EFFECT OF ULTRASOUND AND MICROWAVE PRETREATMENTS ON AEROBIC AND ANAEROBIC DIGESTION AND EPS STRUCTURE OF WASTEWATER SLUDGES

by

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ABSTRACT

The treatment and disposal of excess sludge is one of the critical issues facing modern society, due to the increase in sludge production. The research for producing less sludge is therefore necessary. Recently, many different studies were conducted in order to minimize the sludge during the wastewater treatment line or prior to sludge stabilization. This study investigates the effect of ultrasonic and microwave pretreatment applications prior to aerobic and anaerobic digestion of waste activated sludge in terms of stabilization efficiency, pathogen removal and biogas production. Sludge samples were collected from four different domestic and urban wastewater treatment plants. Ultrasonication and microwave irradiation disintegration methods were applied to specific portion of the sludge samples. In order to evaluate the digestion efficiency, one control reactor was operated. Furthermore, the change in extracellular polymeric substances (EPS) structure and dewatering properties of the sludge with the specified pretreatments were examined.

Both pretreatment applications showed significant enhancement of stabilization for each wastewater sample. In aerobic digestion, pretreated reactors showed better organic reduction with an average of 45% and 42.5% volatile solids (VS) reduction for microwave irradiation and ultrasonication application, respectively while the VS reduction of the unpretreated samples was 31.5% on average. Chemical oxygen demand (COD) removals also increased with disintegration: an average of 31.5%, 33.5% and 21.75% for microwave irradiation, ultrasonication application and unpretreated samples, respectively. The applied disintegration methods resulted in the solubilization of the EPS of the sludge. Therefore the dewaterability of the sludge deteriorated right after the ultrasonication and irradiation applications. However, as the EPS removal was enhanced, dewaterability of the sludge increased during the digestion. Biogas production was increased significantly with both disintegrations. For all different wastewater samples, cumulative biogas productions of microwave irradiated samples were the highest ones. In addition, both aerobic and anaerobic digestion processes pathogen reduction was successfully achieved.

ÖZET

Atık çamurların arıtılması ve bertarafı, gittikçe artan çamur oluşumu nedeniyle modern toplumun en kritik sorunlarından biri haline gelmiştir. Bu sebeple, daha az çamur üretimi üzerine yapılacak araştırmalar yapılması zaruri olmuştur. Son yıllarda çamurun atıksu arıtımı sırasında ya da çamur stabilizasyonu öncesinde hacminin azaltılmasına yönelik pek çok çalışma gerçekleştirilmiştir. Bu çalışmada, havalı ve havasız çürütme öncesinde uygulanan ultrason ve mikrodalga yöntemlerinin, çamurun stabilizasyon verimini, patojen giderimini ve biyogaz oluşumunu nasıl etkilediği araştırılmaktadır. Çamur örnekleri dört farklı evsel ve kentsel arıtma tesisinden temin edilmiş ve her iki uygulama, stabile edilecek çamurun belli bir oranına uygulanmıştır. Stabilizasyon veriminin ölçülebilmesi için ön-arıtma uygulanamamış olan reaktörler de dezentegre edilmiş çamurlarla birlikte işletilmiştir. Bunun yanında, ultrason ve mikrodalga parçalama yöntemlerinin çamurun ekstasellüler polimerik madde (EPS) yapısını nasıl değiştirdiği de araştırılmıştır.

Her iki ön-arıtım yöntemi, kullanılan tüm çamur örneklerinin stabilizasyonunda iyileştirici etki göstermiştir. Havalı çürütme işleminde, dezentegre edilmiş çamurlar, mikrodalga ve ultrason uygulamaları için sırasıyla 45% ve 42.5% oranında uçucu katı madde (UKM) giderimi elde edilmiştir. Bu değer için ön-arıtım uygulanmamış reaktörlerdeki ortalama ise 31.5% oranındadır. Dezentegrasyon yöntemlerinin kimyasal oksijen ihtiyacı (KOİ) gideriminde de etkili olduğu görülmüştür: mikrodalga ve ultrason uygulamalarındaki ortalama KOİ giderim yüzdeleri sırasıyla 31.5% ve 33.5% olmuştur. Kontrol reaktörlerinin ortalama KOİ giderim yüzdesi ise 21.75%'tir. Uygulanan ön-arıtım yöntemleri çamurun içindeki EPS'lerin de yüksek oranda çözünmesini sağlamıştır. Çözünmüş EPS konsantrasyonundaki artış çamurun susuzlaştırılabilirliğini ilk olarak kötüleştirdiyse de stabilizasyon sırasında çözünmüş EPS'lerin giderimi sağlandıkça çamurun susuzlaştırılabilirliği artmıştır. Çamurun havasız çürütülmesi sırasında her iki ön-arıtım yönteminin de biyogaz oluşumunda oldukça belirli bir artış sağladığı ancak dört farklı çamur örneği için de mikrodalga yönteminin daha etkili olduğu görülmüştür. Ayrıca, tüm reaktörlerde patojen giderimi de başarıyla sağlanmıştır.

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Symbol	Explanation	Units Used
EPS	Extracellular Polymeric Substance	
COD	Chemical Oxygen Demand	(mg/L)
DD	Disintegration Degree	(%)
sCOD	Soluble Chemical Oxygen Demand	(mg/L)
CH_4	Methane	(%)
CO_2	Carbon Dioxide	
H_2O	Water	(L)
HRT	Hydraulic Retention Time	(days)
SRT	Sludge Residence Time	(days)
WWTP	Wastewater Treatment Plant	
MW	Microwave Irradiation	
TS	Total Solids	(mg/L)
VS	Volatile Solids	(mg/L)
MLSS	Mixed Liquor Suspended Solids	(mg/L)
MLVSS	Mixed Liquor Volatile Suspended Solids	(mg/L)
TOC	Total Organic Carbon	(mg/L)
DOC	Dissolved Organic Carbon	(mg/L)
TKN	Total Kjeldahl Nitrogen	(mg/L)
NH_4-N	Ammonium Nitrogen	(mg/L)
NO_3	Nitrite	(mg/L)
NO_2	Nitrate	(mg/L)
TP	Total Phosphorous	(mg/L)
SO_4^{2-}	Sulfate	(mg/L)
Cl^-	Chloride	(mg/L)
CST	Capillary Suction Time	(seconds)
TC	Total Coliform	(CFU/100 mL)
FC	Fecal Coliform	(CFU/100 mL)

FS	Fecal Streptococci	(CFU/100 mL)
ORP	Oxidation Reduction Potential	(mV)
DO	Dissolved Oxygen	(mg/L)
VFA	Volatile Fatty Acids	(mg/L)
GC	Gas Composition	
CER	Cation Exchange Resin	
EC	Electrical Conductivity	(mS/cm)
TB-EPS	Tightly Bound EPS	(mg/L)
LB-EPS	Loosely Bound EPS	(mg/L)
tEPS	Total EPS	(mg/L)
sEPS	Soluble EPS	(mg/L)
PS	Polysaccharides	(mg/L)
PN	Proteins	(mg/L)

1. INTRODUCTION

The treatment and disposal of wastewater sludge is the major contributor of the operational costs of wastewater treatment processes which may constitute as much as 35-60% (Neyens et al.,2004; Feng et al., 2009). Gradual increases in water consumption and increased stringency of environmental legislative quality requirements of sludge also cause an enormous increase in sludge production.

Reducing the amount of the sludge during the wastewater treatment by process modifications or sludge treatment by applying pretreatment (disintegration) is one way of dealing with excessive sludge problem and it is called as 'sludge minimization'. By minimizing the amount, investment and operational costs of the sludge treatment are reduced as well as the post-treatment costs.

For sludge stabilization, aerobic and anaerobic digestion processes are the most commonly applied processes. Sludge disintegration is the term used to define the applications which cause sludge cells to rupture for enabling the rapid solubilization of larger particles. Hence, the hydrolysis of sludge during the digestion process is accelerated and digestion efficiency is improved (Carrere et al., 2010; Bougrier et al., 2006; Braguglia et al., 2012).

Sludge disintegration technologies have been available for several decades; however, technological developments have brought some sludge methods to the forefront (Roxburgh et al., 2006). Recent years have seen growing interest in ultrasound application and microwave irradiation as promising techniques for sludge minimization since the use of ultrasound and microwave energy may lead to remarkable volume reduction, lower operation times and savings in energy consumption (Yu et al., 2010; Tiehm et al., 1997; Eskicioglu et al. 2009; Khanal et al., 2007).

In this study, the effects of ultrasonication and microwave irradiation applications on the biodegradability, organic matter and volume reduction, biogas production and methane content during the anaerobic digestion, EPS structure and dewatering properties of the sludge are investigated. In addition, ultrasound and microwave irradiation pretreatments are compared in case of sludge solubilization and their impacts on digestion efficiencies for both aerobic and anaerobic stabilizations. Four different sludge samples collected from urban and domestic wastewater treatment plants used for the experimental analyses in order to observe the effects of the pretreatments more clearly.

2. LITERATURE REVIEW

2.1. Sludge Production and Sludge Waste Problems

Over a century, wastewater has being treated biologically. The process has developed from simple small systems, such as septic tanks and lagoons, into large treatment facilities. As the result of gradually increasing world population, millions of liters of wastewater get treated every day which is provided by the utilization of a variety of microorganisms. Conventional wastewater treatment process transforms organic pollutants into gas, water and biomass. This produced biomass typically contains from 0.25 to 12 percent solids by weight, depending on the operations and processes used (Tchobanoglous & Burton, 1991).

Disposal of the wastewater sludges is one of the major problems related with the wastewater treatment process: Because of its high organic content, it is accepted as 'hazardous material' and therefore surface disposal is strictly prohibited; for land application (i.e. for agricultural use or as fertilizer in the forestlands) there are too many requirements to be met in the sense of organic and inorganic content; the existing cement factories and other incineration facilities fall short to deal with the waste sludge. For all those reasons, the produced biomass needs to be further treated.

Additionally, the processing and disposal of wastewater sludges is one of the most important and complex problems during the treatment since, (i) it contains the substances coming from the primary settling of the untreated water which are responsible of the offensive character of the treated wastewater, (ii) it is composed largely of the organic matter coming from the biological treatment process which is quite resistant for further degradation, (iii) very small portion of the sludge is solid material. Furthermore, the cost of biomass treatment and disposal can account up to 60% of the wastewater treatment plant expenses (Weemaes and Verstraete, 1998). Hence, there is a growing interest in the methods to minimize the biomass to be disposed.

There are different applications to reduce the overall sludge amount in the plant. **Figure 2.1** shows potential locations for sludge minimization technologies in a classical urban wastewater treatment plant. To minimize the sludge production in the water line, pretreatment may be implemented either directly in the aeration tank (T1) or in the sludge recycle line (T2). Direct material pretreatment prior to sludge treatment may be either on primary sludge (T3), to excess waste activated sludge (T4), or to mix sludge coming from primary and secondary clarifiers (T5). Another way is to apply the minimization technology to sludge in the recirculation loop of the digesters (T6).

