentrations and VS removal efficiencies in the anaerobic reactors.

TS and VS removal efficiencies in all anaerobic reactors were higher than the control reactor; however, extreme differences in these values were not observed. It can be said that the organic compounds transferred by ultrasonic, microwave and alkaline pretreatments from the sludge solids into the aqueous phase were quickly biodegradable as compared to the control reactor.

The ratio of VS/TS in the anaerobic reactors decreased from 0.60 on average for control reactor including unpretreated sludge to 0.54 on average for sludge pretreated with ultrasound, microwave, alkaline and combined of microwave-alkaline. This decrease was stated in the study of Bougrier et al. (2006) that particular fraction of sludge became more mineral.

4.3.6. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations of inoculum at the end of the anaerobic digestion decreased from

14220 to 9560 mg/L and from 9360 to 7065 mg/L, respectively, as shown in Figure 4.8. MLVSS reduction indicated that some fraction of the organic phase was solubilized MLSS and MLVSS during anaerobic digestion. MLSS and MLVSS removal efficiencies were achieved to be 32.7% and 25% in inoculum after anaerobic digestion, respectively, as given Figure 4.8. MLSS and MLVSS concentrations could not be measured for the anaerobic reactors containing unpretreated and pretreated sludge because sludge samples having high solids content of 65% were impossible to be filtered in the filtration apparatus.

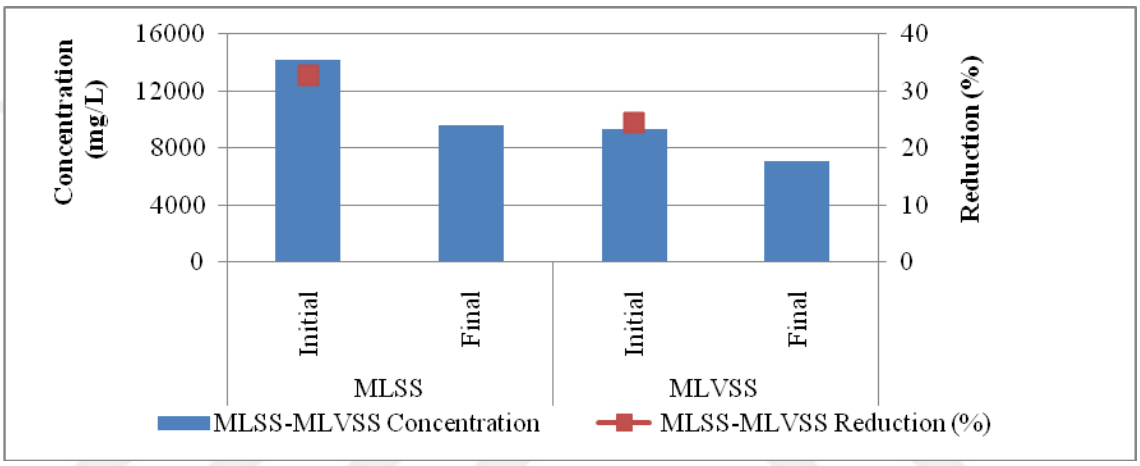

Figure 4.8. MLSS and MLVSS changes in the inoculum.

4.3.7. Total Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

Chemical oxygen demand (COD) is a chemical parameter which is commonly used to characterize organic compounds in the substrate and indicates of the sludge biodegradability. Soluble chemical oxygen demand (sCOD) is the main parameter to demonstrate the degree of hydrolysis and represents the maximum level of sludge solubilization. As sCOD increased, the substance is readily biodegradable to generate methane during anaerobic digestion (Rani et al., 2012).

TCOD and sCOD concentrations of the sludge samples were calculated to determine the degree of sludge hydrolysis and solubilization. Figures 4.9 and 4.10 showed that TCOD and sCOD concentrations in the reactors and removal efficiencies of the reactors before and after the anaerobic digestion.

TCOD removal efficiencies of all pretreated reactors were in the range of 34.3-70.2%. The highest TCOD removal of 70.2% was obtained in the reactor containing combined microwave-alkaline pretreated sludge samples. On the other hand, the lowest TCOD removal efficiency of 34.3% was achieved in the reactor having ultrasonically pretreated sludge at specific energy of 12000 kJ/kg TS. Ultrasonic (at specific energy of 24000 kJ/kg TS), microwave and alkaline pretreatments showed quite similar results in terms of TCOD removal efficiencies such as 65.8%, 69.5%, 68.7%, respectively.

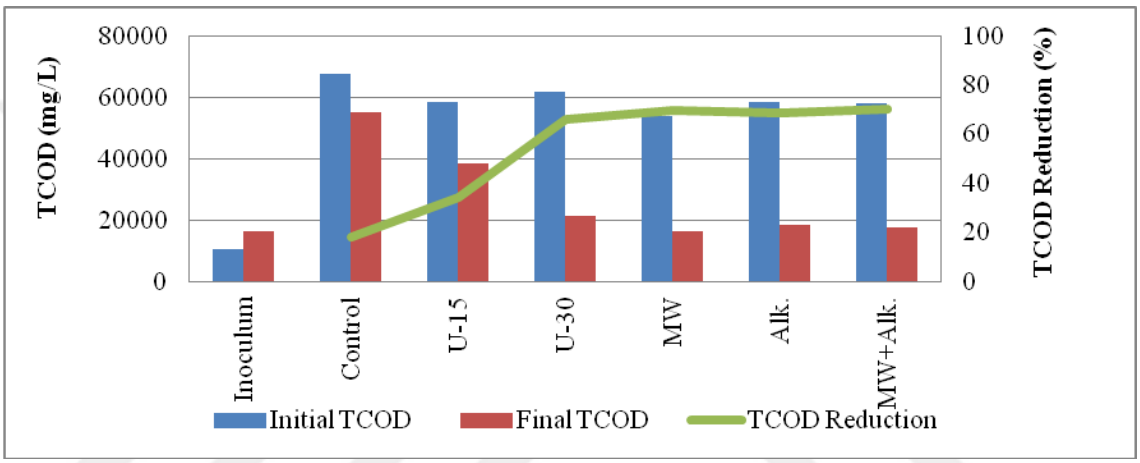

Figure 4.9. TCOD concentrations and TCOD reductions in the anaerobic reactors.

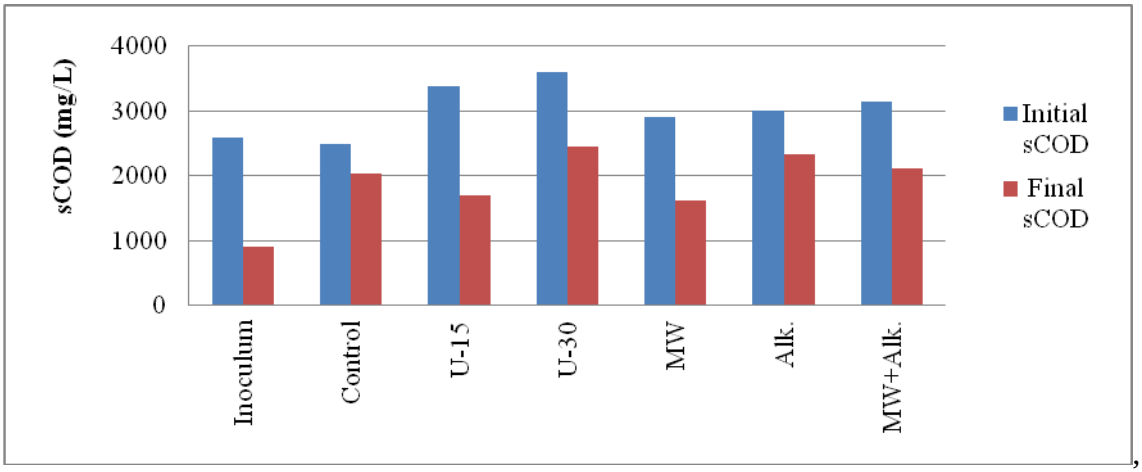

Figure 4.10. sCOD concentrations in the anaerobic reactors.

Pretreatment increased the sCOD concentrations of the sludge samples in the reactors before anaerobic digestion. The reason of these increases in the sCOD concentrations after disintegration was atributed to that intracellular organic material was released to the liquid

phase due to the disruption of chemical bonds in cell walls (Appels et al., 2008; Örmeci and Ahmad, 2009).

A maximum of an approximate 24% increase was achieved in sCOD with the combined pretreatment of microwave and alkaline (Dogan and Sanin, 2009). In this study, the result of sCOD removal in the combined microwave-alkaline pretreatment (32.4%) was in agreement with the findings of Dogan and Sanin, (2009).

Pretreated sludge samples showed important increases in the ratios of sCOD/TCOD when compared to the unpretreated sludge samples. The significant increase in the sCOD/TCOD ratio with all pretreatments used in this study may have resulted from the solubilization of colloidal matters. Prior to anaerobic digestion, the sCOD/TCOD ratio (COD solubilization) of control reactor containing unpretreated sludge sample found to be 3.7%, little bit higher than the expected value. The sCOD/TCOD ratio increased due to the proceeding fermentation during the transfer of sludge samples to the laboratory. Prior to anaerobic digestion, the sCOD/TCOD ratios in the reactors containing ultrasonically pretreated at specific energies of 12000 kJ/kg TS and 24000 kJ/kg TS, alkaline and combined microwave-alkaline pretreated sludge samples were found to be 5.8%, 5.84%, 5.13%, and 5.39%, respectively. At the end of the anaerobic digestion, the sCOD/TCOD ratios increased to 6.2%, 11.6%, 12.7%, and 12.2% with the application of ultrasonic at specific energy of 12000 kJ/kg TS and 24000 kJ/kg TS, alkaline and combined microwave-alkaline pretreatments, respectively. Eskicioglu et al. (2008) reported that sCOD/TCOD was increased from 6% (control) to 15% with MW pretreatment at 96°C. Eskicioglu et al. (2009) found that sCOD/TCOD ratios of 35 + 1% at microwave pretreatment temperatures of 175°C. In this study, sCOD/TCOD ratio increased from 5.41% to 10% for the microwave pretreatment at 175°C. These findings were in accordance with the literature.

4.3.8. Dissolved Organic Carbon

The dissolved organic carbon (DOC) concentrations in all reactors and removal efficiencies of all reactors before and after anaerobic digestion were given in Figure 4.11.

Before anaerobic digestion, similar to sCOD values, the DOC concentrations of all reactors increased with the application of pretreatments used in this study. After anaerobic digestion, the DOC concentrations decreased and higher reductions (87% and 83%) were observed in the reactors containing alkaline pretreated sludge and ultrasonically pretreated sludge at specific energy of 24000 kJ/kg TS, respectively. The higher removal efficiency results indicated that an important fraction of organic matter was hydrolyzed and dissolved in solution. The DOC removal efficiency in the control reactor was found to be 33%. With the application of ultrasonic pretreatment at specific energy of 12000 kJ/kg TS (U-15), the DOC removal efficiency reached to 50.4%, which was much higher than that of microwave (44%) and alkaline pretreatments (40%).

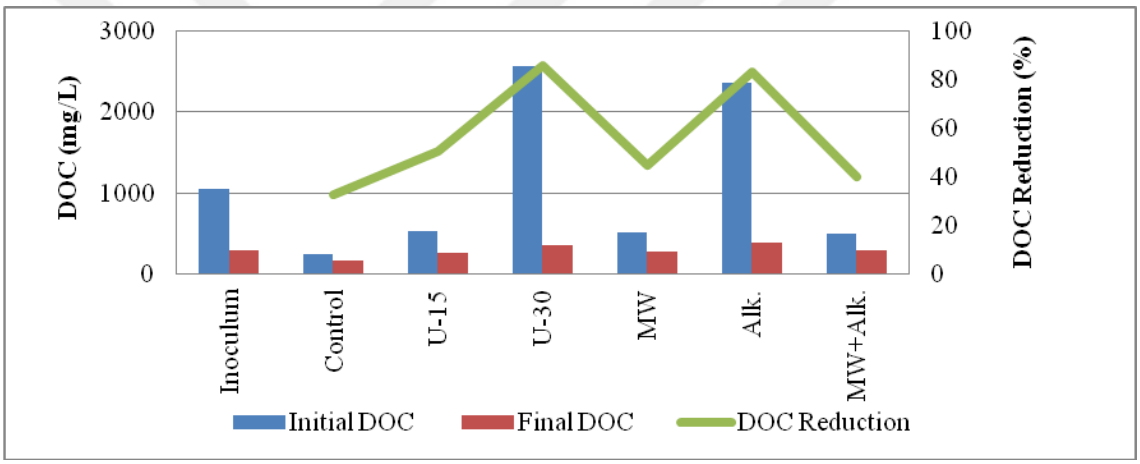

Figure 4.11. DOC concentrations and DOC reductions in the anaerobic reactors.

4.3.9. Total Kjeldahl Nitrogen and Ammonium Nitrogen

Total kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4^+-N) concentrations of the sludge samples in all reactors before and after anaerobic digestion were shown in Figure 4.12 and Figure 4.13, respectively.

TKN is the sum of the organic nitrogen and ammonia nitrogen. Organic nitrogen, proteinaceous materials, is solubilized from particulate organics in sludge by hydrolysis and some of the solubilized organic nitrogen is likely to be degraded. (Kim and Novak, 2011).

Application of pretreatments increased TKN concentrations of the sludge samples due to hydrolysis. At the end of the anaerobic digestion, TKN concentrations of the sludge samples in the reactors decreased and ammonia concentrations increased due to the rapid decomposition of organic material containing nitrogen.

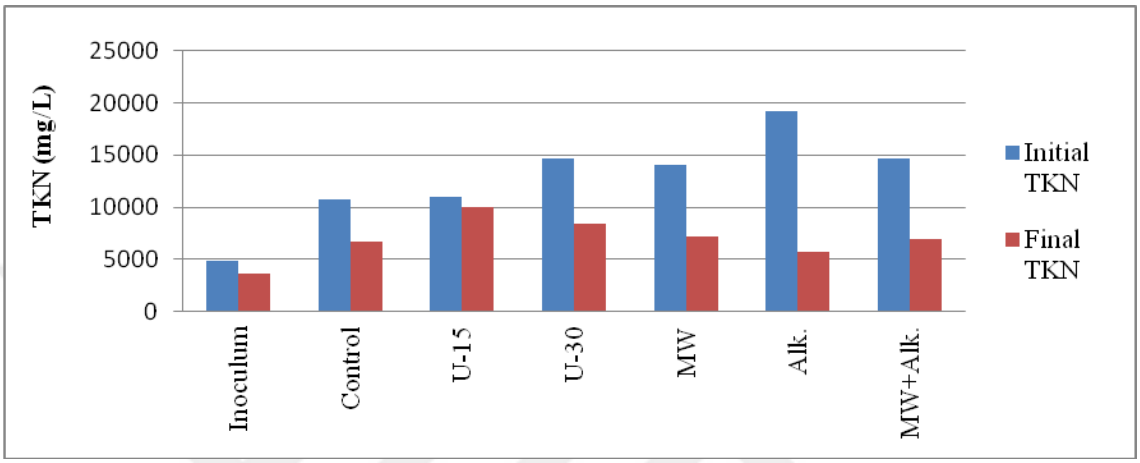

Figure 4.12. TKN changes in the anaerobic reactors.

Ammonia within the anaerobic digestion process can be in two forms of inorganic nitrogen such as ammonium nitrogen (NH_4^+-N) and dissolved ammonia gas (NH_3) (Akin, 2008). NH_4^+-N provides a buffer capacity in an anaerobic reactor, so stabilizes the pH value in the reactor with concentrations of up to 1000 mg/L. Acidogens were hardly affected and methanogens lost 56.1% of their activity in the range of 4000 and 6000 mg NH_4^+-N/L (Fricke et al., 2007). The maximum safe limit for the ammonium nitrogen was 3000 mg/L (Chi et al., 2011).

After anaerobic digestion, NH_4^+-N in the aqueous phase increased because bacterial cells were disintegrated, releasing intracellular organic nitrogen into the aqueous phase, which was subsequently hydrolyzed to ammonia. In this study, NH_4^+-N concentrations of the sludge samples in the reactors was between 2200 and 2662 mg/L at the end of the anaerobic digestion, this indicated that the amount of NH_4^+-N was within a safe level and the methanogens play active role to produce methane gas.

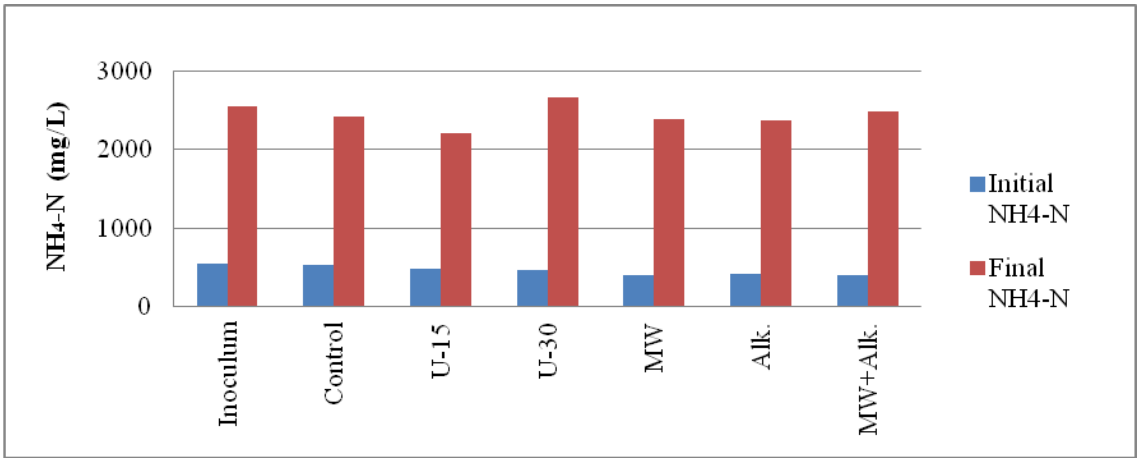

Figure 4.13. NH_4^+-N changes in the anaerobic reactors.

4.3.10. Total Phosphorus

Total phosphorus (TP) concentrations of the sludge samples in the anaerobic reactors were given in Figure 4.14. TP was one of the necessary nutrients in the anaerobic degradation for growth and performance of the microbial population due to the synthesizing energy-storage compounds as well as RNA and DNA. As can be seen from Figure 4.14, TP concentrations increased with the pretreatment applications before anaerobic digestion and improved at the end of the anaerobic digestion because of the phosphorus solubilization by hydrolysis. The increase of TP concentrations in the reactors containing ultrasonically (at specific energy of 24000 kJ/kg TS), alkaline and combined microwave - alkaline pretreated sludge samples were higher than the control reactor having unpretreated sludge.

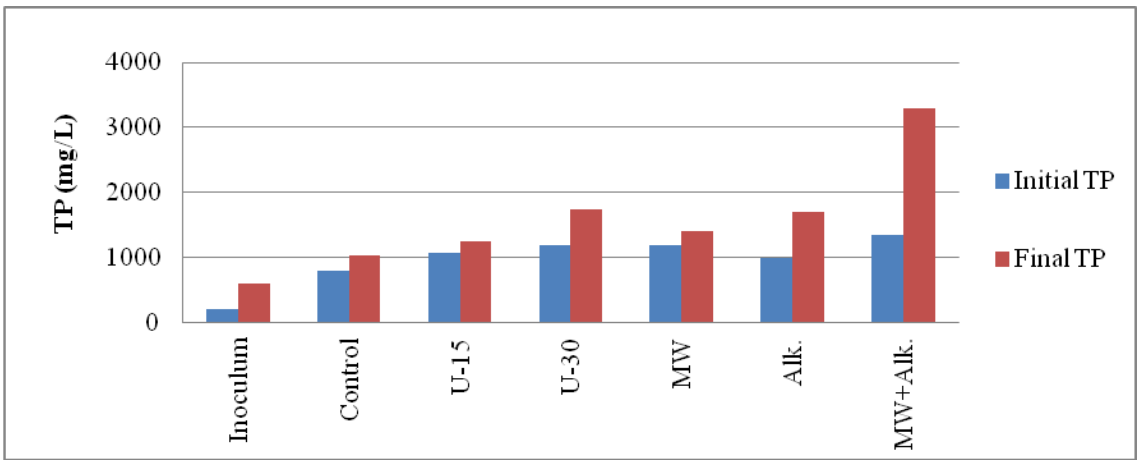

Figure 4.14. TP changes in the anaerobic reactors.

4.3.11. Sulfate and Chloride

Sulfate (SO_4^{2-}) and chloride (Cl^-) concentrations of the sludge samples in the anaerobic reactors before and after anaerobic digestion were demonstrated in Figure 4.15 and Figure 4.16., respectively. Sulfate is used as an electron acceptor in the oxidation of an energy substrate and reduced to hydrogen sulfide by sulfate reducing bacteria in an anaerobic digestion process. Methanogenic microorganisms can be influenced by the presence of the high levels of free-hydrogen sulfide (Appel et al., 2008). The sludge samples had low sulfate concentrations, less than 100 mg/L. No inhibition of sulfate reduction was observed.

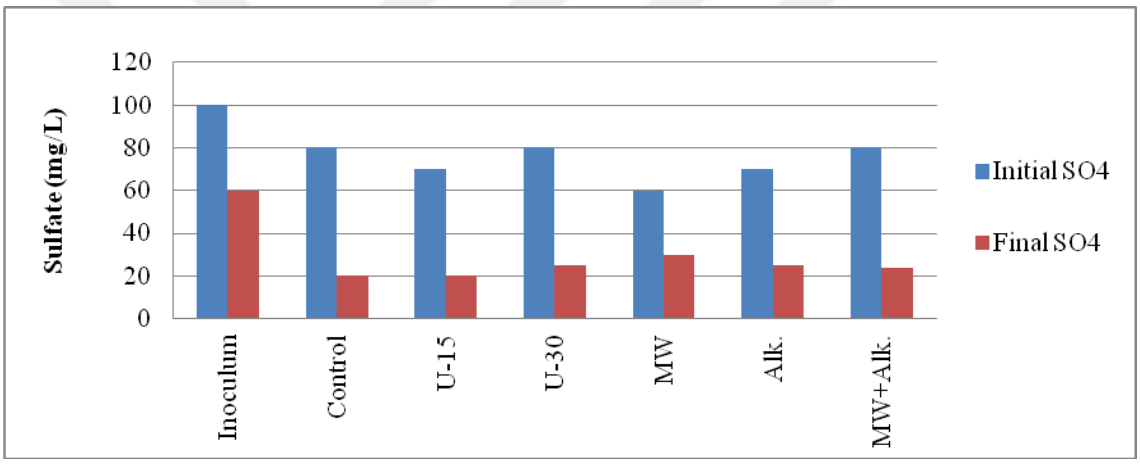

Figure 4.15. SO_4^{2-} change in the anaerobic reactors.

Chloride concentrations of the sludge samples in all reactors showed little increase at the end of the anaerobic digestion as can be seen from Figure 4.16. After pretreatments applied to the sludge samples prior to anaerobic digestion, little increases in the chloride concentrations were observed, excepting microwave pretreated sludge. Considering literature survey for chloride level, it can be noted that there was no inhibitory effect in the all reactors because of the increment in chloride concentration.

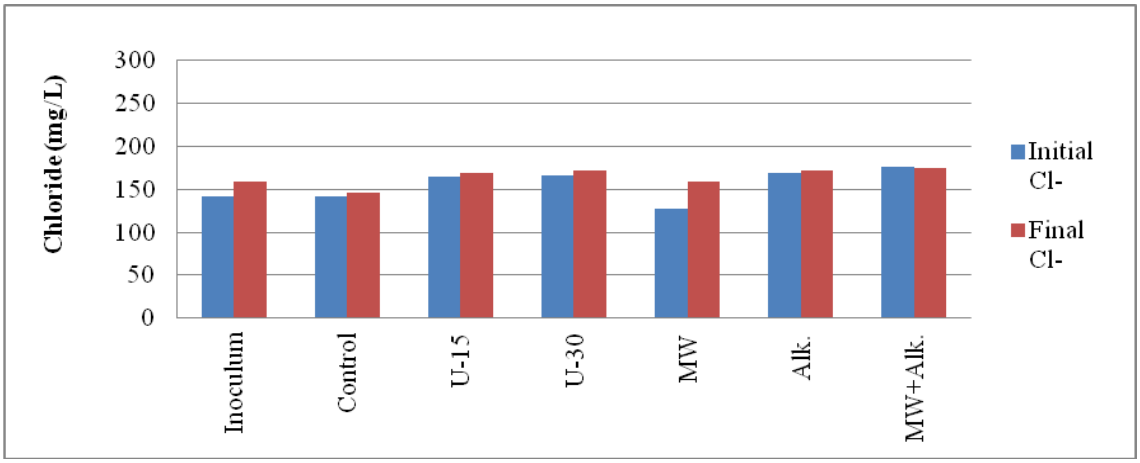

Figure 4.16. Cl^- concentration change in the anaerobic reactors.

4.3.12. Particle Size Distribution

Particle size is an important parameter to be taken into consideration that effects biogas production. Smaller particles provide a large surface area available to the microorganisms, increase the microbial activity and the anaerobic biodegradability, resulting in increased biogas yield (Xiao et. al., 2013).

The values of particle size for unpretreated and pretreated sludges were given in Table 4.12. Pretreatments decreased the mean particle size, d_{50}, due to the dispersion of sludge flocs. Higher particle size reductions were obtained in the pretreated sludge samples compared to the unpretreated sludge.

Table 4.12. Particle size distribution in the anaerobic reactors.

Anaerobic Reactors	Surface Weighted Mean D[3.2] µm	Volume Weighted Mean D[4.3] µm	d(0.9) µm	d(0.5) µm	d(0.1) µm
Inoculum					
Day 0	18.262	38.599	11.116	29.923	71.166
Day 30	16.094	35.980	9.476	27.506	61.699
Control					
Day 0	28.301	77.124	13.983	64.168	190.889
Day 30	21.320	70.780	11.116	35.893	157.409
Ultrasonication - 15 min					
Day 0	25.344	107.221	12.657	45.388	255.999
Day 30	23.878	99.160	12.404	37.079	219.779
Ultrasonication - 30 min					
Day 0	28.935	111.392	13.823	49.600	294.145
Day 30	21.805	63.597	12.376	36.188	136.054
MW					
Day 0	28.026	98.342	13.788	47.444	241.182
Day 30	25.770	83.953	13.189	38.806	182.304
Alkaline					
Day 0	29.755	143.930	14.016	51.437	436.370
Day 30	25.355	104.764	13.111	40.599	260.529
MW+Alkaline					
Day 0	25.699	92.656	12.629	45.616	217.245
Day 30	26.541	93.723	13.080	39.977	258.449

4.3.13. Viscosity

Viscosity of the sludge plays an important role for the filtration resistance (Iversena et al., 2009). The viscosity values of the sludge samples in the reactors before and after anaerobic digestion were given in Figure 4.17. Pretreatments led to a decrease in the viscosity of sludge samples at the beginning of the anaerobic digestion. This decrease in viscosity after sludge pretreatment can be attributed to the enhancement of sludge particles aggregation and weakening of floc strength (Pham et al., 2010). At the end of the anaerobic digestion, viscosity values of the sludge samples in all reactors decreased, the values for all reactors were obtained similar, and digested sludge samples became more viscous.

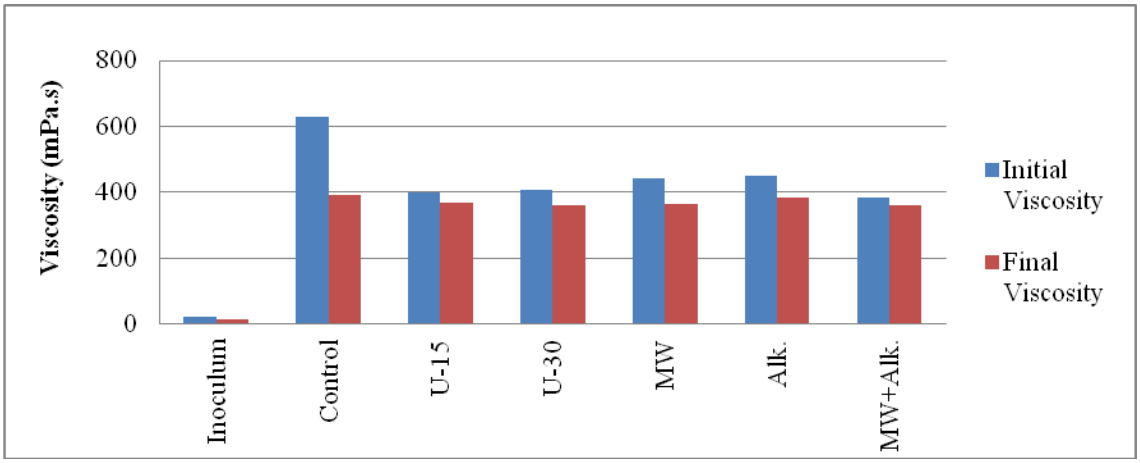

Figure 4.17. Viscosity change in the anaerobic reactors.

4.3.14. Capillary Suction Time

It was imposible to measure the capillary suction time (CST) values of the sludge samples having high solids content of 65%.

4.3.15. Microbiology

The results of the microbiological studies for the reactors before and after anaerobic digestion were given in Table 4.13. Total coliform, fecal coliform, and fecal streptococci bacteria were measured to evaluate the efficiency of the anaerobic digestion process on pathogen removal.

At the beginning of the anaerobic digestion, pretreatments did not show a significant effect of the microbial content of the reactors. Similarly, at the end of the anaerobic digestion, there were no important differences in terms of pathogen removal for the reactors containing pretreated and un-pretreated sludge. Pathogen reductions were achieved to be 99.9% on average for the all reactors. This reduction was one of the indicator of a successful digestion in this study. As can be seen from the Table 4.13., the fecal streptococci bacteria in all reactors were found to be negligible at the end of the digestion. The final values of fecal coliform colonies for the all reactors were lower than that of the U.S. EPA Class B limit ($2x10^6$ colonies/g TS).

Table 4.13. Microbial results of the reactors before and after anaerobic digestion.

Anaerobic Reactors	Total Coliform (TC) CFU/100mL		Fecal Coliform (FC) CFU/100mL		Fecal Streptococci (FS) CFU/100mL	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
Inoculum	$6x10^6$	$1.5x10^4$	$2x10^5$	$2.1x10^2$	$5.1x10^4$	$1x10^2$
Control	$4.2x10^7$	$2x10^3$	$1.8x10^7$	$5.1x10^2$	$3x10^6$	$1x10^2$
U-15	$3.4x10^7$	$1.9x10^3$	$1.5x10^7$	$2.9x10^2$	$3.1x10^6$	≤ 10
U-30	$3.8x10^7$	$1x10^4$	$1.4x10^7$	$4x10^2$	$3.8x10^6$	$2.1x10^2$
MW	$5.8x10^7$	$2.3\ x10^3$	$2.4x10^7$	$2.3x10^2$	$3.3x10^6$	≤ 10
Alk.	$4.2x10^7$	$3x10^4$	$3x10^7$	$1x10^2$	$4x10^6$	55
MW+Alk.	$3.2x10^7$	$1.5x10^3$	$2.2x10^7$	$4.1x10^2$	$4x10^6$	≤ 10

4.3.16. Volatile Fatty Acids

Changes of volatile fatty acids (VFAs) concentration could be used to indicate the performance of anaerobic digestion. In the methane fermentation phase, pH increases to more neutral values because of the conversion of VFAs and hydrogen to CH_4 and CO_2 (Rani et al., 2012). VFAs produced during anaerobic digestion can be consumed by methanogens (Siegert and Banks, 2005). According to the study of Dearman and Bentham (2007), it could be stated that as VFAs concentration decreased, biogas production rate increased. In this study, eight VFAs; acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and caproic acids were explored before and after the anaerobic digestion. The results were given in Table 4.14 and Table 4.15.

VFA concentrations in the reactors containing pretreated sludges increased at the beginning of the anaerobic digestion when compared to the control reactor having unpretreated sludge. As can be seen from the Table 4.14, acetic acid was the most prevalent VFA and propionic acid located at the second place. The maximal acetic acid accumulation occurred in the reactor containing alkaline pretreated sludge samples. VFAs decreased along with the anaerobic digestion and little VFAs left at the end of the digestion because the most microbial cells were destroyed.

Table 4.14. VFAs concentrations in the reactors at the beginning of anaerobic digestion.

VFA (mmolar)	Initial Values						
	Inoculum	**Control**	**U-15**	**U-30**	**MW**	**Alk.**	**MW+Alk.**
Acetic	7.2975	0.961	6.2916	10.2089	3.6993	14.159	5.0827
Propionic	0.1785	0.8932	4.0463	3.2281	2.8732	0.2477	3.9198
Isobutyric	0.4981	0.1383	0.8468	1.0822	0.7601	0.3109	0.9068
Butyric	0.1622	-	0.4372	0.6059	0.2615	0.0412	0.2982
Isovaleric	0.2516	0.0701	0.7788	10.454	0.6691	0.2429	0.7865
Valeric	0.1786	-	0.4628	0.5895	0.3248	0.066	0.4153
Isocaproic	-	0.0171	0.0712	0.1053	0.0382	0.0256	0.111
Caproic	0.0218	-	0.0146	0.0308	0.0086	-	0.0072
Heptanoic	-	-	-	-	-	-	-

Table 4.15. VFA concentrations in the reactors at the end of anaerobic digestion.

VFA (mmolar)	Final Values						
	Inoculum	**Control**	**U-15**	**U-30**	**MW**	**Alk.**	**MW+Alk.**
Acetic	0.107	0.8609	0.8032	1.0587	1.1140	0.8902	0.9294
Propionic	-	-	-	-	0.0238	-	0.0174
Isobutyric	-	-	-	-	-	-	-
Butyric	-	0.0149	0.026	0.0144	0.0291	0.0123	0.01315
Isovaleric	-	-	-	-	-	-	-
Valeric	-	-	-	-	-	-	-
Isocaproic	-	-	-	-	-	-	-
Caproic	-	-	-	-	-	-	-
Heptanoic	-	-	-	-	-	-	-

The ratio of propionic acid to acetic acid was lower than 1.4, which meaned that the system successfully produced methane. The highest propionic acid to acetic acid ratio in the reactor containing ultrasonically pretreated sludge samples with 30 min duration was found to be 0.77 at the beginning of the anaerobic digestion and then this ratio decreased at the end of the anaerobic digestion as well as in all anaerobic reactors.

4.3.17. Carbon to Nitrogen Ratio

The carbon to nitrogen (C/N) ratios of the sludge samples in all reactors were shown in Figure 4.18. The C/N ratio between 20 and 30 was the most acceptable range for an anaerobic digestion process in many studies. In the high C/N ratio, nitrogen was rapidly consumed by methanogens and lower gas was produced. On the other hand, a lower C/N ratio resulted in accumulation of ammonium nitrogen and free ammonia and pH values exceeding 8.5, which was toxic to methanogenic bacteria (Kayhanian et al., 1992, Yen et al., 2007).

In this study, the C/N ratio improved from 9 to 12 on average for the anaerobic reactors. This increase of C/N ratios reduced the risk of ammonia inhibition in the reactors and caused the increasing methane production after anaerobic digestion. Although the C/N ratios of the anaerobic reactors in this study were lower than 20, high productions of methane gas were obtained at the end of anaerobic digestion.

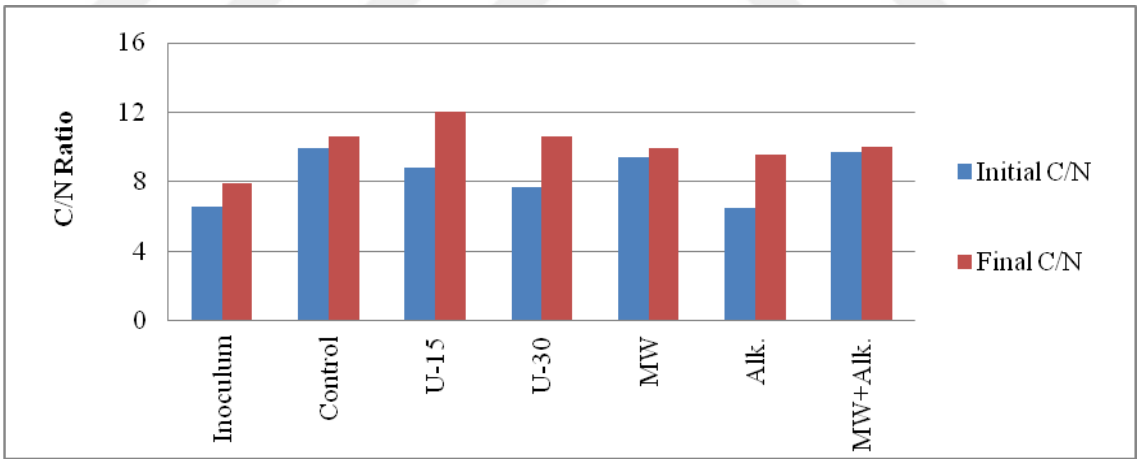

Figure 4.18. C/N ratios in the anaerobic reactors.

4.3.18. Soluble Protein and Carbohydrate

Protein and carbohydrate are two predominant organic matters in the sludge. Soluble protein and carbohydrate concentrations in the reactors and removal efficiencies of the reactors before and after anaerobic digestion were shown in Figures 4.19 and 4.20, respectively.

As the level of soluble protein increased, the efficiency of anaerobic digestion would be improved (Rani et al., 2012). Prior to anaerobic digestion, it was clearly observed that both the solubilization of protein and carbohydrates increased with the application of pretreatments due to release of cellular organics. It should be noted that the soluble protein concentrations were much higher than soluble carbohydrate concentrations in the anaerobic reactors. After anaerobic digestion, soluble protein and carbohydrate concentrations in the reactors containing the pretreated and unpretreated sludge samples were decreased.

Many studies reported that protein concentration in activated sludge exceeded carbohydrate concentration (Frolund et al., 1996; Higgins and Novak, 1997). Wang et al. (2006) found that protein concentration was more predominant than carbohydrate in the aqueous phase of the ultrasonicated sludge. Novak et al. (2003) reported that 3-5 times higher protein concentrations were obtained when compared to carbohydrate concentration in anaerobic conditions. In this study, soluble protein concentrations were approximately 2-3 times greater than carbohydrate concentrations in the anaerobic reactors.

In the study of Liu and Fang, (2002), the ratio of carbohydrate and protein for anaerobic digestion was reported to be 0.2-0.5. The ratio of soluble carbohydrate and protein was found to be in the range of 0.3 and 0.55 for the anaerobic reactors in this study.

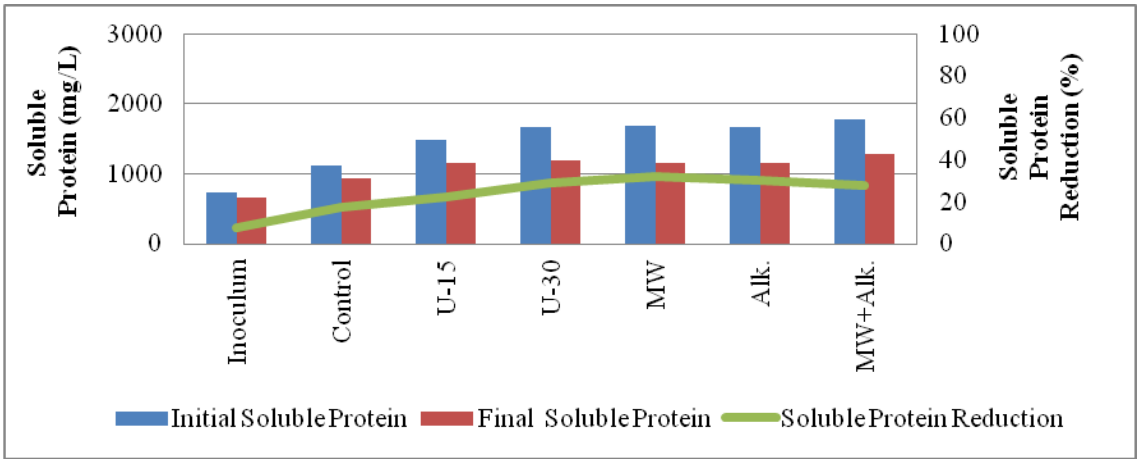

Figure 4.19. Soluble protein concentrations and reductions in the anaerobic reactors.

The highest soluble protein reduction (32%) was achieved in the reactor containing microwave pretreated sludge samples. This reduction for the control reactor was found to be 17%. There were no significant differences in terms of soluble protein reductions between microwave and other pretreatments used in this study. At the end of the anaerobic digestion, the soluble protein removal efficiencies were found to be 22%, 29%, 30%, and 28% for the reactors containing ultrasonically (at specific energy of 12000 kJ/kg TS) and ultrasonically (at specific energy of 24000 kJ/kg TS), alkaline and combined microwave-alkaline pretreated sludge samples, respectively. A similar trend was observed with the carbohydrate reductions.

As provided in Figure 4.20, the removal efficiencies of soluble carbohydrate for all pretreated reactors were higher than that of soluble protein. The highest carbohydrate reduction (48%) was achieved in the reactor containing microwave pretreated sludge samples. Ultrasonic pretreatment at specific energy of 24000 kJ/kg TS showed better removal efficiency compared to that of 12000 kJ/kg TS. Liu and Fang, (2002) reported that the combined microwave-alkaline pretreatment caused more carbohydrate release than each individual method. Dogan and Sanin, (2009) stated that carbohydrate concentration was higher in combined pretreatments compared to individual pretreatment methods. These explanations were similar to the results obtained in the present study.

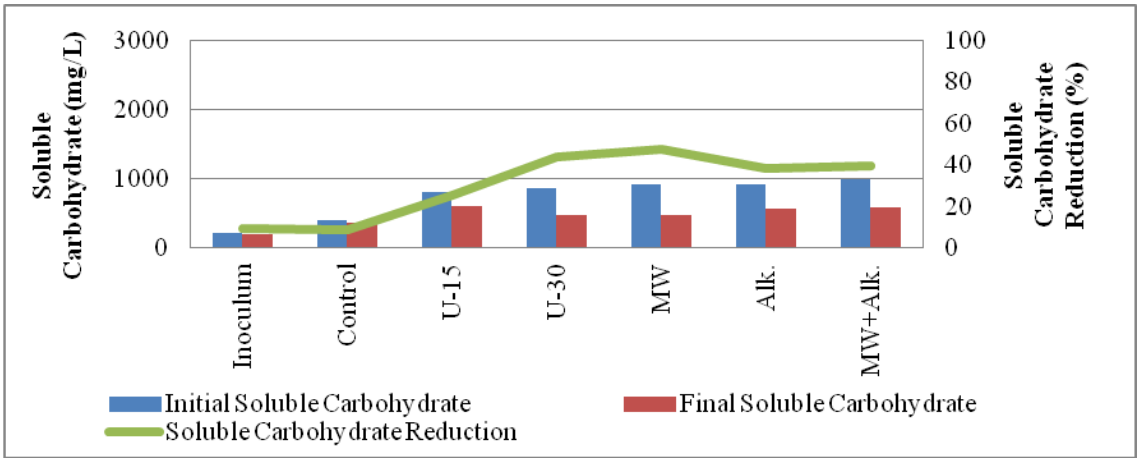

Figure 4.20. Soluble carbohydrate concentrations and reductions in the anaerobic reactors.

4.3.19. Biogas Analyses

One of the aims of this study was to improve biogas production and to enhance methane content of the biogas produced during the anaerobic digestion.

4.3.19.1. Cumulative Biogas Production. The cumulative biogas productions in the anaerobic reactors were given in Figure 4.21. All of the results were presented as the average values obtained from identical reactors. It was observed that there was no significant difference between cumulative biogas productions of the anaerobic reactors up to 5 d due to the acclimatization. After 13 day of the anaerobic digestion, the biogas production rates for the reactors containing pretreated sludges were accelerated due to the disintegration after a while.

As can be seen from the Figure 4.21, the control reactor having unpretreated sludge (13757 mL) produce less biogas than the reactors containing pretreated sludges, this result was not surprising. It can be stated that pretreatment applications improved the biogas production significantly.

The maximum cumulative biogas production from alkaline and microwave pretreated sludge samples were measured to be 19523 mL and 19203 mL, respectively. The cumulative biogas productions were improved by 42%, 40% and 37% in the cumulative biogas production were achieved with the alkaline, microwave and combined microwave-alkaline pretreatments, respectively. A similar data for microwave pretreatment was also reported by Eskicioglu et al. (2008). It was stated that 31±6% more biogas production was observed at 175°C microwave pretreated sludge in anaerobic digestion. Biogas production from ultrasonically pretreated sludge at specific energy of 24000 kJ/kg TS (17976 mL) was higher than that of 12000 kJ/kg TS (17604 mL) due to higher disintegration and solubilization of sludge solids.

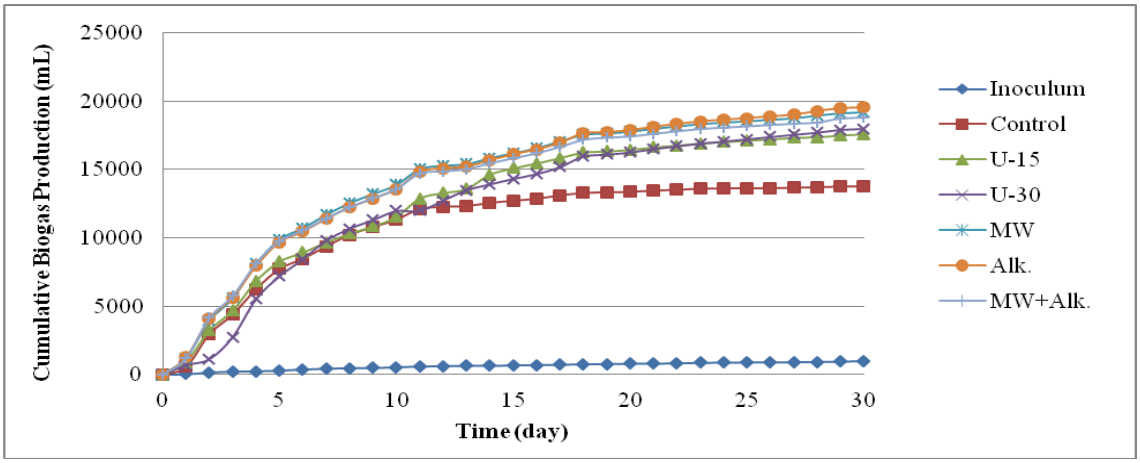

Figure 4.21. Cumulative biogas production in the anaerobic reactors.

4.3.19.2. Methane Production. Biogas consists mainly of methane (CH_4), carbon dioxide (CO_2) and small amounts of water vapor, hydrogen, nitrogen, hydrogen sulphide, unsaturated hydrocarbons and other gases. Major components of biogas include methane and carbon dioxide, typically 55-70% and 30-45% by volume of the biogas, respectively, for anaerobic digestion process. The most important gas is the methane because it is used for energy production. Moreover, the methane content of the biogas indicates the stability, performance of the digester, the biological activity and organic material conversion in anaerobic reactors (Gray, 2005).

Methane percentages of the reactors were given in Figure 4.22. All of the methane percentage results were given in Appendix B. The methane production results were not in one to one correlation with the biogas production results. Pretreatments used in this study improved the methane production. The methane contents of biogas in the reactors were obtained in the range of 50-70% throughout this study. The methane production increased in the reactors containing pretreated sludge samples during the anaerobic digestion, and then decreased at the end of the operation period, the reason being that pretreated sludge hydrolyzed much organic material into solution, and the anaerobic digestion process used it immediately. There was no rapidly change in the methane content of the control reactor containing unpretreated sludge from the 17th day to at the end of the operation period.

At the end of anaerobic digestion, the highest (70%) methane content was observed in the reactor including microwave pretreated sludge. The alkaline and combined microwave-

alkaline pretreatments had same methane percentage (62%) at the 21th of the reactor operation. Valo et al. (2004) stated that added chemicals to microwave pretreatments limit the effect of pretreatment methods. In the study of Valo et al. (2004), microwave pretreatment led to 6.6% higher methane production over combined pretreatment of microwave and alkaline.