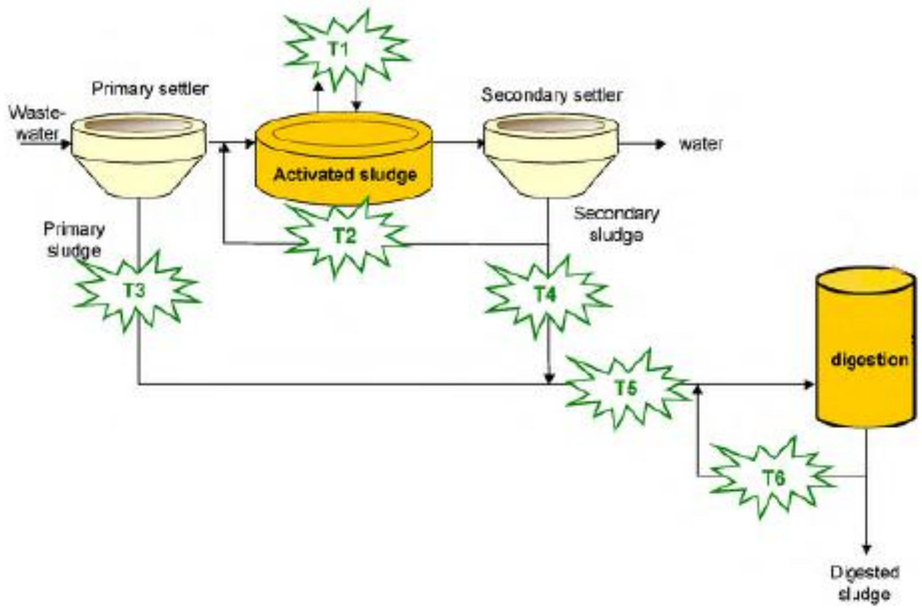


Figure 2.1 Potential locations for sludge minimization applications (Carrere et al.,2010).

2.2. Sludge Minimization in Water Line

There are two ways to minimize the overall amount of sludge to be disposed: (i) to reduce the amount of sludge during the treatment of wastewater, in other words, to produce less sludge via different technologies at the source, (ii) to improve the sludge reduction during the sludge treatment process with enhancing the stabilization process of the sludge.

Different improving technologies to reduce the sludge production in water line are shown in Table 2.1.

Table 2.1 Different sludge minimization applications in the water line.

Technology	Application	Advantages	Disadvantages
Magneto- Ferrite Method (Kabir et.al., 2008)	Ferrite particles are added to some portion of activated sludge in the recycle line and it is subjected to changing magnetic flux produced by a pair of moving magnets. Collisions occur in between ferrite particles and the floc of activated sludge which can possibly crush the cell wall of the microbes.	- 42% reduction in sludge production in literature for lab scale applications - Ferrite particles are easily separated from the sludge - Low construction costs - Easy to operate	- Needs further investigation - Uncertain effects for real applications
Cannibal® (Roxburgh et al., 2006)	The process uses cyclical conditions to maintain anoxic and anaerobic microbial community in the bioreactors with intermittently applied air. The unadapted community becomes the carbon source for the attuned ones.	- High sCOD release - Proven technology (several full scale applications) - 50% reduction in sludge production in the literature	- May not be cost effective for large facilities - Issues with phosphorus mass balance remain unresolved - May not be compatible with biological phosphorus removal
Fungal Treatment (Fakhru’l Razi et al., 2007)	Some portion of the waste activated sludge is treated in the bioreactors containing high amounts of specific fungi and microorganisms	- 30% sludge reduction in literature - Easy to construct and operate - 100% biological process	- Odor problems
IDI Biolysis (Roxburgh et al., 2006)	Chemical or enzymatic stressing is used to make the sludge biodegradable, limit the microbial growth and increase the energy requirements for bacteria metabolism.	- Improved capacity and performance in the secondary clarifiers - 70% sludge reduction	- High construction costs - High operational costs because of the high oxygen requirement for ozonation process.
Membrane Bioreactors (MBR) (EPA, 2007)	Wastewater passes through the membrane filters which are made of cellulose or another polymeric structure. The accumulation of the fine particles on the filters is prevented by cross flow.	- High solids removal - Smaller reactor volumes	- High costs of construction and operation - High energy requirement for aeration - Worsens the settleability of the sludge
Extended Aeration (Oveido et al., 2003)	With long sludge retention time microbial community is force to starve. Starvation results in cellular lysis and microorganisms use as food proteins and polysaccharides excreted in to the medium.	- High sludge reduction efficiencies - Easy to construct and operate	- Large tanks needed - High energy requirement for longer aeration period

The sludge minimization technologies described above provide reduction in the amount of produced sludge at the end of the wastewater treatment process. After the application of some of the mentioned technologies no further treatment for the sludge is required or just a dewatering process is enough for the ultimate disposal. However, for conventional wastewater treatment plants, the produced biomass needs to be treated properly before disposal or beneficial use.

2.3. Sludge Treatment

The largest volume of the resulting material from the wastewater treatment process is sludge. Since it contains high quantities of organics, nutrients, pathogenic microorganisms and water, a further treatment is necessary. Different pathways for sludge treatment are possible and most of the process available is given in Figure 2.2. However, the most essential units of the sludge treatment are: (i) stabilization -to reduce organics and pathogens- and (ii) dewatering -to reduce its high water content- processes.

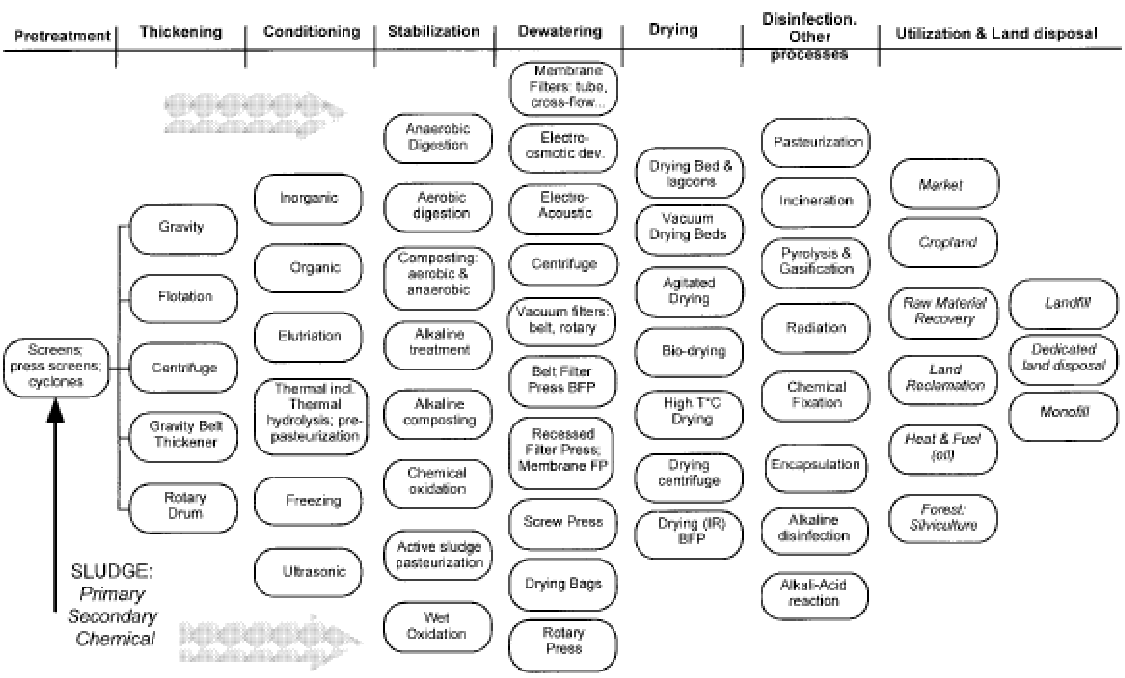


Figure 2.3 Most of the unit processes available for sludge treatment (Oleszkiewicz&Mavinic, 2002).

2.3.1. Sludge Stabilization

Stabilization defines the operations and processes carried out on sludge to minimize its damage to the environment when it is disposed of and therefore, it is one of the most important issues in sludge management. Stabilization aims:

- To achieve the removal of pathogenic microorganisms
- To eliminate the odor problem
- To reduce the organic content and therefore the volume of the sludge
- To obtain a homogenous product

The most effective sludge stabilization methods to be applied with today's technology are (Oleszkiewicz&Mavinic, 2002; Houdkova et al., 2008; Murray et al., 2008):

- **Aerobic Digestion**
- **Anaerobic Digestion**
- Composting
- Alkali Treatment
- Thermal Processes

Among these stabilization processes aerobic and anaerobic stabilization are the most utilized ones because of their possible modification options, low construction costs and high stabilization efficiencies.

2.3.1.1. Aerobic Digestion

The simple principle of the aerobic digestion is that; if a wastewater is aerated for a long time, theoretically all biooxidizable organic materials will be degraded, which is the same principle for extended aeration systems. The pathway of aerobic digestion process is shown in **Figure 2.4**. During the extended aeration period, microbial community gets in the endogenous respiration phase because of the lack of the food. With the absence of an external energy source, heterotrophic bacteria use its own protoplasm to generate energy and the dead cells become the food source for the remaining community. Microorganisms hydrolyze biodegradable organic matters into biodegradable soluble organic matters and then convert these soluble matters into water and carbon dioxide. Consequently, the volume of the sludge is decreased and the organic content is reduced.

Because of its energy requirement for the aeration of the system, aerobic digestion cannot be considered as an economically advantageous system. However, it is less costly solution for municipal treatment plants below 50000 population equivalent compared to anaerobic stabilization of the sludge (Tas, 2010).

When considered the energy efficiencies and volume reduction of the sludge, aerobic digestion applications has become a less preferable option for larger wastewater treatment plants. Instead, in recent years, anaerobic digestion is considered as a robust process for the treatment of organically rich materials and has been emerging with an annual growth rate of 25% (Appels et al., 2011).

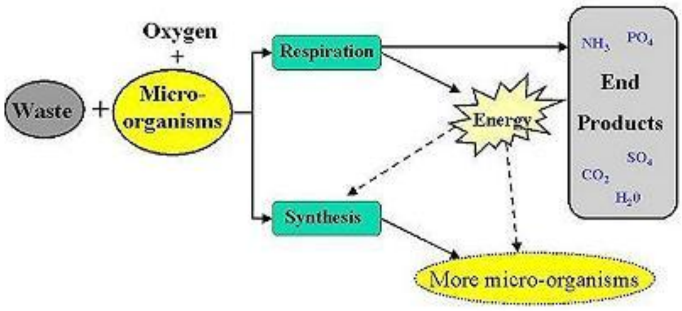


Figure 2.4 Schematic representation of aerobic digestion process (http://water.me.vccs.edu/).

2.3.1.2. Anaerobic Digestion

Anaerobic digestion is a process in which the sludge is kept in oxygen-free conditions and therefore, degraded by the anaerobic microorganisms. As a result of the activity of these specialized microorganisms, methane and carbon dioxide is produced. Not only sewage sludge but other waste materials such as manure, crop waste and organic fraction of the solid waste are processed with anaerobic digestion since a continuous power generation from these sources can be guaranteed (Raposo et al., 2011; Appels et al., 2011).

In literature, anaerobic digestion is proved to reduce the organic content of the sludge by 50% and furthermore, biogas is generated as a result of the process which can be used as renewable energy to replace fossil energy sources (Davidsson et al., 2006). Additionally, the digestate remaining after the anaerobic stabilization process contains has lower volumes and high solids percent and on account of carbon removal in the form of methane and carbon dioxide, the end product shows a substantially better biological stability when compared to other stabilization processes (Tiehm et al., 1997).

In anaerobic digestion of an organic material, various types of microorganisms are involved in the process such as primarily prokaryotics, different types of anaerobic and anoxic bacteria and strictly anaerobic methanogens. Path of the anaerobic digestion is shown in **Figure 2.5**. During the process, series of complex biochemical reactions take place. These reactions may be divided in four main steps:

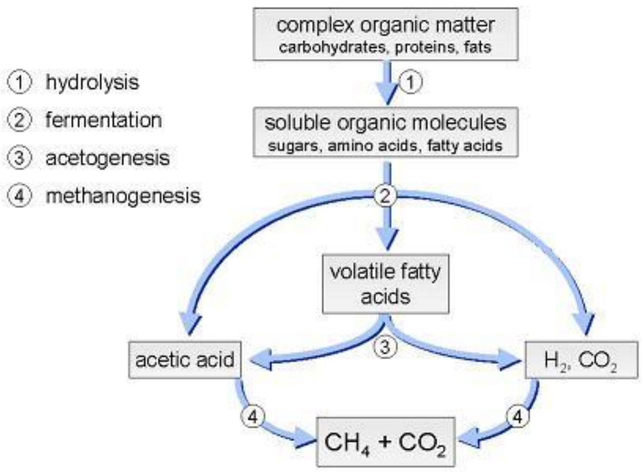


Figure 2.5 Schematic representation of anaerobic digestion process (http://water.me.vccs.edu/).

Hydrolysis: In this step, complex organic matters such as lipids, polysaccharides, proteins and nucleic acids are decomposed into simple soluble organic molecules like sugar, fatty acids and amino acids using water to split the chemical bonds between the substances. In this phase of the digestion, environmental conditions like temperature and pH; biodegradable organic matter concentration and biomass nature determines the success of the process.

For anaerobic digestion of waste activated sludge, hydrolysis is considered as the “rate-limiting step” since it often controls the degree of COD conversation to methane (Lee et al., 2011). Therefore, pretreatment methods are applied to the sludge prior to digestion in order to enhance the hydrolysis and speed up the process.

Acidogenesis: During this step the chemical decomposition of carbohydrates takes place. Volatile fatty acids (VFA) are produced by acidogenic (fermentative) bacteria along with ammonia, carbon dioxide, hydrogen sulfide and other by-products.

Acetogenesis: The products of acidogenesis step are converted into acetate (acetic acid), hydrogen and carbon dioxide by acetogenetic bacteria in acetogenesis. This conversion is controlled mainly by the partial pressure of hydrogen in the mixture.

Methanogenesis: This is the last step of the anaerobic digestion in which the intermediate products formed during the previous steps of the process are converted into methane, water and carbon dioxide. One of the two types of methanogens is acetoclastic microbes that convert acetate to methane gas whereas, H_2-oxidizers convert H_2 and CO_2 into methane. Acetoclastic methanogens are more sensible for temperature changes and they provide the two-third of the total methane production in the system (Lettinga et al., 1999).

Methanogenesis also can be a rate-limiting step if methanogens are washed out from the system with short sludge retention times (SRT). They are slow growers which can also be inhibited by the accumulation of short chain organic acids in the system resulting in the decrease of the pH (Lee at al., 2011).

Operation of the anaerobic systems is more complex when compared with aerobic systems. The environmental conditions such as temperature, pH and alkalinity; operational conditions like organic loading rate, food to microorganism ratio, hydraulic and solid retention times and microbial activity should be monitored and controlled properly. Anaerobic digestor systems are conventionally operated at mesophilic temperatures (30-38°C) and optimum pH of the system is kept between 6.8 and 7.4 in order to maintain all the varying types of microbes. Recently, there are many configurations of the anaerobic digestion systems are utilized which are operated in mesophilic, thermophilic (50-70^{o}C) or combined systems (Tchobanoglous et al., 2003).

The benefits of an anaerobic system can be specified as:

- Anaerobic digestion is a method that provides biological conversion in an aqueous environment. This means biomass sources with very low solid contents like sewage sludge can be processed.
- Anaerobic digestion of the sewage sludge has the highest biogas production capacity worldwide. Although the biogas yields are strongly dependent on the sludge composition, theoretically, it is 590 liters of biogas per kilogram of organic dry solids (Appels et al., 2011). Additionally, the produced biogas (CH_4, CO_2 and trace gases such as H_2, N_2 and H_2S) is environmentally friendly because of the low emissions of hazardous pollutants.

- The product of the digestion process is nitrogen rich and therefore can be used for the organic amendment purposes.

- It is not only feasible for large-scale WWTPs but can also be modified for small scale installations. This may provide opportunities for this system to be applied in developing countries and rural areas for energy supply is limited (Appels et al., 2011) .

A disadvantage of anaerobic digestion systems is the slow degradation rate of sewage sludge. Conventionally, sludge residence times of the anaerobic reactors are about 20 days (Tiehm et al.,1997). This digestion period requires large digesters and consequently high construction and operational costs. One way of dealing with that problem is increasing the rate of hydrolysis step and this is achieved by pretreatment applications.

2.4. Sludge Minimization in Sludge Line

In order to improve the digestion efficiency of the sludge, the most logical approach is to disrupt the microbial cells in the sludge. Sludge pretreatment is therefore introduced to enhance the solubilization of sludge by converting slowly biodegradable, particulate organic matters to low molecular weight, readily biodegradable compounds. This procedure is called sludge disintegration (Weemaes & Verstraete, 1998).

2.4.1. Sludge Disintegration Mechanisms

The aim of sludge disintegration is the release of the organic substances inside and outside the sludge cells through disruption of their cell walls. As a result of this process, soluble chemical oxygen demand is increased. When sludge cells are ruptured, contents of these microbial cells are released into the medium and provide an autochthonous substrate to other microorganisms to be used for their metabolism. This microbial growth is called as "lysis-cryptic growth" (Chu et al.,2009). A representation of this process is shown in **Figure 2.6**.

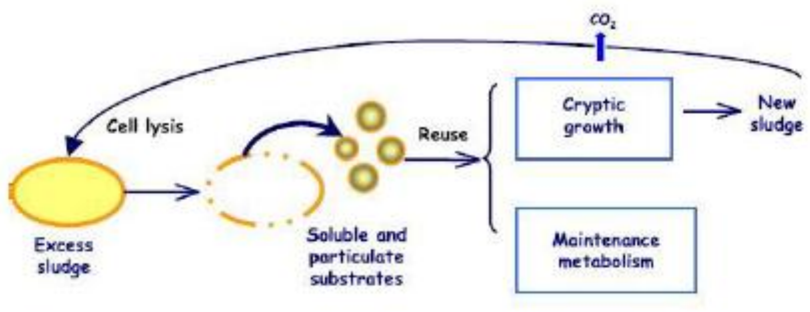


Figure 2.6 Schematic diagram of cell lysis and cryptic growth (Chu et al.,2009).

Hydrolysis has long been accepted as the rate limiting step of the digestion process, as mentioned previously. Cell lysis technologies, therefore, aim to speed up this step by lysing the cellular material. Some technologies rely primarily on physical cell lysis (ultrasonication, pulsed electric fields, ball mills) whereas some of the methods combine chemical and physical processes (Microsludge, ozonation, microwave irradiation).

2.4.2. Sludge Disintegration Methods

Different studies for sludge minimization have been available for several decades. However, recent developments have driven some of these methods forward. Among varying sludge disintegration studies, mostly the technologies proven to be successful are investigated in this study.

2.4.2.1. Mechanical Disintegration

Mechanical sludge disintegration technologies mainly rely on the disruption of the microbial cell walls by shear stress. Mechanical stress of the solids causes tensions and deformations. In order to achieve the cell lysis, the applied stress should be higher than the strength of the cell wall (Neyens et al., 2003). Technologies using mechanical disintegration are as following:

Microsludge™: This technology is a recently developed patented process and it is comprised of two steps: (i) by application of a chemical/physical procedure weakening the microbial cells is employed, (ii) sludge is subjected to high pressures in order to achieve complete disruption of the microbial cells. For the first step sodium hydroxide to raise pH to between 9 and 10 is used. Shear stress might also be applied to weaken the cell walls. In the second step of the process, approximately 82700 kPa pressure is applied by passing the liquied through very narrow channels to the homogenizers. The pressure drop cause cells to disrupt. VS solubilization of 78% with 13 days of HRT is reported with this system (Roxburgh et al., 2006).

Pulsed Electric Field: In this process, sludge cells are disrupted with high voltage and frequency electric pulses. Sludge is placed between two electrodes and high voltage (10-50 kV/cm) is applied to the liquid medium with short periods (10 nsec - 20 μs). There are several full scale installations with more than 10% increase in organic removal and 55-60% increase in biogas production (Salerno et al., 2009).

Lysis-centrifuge: This procedure operates directly on the thickened sludge in a dewatering centrifuge and after that, it is then mixed with the liquid stream. There are several full-scale applications in Europe which have different population equivalents. 15-26% increase in biogas production is reported (Carrere et al., 2010).

High Pressure Homogenizer: In this method, sludge is subjected to high pressures up to 900 bar and after that goes through an homogenization valve under strong depresurrisation. Full scale applications exist with the application of 150 bar prior to digestion. 30% increase in biogas production and 23% enhanced reduction of sludge volume was achieved (Carrere et al., 2010).

Grinding: Disruption of the sludge is provided by stirred ball mills. An increase of batch biogas production by 60% is reported in literature (Carrere et al., 2010).

Ultrasonication: The application of high intensity ultrasound has significant potential for improving the solid reduction in sludge stabilization process. The process uses the sound with frequencies between 20 kHz and 10 MHz which are above the range of human hearing. The lower end of this sound range generates compaction and rarefaction waves which lead to the formation of cavitation bubbles in the liquid medium. These bubbles create high mechanical shear forces which are used to disintegrate the solids in the sludge. Applications of this method will be investigated in detail in Chapter 2.5 (Roxburgh et al., 2006).

2.4.2.2. Chemical Disintegration

Oxidation: In this process, microbial cell walls are intended to be cracked with oxygen. However, for a successful application elevated temperatures and pressures are essential (Weemaas & Verstrate,1998). The widely used chemical disintegration method of the sludge is ozonation which leads to partial sludge solubilization and yield increases with ozone. However, too high ozone doses will cause reduced solubilization due to further oxidation of the solubilized components. Moreover, the application of higher doses might deteriorate the methanogenic activity and therefore decrease the biogas yields. An optimum dose for ozonation is determined to be between 0.1 g O_3/g COD and 0.2 g O_3/g COD (Carrere et al., 2010). Fenton is also a strong oxidative agent for sludge cells. Catalyzer effect of Iron (II) salt and oxidation effect of hydrogen peroxide is utilized in this process.

Alkali Treatments: Addition of strong acids or alkali products may also be used for cell lysis to take place in the sludge. These chemicals allow macromolecules like lipids, hydrocarbons and proteins to hydrolyze into smaller soluble compounds like aliphatic acids, polysaccharides and amino acids. Alkali products such as lime and sodium hydroxide are commonly used for alkali treatment. In this process, pH is increased up to 12 and kept under that pH level at least for 24 hours. One main disadvantage of the procedure is that the dewaterability of the sludge is totally deteriorated (Weemaes and Verstraete,1998).