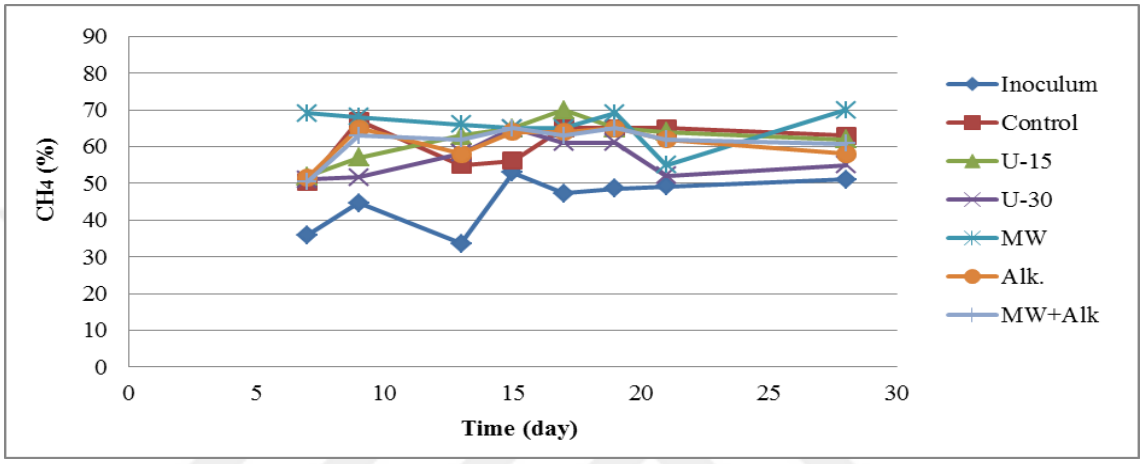

Figure 4.22. Methane content of the reactors during the anaerobic digestion.

The methane percentage which was produced in the reactor containing ultrasonically pretreated sludge samples (at specific energy of 12000 kJ/kg TS) was at its highest level (70%) at the 17th day of the anaerobic digestion. Ultrasonic pretreatment at specific energy of 12000 kJ/kg TS had higher improvement than that of 24000 kJ/kg TS. At the end of the anaerobic digestion, the methane production was improved by 55% and 43% in the reactor containing microwave and alkaline pretreatments, respectively, compared to control reactor including unpretreated sludge samples. The highest biogas and methane productions were achieved with microwave and alkaline pretreatments with 16.3% and 18.9% improvements over control reactor, respectively (Dogan and Sanin, 2009).

4.3.19.3. Biogas Yields. Biogas and methane yields in the reactors were presented in Figure 4.23 as Lbiogas/$gVS_{removed}$, Lbiogas/gVS_{added}, $LCH_4/gVS_{removed}$, and LCH_4/gVS_{added}. Pretreatments increased both biogas and methane yields due to increase in solubilisation of complex organic matter. After the application of ultrasonic, alkaline, microwave, and combined microwave-alkaline pretreatments to the sludge samples, methane yields were improved by 40%, 56%, 77%, and 42%. Microwave pretreatment showed the highest

biogas and methane yields of 0.52 Lbiogas/gVS_{added} (2.80 Lbiogas/$gVS_{removed}$) and 0.37 LCH_4/gVS_{added} (1.96 $LCH_4/gVS_{removed}$), respectively.

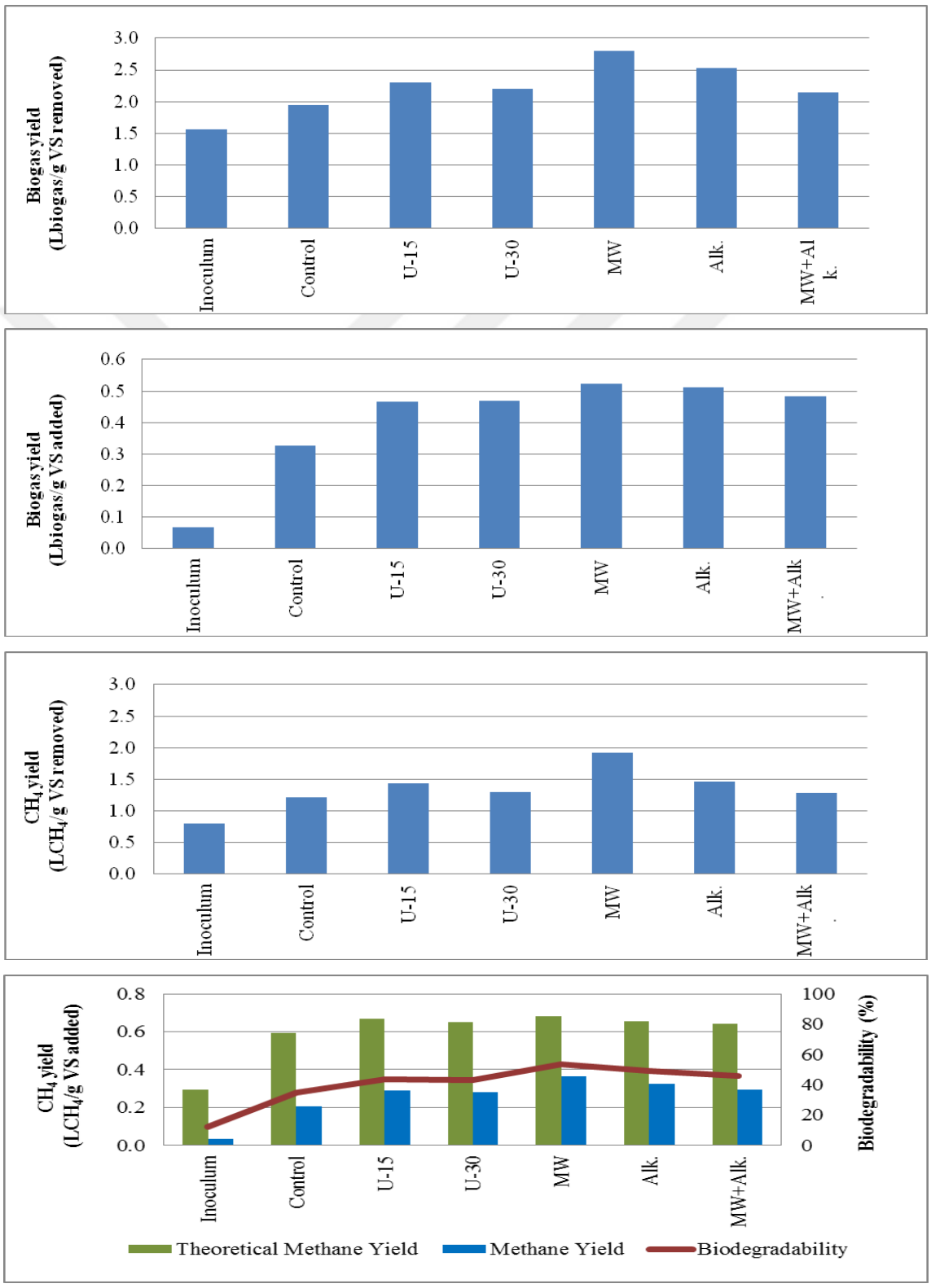

Figure 4.23. Biogas and methane yields of anaerobic reactors.

4.4. Aerobic Digestion Experiments

WAS was taken from the recycle line of Kullar Municipal wastewater treatment plant in Kocaeli. A total of 12 plexiglass aerobic semi-continuous reactors with 10 L of working and 15 L of total volume were operated for 30 days of digestion period. Three of the reactors names as C1, C2, and C3 were operated as control reactors and fed only with unpretreated sludge samples. The other nine reactors were fed with partially pretreated sludge samples.

Ultrasonication, microwave, and alkaline pretreatments were applied to 10%, 20%, and 25% of sludge samples (Sludge pretreatment ratio, SPR, of 10%, 20%, 25%) to see effect of pretreatment application volume on the aerobic stabilization efficiency.

4.4.1. pH and Temperature

pH is an important parameter in order to control nitrification system. Suitable range for pH should be between 6 and 9 in aerobic digestion process, even though optimal range is much narrower being from 7.5 to 8 (Tchobanoglous et al., 2004). During the operation period, pH values of the aerobic reactors containing unpretreated (control) and pretreated sludge samples measured daily and varied from 6.02 to 8.21. The results were depicted in Figure 4.24.

The operation temperature is important for the aerobic microbial activity. The temperature should be in the range of 25-35°C for nitrification and suitable microbial activity in aerobic digestion. (Tchobanoglous et al., 2004). In this study, the operation temperature ranged from 21-26°C for the aerobic reactors, the general range was 25±3°C. The temperatures of the pretreated and unpretreated sludge samples in aerobic reactors during operation time were presented in Figures 4.25.

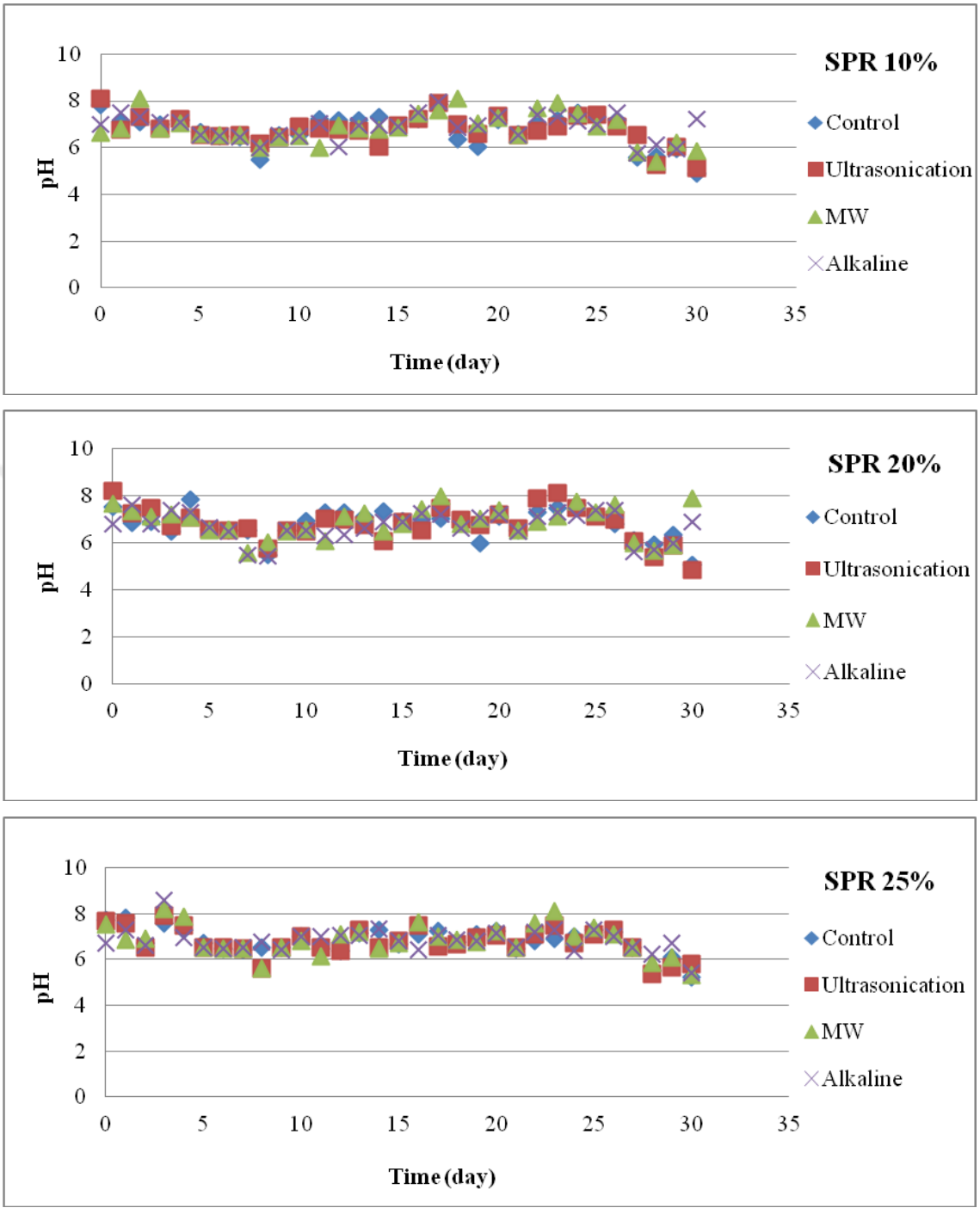

Figure 4.24. pH changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

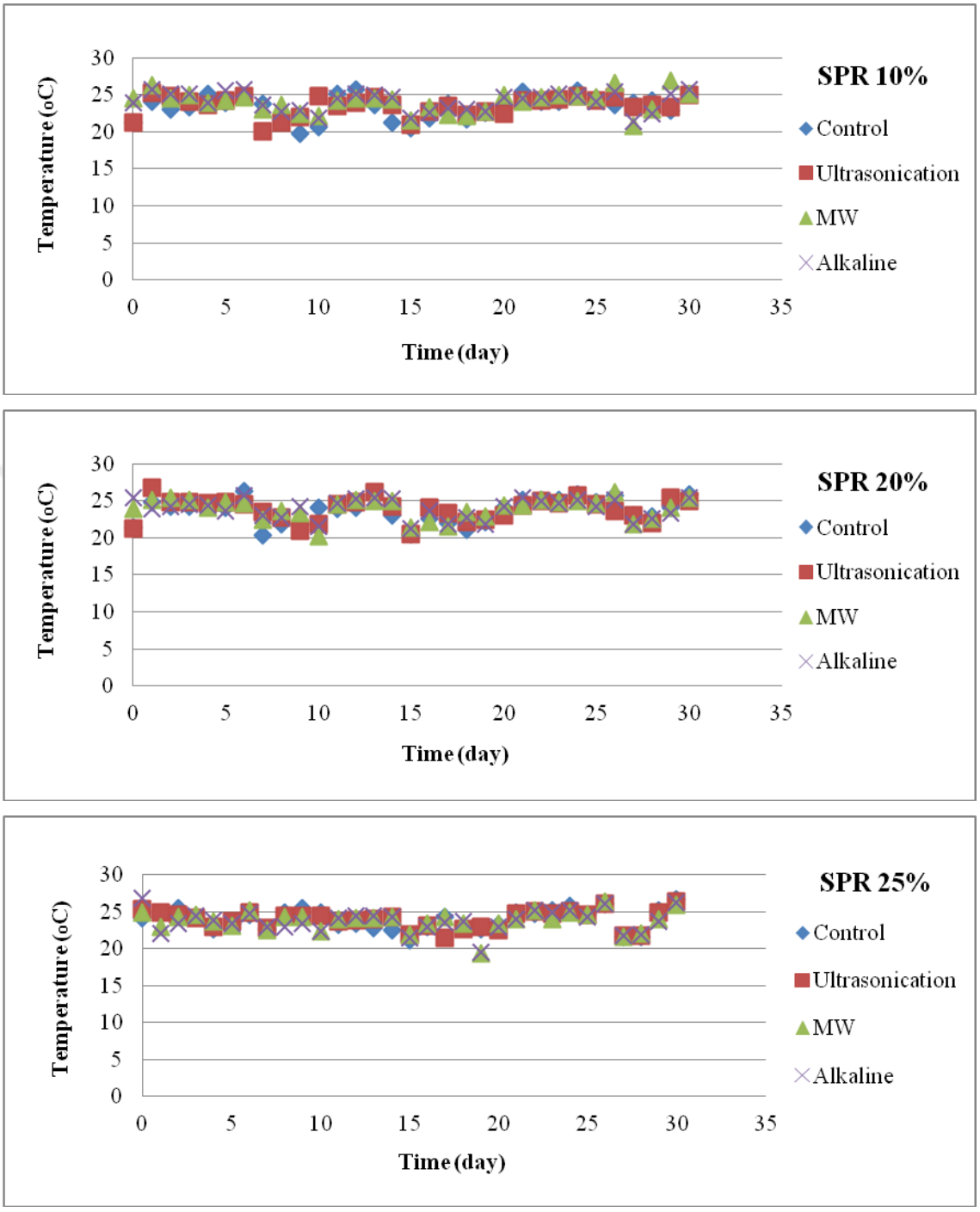

Figure 4.25. Temperature changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.2. Dissolved Oxygen

Oxygen plays an important role in aerobic digestion process because the microorganisms remove the production of bad odors in the system. Dissolved oxygen (DO)

concentrations should not be less than 1 mg/L for aerobic digesters (Tchobanoglous et al., 2004). DO values were kept at levels higher than 2 mg/L in the aerobic reactors. The DO concentrations of the sludge samples in the reactors were given in Figure 4.26.

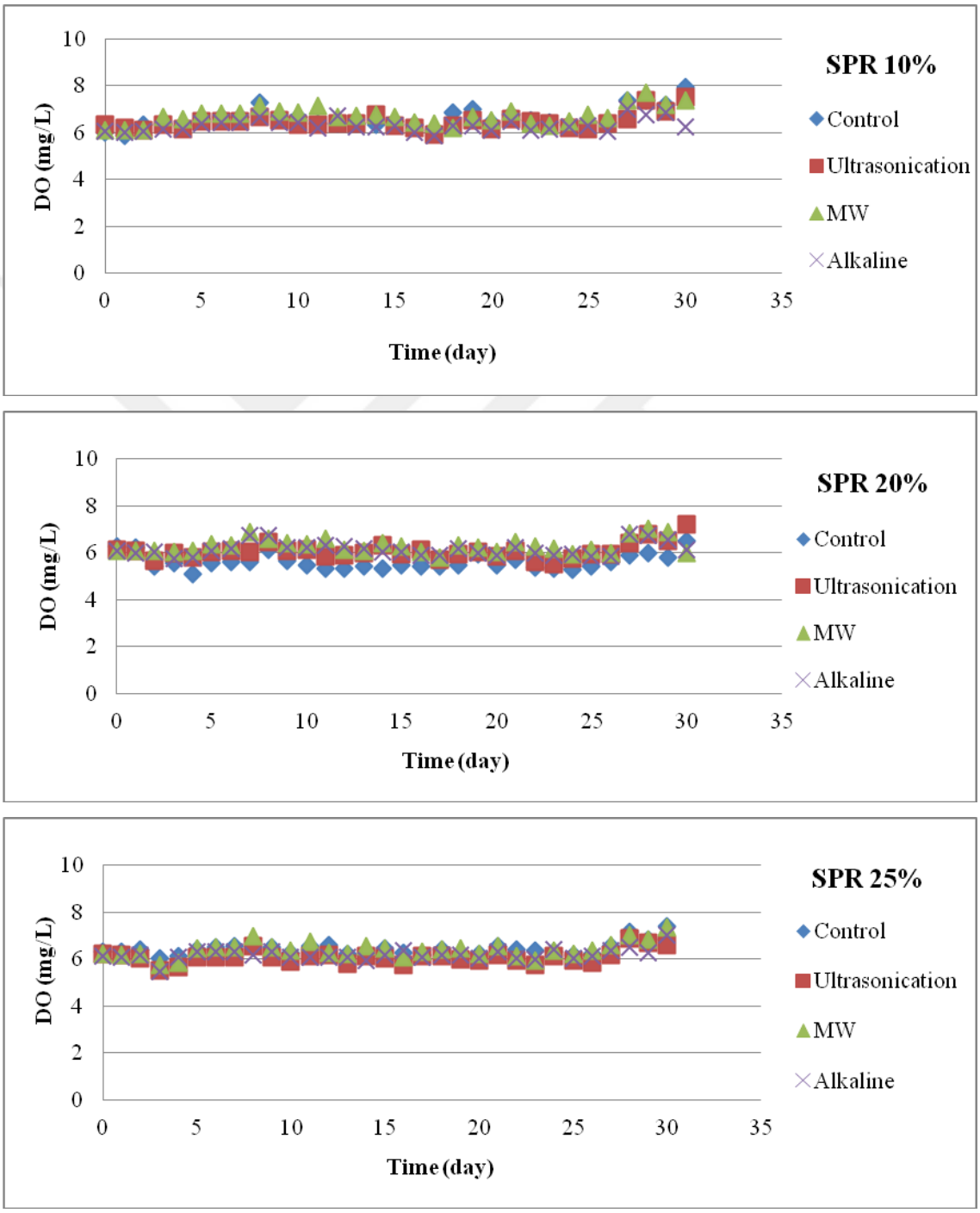

Figure 4.26. DO changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.3. Oxidation Reduction Potential

Oxidation reduction potential (ORP) was measured daily in order to evaluate the performance of the reactors. The ORP data were given in Figure 4.27. ORP values in all of the aerobic reactors were higher than 100 mV.

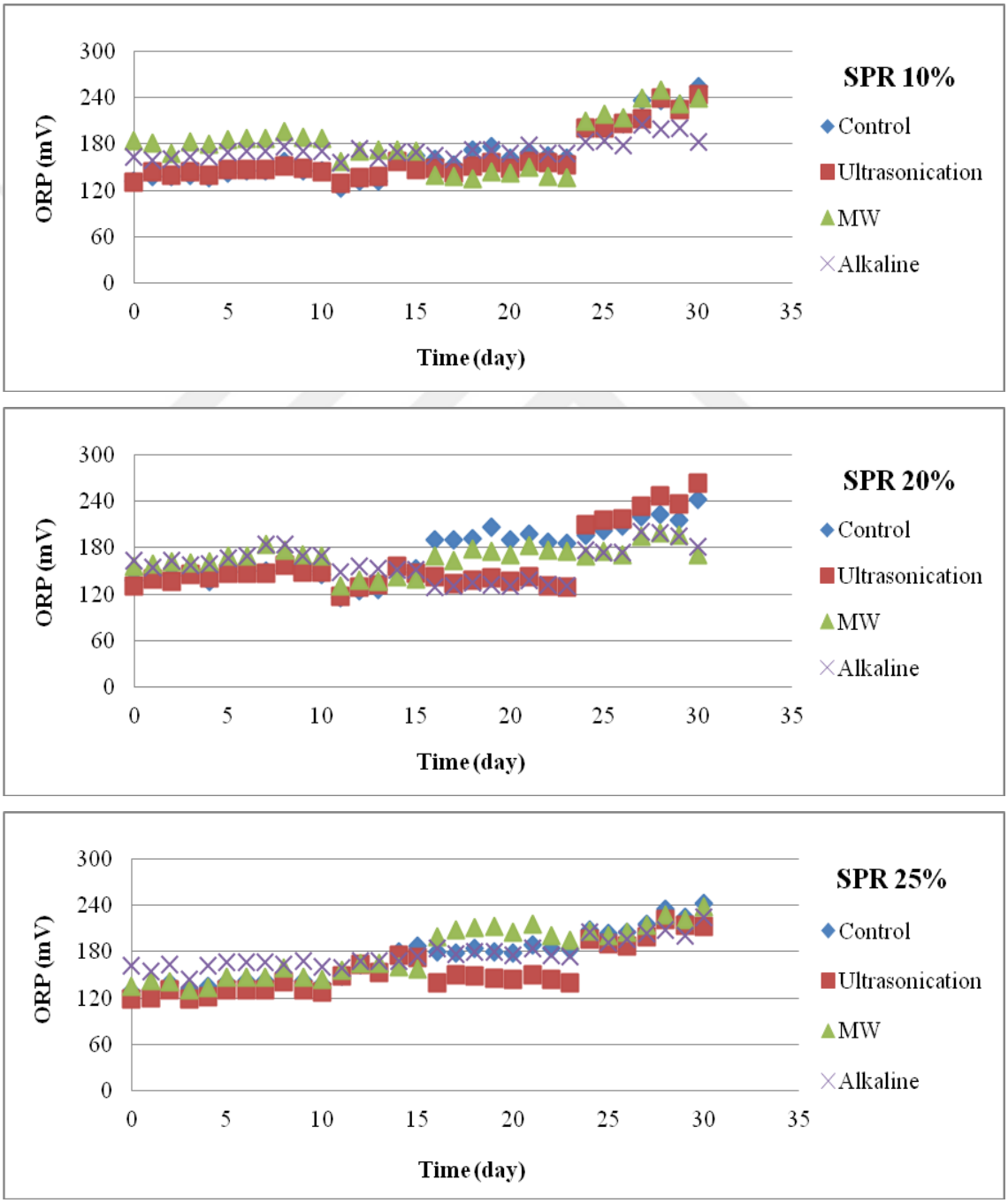

Figure 4.27. ORP changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.4. Electrical Conductivity and Salinity

Electrical conductivity (EC) is used to monitor and control aerobic process since this parameter is a general indicator of water quality. The variation of the ion content (H^+, OH^- and nutrients such as phosphate and nitrate) in the wastewater cause to change in the conductivity (Levlin, 2009). The EC values of all aerobic reactors were almost the same up until 20th day of aerobic digestion and varied between 2 and 14 mS/cm. The results were given in Figure 4.28. A decrease was observed in the EC values of the reactors containing 10% and 20% pretreated sludge samples after 20th day of the operation period. The cause of this decrease would be removal of nutrients. Ammonium nitrogen is especially affecting the EC values.

The trend of EC and salinity in the aerobic reactors were similar as expected. The salinity data was shown in Figure 4.30. Salinity values changed between 1.2 and 10.2 ‰ for the aerobic reactors. Similar to EC values, salinity values of the reactors for SPR of 10% and 20% increased after 20th day of the operation period. However, little increases were observed in the salinity values of the reactor for SPR of 25%.

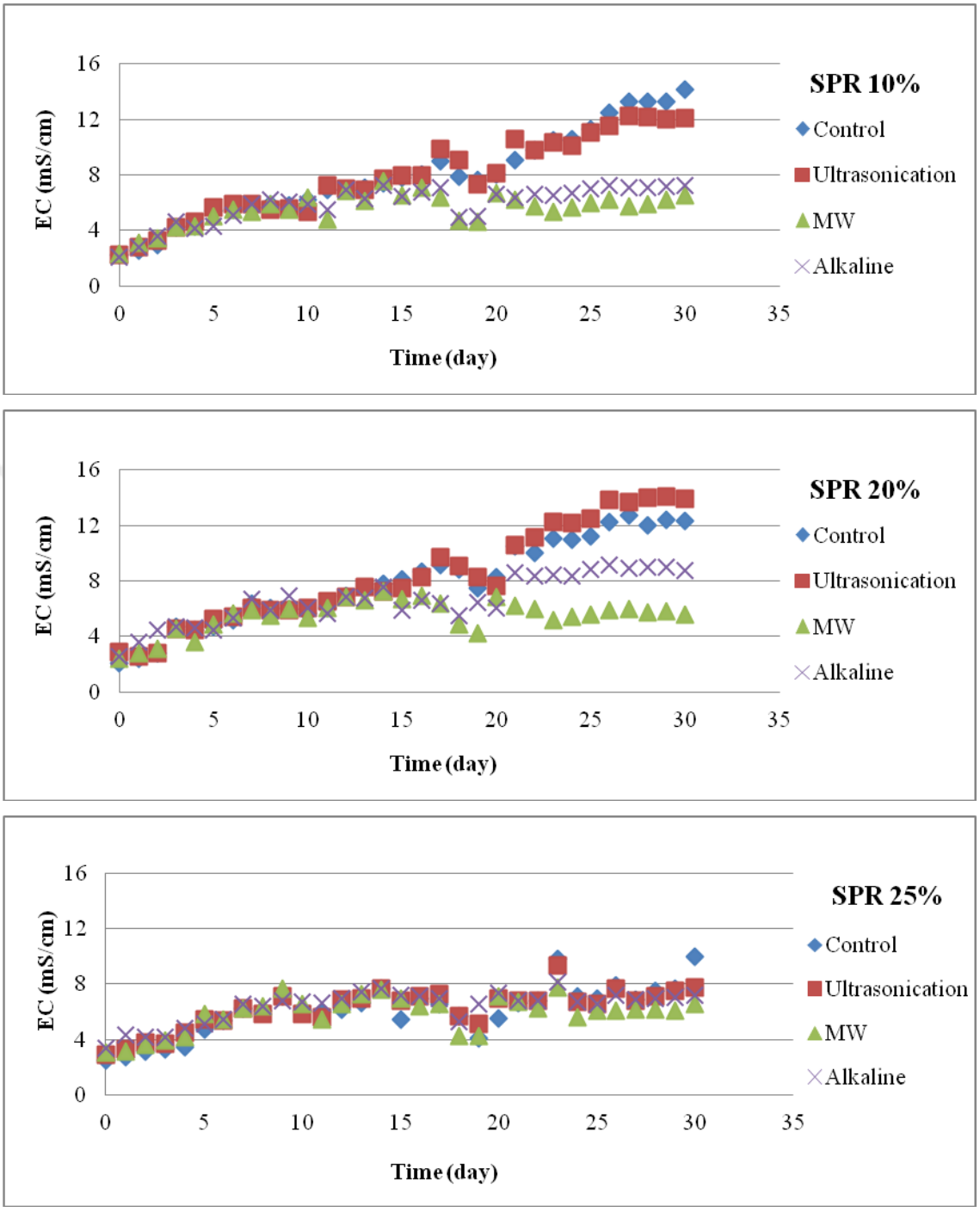

Figure 4.28. EC changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

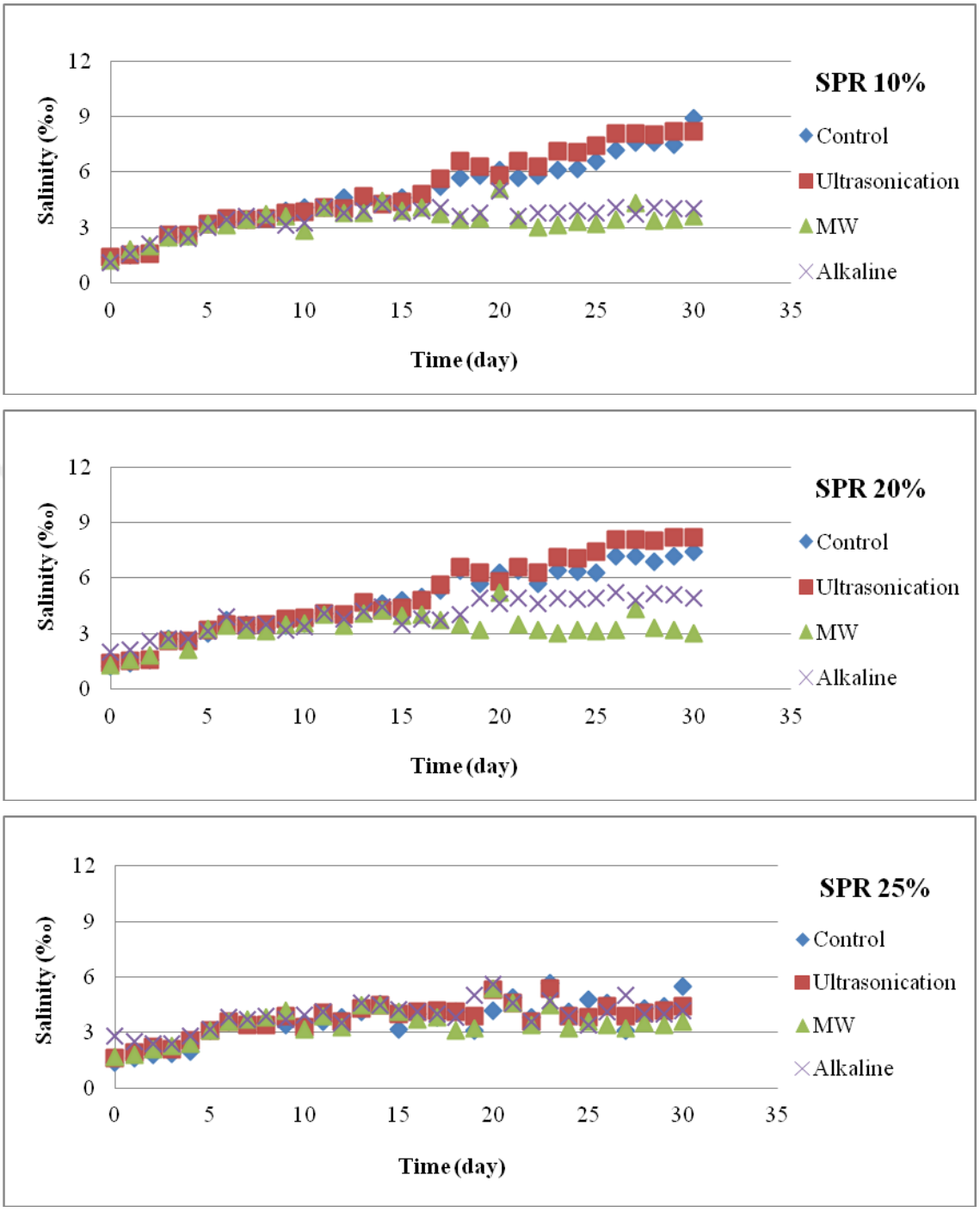

Figure 4.29. Salinity changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.5. Alkalinity

Alkalinity is an indicator that is useful in monitoring the performance of aerobic digestion process. It can be defined as the ability of a water to neutralize acid or to absorb

hydrogen ions. The alkalinity concentrations were shown in Figure 4.30. The alkalinity concentrations in the reactors decreased during the operation time. The cause of this decrease can be explained as existence of oxygen and the production of hydrogen ions in the system. Initial alkalinity concentrations ranged between 750 and 1750 mg $CaCO_3$/L and final alkalinity concentrations were in the range of 125 to 800 mg $CaCO_3$/L.

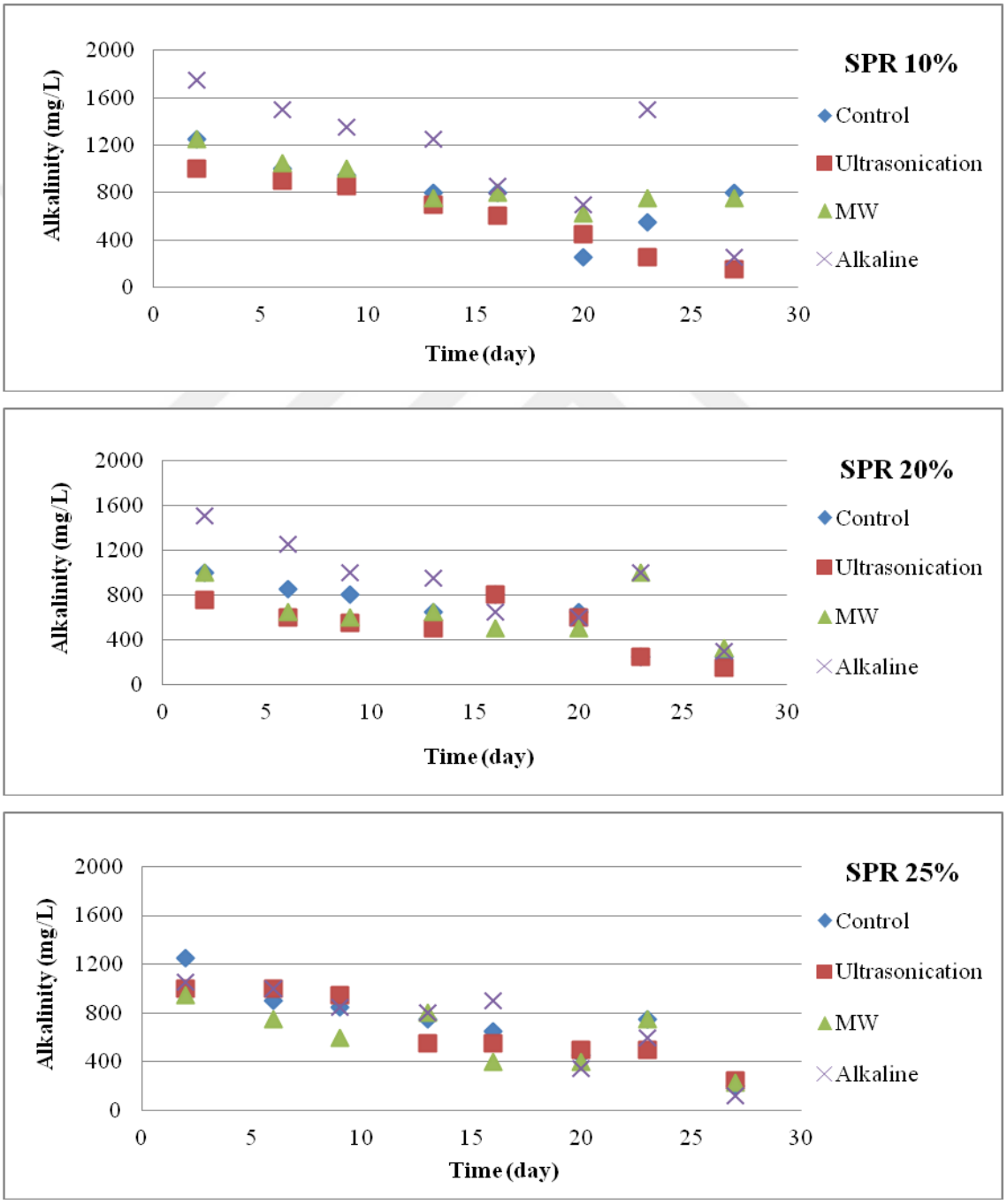

Figure 4.30. Alkalinity concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.6. Total Solids and Volatile Solids

Total solids (TS) and volatile solids (VS) concentrations in the reactors were analyzed three times a week and the results were shown in Figures 4.31 and 4.33, respectively. In addition, TS and VS removal efficiencies of the reactors were given in Figures 4.32 and 4.34, respectively.

Aerobic digestion decreased the TS and VS contents of the sludge samples. Pretreatment played an important role in the decrease of TS content. The highest TS reduction was obtained from alkaline pretreatment applied to 25% of sludge sample. At the end of the 30th day of the operation, TS values decreased to 25%, 33%, 35% and 42% for the reactors containing unpretreated sludge (control reactor), ultrasonication, microwave and alkaline pretreated (SPR:25%) sludge samples, respectively. Therefore, the highest TS removal (42%) was achieved in the reactor including 25% of alkaline pretreated sludge.

For alkaline pretreatment, the TS removal efficiency increased when the portion of sludge to be treated increased. However, this trend was not same for ultrasonic and microwave pretreatment. Reactors containing 10% ultrasonically pretreated sludge samples showed better TS removal performance than reactors having sludge pretreatment ratio, SPR, of 20% and 25%. On the other hand, in microwave pretreatment, the highest VS removal was achieved in reactor containing 20% pretreated sludge.

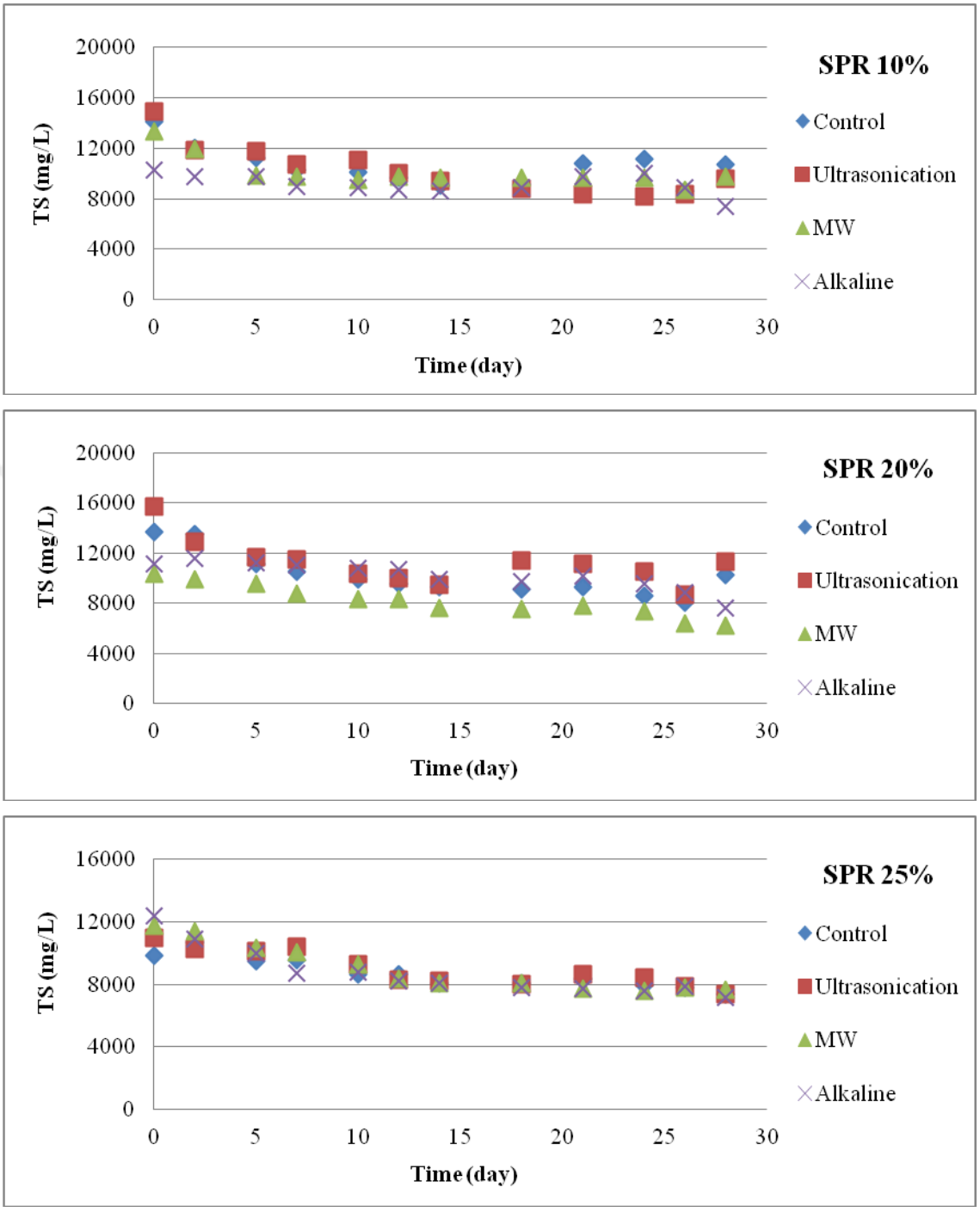

Figure 4.31. TS concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

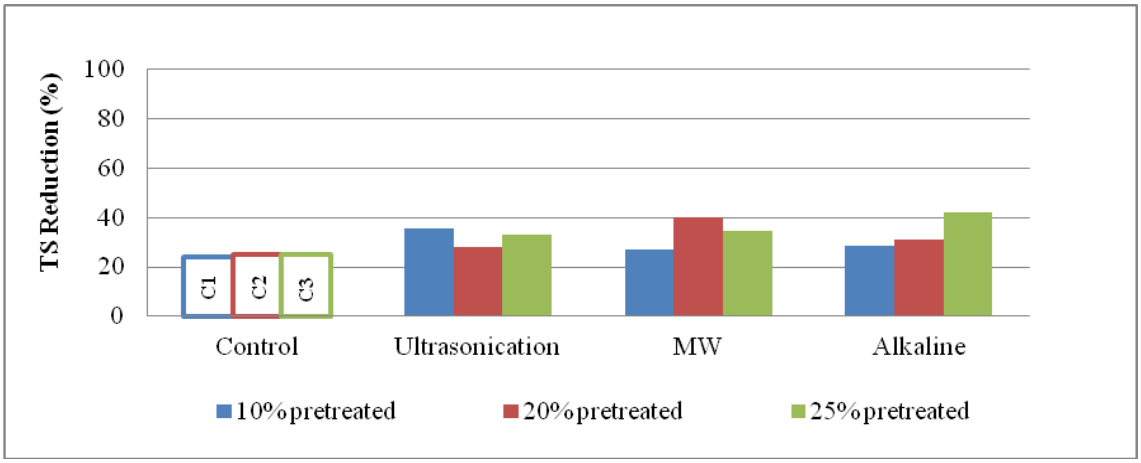

Figure 4.32. TS removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

The reduction of VS in this study was slightly higher than that of TS because most organic matter in the sludge was mineralized during the aerobic digestion process. VS reductions in the control reactors (C1, C2, and C3) were found to be 31%, 33%, and 34%. The results showed that pretreatments improved the sludge minimization. Ultrasonication pretreatment increased the VS reductions of the reactors having SPR of 10%, 20%, and 25% to 52%, 44%, and 42%, respectively. In the same way, VS reductions of the reactors for microwave and alkaline pretreatments, in the same order, were found to be 45%, 46%, 39% and 38%, 41%, and 57%, respectively. When, SPR, the portion of sludge to be pretreated increased, VS reduction decreased in ultrasonic pretreatment and increased in alkaline pretreatment. This decrease in ultrasonication pretreatment can be explained with decreased efficiency of disintegration due to increased sludge volume lowering the applied specific energy. It seems, in VS reduction, ultrasonication pretreatment was more effective for SPR values of 10%. The highest VS removal efficiency was obtained from alkaline pretreatment for SPR of 25%.

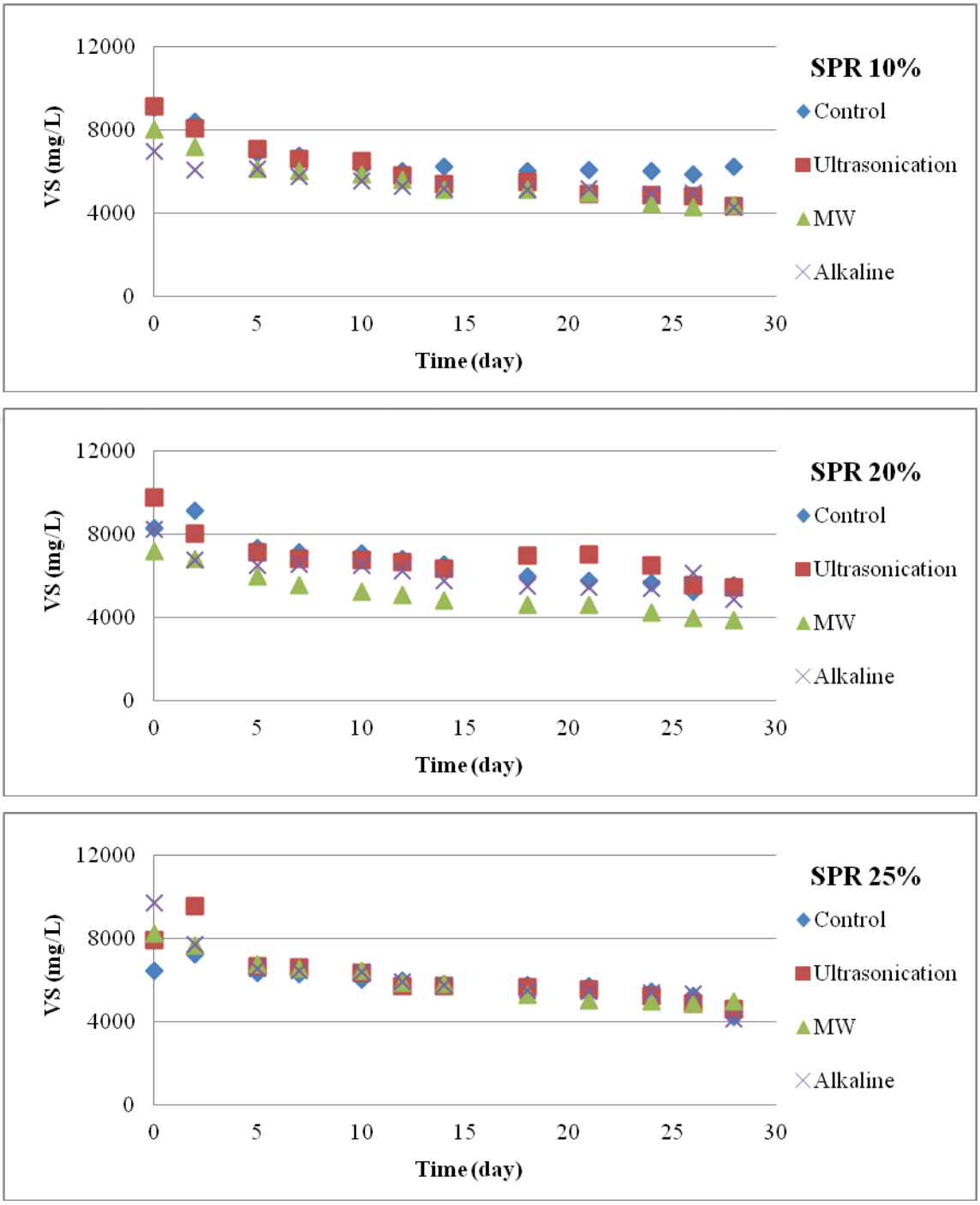

Figure 4.33. VS concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

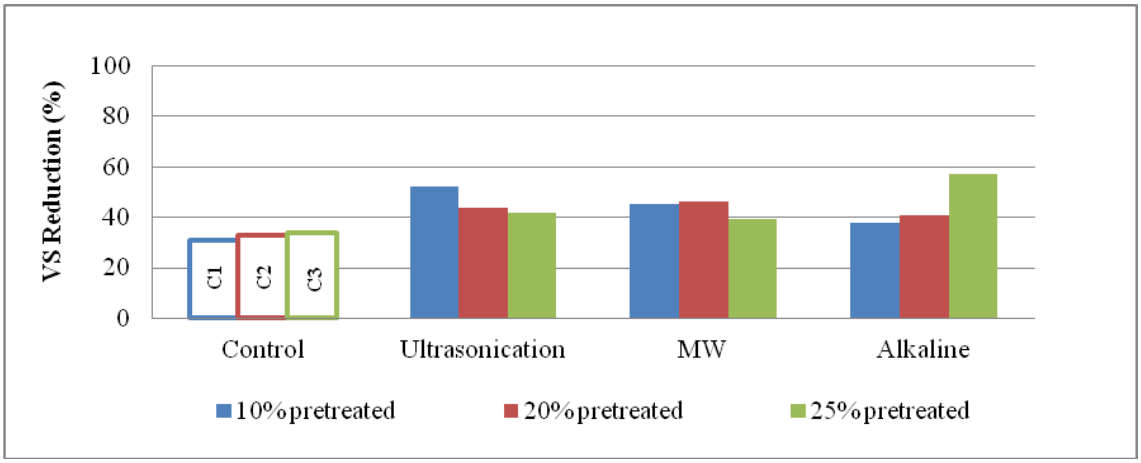

Figure 4.34. VS removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.7. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were used as an indicator of sludge reduction. MLSS and MLVSS concentrations in the reactors were given in Figures 4.35 and 4.37, respectively. Figures 4.36 and 4.38 show the MLSS and MLVSS removal efficiencies of the reactors, respectively.