2.4.2.3. Thermal Hydrolysis

Thermal hydrolysis was first designed to improve the dewaterability of sludge since it allows degradation of the sludge gel structure and release of linked water. This results in enhanced sludge dewaterability after the heat treatment in the range of 150^{o}C- 200^{o}C. Recently, the solubilization effect of heat treatment is utilized for sludge disintegration purposes with the association of pressure. Literature shows that the optimal temperature for the process is in the range of 160^{o}C- 180^{o}C and the pressure adjoining is between 600 and 2500 kPa. Treatment times from 30 to 60 minutes are common. However, studies show that time does not have significant effect on disintegration efficiencies (Weemaes and Verstraete,1998); Carrere et al., 2010). Qiao et al. (2011) reports ultimate biogas increase by 67.8% and 65.5% increase in methane production with heat treatment at 170^{o}C for 1 hour (Qiao et al., 2011)

Cambi™: The Norwegian Company developed a sludge minimization system featuring thermal hydrolysis. First, the thicken sludge with solid content of 15-20% is heated at 80°C- 100°C and homogenized in the grinders. The homogenized sludge then heated up to 160°C- 180°C with the steam and high pressure is applied to the sludge. Sludge is kept under these conditions for approximately 30 minutes. At this point, solubilization of the sludge has increased by 30%, dependent on the sludge type. Finally, thermally treated sludge is taken into another tank and the sudden pressure drop causes sludge cells to disrupt. More than 10 installations of the process is present in which an increase in biogas production and reduction of organic matter around 60% and an increase of digestor capacity with higher organic loadings achieved is reported (Carrera et al, 2010; Weemas et al., 2008).

Microwave Irradiation: MW irradiation can produce focused direct heat rapidly which lowers the energy losses while transmitting the energy in conventional heating. The mechanism of microwave irradiation has two separate effects; (i) thermal effect which describes the changes occur due to increase of the heat and (ii) athermal effect which means the changes modulated with square wave of different pulse repetition frequencies which is irrelevant to the heat of the system (Banik et al., 2003). Applications of this method will be investigated in detail, in Chapter 2.6.

2.4.2.4. Biological Disintegration

Biological treatment refers to a broad range of applications including modifications of aerobic/anaerobic systems and different processes (mesophilic/thermophilic). However, as a disintegration method, enzyme addition will be investigated in this part.

Enzyme Addition: During the hydrolysis step of the sludge degradation, microorganisms excrete enzymes such as cellulose, protease and lipase in order to convert the macro molecules into small weighted readily degradable matters. Addition of these enzymes to the system significantly increases the solubilization of the sludge since they catalyze the degradation of organic substances in sludge as a function of the substrate. In the literature, studies with 0.5% enzyme addition by volume showed efficient disintegration of sludge (Dey et al., 2006).

2.5. Ultrasonication as a Sludge Disintegration Method

Utilization of sonication technology was first developed in 1960s with laboratory-scale research but did not considered as a cost-effective system for that time because of the lack of technological improvements and proper equipment (Roxburgh et al., 2006). Today, however, ultrasound is used in a wide range of industrial applications as well as wastewater treatment, in many different frequencies and forms. The mechanism of the ultrasound technology and various studies conducted to observe the effects of the system on sewage sludge disintegration is reviewed in this section.

2.5.1. Mechanism of Ultrasonic Disintegration

Sonication generates cavitation (implosion) processes in liquid mediums which create localized hot spot with conditions similar to the sun, reaching temperatures over 1000^{o}C and pressure up to 500 bar. These implosions also cause jet streams to form and the speed of these streams may reach up to 400 km/h. All these movement created in the medium cause the weakening and eventually corruption of the walls of the microbial cells and therefore the solubilization takes place (Roxburgh et al, 2006). This phenomena is occurring with two key mechanisms: (i) cavitation, formed due to ultrasound waves and (ii) chemical reactions due to formation of radicals.

2.5.1.1. Cavitation Mechanism

Ultrasound is sound wave with frequencies between 20 kHz and 10 MHz which are inaudible by humans. With the propagation of this high frequency ultrasound in the sludge medium, repeating patterns of compression and rarefactions are generated. Compression exerts a positive pressure on the medium and therefore pushes the molecules together. The rarefactions are spots of excessively large negative pressure in which the molecules are pulled apart. As a result of this large negative pressure, microbubbles –aslo called as cavitation bubbles- are formed. As described before, the growing cavitation bubbles with the repetition of the movement of the molecules in the medium collapse at some point after losing its stability. With this collapse shock waves are produced which is followed by high local temperatures and pressures (Khanal et al., 2007; Roxburgh et al, 2006; Pilli et al. 2011). The schematic representation of cavitation phenomena is shown in Figure 2.7.

The success of the ultrasonication disintegration is mainly dependent on the cavitation phenomena. The temperature of the liquid, viscosity of the fluid, ultrasonic intensity, ultrasonication duration and the frequency of the vibration (usually better at the lower end between 20 and 40 kHz) are some of the many factors effecting cavitation (Pilli et al., 2011; Bourgrier et al., 2005).

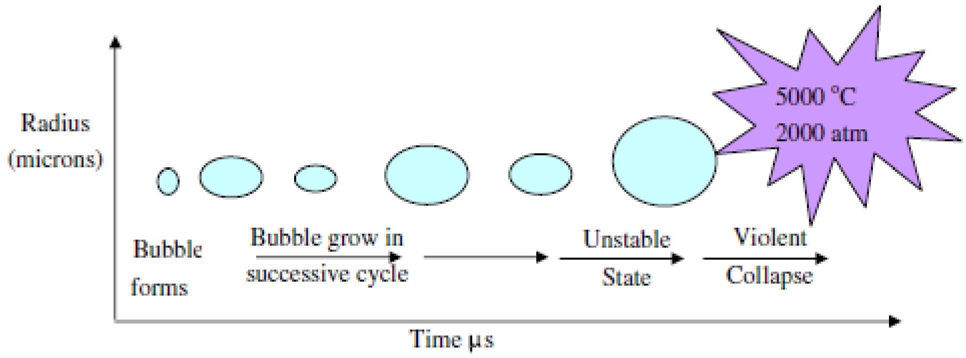


Figure 2.7 Development and collapse of the cavitation bubble (Pilli et al, 2011).

2.5.1.2. Chemical Reactions

Extreme local conditions created by cavitation mechanism result in the formation of OH•, HO_2•, and H• radicals and hydrogen peroxide. Among the hydro-mechanical shear forces of the microbubbles generation oxidizing effect of these radicals and additionally, with the elevated local temperatures decomposition of volatile hydrophobic substances in the sludge also cause cell lysis of the sludge (Wang et al., 2005).

According to Weemaes and Verstraete (1998), sonication treatment is the most powerful mechanical method for cell disruption and at high levels of power 100% sludge disintegration may be achieved (Weemaes and Verstraete, 1998). However, a threshold for specific energy is often reported for ultrasonication disintegration. When considered the energy consumption and the digestion improvement of the sludge a specific energy ranges from 1000 to 16000 kJ/kg total solids (TS) depending on the TS concentration of the sludge (Carrera et al., 2010).

2.5.2. Application of Ultrasonic Sludge Disintegration

There are many studies conducted to investigate the ultrasonic disintegration of the sludge by various authors. Their findings are reviewed as following:

Yin et al. (2004) states that sludge disintegration is mostly effective at low-frequencies. Large cavitation bubbles are created by low frequency ultrasound waves and these bubbles generate the jet streams to torn the cells apart. Furthermore short durations of ultrasonication cause deagglomeration of sludge cells but cannot achieve the disruption of the cell wall. Longer sonication durations with lower frequencies will result in higher disintegration efficiencies (Yin et al., 2004). On the other hand Carrera et al (2010) reports that for a given ultrasonic specific energy, power input is also more significant than application duration (Carrera et al., 2010).

Tiehm et al. (1997) applied ultrasound to the sludge prior to anaerobic digestion and investigate the effects of the pretreatment. The sewage sludge was subjected to ultrasound disintegration for 64 seconds in a high performance ultrasound reactor which operated at a frequency of 31 kHz and power of 3.6 kW. Temperature of the sludge is elevated to 45^{o}C from 15^{o}C with the application. The pretreated samples are digested anaerobically under semi-continuous mesophilic conditions (37^{o}C) with four different residence times: 8, 12, 16 and 22 days whereas the unpretreated waste is digested conventionally for 22 days. Final volatile solids (VS) reduction in the unpretreated sludge sample was found to be 45.8% and 50.3% in the pretreated sample after 22 days of digestion. Reduction of volatile solid after the digestion in the pretreated sample with the residence time of 8 days was 44.3%. This indicates that similar level of solid reductions can be obtained in shorter residence times. Moreover, the production of biogas was significantly increased since there was more input of organics to the semi-continuous system with higher organic removal efficiencies. Therefore, it was concluded that ultrasound pretreatment is a promising tool to enhance the digestion efficiencies and reduce the digester volumes for full scale applications (Tiehm et al.,1997).

Braguglia et al. (2012) investigates the difference between the ultrasonication and ozonation pretreatments on anaerobic digestion of sludge. Specific energy supplied to ultrasonicated sample was approximately 2500 kJ/kg TS and the specific energy for ozonation was approximately 4500 kJ/kg TS with the ozonation dose of 0.07 g O_3/g TS. Digesters were operated semi-continuously with HRT of 10 days. VS removal in the ultrasonicated digestion reactor was 32% whereas it was 27% in the ozonated reactor. COD removals of the two pretreatments were significantly different; 74% COD removal was achieved with ultrasonic disintegration while the COD removal obtained by ozonation was 57%. In terms of biogas, the sonicated sludge produced 26% more biogas when compared with unpretreated sample whereas ozonated sample produced even less biogas than the control reactor. Authors concluded that ultrasound was a more efficient system for sludge disintegration with respect to ozone (Braguglia et al.,2012).

Another study which compares the ultrasonication disintegration with ozonation was conducted by Bourgrier et al. (2006). Additionally, thermal pretreatment was also evaluated in this study. For sonication two different specific energies (6250 kJ/kg TS and 9350 kJ/kg TS); for ozonation two different ozone doses (0.1 and 0.16 g O_3/g TS) and for thermal treatment two different temperatures (170 and 190 ^{o}C) are applied. Ultrasonication of the system was realized in an ultrasonic homogenizer frequency of 20 kHz and a supplied power of approximately 225 W. COD solubilization and also TS solubilization was analyzed. For each of the three systems COD and TS solubilization were almost the same but quite different from each other when compared. The highest solubilization was achieved with thermal treatment with 40%. It is followed by ozonation (20%) and ultrasonication (15%). When dewaterability of the samples were investigated it was found that by decreasing particle size, sonication results in the deterioration of the sludge filterability. When compared anaerobic biodegradability of the samples, the enhancement of biogas production was provided by ultrasonic disintegration and thermal treatment. The similar enhancement of biogas even though the different solubilization of the samples were explained as ultrasound cause the particle size reduction and this allowed the enhancement of the particulate fraction biodegradability while thermal treatment mainly effect the solid solubilization and not he particulate biodegradability (Bourgrier et al., 2006).

Feng et al. (2009) evaluated the physical and chemical characteristics of the ultrasonically treated sludge and therefore applied different ultrasonic specific energies varies between 0 and 26000 kJ/kg TS to find the optimum application conditions. A positive correlation between applied energy dose and sCOD is found and stated that ultrasonication increases the solubilization of COD significantly. At the maximum specific energy applied in the study of 26000 kJ/kg TS, a 1223% increase in sCOD was observed. Specific energies lower than 1000 kJ/kg TS showed poorer COD solubilization with 120% whereas sCOD/COD was only 4% (Feng et al., 2009). Effects of different specific energies on the EPS structure of the sludge will be reviewed in section 2.7.

Yu et al. (2008) studied the extracellular structure of the sludge among the effect of ultrasonication on solid content of the sludge. Applied ultrasonic density ranged from 0 to 15 kW/L and the application duration from 0 to 20 minutes. After disintegration, sludge was aerobically digested at room temperatures. sCOD/COD ratios showed great solubilization took place during the pretreatment and commented as increase in sCOD might be the result of sludge destruction and this can lead the release of colloidal and soluble organics into the liquid medium. Moreover, TSS and VSS removals of pretreated sludge were 30.9% and 35.0%, respectively while the values were 20.9% for both removals in the control reactor (Yu et al., 2008).

Ultrasonication effects on methane production and organic degradation were investigated in Davidsson and Jansen’s study (2006). Applied ultrasonic density was 0.05 kW/kg TS and the wave frequency was maximum 50 kHz. Digestion of the samples was operated in continuous pilot-scale digestion reactors at mesophilic temperatures with the HRT of 13 days. Although the COD solubilization was found to be very low, the methane potential for the ultrasonicated sludge was significantly higher than the control reactor. Analyses indicated that even the sCOD of the sample was not increased methane potential of the sample might still be enhanced with ultrasonication (Davidsson & Jansen, 2006).

2.6. Microwave Irradiation as a Sludge Disintegration Method

Microwaves are used in a wide range of application in environmental engineering including decomposition of organic materials, soil remediation, inactivation of microorganisms and waste processing and sterilization. The reasons behind the utilization of microwave irradiation are rapid heating, ease of the application, high efficiencies to balance the capital and operational cost and the compactness of the system.

2.6.1. Mechanism of Microwave Irradiation

The microwave region corresponds to the wavelength of 1 mm to 1m with frequencies of 300 GHz to 300 MHz, respectively. Domestic and industrial microwaves generally operate at a wavelength of 12.2 cm which corresponds to 2.45 GHz and energy of 1.02×10^{-5} eV (Jacob et al., 1995).

The principle of the microwave irradiation is that, when a compound is subjected to microwave application, electric field created by the microwaves exerts a force on the charged particles of that compound. If those charged particles are free to move, an electric current is induced. However, if they are unable to move, they may just reorient themselves in that electric field (2450 million times/s). This phenomenon is called as dielectric polarization. Due to the different behaviors of the charged particles of the compound, the dielectric polarization is the sum of four components: electrons (electronic polarization), nuclei (atomic polarization), permanent dipoles (dipolar polarization) and charges at interfaces (interfacial polarization) (Banik et al., 2003; Jacob et al., 1995).

Jacob et al. (1995) explains the microwave heating affect as:

> “The electric field reversal of the microwave radiation causes the reversal of the dielectric polarization. The atomic and electronic polarization and depolarization occur at a faster time scale than the electric field reversal of a microwave and therefore do not contribute to the dielectric heating effect (The resonant frequency of the electronic and atomic polarization

> is in the ultraviolet/ visible and infrared frequency, respectively.). The time scale of orientation and disorientation of permanent dipoles in a molecule is similar to the time of electric field reversal in the microwave region. In the microwave region, therefore, the dipoles rotate to align themselves in phase with the reversing electric field. The resulting polarization lags behind the changes of the electric field and causes dielectric heating of the material. The extent of this dipolar polarization depends on the power of the field, strength of the dipole moment, and the mobility of the dipole. The interfacial polarization contributes to dielectric heating when conducting particles are suspended in a non-conducting medium in an in-homogenous material. Thus both conduction and dielectric polarization are vehicles of microwave heating in matter."

However, it is known for some time now, microwave irradiation cause various types of alterations and transformations on the molecular structure of the irradiated compound which is not caused by the thermal effect of the microwave. These are called "athermal effects". The polarized parts of macromolecules align with the poles created with electromagnetic field and as a result, breakage of hydrogen bonds leading to denaturation and cell death occur. Some of the athermal effects of the microwave which provide enhancement of the reaction rate in the compound are (Jacob et al., 1995):

1. Creation of hot spots(localized heating effects
2. Molecular agitation
3. Improved transport properties of molecules
4. Other reasons such as specific pulsing of microwaves

2.6.2. Applications of Microwave Irradiation Disintegration

There are many studies conducted to investigate the microwave irradiation disintegration of the sludg by various authors. Their findings are reviewed as following:

To observe if there are any athermal effects of microwave irradiation on solubilization and biogas production potential of the sludge, Eskicioglu et al. (2006) applied microwave irradiation (MW) and conventional heating (CH) to the thickened sludge with same temperatures. As expected, COD, protein, sugar and total volatile fatty acids (TVFA) concentrations of soluble phases increased after MW and CH. However, different level of release of COD and biopolymers was for MW and CH were obtained, the even though the sludge was pretreated to a similar temperature (96±2 ^{o}C). sCOD and soluble sugar concentration increased 361±45% and 308±2%, respectively wheraes sCOD of MW-irradiated TWAS was 143±34% higher and soluble sugar was 15±0% higher than the control. Thickened sludge pretreated with MW resulted in higher soluble protein (157±20% higher compared to control) and higher soluble TVFA (381±32% higher compared to control) concentrations in the when compared to CH (43±22% increase in soluble protein and 235±34% higher soluble TVFA compared to controls). The authors stated that this is possible that extended duration of exposure of CH to achieve a given temperature compared to MW exposure caused this difference. Higher duration of heat exposure led higher increase in sCOD and soluble sugar concentrations with conventional heating of the sludge. It also possibly caused higher level of denaturation of soluble proteins to ammonia and loss of volatile TVFA (Eskicioglu et al., 2006).

To investigate the athermal effects of the microwave irradiation, Eskicioglu et al. (2007) applied microwave irradiation (MW) and conventional heating (CH) to the thickened sludge with same temperatures, this time with same heating profiles. To observe the athermal effects of the microwave irradiation in a more distinguishable way, temperatures were kept lower than death point of bacteria (50, 75 and 96^{o}C). Results showed that MW and CH pretreatments with identical heating profiles caused similar sCOD/COD values for the sludge. sCOD/COD values for 50^{o}C was 1470% and 1570% for MW and CH, respectively. The ratio was 1970% and 1971% for 75^{o}C; and 2271% and 2471% for 96^{o}C for MW and CH, respectively. However, irradiated samples produced higher biogas than conventionally heated ones, consistently indicating that a MW athermal effect exists (Eskicioglu et al., 2007).

In Ahn et al. (2009)'s study, to evaluate the effect of microwave irradiation on the disintegration degree and the acidogenic features of the sludge, individual samples were irradiated for 0 (control), 3, 5, 7, 9, 11, or 15min and digested in continuous mesophilic conditions. As microwave irradiation time was increased from 0 to 15min, the SCOD concentration increased from 0.38 to 5.52 g l^{-1} and SCOD/COD increased from 0.02 to 0.22 The soluble protein, carbohydrate, and lipid concentrations also increased due to sludge disintegration by microwave irradiation. This result indicates that microwave irradiation increases sludge solubilization significantly. The magnitude of VS reduction in pretreated sludge increased with microwave irradiation time. % VS reductions were 25%, 32%, 34%, 35%, 37% and 44% for 0, 3, 5, 7, 9, 11, or 15 minutes of microwave application, respectively. Microwave irradiation also proved to increased the biochemical acidogenic potential (BAP) of the sludge. However, BAPs of the sludge samples were not differ significantly for microwave irradiation times ranging from5 to 15min so that the optimum microwave irradiation time for BAP of sludge was 5min when considered the energy efficiency (Ahn et al., 2009).

Sludge was pretreated with microwave irradiation for different temperatures to observe the sludge solubilization and methane potential of the sample after anaerobic digestion in Eskicioglu et al. (2009)'s study. Sludge was irradiated for 10 minutes with temperatures of 120, 150 and 175°C. When considered the solubilization of COD, there observed a linear relationship between the sCOD and temperatures. While sCOD/COD ratio of the unpretreated sludge was 9±1 %, the value for 120°C irradiation was 24±3 % , for 150°C it was 28±1 % and for 175°C it was 35±0 %. After found out 175°C of microwave irradiation to be optimum, the sample was anaerobically digested for 18 days. It was reported that 31±6 % higher biogas production is obtained when controlled with the unpretreated sample (Eskicioglu et al., 2009).

In Tang et al. (2010)'s study, a domestic microwave oven was used to provide MW irradiation for sludge pretreatment. A thoroughly mixed sample of 200–800 mL volume in a polyfluortetraethylene container was placed in the microwave and exposed to MW irradiation for 30–300 s at power settings of 400–800 W. By using the supplied power and time of exposure, three different specific energies were calculated: 20, 60 and 120 J/mL sludge. It was observed that biogas production was significantly enhanced with all three specific energies. However, the biogas enhancement of the sample with the specific energy of 60 J/mL was very close to the one with 120 J/mL (Tang et al., 2010).

Effect of output power, irradiation temperature and solid content of the sludge for microwave disintegration were investigated in Park et al. (2010)'s study. The ranges of independent variables were 400–1600 W, 60–120 ^{o}C, and 1–3% TS. Each treatment with a center point (i.e. 1000 W, 90 ^{o}C, and 2% TS) was replicated 3 times. By using this center points, the effect of an interaction between variables was tried to be found, using models. At the end of the study, an output power of 400W with the temperature of 102^{o}C and total solid concentration of 2.3% was found to be the optimum level for microwave irradiation application (Park et al., 2010).

In Yu et al. (2010)'s study, 400 mL of sludge samples were put into the reactor and treated with an array of 500, 750, and 900W microwave irradiation energy for various durations, e.g., 0, 20, 40,.. 140 s. After this disintegration application, sludge samples with and without microwave irradiation were analyzed for settling velocity (SV), VSS, and COD solubilization. The results showed that sCOD increases gradually with disintegration. However, increasing rate of sCOD became smaller with the elevating microwave energy and contact time. Furthermore, a positive correlation between SCOD and the contact time was observed. The sCOD/COD ratio increased from 0.0622 (raw sludge) to 0.1571, 0.1581 and 0.1611 at energies of 500 W, 750W and 900 W, respectively, with the exposure time of 140 seconds. It was also proven that with the same durations, higher irradiation powers cause a higher increase in sludge temperature. Therefore, instead of longer irradiation durations higher powers are more effective. The study have shown that 900Wand 60s was the ideal microwave condition for improving sludge solubilization (Yu et al., 2010).

Toreci et al. (2009) applied high temperature microwave irradiation prior to mesophilic anaerobic digestion of the sewage sludge with three different SRTs (5, 10 ana 20 days). The authors concluded that high temperature MW disintegration with 3.75^{0} C/min MW intensity improved the solubilization of sludge. However, for 20 days SRT, an enhancement for the extend of digestion did nor observed. At 10 days SRT with the same irradiation temperature and intensity, biogas yield was increased from 676 to 839.6 L biogas/kg VS$_{destroyed}$ whereas the VS removal was decreased from 49.9% to 43.4 compared to unpretreated sample with 20 days SRT. Furthermore it was stated that even though it enhanced the solubility of the sludge, low irradiation intensity had negative effect on mesophilic anaerobic digestion.