Under aerobic conditions, sludge reduction was enhanced by ultrasonication, microwave and alkaline pretreatment of the sludge. The reactors fed with 10%, 20%, and 25% of ultrasonically pretreated sludge achieved higher MLSS removal efficiencies of 38%, 33%, and 36%, respectively, as compared to 26%, 27%, and 26% in the control reactors (C1, C2, and C3) at the end of aerobic digestion. These results are in accordance with the literature. Yu et al. (2010) achieved 40% of MLSS reduction with the ultrasonic pretreatment applied to sludge at 9500 kJ/kg TS of specific energy. In the microwave pretreatment, the MLSS reduction increased about 19% to be 45% for SPR of 20% and 25%. There was almost no change in the MLSS reduction for SPR 10%. The contribution of alkaline pretreatment applied to 10%, 20% and 25% of sludge samples was 4%, 8% and 20% on MLSS reduction, respectively. The best pretreatment in term of MLSS removal improvement was alkaline pretreatment (SPR:25%) under aerobic conditions. Same as the

VS removal, the highest MLSS removal efficiencies was also obtained from alkaline pretreatment for SPR of 25%.

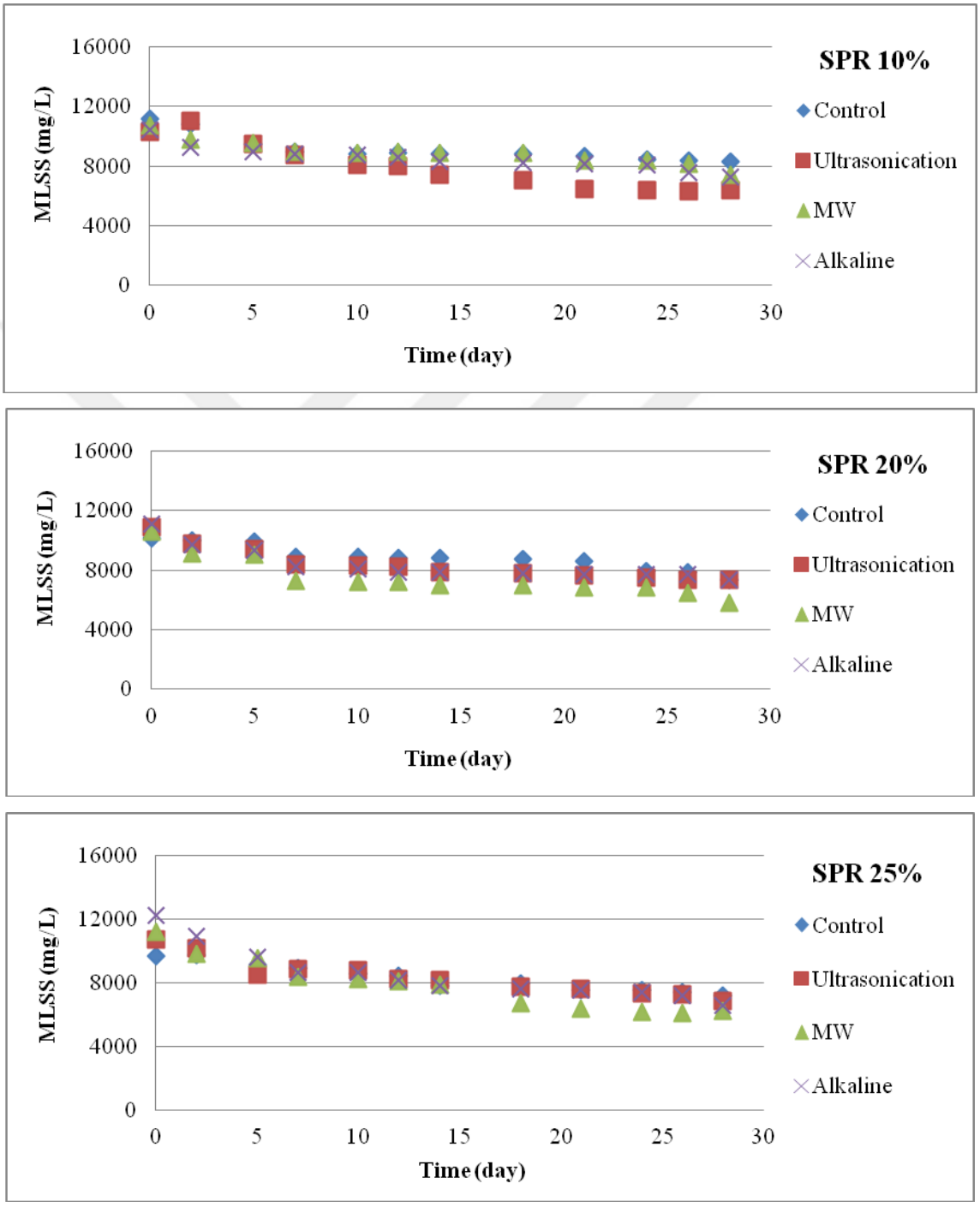

Figure 4.35. MLSS concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

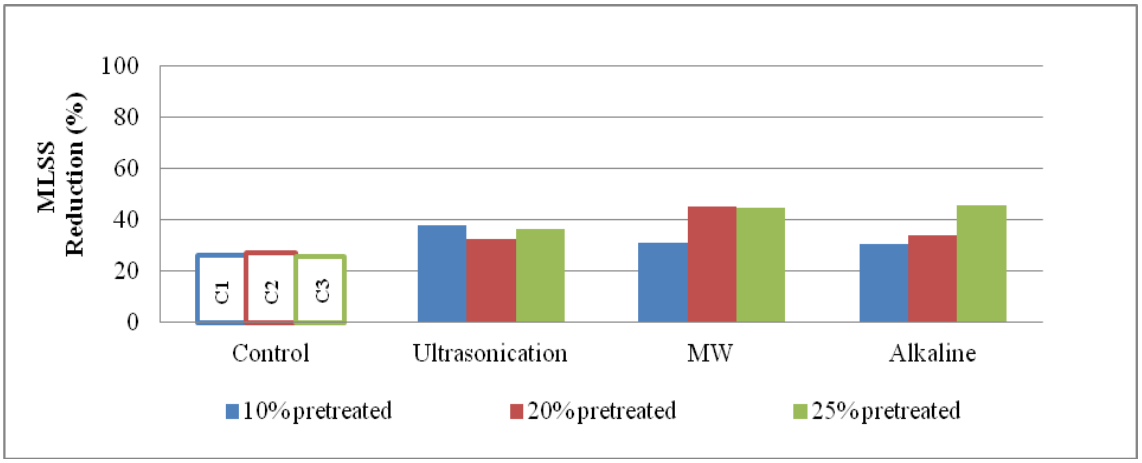

Figure 4.36. MLSS removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

MLVSS reductions in the reactors showed very similar trends with MLSS reductions. MLVSS removal efficiency was the main parameter used in the evaluation of sludge degradation. It was found that all these pretreatments improved the removal of MLVSS degradation rate and this value increased from 27% to 32%, 38%, and 50% with ultrasonication, microwave, and alkaline pretreatments applied to 25% of sludge, respectively, where, the alkaline pretreatment gave the best result under aerobic conditions. Chang et al. (2011) stated that 40-50% MLVSS reduction can be achieved during aerobic digestion of sludge at SRT of 10-15 d.

In this study, it was seen that ultrasonication pretreatment gave a MLVSS removal of 40%, 34%, and 38% for sludge pretreatment ratios (SPR) of 10%, 20% and 25%, respectively, whereas, in microwave pretreatment, MLVSS removal efficiencies were found to be 33%, 46%, and 48% for SPR of 10%, 20%, and 25%, respectively. The MLVSS reductions were found to be 28%, 30% and 27% for the control reactors (C1, C2, and C3), respectively (Figures 4.38). When the portion of pretreated sludge increased, sludge reduction in terms of MLVSS increased for microwave and alkaline pretreatment. MLVSS removal efficiencies were found to be in order with literature values. In the study of Cokgor et al. (2012), the MLSS and MLVSS concentration of the aerobically stabilized sludge was reduced leading to 22% and 28% of removal efficiency (Cokgor et al., 2012).

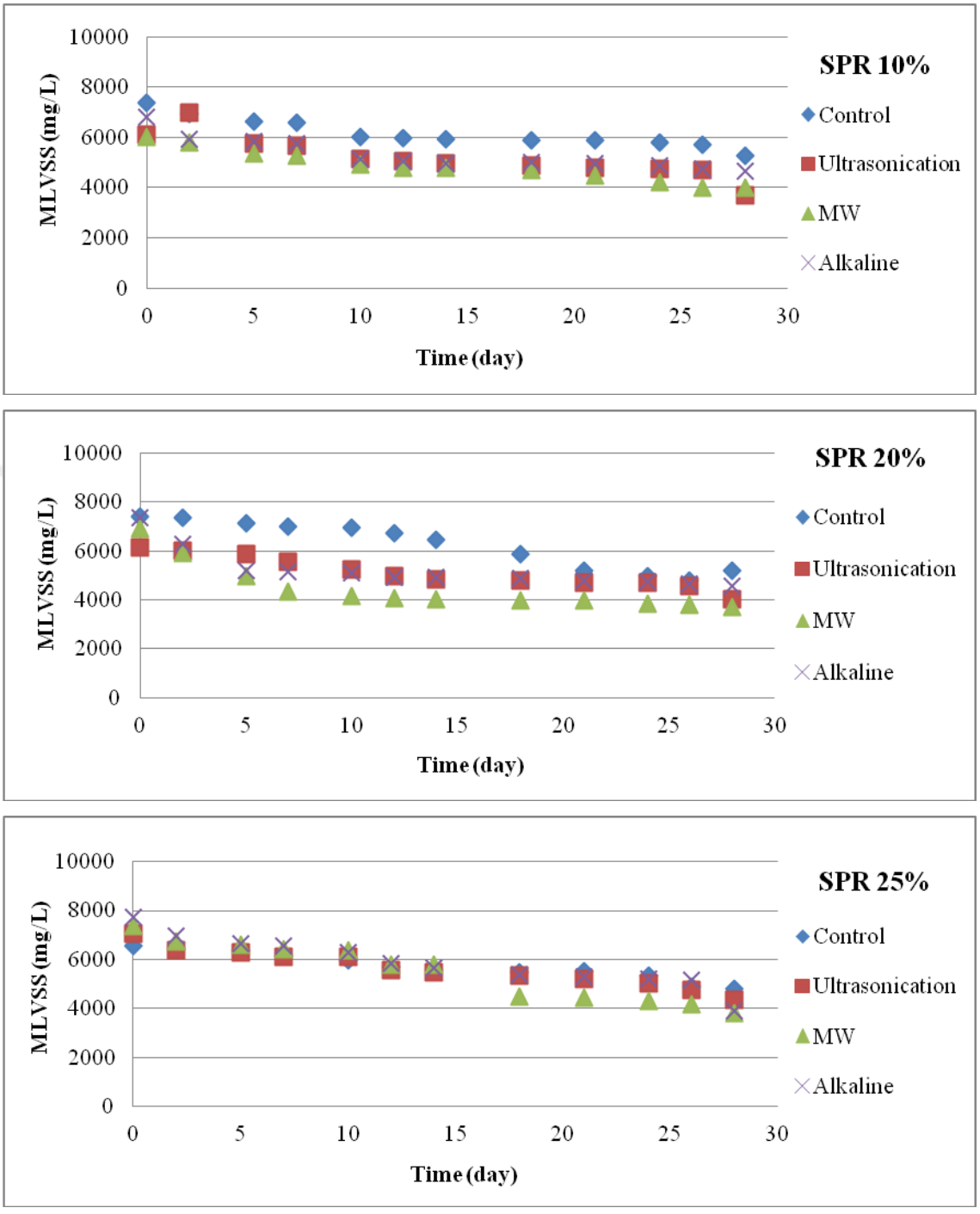

Figure 4.37. MLVSS concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

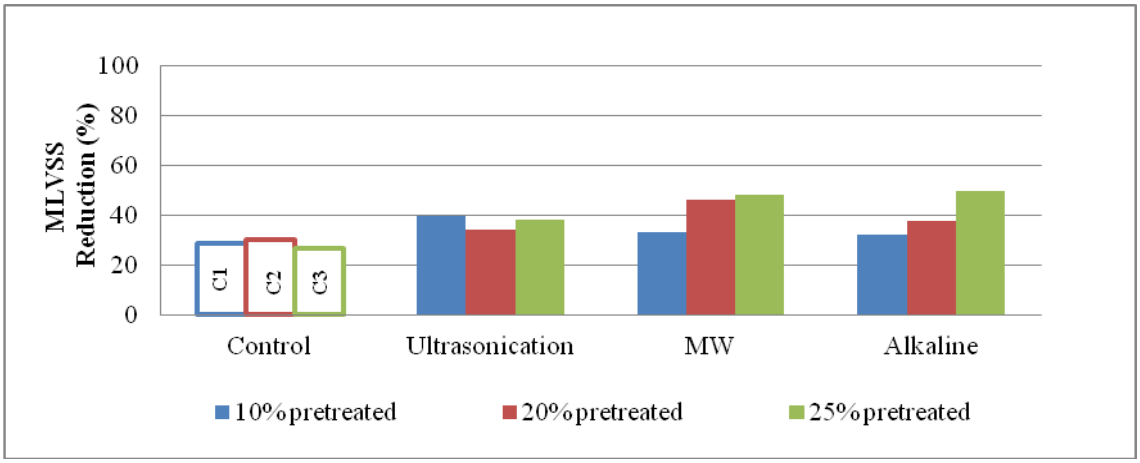

Figure 4.38. MLVSS removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

MLVSS/MLSS ratios of the reactors were given in Figure 4.39. High MLVSS/MLSS ratio showed that the sludge contained high microbial activity (Erden et al., 2010). MLVSS/MLSS ratio values decreased for all reactors since organic matter contents of sludge decreased during the aerobic digestion, but no significant changes were observed at the end of aerobic digestion. MLVSS/MLSS ratio showed a decline from 0.68, 0.66, 0.66, 0,63 at the beginning to 0.67, 0.60, 0.59, 0,58 at the end of aerobic digestion of the reactor containing unpretreated (control), ultrasonic, microwave, and alkaline pretreated sludge for SPR of 25%, respectively. The highest level of sludge degradability was obtained in the reactor fed with alkaline pretreated sludge for SPR of 25%. The MLVSS/MLSS values obtained from this study were in agrement in literature. Summary table of overall solid reductions were given in Table 4.16.

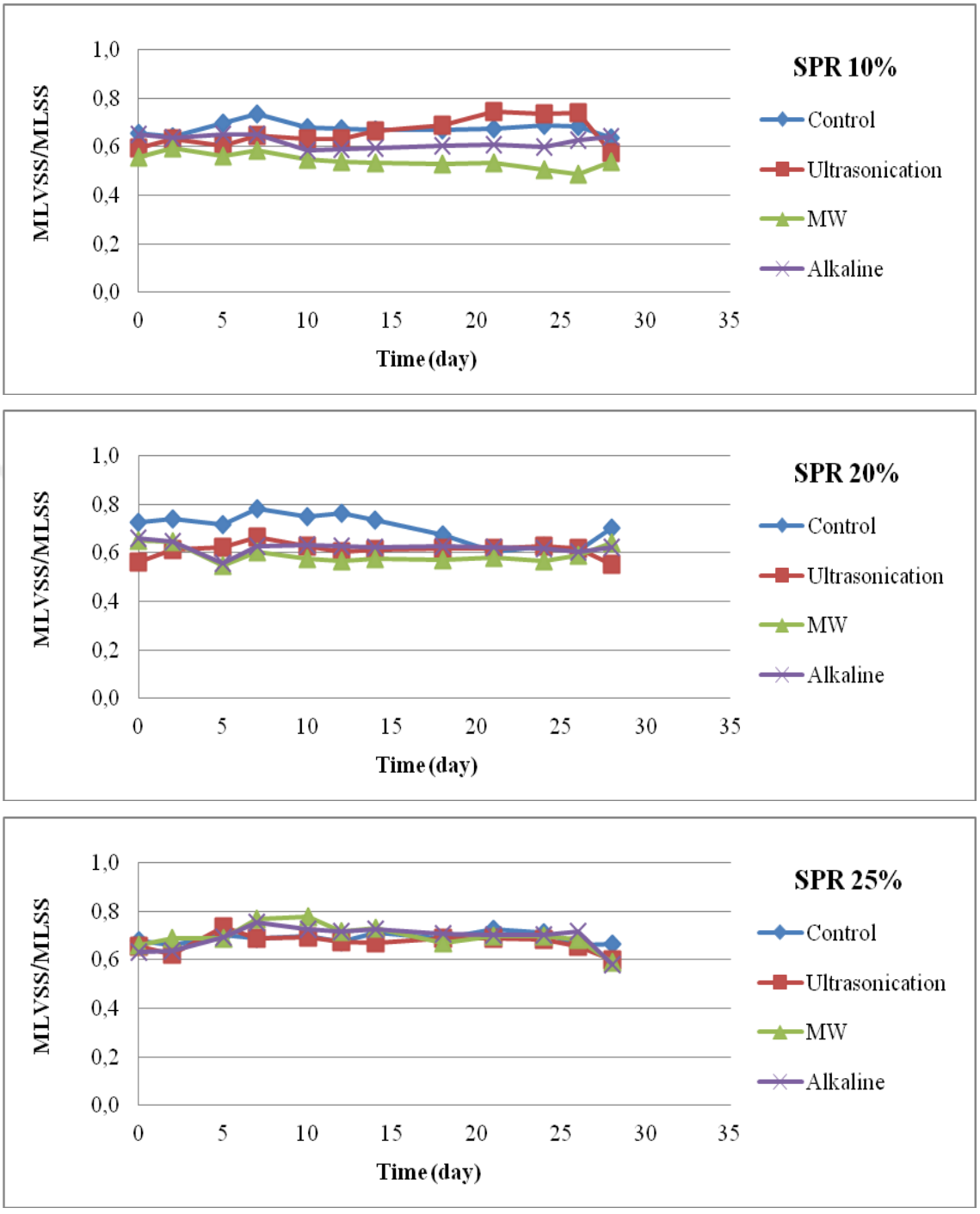

Figure 4.39. MLVSS/MLSS ratios in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

Table 4.16. Summary table of overall solids reductions in the aerobic reactors.

SPR (%)	Aerobic reactors	TS (%)	VS (%)	MLSS (%)	MLVSS (%)
10	Control	24	31	26	28
	Ultrasonication	36	52	38	40
	Microwave	27	45	31	33
	Alkaline	28	38	31	32
20	Control	25	33	27	30
	Ultrasonication	28	44	33	34
	Microwave	40	46	45	46
	Alkaline	31	41	34	38
25	Control	25	32	26	27
	Ultrasonication	33	41	36	38
	Microwave	35	39	45	48
	Alkaline	42	57	46	50

4.4.8. Total Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

Chemical oxygen demand (COD) was measured to determine the quantity of oxygen used in decomposition of organic matter and the oxidation of inorganic materials in the sludge samples. Soluble chemical oxygen demand (sCOD) was analyzed to find organic content in the soluble phase of the samples.

TCOD and sCOD concentrations in the aerobic reactors were given in Figures 4.40 and 4.42, respectively. Figure 4.41 and Figure 4.43 presented the TCOD and sCOD removal efficiencies, respectively.

The TCOD concentrations of all reactors slowly decreased in course of the aerobic digestion. The TCOD removal efficiency of the control reactor (C3) containing unpretreated sludge was about 29%. The highest TCOD removal efficiencies were found to be 37%, 39%, and 40%, respectively, for ultrasonication, microwave, and alkaline pretreatments applied to 25% of sludge samples. However, there were not a big difference in the TCOD removal efficiencies of 33%, 37%, and 39% in the reactors containing 10%, 20% and 25% ultrasonically pretreated sludge, respectively.

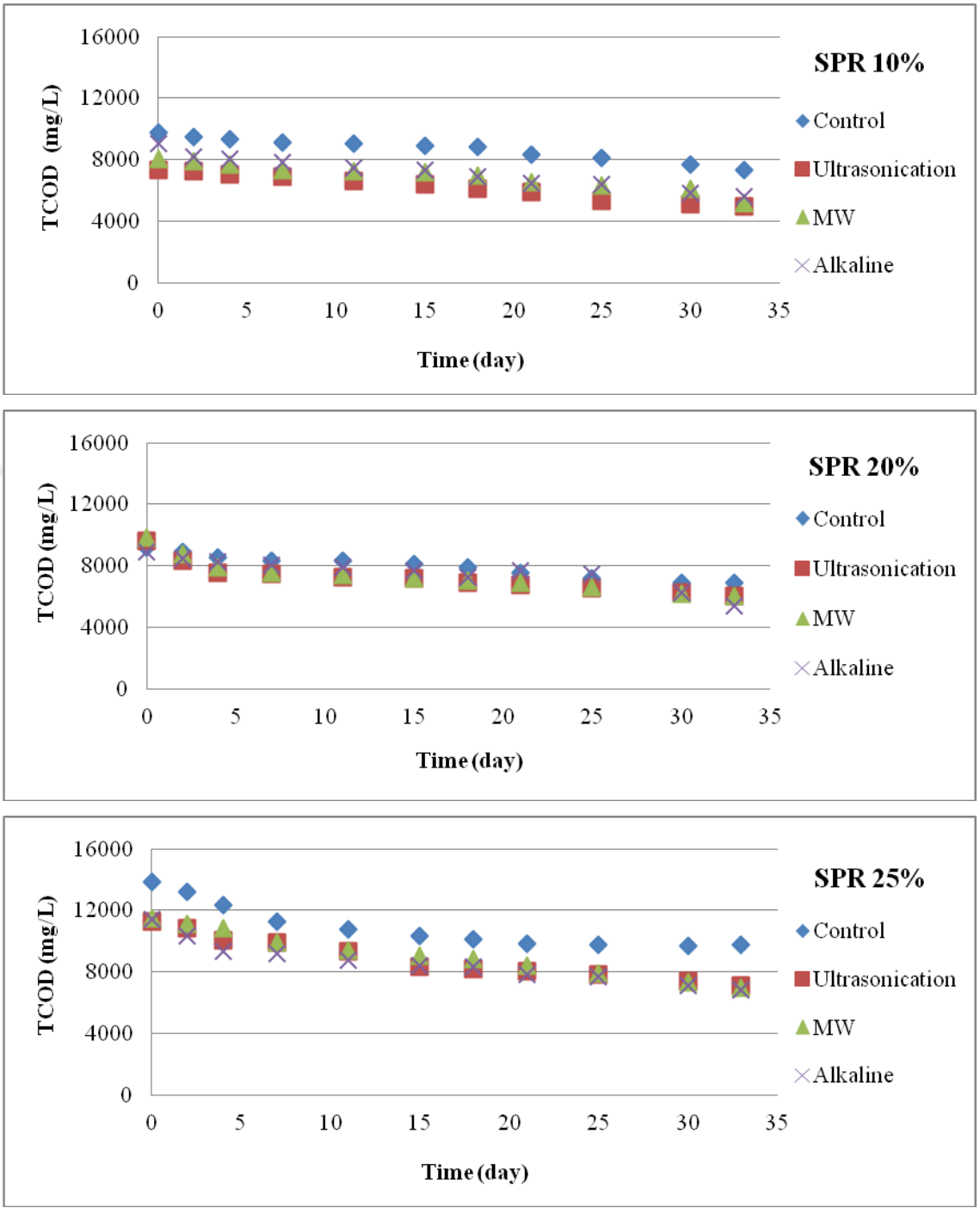

Figure 4.40. TCOD concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

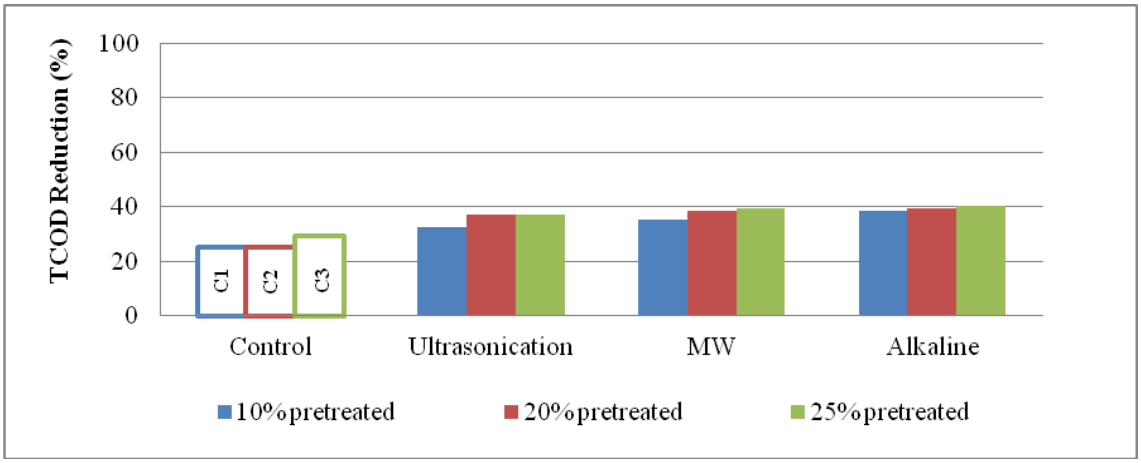

Figure 4.41. TCOD removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

The sCOD concentrations of the sludge samples disintegrated with ultrasonic, microwave and alkaline pretreatments extremely increased. These increases in the SCOD concentrations after the pretreatments were explained as the floc disintegration, the release of intracellular material and other organic constituents into the solution.