2.7. Extracellular Polymeric Substances and Sludge Disintegration

Extracellular polymeric substances (EPS) are metabolic products that accumulate on the surface of the cell wall of the microorganisms and combine the biomass and water together and therefore bioaggregates, such as biofilms and sludge flocs are formed (**Figure 2.8**.) EPS account for about 50-80% of the organic matter in a conventionally treated waste activated sludge (Wang et al.,2013; Yin et al., 2004; Shao et al., 2010).

In a biological wastewater treatment system, the large portion of the biomass is found in this bioaggregates. The presence of this gel like, three-dimensional high molecular weight mixture of polymeric, substances have a significant influence on physical and chemical properties of the waste such as flocculation, biodegradability, filterability, settling properties and adsorption ability (Sheng et al., 2010).

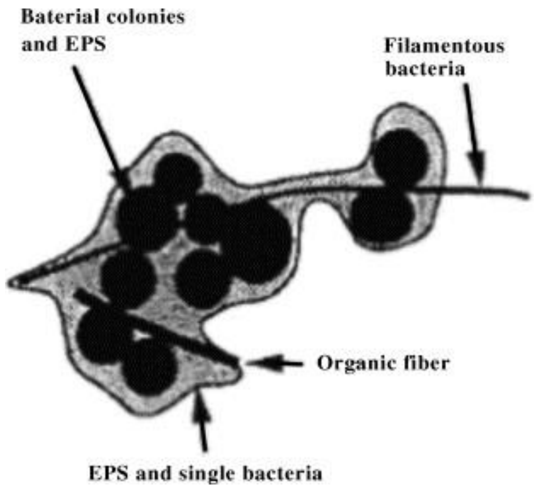


Figure 2.8 EPS cloud and the cells settled in it (Yin et al., 2004).

Extracellular polymeric substances include the various high weighted molecules like polysaccharides, proteins, nucleic acids (DNA and RNA), phospholipids and other polymeric compounds. These compounds keep the biomass together by weak physicchemical interactions such as electrostatic and hydrophobic forces, Van der Waals and hydrogen bonds. The total binding force of the EPS strongly depends on the contribution of each of these interactions and therefore the EPS structure of each medium may differ (Garnier et al., 2005).

There are many recent studied, focusing on the effects disintegration processes, on the EPS structure of the sludge. Some of them are reviewed here:

He et al. (2008), have investigated the extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasonic pretreatment. In the study, in order to understand the mechanisms of this impact, sludge flocs were divided into four layers, i.e. (1) slime, (2) loosely bound extracellular polymeric substances (LB-EPS), (3) tightly bound EPS (TB-EPS) and (4) pellet. Extracellular proteins, polysaccharides and five types of hydrolytic enzymes (protease, a-amylase, a-glucosidase, alkaline-phosphatase and acid-phosphatase) from sludge flocs were investigated to determine their influence on sludge aerobic digestion after ultrasonic pretreatment. Results suggested that most of the extracellular enzymes (except a-amylase) were present in pellet and TB-EPS layers, with minor quantities detected in LB-EPS and slime layers, and almost none detected in bulk solution. Ultrasonic pretreatment enhances enzymatic activities and promotes the shifts of extracellular proteins, polysaccharides and enzymes from inner layers of sludge flocs, i.e., pellet and TB-EPS, to outer layers, i.e., slime, to increase the contact and interaction among extracellular proteins, polysaccharides and enzymes that were originally embedded in the sludge flocs, resulting in improved efficiency in aerobic digestion (He et al., 2008).

Lei et al. (2010), have conducted a study, focusing on the influence of microwave irradiation on the sludge dewaterability. After sludge disintegration by microwave irradiation, the EPS and cellular substances were released into the aqueous phase, leading to an increase in protein and polysaccharide levels. It is concluded that, “the interactions of the very weak forces binding EPS components together, which are very important to the colloidal stability of flocs, are also disrupted during microwave irradiation.” Therefore, the higher microwave contact time could not only break the flocs and release extracellular materials but also completely destroy cells and release intercellular materials from cells into the aqueous phase. It is also mentioned that, the relationship between EPS concentration and sludge dewaterability, is not always directly proportional. This means that increasing the EPS concentration does not always decreased the sludge dewaterability (Lei et al., 2010).

Laboratory-scale anaerobic and aerobic digestion studies were conducted in the study of Novak et al.(2003) using waste activated sludges from two municipal wastewater treatment plants in order to gain insight into the mechanisms of floc destruction that account for changes in sludge conditioning and dewatering properties when sludges undergo anaerobic and aerobic digestion. Batch digestion studies were conducted at 20°C and the dewatering properties, solution biopolymer concentration and conditioning dose requirements measured. The data indicated that release of biopolymer from sludges occurred under both anaerobic and aerobic conditions but that the release was much greater under anaerobic conditions. In particular, the release of protein into solution was 4–5 times higher under anaerobic than under aerobic conditions. Both the dewatering rate, as characterized by the specific resistance to filtration and the amount of polymer conditioning chemicals required was found to depend directly on the amount of biopolymer (protein + polysaccharide) in solution (Novak et al., 2003).

In the study on Shao et al. (2009), effect of proteins, polysaccharides, and particle sizes on sludge dewaterability has been investigated. Four batch experiments of hydrolysis and acidification were carried out to investigate the distributions of proteins (PN) and polysaccharides (PS) in the sludge, the PN/PS ratio, the particle sizes, and their relationship with sludge dewaterability. The results showed that PN was mainly partitioned in the pellet (80.7%) and TB-EPS (9.6%) fractions, while PS distributed evenly in the four fractions. During hydrolysis and acidification, PN was transferred from the pellet and TB-EPS fractions to the slime fraction, but PS had no significant transfer trends. The mean particle sizes of the sludge flocs decreased with hydrolysis and acidification (Shao et al.,2009).

In Yu et al. (2010)'s study mentioned in Section 2.6.1., dewaterability properties of the sludge was also analyzed with different irradiation power and durations. To evaluate the dewaterability of the sludge, capillary suction time (CST) of the samples was measured. Initial CST of the sludge was 92.5. After microwave disintegration, the lowest CST values which were 53.0, 59.2 and 62.4 were observed for the contact time of 60, 100 and 200 seconds. When contact time extened, CST values were measured to be even worse than the initial value. This indicates that microwave irradiation has a positive effect of filterability. However, after a certain degree, dewaterability properties are worsened (Yu et al., 2010). This may result from the complete distruction of floc structure and the release of intra and extracellular compounds. Novak et al. (2003) also confirms that. It was stated thatunattached biocolloids remained in the solution are the responsible for the deterioration of sludge dewaterability properties (Novak et al., 2003).

Wang et al. (2006) investigates the components of released liquid after the ultrasonic disintegration of the sludge. Total content of protein was 694.4 mg/L while the total content of the polysaccharide was 143.1 mg/L which means the proteins correspond the larger portion of the EPS. The behavior of these two components were investigated and stated that the releasing of protein was fast during the first 20 minutes, than the rate of release was decreased as time increased. Since polysaccharide is easy to hydrolyze, it increased with time (Wang et al., 2006).

Eskicioglu et al. (2006), investigated athermal effects of microwave and also conducted analysis for sugar and protein solubility with microwave irradiation and conventional heating. With the same temperature (96^{o}C) solubilization of sugar was 300% and 10% whereas solubilization of proteins was 50% and 170% for CH and MW respectively (Eskicioglu et al., 2006) . This was explained in Eskicioglu et al. (2007)'s following study with caramelization or Maillard reactions occurring at temperatures over 80^{o}C. These reactions occur between amino acids and reducing sugars at high temperatures in polymerization takes place (Eskicioglu et al., 2007).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Sewage Sludge

The activated sludge samples were obtained from recirculation unit of the 5 different wastewater treatment plants. The sludge samples used in aerobic and anaerobic digestion processes were supplied from two different regions of Turkey which are Marmara and Blacksea Regions. For each regions two different types of treatment plants were chosen to collect the sludge; a domestic WWTP and an urban WWTP. The treatment facilities from Marmara Region are located in İzmit Kullar, İstanbul Bahçeşehir wheras the plants from Karadeniz Region are located in Samsun Bafra, and Düzce Akçakoca. Among them, İzmit Kullar and Düzce Akçakoca are urban treatment plants, whereas İstanbul Bahçeşehir and Samsun Bafra are domestic. For EPS analyses of the sludge, sample was collected from the recycle line of Paşaköy Advanced Biological Wastewater Treatment Plant which is located in İstanbul.

3.1.2. Seed Sludge (Inoculum)

The seed sludge used in this study was taken from the anaerobic digesters of Frito Lay Factory, located in Kocaeli, Turkey. Anaerobic digesters of Frito Lay treat the wastewater that originates from the production of potato chips.

3.1.3. Chemicals

All chemicals used in this study were of analytical grade and mainly supplied from Sigma-Aldrich, Hach and Merck.

3.1.4. Instrumental Equipments

Instruments used in this study to conduct the analyses are given in **Table 3.1**.

Table 3.1 Instrumental equipments used in the experiments.

Instrumental Equipment	Experimental Analysis	Trademark/Model
Ultrasonic Homogenizer	Disintegration	Bandelin Sonopuls HD 3400
Microwave Digestion System	Disintegration	Berghof Speedwave MWS+3
pH meter	pH	WTW 3110
Conductivity and Salinity	EC and Salinity	WTWLF 320
Dissolved Oxygen Meter	Dissolved Oxygen	Hach HQ 30d
Analytic Balance	TS, VS, MLSS, MLVSS	Scaltec (SBA 31)
Drying Oven	TS,VS, MLSS, MLVSS	NÜVE (FN 500)
Muffle Furnace	TS,VS, MLSS, MLVSS	Protherm
Filtration Apparatus	MLSS, MLVSS	Schott Duran
Centrifuge	sCOD, DOC, NH_3,NO_2^-,NO_3^-, SO_4^{2-}	Hettich Zentrifugen 16A
COD Digester	COD, EPS-Carbohydrates	Velp Scientifica Eco 25 Thermoreactor
Spectrophotometer	COD, TP, TKN, NH_3,NO_2^-,NO_3^-, SO_4^{2-}, EPS-Carbohydrates	HACH DR/2010
Organic Carbon Analyzer	TOC, DOC	Shimadzu TOC-V CSH
Digesdahl Digestion Apparatus	TKN, TP	HACH, 1999
Particle Size Analyzer	Particle Size	Malvern The Mastersizer 2000 (with Hydro2000MU unit)
Capillary Suction Timer	CST	Triton 304M
Viscometer	Viscosity	Brookfield Rvdv Prime
Gas Chromatograph	VFA	Perkin Elmer Clarus 600
Milligas Counter	Biogas Production	Ritter MGC 1
Gas Chromatograph	Biogas Composition	Agilent HP 6850
Temperature Arranged Centrifuge	EPS Extraction	Beckman Coulter Allegra 64R
Vortex	EPS	DragonLab MX-S
Homogenizer	EPS	Heidolph Silent Crusher M
UV Spectrophotometer	EPS-Protein	Shimadzu UV-160A

3.2. Methods

3.2.1. Experimental Set-up and Procedure

Experimental set-up for aerobic and anaerobic digestion processes and applied pretreatment methods are described in the following sections.