It looks pretreatment increased the rate of organic removal by speeding up the solubilization of the organics. The biodegration of the organics solubilized by the pretreatment happened in first 3 days of the digestion and the sCOD decreased from 270-300 to 60-70 mg/L. After the 3rd d, organic removal rate slowed down and sCOD concentrations of the sludge samples increased slightly. The sCOD reduction rate of the control reactor (containing only sludge sample) was 20%. However, sCOD reduction rates in reactors containing pretreated sludge samples increased more than 2 folds to be in the range of 40-63%. When the ratio of pretreated sludge (SPR) increased, the rate of sCOD removal also increased. In this study, the findings on sCOD reduction were in good agreement with literature.

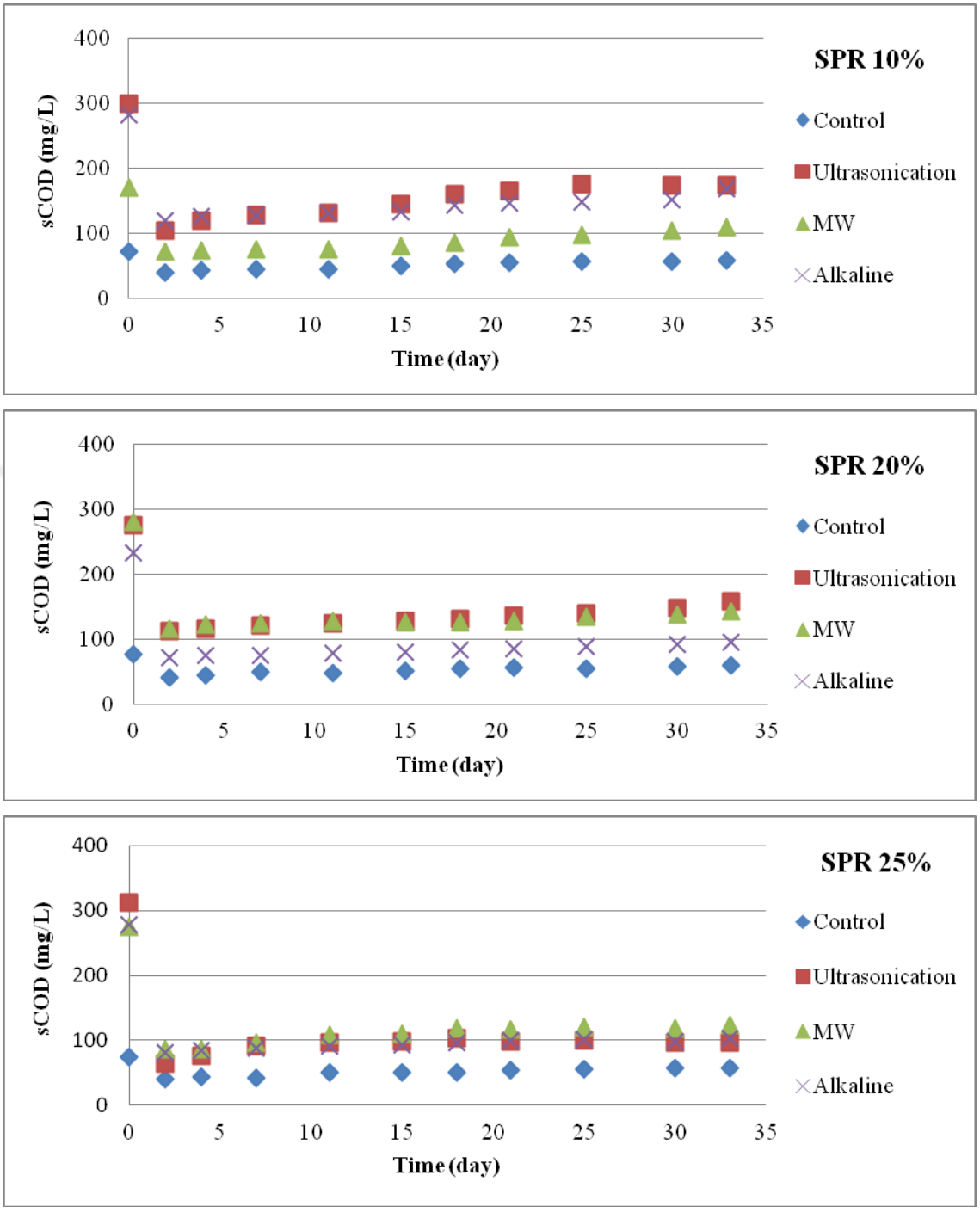

Figure 4.42. sCOD concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

The sCOD/TCOD ratios in the reactors were calculated to evaluate the solubilization of organics in the sludge solids during pretreatment. The ratio of sCOD/TCOD after ultrasonic, microwave and alkaline pretreatments applied to 25% of sludge was found to be 2.76%, 2.39%, and 2.43%, whereas this ratio for the unpretreated sludge was 0.53%. The

sCOD/TCOD ratios in control reactors (C1 and C2) were found to be 0.73% and 0.84% for the pretreatment applied to 10% and 20% of sludge, respectively. For ultrasonic, microwave, and alkaline pretreatments for SPR of 10% and 20% were found to be 4.06%, 2.12%, 3.10%, and 2.86%, 2.85%, and 2.60%, respectively. The sCOD/TCOD ratios after pretreatments increased due to the solubilization organics in sludge samples. Dogan and Sanin (2009) observed that the significant increase in sCOD/TCOD ratio from 0.5% to 18% after microwave pretreatment at 160°C for 16 min. In addition, the sCOD/TCOD ratio increased from 0.6% (raw WAS) to 28.4 for alkaline pretreatment at pH 12. The highest sCOD/TCOD ratio was found as 17.5% at 120°C temperature and 2% TS concentration in the study of Park et al. (2010). These results were relatively higher than the results of this study. This can be explained with sludge pretreatment application ratios used in the studies. In the study of Park et al. (2010), pretreatment applied to half of the sludge samples (SPR:50%).

4.4.9. Dissolved Organic Carbon

Dissolved organic carbon (DOC) concentrations in the reactors were given in Figure 4.43. DOC concentrations were lower than sCOD concentrations because DOC included carbon fraction of the organic content. At the end of the aerobic digestion, DOC concentrations in the reactors in which ultrasonic, microwave and alkaline pretreatments applied to 10% of sludge samples increased by 186%, 117%, and 93% respectively. The increase in DOC concentration in the control reactor was about 59%. In the reactors containing 25% ultrasonically, microwave, and alkaline pretreated sludges, DOC concentrations increased to 124%, 96%, and 84% respectively. The DOC content in the control reactor of this set was 75%. As shown in Figure 4.44, the better of DOC increases were observed in the reactors containing 10% and 25% of pretreated sludge samples compared to the reactors fed with 20% of pretreated sludge samples.

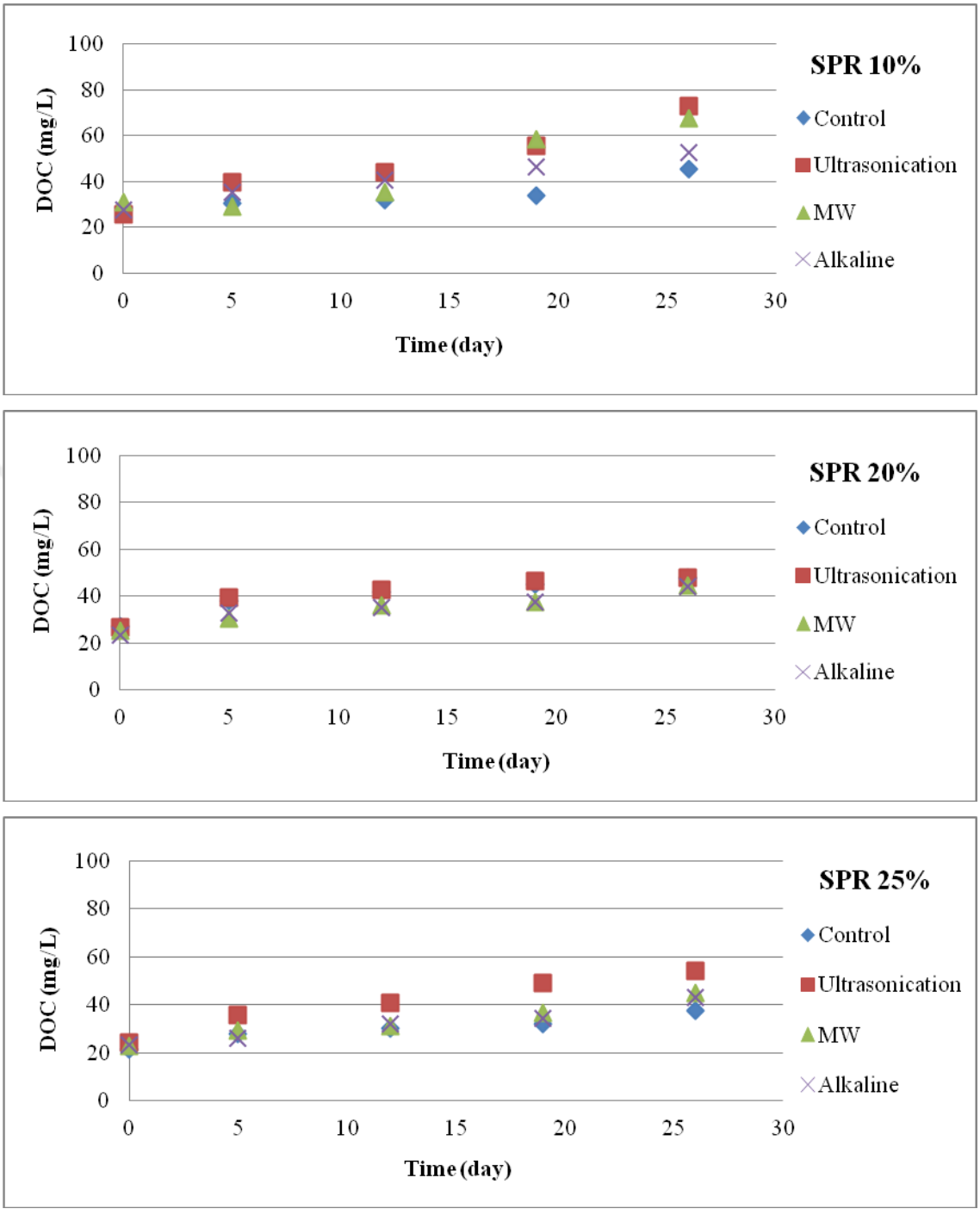

Figure 4.43. DOC concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.10. Total Kjeldahl Nitrogen and Ammonium Nitrogen

Total kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4^+-N) concentrations of all reactors were presented in Figures 4.44 and 4.45, respectively.

The initial TKN concentrations of the reactors in which ultrasonication, microwave, alkaline pretreatments having SPR of 25% were in the range of 1500 to 1900 mg/L, whereas that was found to be 500 and 1000 mg/L when 10% and 20% of sludge samples were disintegrated with same pretreatments. TKN concentrations in all reactors decreased at the end of the aerobic digestion because of the nitrification process.

When the ratio of pretreated sludge (SPR) increased, TKN removal efficiencies increased. The highest TKN removal efficiency was observed to be 77% in the reactor containing 25% of alkaline pretreated sludge. TKN removal efficiencies of the reactors containing unpretreated sludge (control reactor), ultrasonication and microwave pretreated sludge samples (SPR:25%) were found to be 43%, 56% and 60%, respectively.

In aerobic digestion, soluble organic nitrogen, which can be mineralized to ammonium nitrogen (NH_4^+-N) consists of endogenous respiration of proteinaceous amino acids. Aerobic bacteria use NH_4^+-N as an energy source in the synthesis of new cell material under proper conditions such as solids retention time, oxygen concentration, temperature and pH (Anderson and Mavinic, 1993).

As apparent from Figures 4.45, NH_4^+-N concentrations decreased in all reactors. The concentration of NH_4^+-N in the reactors in which pretreatments applied to 25% of sludge samples was higher when compared to the control reactor due to the biodegradation of solubilized sludge solids at the end of the aerobic digestion. As can be seen after the 20th day of aerobic digestion, NH_4^+-N values less than 2.5 mg/L were obtained in all reactors.

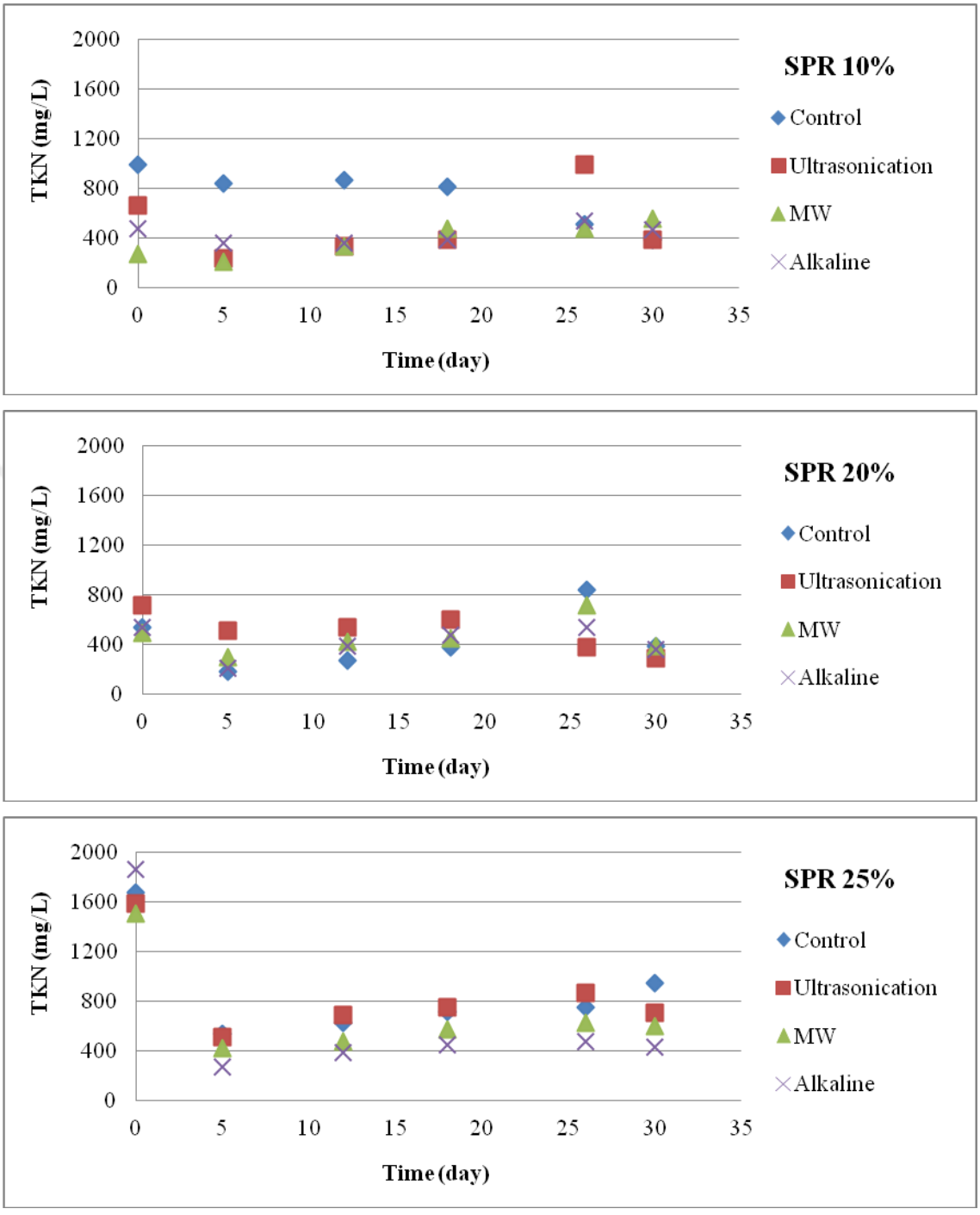

Figure 4.44. TKN concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

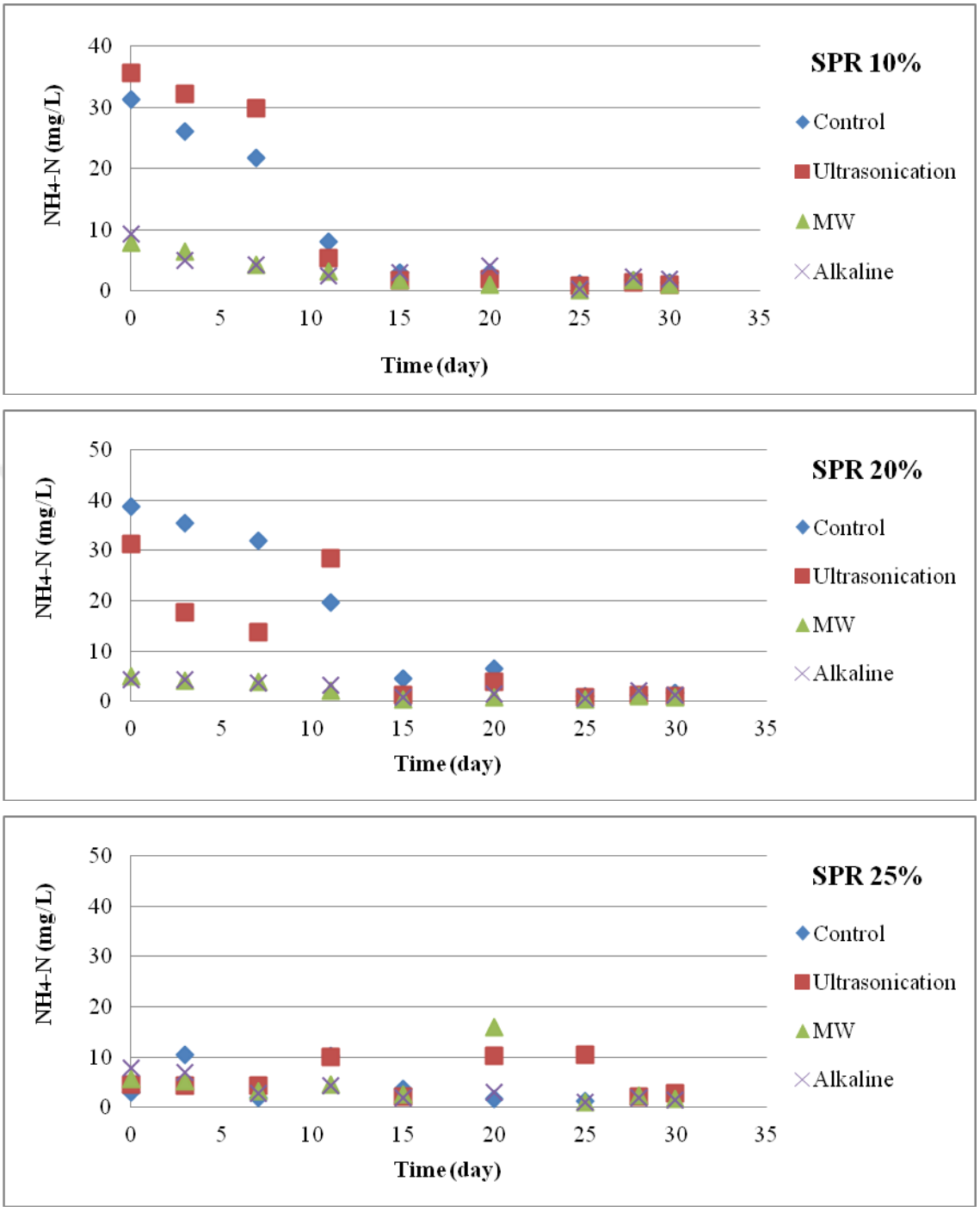

Figure 4.45. NH_4^+-N concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.11. Nitrite and Nitrate

Nitrite (NO_2^--N) and nitrate (NO_3^--N) concentrations for the aerobic reactors were given in Figures 4.46 and 4.47, respectively. Nitrite concentrations in all reactors decreased

where the nitrate concentrations increased due to nitrification reaction during aerobic digestion. The higher nitrate concentration occurred with the lower ammonia concentration because nitrifying bacteria oxidized ammonia nitrogen to nitrate nitrogen during aerobic condition (Ros and Zupancic., 2002).

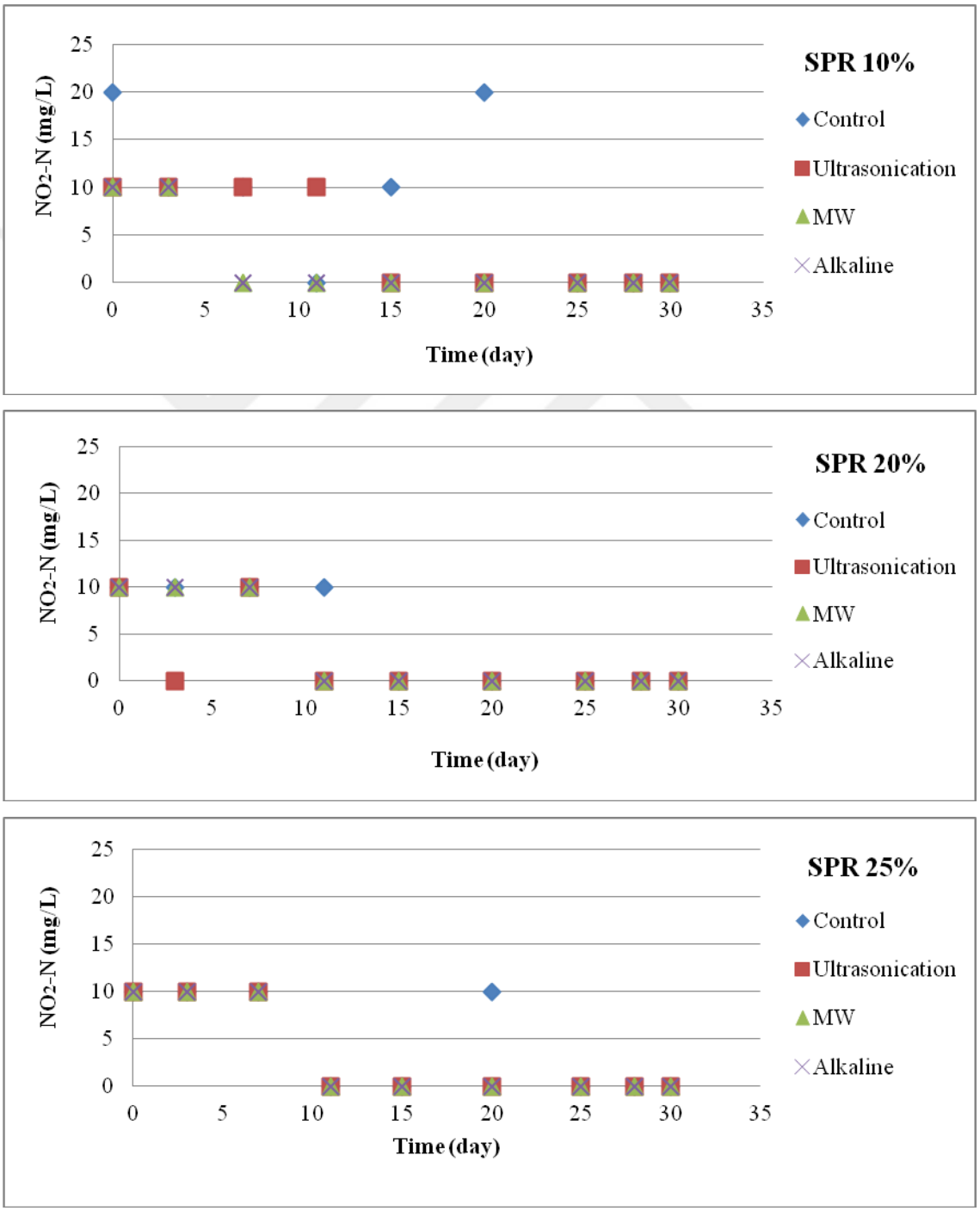

Figure 4.46. NO_2^--N concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

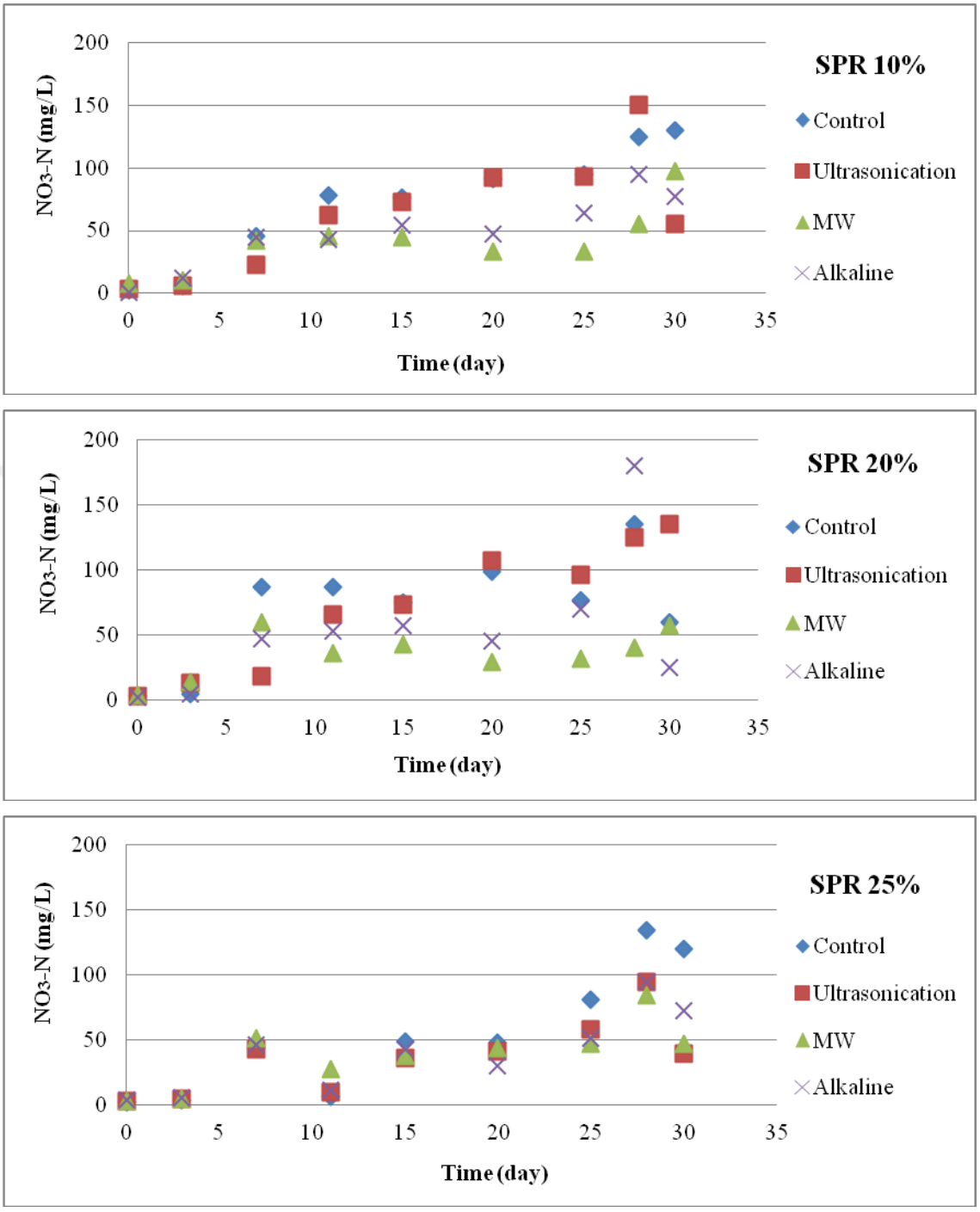

Figure 4.47. NO_3^--N concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.12. Total Phosphorus

Total phosphorus (TP) concentrations of the aerobic reactors were given in Figure 4.48. Initial TP concentrations of the reactors were in the range of 200 to 300 mg/L. At the

end of the aerobic digestion, final TP concentrations of all reactors remained almost constant at the same range. TP remains constant during aerobic digestion because of endogenous respiration (Ramadori, 1987).

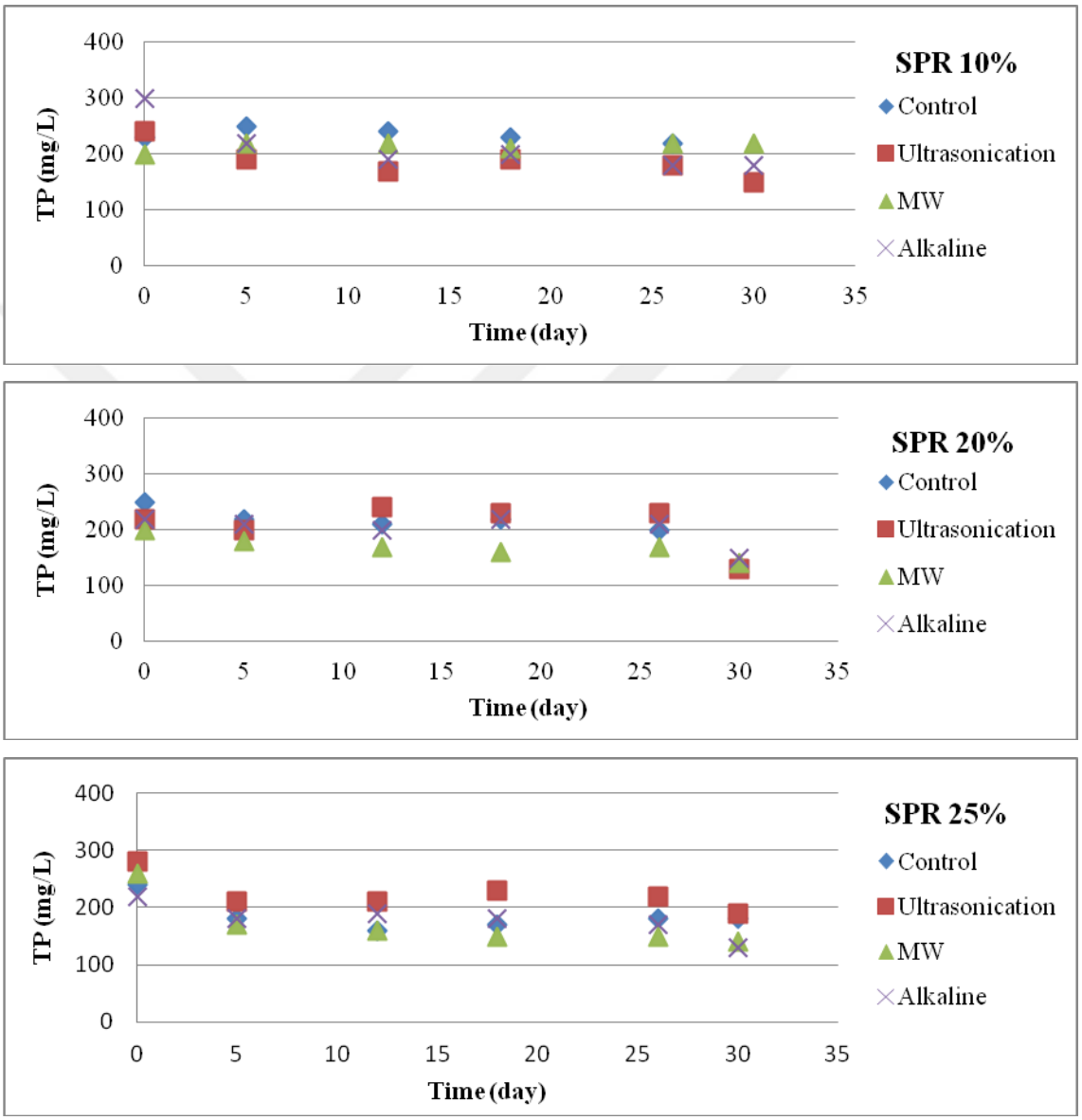

Figure 4.48. TP concentration change in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.13. Particle Size Distribution

The particle size results were given in Table 4.17. Pretreatments applied to the sludge samples prior to aerobic digestion led to a decrease in the mean particle size, d (0.5). The

sludge particles become smaller with pretreatments due to the disintegration of sludge flocs and complete breakage of the sludge cell (Chang et al., 2011). Therefore, finer particles improved the digestibility of sludge (Kennedy et al., 2007).

Table 4.17. Particle size distribution in the aerobic reactors.

SPR (%)	Aerobic Reactors	Surface Weighted Mean D[3.2] µm	Volume Weighted Mean D[4.3] µm	d(0.9) µm	d(0.5) µm	d(0.1) µm
10	**Control**					
	Day 0	40.929	81.004	156.215	68.780	22.550
	Day 30	14.059	67.183	114.798	30.225	7.701
	Ultrasonication					
	Day 0	33.064	68.614	131.399	55.775	17.622
	Day 30	16.988	51.699	97.734	32.758	10.400
	MW					
	Day 0	34.201	79.269	152.188	59.302	17.717
	Day 30	23.932	66.916	132.724	41.901	15.139
	Alkaline					
	Day 0	33.018	76.232	147.365	57.109	16.949
	Day 30	25.038	67.808	126.562	42.612	14.833
20	**Control**					
	Day 0	40.744	96.646	191.612	71.224	20.932
	Day 30	19.872	65.530	114.596	37.324	12.158
	Ultrasonication					
	Day 0	35.186	73.520	142.080	59.455	18.661
	Day 30	22.427	68.475	127.493	40.403	12.981
	MW					
	Day 0	34.424	74.928	149.113	59.415	17.633
	Day 30	33.928	74.957	141.438	55.286	16.102
	Alkaline					
	Day 0	35.137	75.555	147.584	60.344	18.237
	Day 30	32.117	68.910	127.288	50.282	18.525
25	**Control**					
	Day 0	42.344	96.632	196.774	70.737	22.211
	Day 30	26.610	59.605	109.848	41.306	15.563
	Ultrasonication					
	Day 0	40.419	95.740	190.654	68.229	21.076
	Day 30	25.888	54.913	99.548	42.658	15.852
	MW					
	Day 0	39.684	95.011	189.962	65.458	21.148
	Day 30	31.827	64.427	128.272	50.090	18.501
	Alkaline					
	Day 0	38.980	93.853	187.195	69.933	21.118
	Day 30	29.383	69.729	136.391	50.308	17.471

4.4.14. Viscosity

Viscosity is the basic rheological parameter influencing biodegradability of sludge and mass transfer in aerobic digestion. The sludge dewaterability may be closely related to the viscosity of sludge. Low viscosity means that easy to dewater by filtration (Pham et. al., 2010). The viscosity measurements were given in Figure 4.49. The viscosity values in all reactors decreased at the end of aerobic digestion. The decrease in viscosity of ultrasonication, microwave, and alkaline pretreated sludges can be attributed to shearing forces weaking sludge floc structure and disintegrating the cells. In addition, the viscosity of disintegrated sludge decreased with the increasing of temperature (Travnicek et al., 2012).

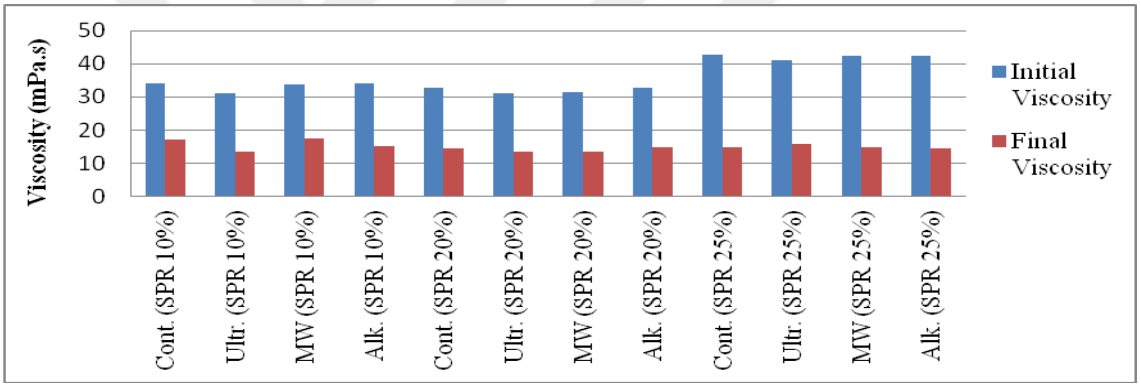

Figure 4.49. Viscosity changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.15. Capillary Suction Time

Filterability of sludge was assessed by measuring capillary suction time (CST). Vesilind, (1988) put forward to the lower CST, the better filterability and the better dewaterability. The CST results of the reactors were presented in Figure 4.51. Before the aerobic digestion, the inital CST values in the reactors containing unpretreated (control) and ultrasonication, microwave and alkaline pretreated sludges were very close to each other, almost same, except the ultrasonically pretreated sludge samples with SPR of 10% and 20%. CST of these samples increased up to 21st day of digestion and then decreased to a value close to the other samples at the end of digestion period.

The filterability constant values of the pretreated and unpretreated sludge samples in the aerobic reactors were given in Table 4.18. Prior to aerobic digestion, filterability constants (X) of the sludge samples decreased with the applications of pretreatments. After aerobic digestion, deterioration in the sludge dewaterability and filterability was observed because of a further decrease in the filterability constants (X) of the sludge samples. Before aerobic digestion, the ultrasonically pretreated sludge samples showed the highest deterioration in sludge dewaterability. The dewaterability of alkaline and microwave pretreated samples improved when compared to control sample for SPR:20% and 25%.

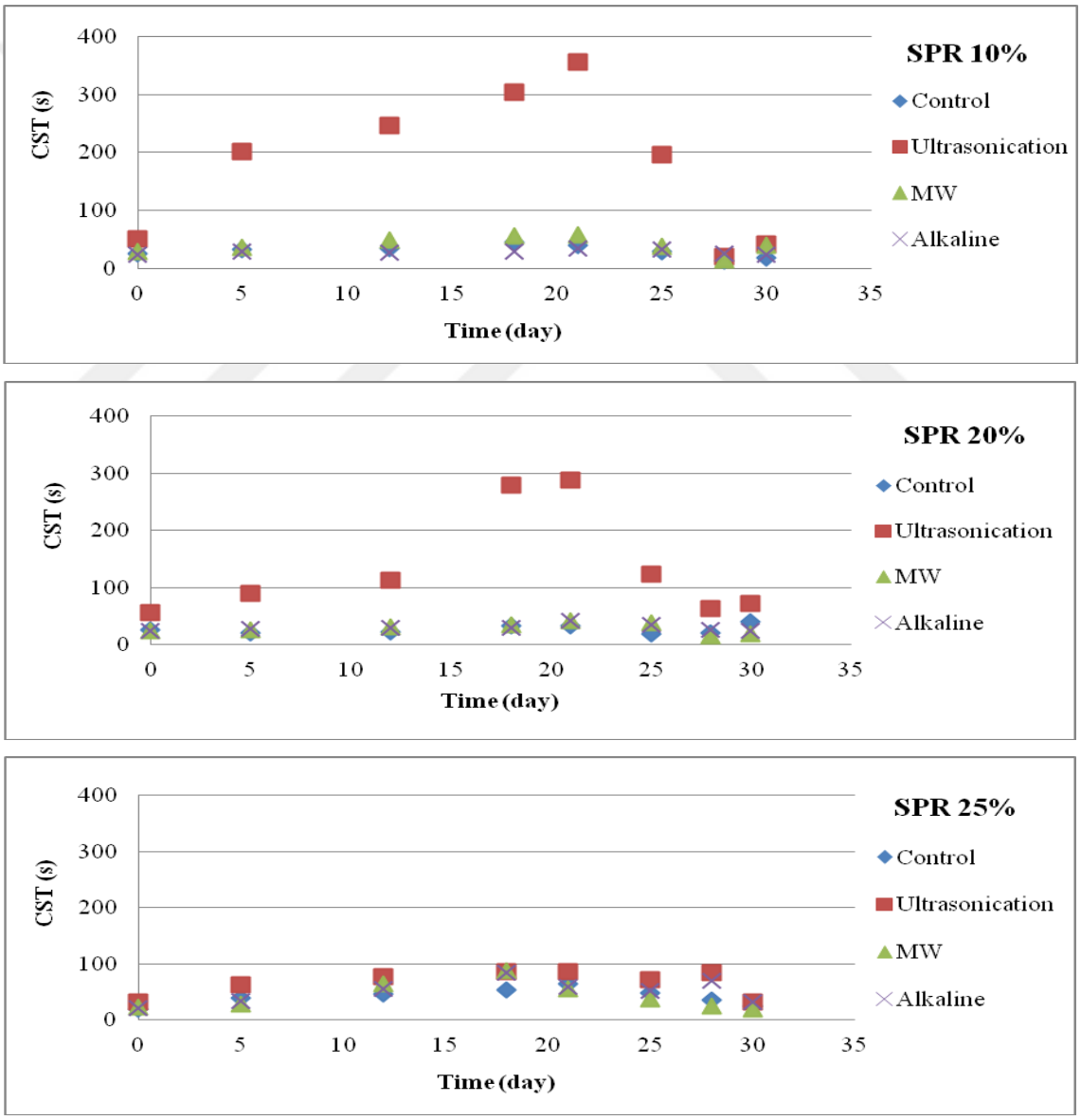

Figure 4.50. CST changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

Table 4.18. Filterability constant changes in the aerobic reactors before and after digestion.

SPR (%)	Aerobic Reactors	Filterability constant (X) ($kg^2/s^2.m^4$)	
		Initial	Final
10	Control	1.85	1.22
	Ultrasonication	0.86	0.38
	Microwave	1.61	0.50
	Alkaline	1.72	0.54
20	Control	1.93	0.42
	Ultrasonication	0.83	0.25
	Microwave	1.49	0.50
	Alkaline	1.77	0.55
25	Control	2.66	0.52
	Ultrasonication	1.54	0.43
	Microwave	2.49	0.68
	Alkaline	2.59	0.38

4.4.16. Sludge Volume Index

Sludge volume index (SVI) is measured to determine criterion for settling characteristics of sludge. The sludges having the SVI value between 50 and 100 mL/g presents good settling characteristic (Dick and Vesilind 1969; Janczukowicz et al., 2000; Jin et al., 2004). The SVI changes in the aerobic digestion process were shown in Figure 4.51. The initial SVI of the control sludge sample was about 60 mL/g. Applied pretreatments disintegrated the sludge flocs and increased the SVI values of the samples to a range of 80-91 mL/g. After the aerobic digestion of the sludge samples, SVI values further decreased to range of 55-78 mL/g (Table 4.19) as a result of easily decomposition of organics solubilized by the applied pretreatments.

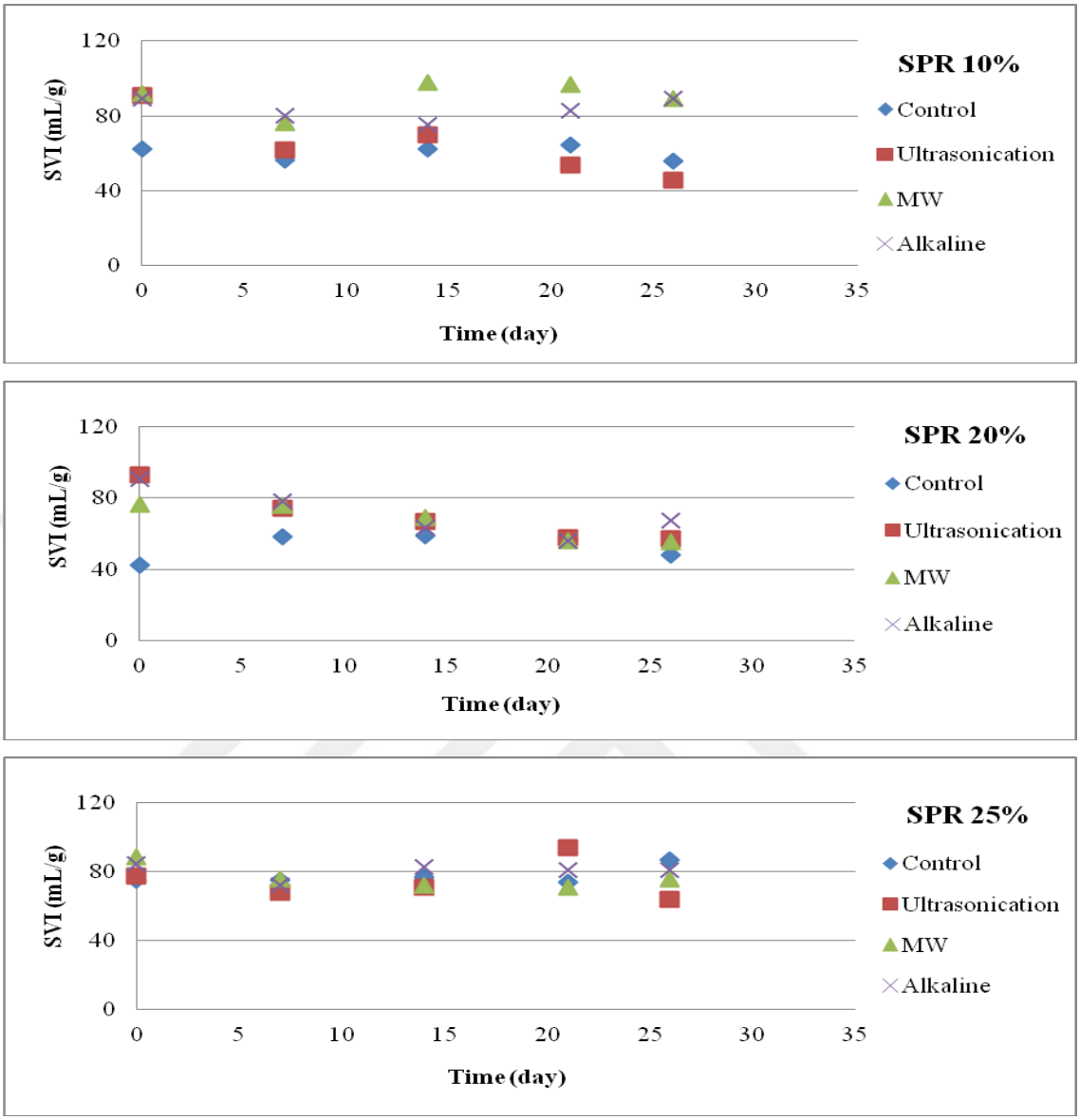

Figure 4.51. SVI changes in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

Table 4.19. SVI changes in the aerobic reactors before and after digestion.

SPR (%)	Anaerobic Digestion	Control	Ultrasonication	Microwave	Alkaline
10	Before digestion	62	91	92	89
	After digestion	55	45	89	89
20	Before digestion	42	93	77	91
	After digestion	48	57	55	67
25	Before digestion	75	78	89	84
	After digestion	87	64	76	81

4.4.17. Microbiology

Total coliform (TC), fecal coliform (FC), and fecal streptococci (FC) are present in human waste or feces of warm-blooded animals. These pathogens were analyzed in the control and pretreated reactors before and after aerobic digestion. The microbial data in the aerobic reactors were given in Table 4.20.