3.2.1.1. Aerobic Digestion Reactors

Aerobic digestion of the sludge samples were set-up as batch reactors which were operated for 21 days. Two different disintegration methods were applied to four different sludge samples. With the addition of unpretreated control samples, a total number of 12 reactors were operated. All reactors were aerated by air blowers and air stones were placed at the end of the air lines in order to make sure the air flow was not blocked. Air was distributed by diffuser systems and dissolved oxygen concentrations in the reactor were kept above 2 mg/L at all times.

Aerobic reactors had the volume of 5 liters which were full with 4 liters of sludge that ¼ of the samples were disintegrated. For each sludge sample three different reactors were set including control reactor (only the sludge itself, without any pretreatment), ultrasonication reactor (1 liter of ultrasonication applied sludge and 3 liters of unpretreated sludge) and microwave reactor (1 liter of microwave radiation applied sludge and 3 liters of non-treated sludge). All sludge samples were adjusted for 1% solid content in order to make the result comparable.

3.2.1.2. Anaerobic Digestion Reactors

Same as aerobic stabilization, reactors were designed as batch reactors in the anaerobic digestion process. Biogas productions in the reactors were checked and digestion operation was terminated on Day22 when the biogas productions were almost stopped.

Before the set-up of the reactors, TS contents of the reactors are adjusted to 1.5-2 % by gravitational thickening and inoculums/substrate ratio was determined to be the 1/3. Amber coloured glass reactors of 2.5 liters were used for anaerobic digestion with the active operation volume of 1600 mL. Total of 12 reactors were operated including control reactors. Anaerobic reactor set-up is given in **Table 3.2**.

Amber bottles were closed with bottle caps which were specially designed as two pipe lines coming from the reactor. One of the lines was for collecting the gas sample and the other one was directly connected to the miligas counter in order to measure the gas production of the reactor. Impermeability of the reactors was provided by silicosining the caps. In order to evacuate all the oxygen in the reactors nitrogen gas was passed through the reactors for 5 minutes. After that all reactors were placed in water baths with a constant temperature of 37°C. Reactors were shaken daily to prevent gravitational settling hinder the microbial activity.

Table 3.2 Content of the anaerobic digestion reactors

Sludge Sample	**Description**	**Seed Sludge (ml)**	**Recycled Sludge (ml)**	**Pretreated Sludge by Microwave (ml)**	**Pretreated Sludge by Ultrasound (ml)**
İzmit Kullar WWTP	Control	400	1200	-	-
	Ultrasound	400	800	-	300
	Microwave	400	800	300	-
İstanbul Bahçeşehir WWTP	Control	400	1200	-	-
	Ultrasound	400	800	-	300
	Microwave	400	800	300	-
Samsun Bafra WWTP	Control	400	1200	-	-
	Ultrasound	400	800	-	300
	Microwave	400	800	300	-
Düzce Akçakoca WWTP	Control	400	1200	-	-
	Ultrasound	400	800	-	300
	Microwave	400	800	300	-

3.2.1.3. Pretreatment Applications

Ultrasonication Applicaton

Ultrasonic experiments were performed by using an ultrasonic homogenizer (Bandelin-Sonopuls HD 3400) shown in **Figure 3.1**. The ultrasonic homogenizer used in the study is equipped with a generator (GM 3400), an ultrasonic converter (UW 3400), a booster horn (SH 3425) and a probe (VS 200 T). The generator converts the received power (50 or 60 Hz) into high-frequency power at a frequency of 20 kHz. The ultrasonic converter connected to the generator transforms the high-frequency power from the generator to ultrasound, converting it to a form of mechanical energy. This is achieved through an efficient and robust PZT ultrasonic transducer system. Hence, the tip of the probe also vibrates at a frequency of 20 kHz and transfers these vibrations with high power density to the sonicated sample (Bandelin, 2009). The ultrasonic unit used in this study has a constant frequency of 20 kHz.

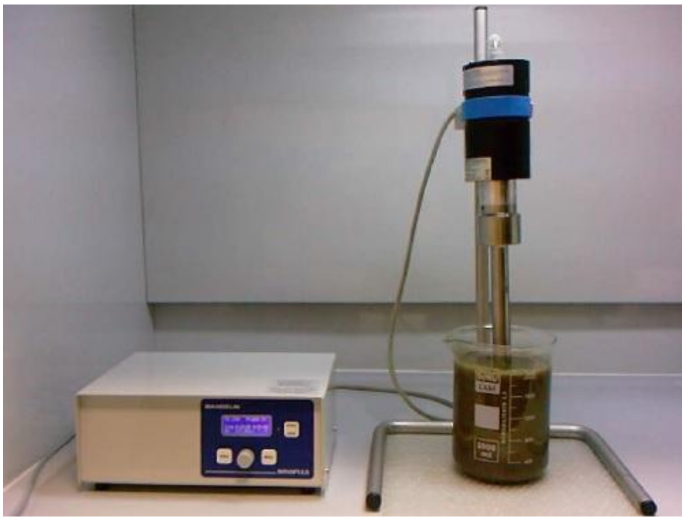


Figure 3.1 The ultrasonic homogenizer used in the study (Bandelin-Sonopuls HD 3400).

The amplitude was set to 70% and the energy-output was 200 W. Optimization studies for the ultrasonication applications will be explained in Chapter 4 in detail. The full specifications of the used ultrasonic equipment are listed in Table 3.3.

Table 3.3 Characteristics of the ultrasonic equipment.

GM 3400 Generator	
Power Supply	230V~50/60Hz (alternatively115V~50/60Hz)
Ultrasonic frequency	20 kHz
Maximum Power	400 W
Power setting range	60 – 300 W
Weight	3.1 kg
Dimentions (l× w × h)	324 × 230 × 131 mm
Time setting range	0:00:01-9:59:59 (h:mm:ss) or continuous operation
Amplitude setting range	10 – 100 % , 1 % increments
UW 3400 Ultrasonic Converter	
Frequency	20 kHz
Weight	2.2 kg
Dimensions	Ø 90 ×180 mm
Degree of protection	IP 20
VS 200 T Probe	
Diameter	Ø 25 mm
Connection to standard horn	SH 3425
Volume range	100 – 2500 mL
Maximum admissible amplitude setting	100%
Immersion depth (recommended)	10 – 20 mm

Specific Energy

The specific energy input is defined as the energy input per unit of sludge (as TS) to achieve a certain degree of disintegration (Khanal et al., 2007). Specific Energy (E_s) is a function of ultrasonic power, ultrasonic duration, and volume of sonicated sludge and TS concentration, and can be calculated using the following Equation 3.1 (Bougrier et al., 2005):

$$E_s = \frac{P * t}{V * TS} \quad (3.1)$$

where,

E_s : the specific energy input in kWs/kg TS (kJ/kg TS)
P : the ultrasonic power in kW
t : the ultrasonic duration in seconds
V : the volume of sonicated sludge in liters
TS : the total solids concentration in kg/L

Microwave Application

Thermal disintegration of the sludge is supplied by microwave irradiation using Berghof Microwave System (MWS+3) shown in Figure 3.2. After the optimization studies, sludge samples were subjected to 175°C during 10 minutes. Details of the optimization studies will be described in Chapter 4. The full specifications of the used microwave equipment are listed in Table 3.4.

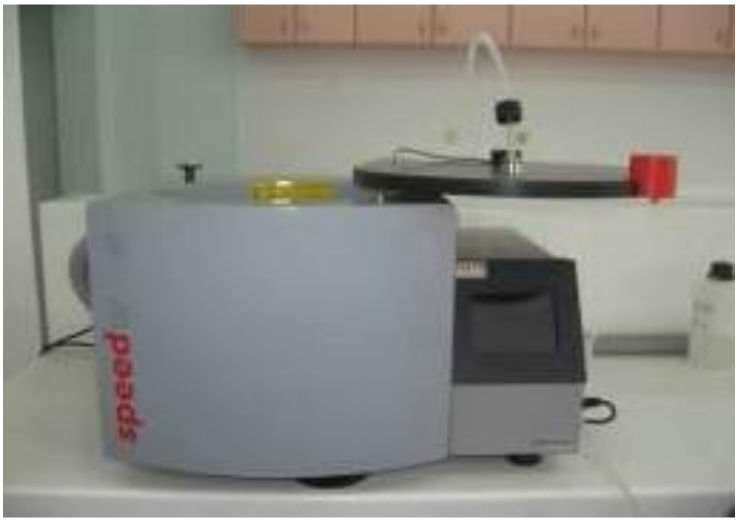


Figure 3.2 The microwave system used in the study (Berghof Speedway MWS+3).

Table 3.4 Characteristics of the microwave equipment.

Power Supply	230 V/50 Hz/ 1,350 W
Microwave Output	1000 W
Frequency	2450 Hz
Weight/dimensions (W x D x H)	Standard device: approx. 14 kg/ 520 x 460 x 330 mm Control unit: approx. 0.5 kg/ 188 x 35 x 114 mm
Oven chamber	Approx. 27 Liter/ 350x x340 x 215 mm (W x D x H)
Noise Level	<60 dB
Ambient Conditions	15- 35 ^oC / 85 % relative air humidity
Temperature measurements	Measurement range 50-260 ^oC, accuracy 1^oC at 200 ^oC

Disintegration Degree

The degree of disintegration (DD_{COD}) was defined by Müller and Pelletier as the comparison between SCOD after pretreatment and the maximum SCOD obtained by alkaline hydrolysis ($SCOD_{NaOH}$), as presented in Equation 3.2. For alkaline hydrolysis, sludge was mixed with NaOH (in the ratio of 1 mol/L) for 24 h at room temperature as described by Müller and Pelletier's formula (1998):

$$DD_{COD} = \left[\frac{SCOD_{Pretreated} - SCOD_0}{SCOD_{NaOH} - SCOD_0} \right] \times 100\% \tag{3.2}$$

where,

$SCOD_{Pretreated}$: the COD in the supernatant of the disintegrated sample (mg/L)

$SCOD_0$: the COD in the supernatant of the original (unpretreated) sample (mg/L)

$SCOD_{NaOH}$: the COD in the supernatant of the alkaline hydrolyzed sample (mg/L)

3.2.2. Analytical Methods

pH, Oxidation Reduction Potential and Temperature: All pH, ORP, and temperature measurements were conducted by using WTW pH 3110 portable pH meter. The pH meter was calibrated regularly with pH standard buffer solutions.

Conductivity and Salinity: Conductivity and salinity measurements were performed by using WTWLF 320portable conductivity/salinity/TDS/temperature meter.

Dissolved Oxygen: Dissolved oxygen content of the aerobic reactors was measured by Hach HQ 30d portable dissolved oxygen meter.

Total Solids and Volatile Solids (TS & VS): TS and VS analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) using method 2540B and 2540C, respectively. Initially, evaporating dishes were brought to a constant weight by heating in muffle furnace (Protherm PLF 110/8) for 1 hour at 550^{o}C. This procedure was repeated until the error between the two consecutive measurements was less than 1%. For TS measurement, 30 mL of well-mixed sludge sample was put in evaporating dish, its weight is reported and then evaporated and dried in oven (Nüve FN 500) for 24 hours at 105^{o}C. Afterwards, the evaporating dish was cooled in desiccator for 30 minutes and weighed. For VS measurement, sample was further ignited in the muffle furnace at 550^{o}C for 1 hour, cooled in desiccator and weighed. TS and VS concentrations of the samples were found by calculations.