As can be seen in Table 4.20, most of the TC, FC, and FS bacteria were reduced in all reactors. This reduction showed a good quality and successfull stabilization for all of the reactors. This table indicated the positive effect of ultrasonic, microwave and alkaline pretreatment on TC, FC, and FS reduction. Besides, the results showed that aerobic digestion gave higher reductions in these pathogens.

Table 4.20. Microbial results of the reactors before and after aerobic digestion.

SPR (%)	Aero. Reac.	Total Coliform (TC) CFU/100mL			Fecal Coliform (FC) CFU/100mL			Fecal Streptococci (FS) CFU/100mL		
		Initial	**Fin.**	**Rem.**	**Initial**	**Fin.**	**Rem.**	**Initial**	**Fin.**	**Rem.**
10	Cont.	$1.3x10^3$	4	99.7	$1.3x10^2$	2	99.8	350	3	99.1
	Ultr.	$3.3x10^3$	45	98.6	$4.6x10^2$	21	99.5	670	-	100
	MW	$1.3x10^3$	11	99.2	$2.7x10^2$	7	99.7	550	3	99.5
	Alk.	$1.6x10^3$	33	97.9	$1.2x10^2$	12	99.0	540	2	99.6
20	Cont.	$1x10^3$	8	99.2	$2.4x10^2$	4	99.8	480	3	99.4
	Ultr.	$2.3x10^3$	3	99.9	$1.7x10^2$	2	99.9	570	-	100
	MW	$2x10^3$	18	99.1	$1.5x10^2$	4	99.7	37	-	100
	Alk.	$4.3x10^2$	27	93.7	59	9	98.5	310	7	97.7
25	Cont.	$1.5x10^3$	23	98.5	78	14	82.1	230	15	93.5
	Ultr.	$1.6x10^3$	9	99.4	67	4	94.0	390	3	99.2
	MW	$2.8x10^3$	10	99.6	252	3	98.8	420	1	99.8
	Alk.	$1.7x10^3$	12	99.3	70	5	98.0	450	-	100

At the end of digestion period, 94-100% removals in the TC, FC, and FS numbers were observed in ultrasonic pretreatment applied to 20% of sludge. It can be noteworty that the reactor fed with 20% of ultrasonicated sludge was more stable than the control reactor. Khanal et al. (2007) observed that the level of fecal coliform was reduced as 42% with

ultrasonic pretreatment using ultrasonic power input of 1.5 kW, frequency of 20 kHz and TS content of 3%. There were no big differences observed in the pathogen reduction for microwave pretreatment, when the pretreated portion of sludge increased from 10% to 25%. After aerobic digestion, a complete removals of FS pathogens were achieved in the reactor containing 10% and 20% of ultrasonically pretreated sludge, fed with 20% of microwave pretreated sludge, and including 25% of alkaline pretreated sludge.

4.4.18. Carbon to Nitrogen Ratio

The carbons to nitrogen (C/N) ratios in all aerobic reactors were presented in Figure 4.52. The C: N ratio of 10:1 was suggested for aerobic digestion process in literature. The C/N ratios in the reactors were measured to be in the range of 9-16 at the beginning and 7-13 at the end of the aerobic digestion, indicating within the ideal ranges.

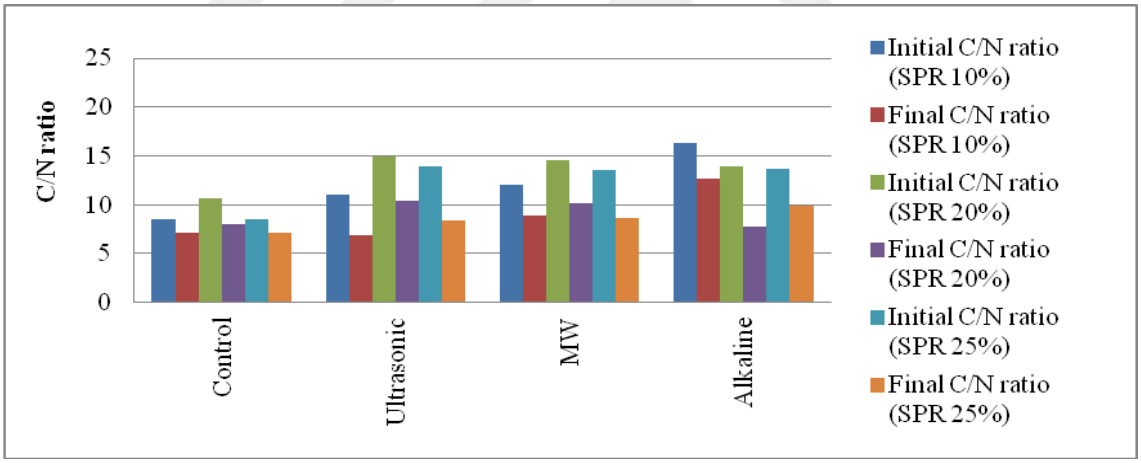

Figure 4.52. C/N ratio in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

4.4.19. Soluble Protein and Carbohydrate

The concentrations in soluble protein and carbohydrate for each aerobic reactor before and after aerobic digestion were given in Figures 4.53 and 4.55, respectively. The removal efficiencies of the soluble protein and carbohydrate were shown in Figures 4.54 and 4.56 respectively.

Ultrasonic, microwave and alkaline pretreatments applied prior to aerobic digestion led to an improvement in the soluble protein and carbohydrate concentrations of sludge samples. Carbohydrates in the sludge have a higher tendency for solubilization and biodegradation compared to proteins. 440% and 708% solubilization of protein and carbohydrate was achieved in the aerobic reactor containing microwave pretreated sludge samples at SPR of 25%. The soluble protein and carbohydrate concentrations of the sludge samples decreased in the reactors including pretreated sludge samples during aerobic digestion while that of the control reactor containing unpretreated sludge samples almost did not change. After the aerobic digestion, the highest reductions in soluble protein and carbohydrate contents were obtained in sludge samples pretreated with microwave irradiation.

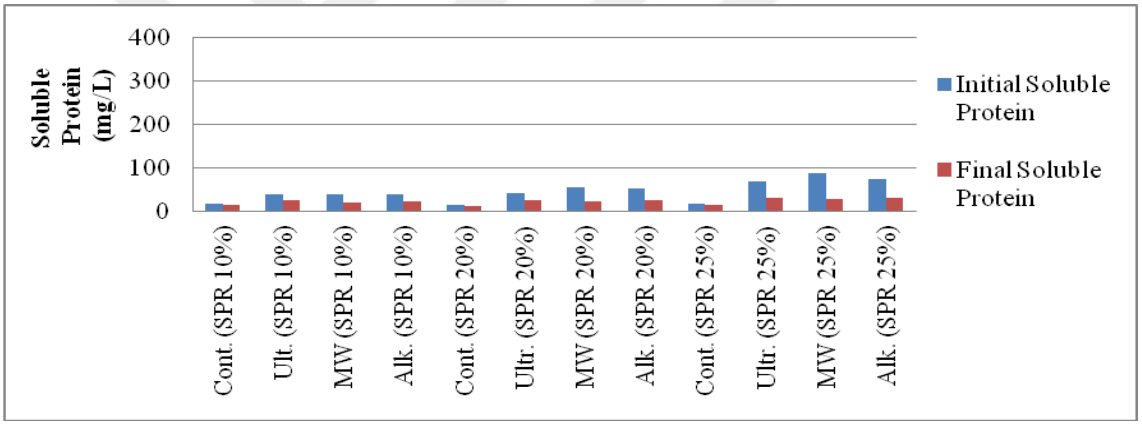

Figure 4.53. Soluble protein concentrations in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

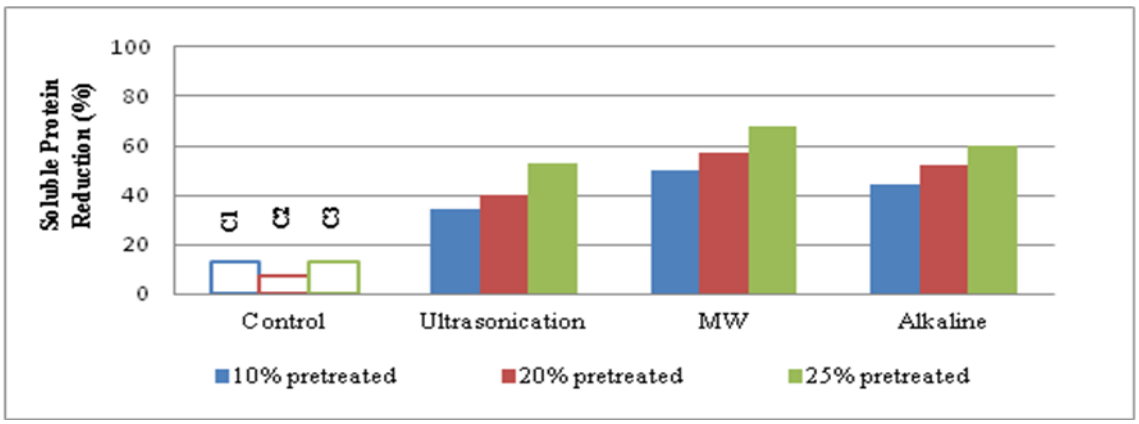

Figure 4.54. Soluble protein removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

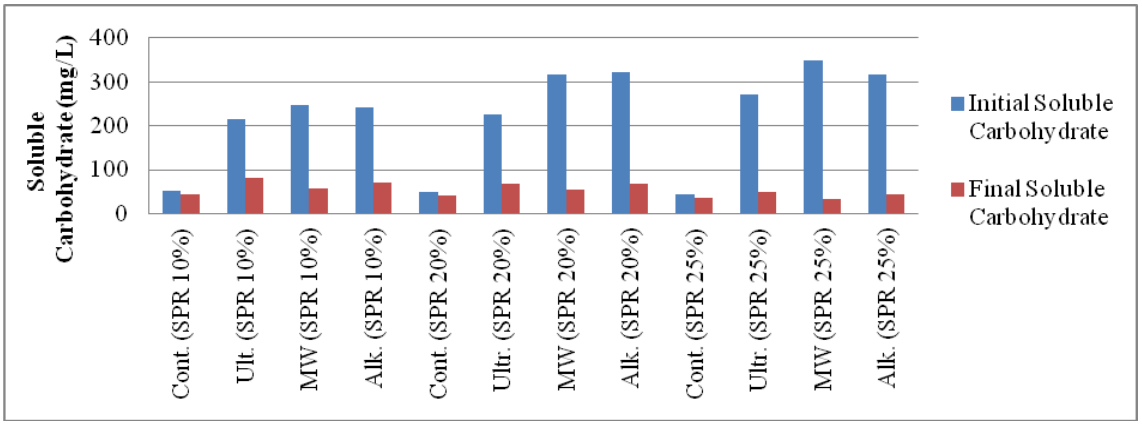

Figure 4.55. Soluble carbohydrate concentrations in the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

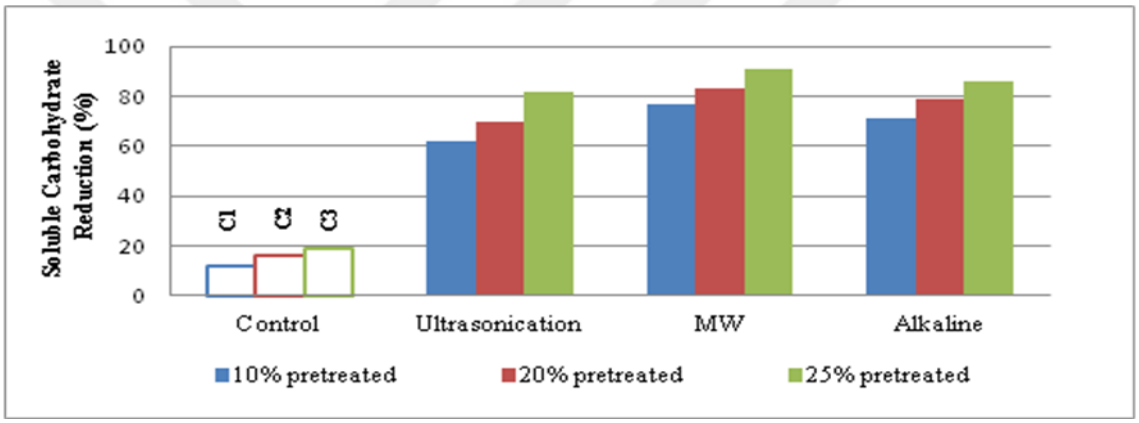

Figure 4.56. Soluble carbohydrate removal efficiencies of the aerobic reactors for sludge pretreatment ratios (SPR) of 10%, 20% and 25%.

5. CONCLUSIONS

This study investigated the effects of ultrasonic, microwave, and alkaline pretreatments on the aerobic and anaerobic digestion of wastewater sludges. The conclusions were given separately for aerobic and anaerobic digestion processes:

- Sludge pretreatment increased the efficiencies of aerobic and anaerobic digestion by disintegrating the sludge floc structure and improving the solubility of the organic solids in sludge. Solubility of organics increased with increasing specific energies (8000-48000 kJ/kg TS) in ultrasonication, rising temperature (100 to 175ºC) in microwave and increasing pH (10 to 12) in alkaline pretreatments.

- Applied pretreatments increased the organic removal efficiencies, minimized the sludge production in the aerobic and anaerobic digestion processes.

- After pretreatments applied to sludge samples, solubilization of EPS protein and carbohydrate increased by about 1.5-5 folds and 1.5-7 folds, respectively.

5.1. The Main Results of Anaerobic digestion process

- Ultrasonication (at specific energy of 12000 and 24000 kJ/kg TS), microwave, alkaline, and combined microwave-alkaline pretreatments applied to sludge samples before anaerobic digestion increased sCOD/TCOD ratios of the samples by 57%, 58%, 46%, 39%, and 46%, respectively.

- Pretreatments minimized the sludge production in anaerobic digestion by 20%-26%.

- In the anaerobic digestion, the biogas and methane productions were greatly improved by the application of ultrasonic, microwave, alkaline and combined microwave-alkaline sludge pretreatments. The highest biogas yields of 0.51 and

0.52 Lbiogas/gVS_{added} were achieved in the reactors containing alkaline and microwave pretreated sludge samples, respectively. On the other hand, the highest methane yield of 0.37 LCH_4/gVS_{added} was obtained by the application of microwave irradiation. After the application of sludge pretreatments, the biogas yields were improved about 55 to 58% compared to unpretreated sludge sample. Ultrasonication, alkaline and microwave pretreatments increased the methane yields by 40%, 56%, and 77%, respectively.

5.2. The Main Results of Aerobic Digestion Process

- At the end of aerobic digestion, sCOD/TCOD ratios in the reactors improved from 0.53% to 2.76%, 2.39% and 2.43% by ultrasonication, microwave, and alkaline pretreatments applied to the 25% of the sludge samples, respectively.

- Pretreatments applied to sludge sample increased the VS removal efficiency as a function of sludge pretreatment ratio (SPR). The highest VS and MLVSS removal efficiencies were obtained from alkaline pretreatment applied to 25% of the sludge sample. After the alkaline pretreatment, the VS and MLVSS reductions improved from 32% and 27% to 57% and 50% respectively, indicating an important reduction in sludge production.

- Pretreatments applied to sludge samples prior to aerobic digestion caused to a decrease in the sludge filterability constants (X), indicating deterioration in the sludge dewaterability, by reducing the particle size and releasing the intracellular materials out. The aerobic digestion led to a further decrease in the filterability constants (X) of the sludge samples.

- The highest deterioration in sludge dewaterability was observed in ultrasonically pretreated sludge samples before the aerobic digestion. After the aerobic digestion of sludges with SPR:10%, the highest dewaterability was measured in the control sample followed by the alkaline, microwave and ultrasonically pretreated samples. When the sludge pretreatment ratio increased to 20% and 25%, the dewaterability

of alkaline and microwave pretreated sludge samples improved compared to unpretreated sludge sample (control).

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APPENDIX A

Calibration Curves

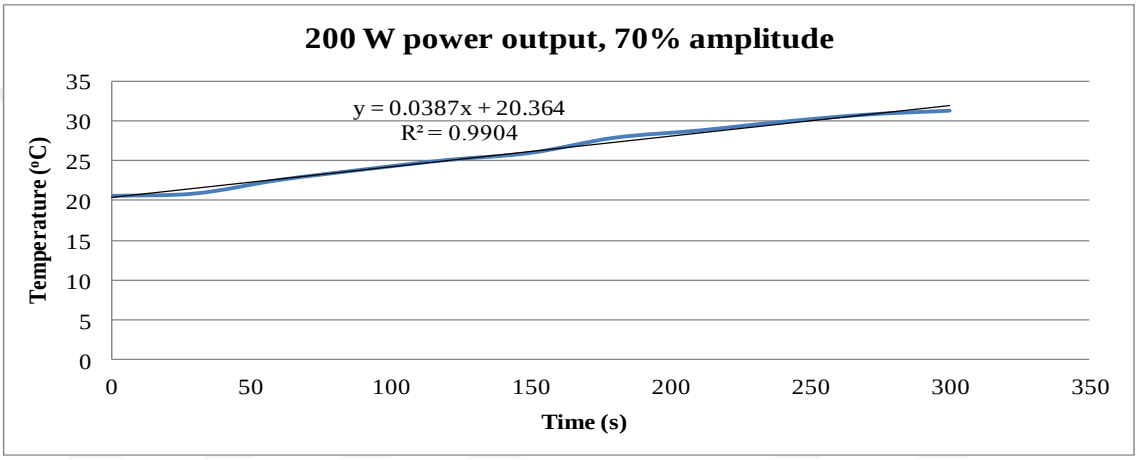


Figure A.1. The temperature against time obtained from calorimetric dosimetry.

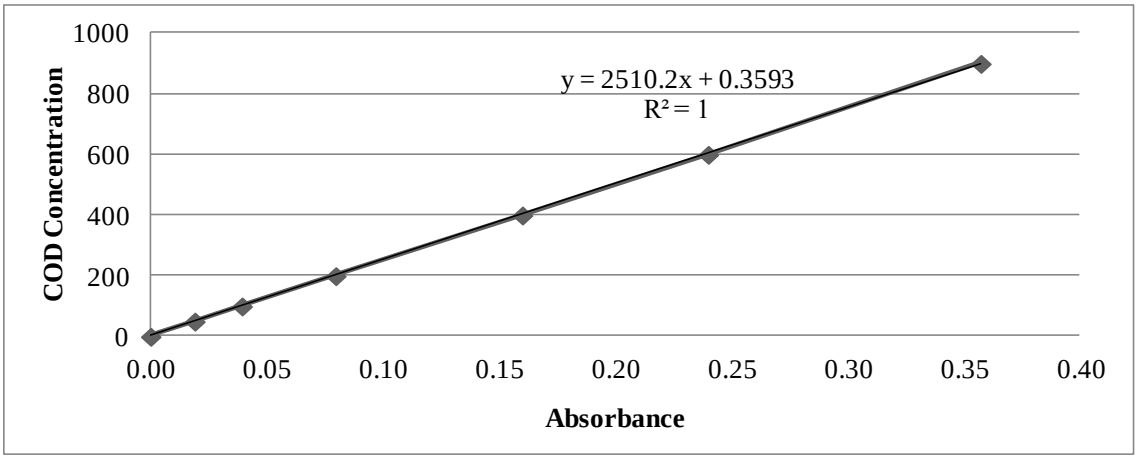


Figure A.2. The calibration curve prepared for COD analysis.

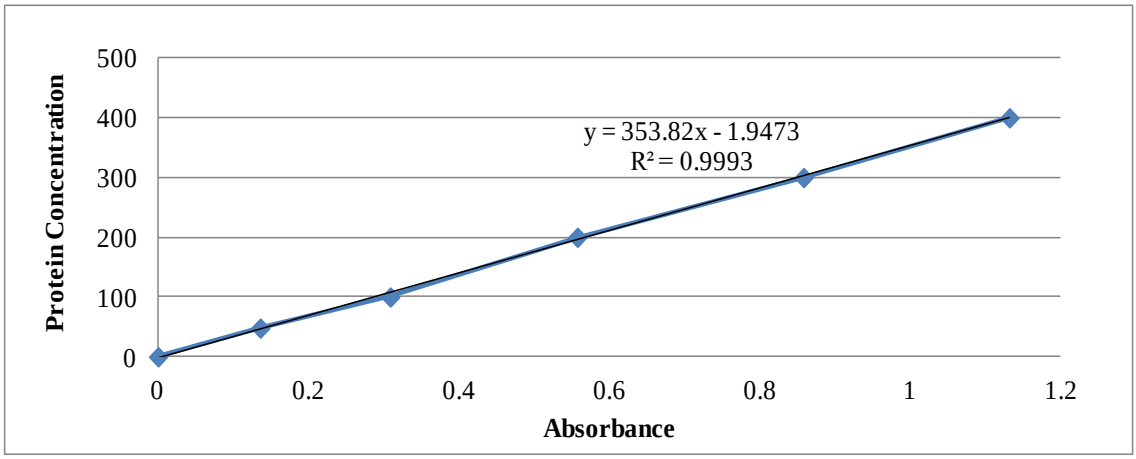


Figure A.3. The calibration curve obtained for the protein analysis.

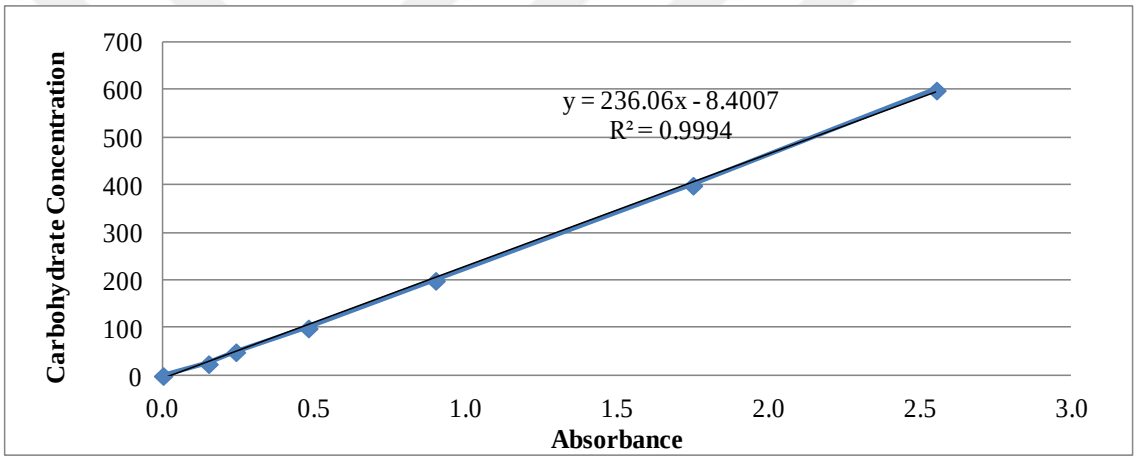
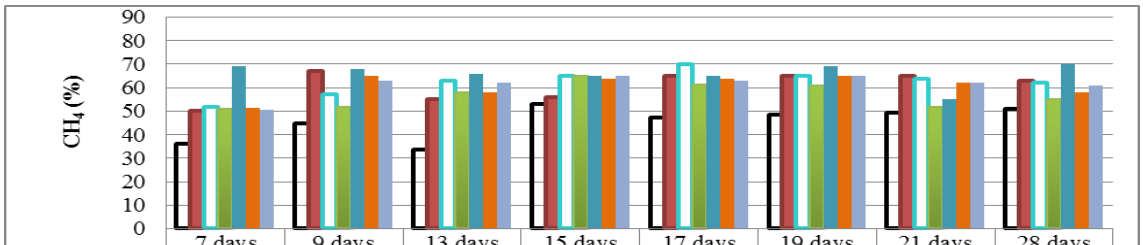


Figure A.4. The calibration curve obtained for the carbohydrate analysis.

APPENDIX B

Methane Percentages



	7 days	9 days	13 days	15 days	17 days	19 days	21 days	28 days
Inoculum	36	45	34	53	47	49	49	51
Control	50	67	55	56	65	65	65	63
U-15	52	57	63	65	70	65	64	62
U-30	51	52	58	65	61	61	52	55
MW	69	68	66	65	65	69	55	70
Alk.	52	65	58	64	64	65	62	58
MW+Alk	51	63	62	65	63	65	62	61

Figure B.1. Methane content of the reactors during anaerobic digestion.