Mixed Liquor Suspended Solids and Mixed Liquor Suspended Solids (MLSS & MLVSS): MLSS and MLVSS were determined in accordance with the Standard Methods (APHA, 2012) using the Method 2540D and 2540E, respectively. Initially, filter papers and crucibles that were used in MLSS and MLVSS analyses were brought to a constant weight.

For MLSS measurement, 10 mL sludge sample was filtered through a pre-weighed glass fiber filter paper (Sartorius Stedim Glassfibre Prefilter) and dried in oven for 1 hour at 105^{o}C. The filter paper was cooled in desiccator for 30 minutes and weighed. For MLVSS calculation, filter paper was further ignited in muffle furnace for 1 hour at 550^{o}C, cooled in desiccator and weighed. MLSS and MLVSS concentrations of the samples were found by calculations.

Chemical Oxygen Demand and Soluble Chemical Oxygen Demand (COD & sCOD): COD and sCOD analyses were performed using dichromate closed reflux colorimetric method according to the Standard Methods for the Examination of Water and Wastewater, Method 5220D. Samples were refluxed with sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$) in Velp Scientifica COD Digester (Eco 25 Thermoreactor) at 150^{o}C for 2 hours. Sludge samples were diluted if necessary. For sCOD analyses, supernatant portion of the sample were used which were obtained by centrifugation in Hettick Rottina 380 centrifuge device at 9000 rpm for 30 minutes. Absorbance values of the refluxed samples were measured at 600 nm by using HACH DR/2010 Portable Data Logging Spectrophotometer. In order to prepare the calibration curve for determination of the corresponding concentration value of the absorbance, potassium hydrogen phthalate (KHP) solutions were used.

Alkalinity: Alkalinity analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater (APHA,2012). Sludge samples were diluted if necessary, then added to an Erlenmeyer flask with a magnetic stirrer. The initial pH of the sample was measured and the sample was titrated with 0,02 N sulfuric acid (H_2SO_4) until its pH reached 3.5. Alkalinity concentrations of the samples were found by calculations.

*Total Organic Carbon and Dissolved Organic Carbon (TOC & DOC):*TOC and DOC of the samples were measured by Shimadzu TOC-V CSH total organic carbon analyzer. Before the analyses, sample eluates were prepared according to their TS contents. For this, samples were left at shakers for 24 hours and the analyses were conducted using the liquid volume that contains the organic content of the samples. For TOC measurement, samples were diluted if needed. For DOC measurement, samples were centrifuged for 30 minutes at 9000 rpm and the supernatant of the centrifuge was filtered from Sartorius Stedim cellulose acetate filter (0.45µm pore size).

Total Kjeldahl Nitrogen(TKN): Sample digestion was performed using the General Digesdahl Digestion procedure via ignition 25 mL sample to 440°C followed by concentrated hydrochloric acid and after that by dilution it to 100 mL as defined in Hach DR/2010 Procedures Manual (Hach, 1997). Sample pretreatment were carried out at Digesdahl Digestion Apparatus. Digested sample was analyzed using the Nessler Method (Method 8075) for TKN measurement according to Hach DR/2010 Procedures Manual (Hach, 1997). TKN concentrations were calculated using the following Equation 3.3 (Hach, 1997):

$$TKN\ (mg/L) = \frac{A \times 75}{B \times C} \tag{3.3}$$

where,

A : mg/L reading from instrument

B : mL sample amount (sample volume used for digestion)

C : mL analysis volume

Total Phosphorous (TP): Same digested sample prepared as mentioned above (in TKN measurement) was used for TP experiment. Digested sample was analyzed for TP measurement using Ascorbic Acid Method (Method 8048) in accordance with Hach DR/2010 Procedures Manual (Hach, 1997). PhosVer 3 Phosphate Powder Pillow kits were used for this analysis. TP concentrations were calculated using the following Equation (3.4) (Hach, 1997):

$$Total\ P\ (mg\ /\ L) = \frac{A \times 2500}{B \times C}$$

where,

A : mg/L reading from instrument

B : mL sample amount (sample volume used for digestion)

C : mL analysis volume

Ammonia Nitrogen (NH_3-N): For the ammonia analysis, Nessler Method (Method 8038) that was given in HACH Procedures Manual (Hach, 1997) was followed. Centrifuged sludge samples were diluted if needed and completed to 25 mL by deionized water. 3 drops Mineral Stabilizer, 3 drops Polyvinyl Alcohol Dispersing Agent and 1 ml Nessler Reagent were added to the sample. After each addition, sample was inverted several times to mix. Same steps were followed for the blank (deionized water) to zero the sample in the spectrophotometer. Following the one-minute reaction period, sample was poured into 10 mL sample cell, and the ammonia nitrogen concentration was read at 425 nm in the Hach DR/2010 spectrophotometer.

Nitrite and Nitrate Nitrogen (NO_2^--N & NO_3^{--}-N): For nitrite and nitrate analyses, supernatants of the centrifuged sludge samples were used. Centrifuged samples were diluted if necessary and spectrophotometer was used for the concentration determination. The procedure that was given in HACH Procedures Manual (Hach, 1997) was followed for both analyses. For nitrite readings, NitriVer 2 nitrite reagent powder pillow was added to the sample, and the sample was shaken vigorously to dissolve. After ten minutes of reaction, sample was placed in the spectrophotometer and wavelength of 585 nm, sample was analyzed. For nitrate readings, NitraVer 5 nitrate reagent powder pillow was added to the sample, and shaken vigorously. Then, five minute of reaction was waited. After that, sample was placed in the spectrophotometer. For nitrate measurement wavelength of 500 nm was used.

Sulfate (SO_4^{2-}): SulfaVer 4 Method that was given in HACH Procedures Manual (Hach, 1997) was followed for sulfate analysis. For sulfate analyses also the supernatants of centrifuged samples were used. SulfaVer 4 Reagent powder pillow were added to the samples and sample cells was swirled vigorously. After five-minute reaction period results were measured at 880 nm in the spectrometer. Same steps were completed for the blank by using deionized water in order to zero the sample in the spectrophotometer.

Chloride (Cl^-): The amount of chloride in sludge samples was determined by silver nitrate method (Mohr Argentometric Method) described in Standard Methods for the Examination of Water and Wastewater (APHA, 2012). In the method, silver nitrate ($AgNO_3$) was used as the titrant and potassium chromate (K_2CrO_4) was used as the end point indicator. Supernatant of the centrifuged sludge sample was added to an Erlenmeyer flask and completed with deionized water until 100 mL volume. Potassium chromate was added to the flask and as a result the color of the solution turned into yellow. The sample was titrated with silver nitrate to the first appearance of red color. Standardization of the silver nitrate solution was made by titration with NaCl. Same steps were completed for the blank by using deionized water. The amount of titrant used was noted down and the calculations were made to find the chloride concentration.

Particle Size Distribution: Particle size analyses were carried out using Malvern Mastersizer 2000 (with the wet dispersion unit of Hydro2000MU). The Mastersizer 2000 uses the technique of laser diffraction to measure the size of particles. The data is analyzed to calculate the size of the particles that created the scattering pattern. The software controls the system during the measurement process and analyzes the scattering data to calculate the particle size distribution.

Water was used as dispersant liquid and its refractive index was 1.33. Sludge samples had refractive index of 1.5. The stirrer and pump speed were kept at 600 rpm, which is the minimum pump/stirrer speed available, to minimize the damage of sludge particles. Sampling was made using a pasteur pipette. 1000-mL low form beaker which had 800 mL deionized water in it was used as dispersion tank. For analysis, each sample was diluted in this dispersion tank and introduced into the measuring cell. Each sample was analyzed in triplicate.

The principle of operation is as following: The dispersion unit uses a 1000-mL “low form” beaker that holds the sample/dispersant liquid. A stirrer, controlled from the keypad, agitates the sample and stops it from settling or separating out. The pump, controlled from the keypad, forces the sample from the “to cell port” of the dispersion unit to the flow cell located in the optical bench (Mastersizer 2000), via the sample tubing. The sample is pumped through the flow cell and then returns to the beaker via the sample tubing and the “from cell port” (Malvern, 2007).

Viscosity: In this study, viscosity measurements were carried out using a Brookfield RVDV-I PRIME digital viscometer. For making measurements, viscometer was turned on, leveled and autozeroed. The level was adjusted using the three feet on the bottom of the base and confirmed using the bubble on the top of the head. The level was set prior to autozeroing and was checked prior to each measurement. 500 mL of sludge samples were filled in 600 mL Low Form Griffin Beaker. In the viscosity experiments, the operating speed was 100 rpm.

Capillary Suction Time: CST analyses were conducted by using Triton Electronics Ltd. Type 304M capillary suction timer. CST papers used were at a size of 7x9 centimeters. 0,2 mL sample was taken and added to the CST sample cylinder with pasteur pipettes. The sample cylinder had a 1,8 cm diameter. The time that is needed to reach the second sensor from the first sensor was calculated by the analyzer and it was given in seconds.

Microbiology: In sludge samples, Total Coliform (SM9222B), Escherichia coli (SM9222D), Fecal Streptococci (SM9230C), and Salmonella analyses were conducted by using membrane filtration technique, which are all indicated in Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Since the samples had high bacterial concentration, high dilutions were necessary in order to estimate the microbial population.

*Volatile Fatty Acids :*VFA analyses were conducted in Perkin Elmer Clarus 600 Gas Chromatograph equipped with a flame ionization detector and a 30 meter 0,32 mm I.D. column. Samples were centrifuged at 9000 rpm and then filtered from Sartoris Stedim Minisart syringe filter (0.45μm pore size). Until the measurement, samples were stored at +4^{o}C with the addition of phosphoric acid.

Biogas Production and Composition: Biogas productions of the anaerobic reactors were recorded daily by Ritter MGC Milligas Counters software application. The produced biogas was collected with a silicon pipe which directly goes to the Milligas counters. Counters are full of special packing liquid. When the gas came into the device it created a bubble which moves the revision screw and this directly reflects on the signal output.

The biogas composition of the anaerobic reactors was determined by using Agilent HP 6850 Gas Chromatograph (GC). In this equipment, helium gas was used as the carrier gas. Gas sample was collected from the reactors by Hamilton Agilent 2.5 mL syringe and immediately injected to the GC.

Extracellular Polymeric Substances: In order to determine the extracellular polymeric substances (EPS) in the sludge it first needs to be extracted. However, there is not a standard method for the extraction of EPS in the literature. Therefore, the most suitable technique for the extraction was chosen. The applied method is Cation Exchange Resin (CER), which is similar to the description of Frølund et al. (1996). DOWEX was used as the strongly acidic CER. The extraction procedure was completed as described below.

Prior to the extraction, DOWEX was washed with phosphate buffer saline (PBS) solution in order to rinse the dust. 35 g DOWEX was weighed per sample. 100 mL PBS solution and 35 g DOWEX was mixed in a flask, which was covered with alu-folio and stirred for 1 hour. After 1 hour, DOWEX was filtered and left to dry.

Sludge sample was collected from the reactor in a volume which will give 0,5 g MLVSS / 35 g DOWEX and it was centrifuged in Beckman Coulter Allegra 64R centrifuge for 15 minutes at 12.000 xg, +4^{o}C. Supernatant of the centrifuged sample was collected and stored at -20^oC for further analysis. These samples were used to estimate the soluble EPS of the sludge.

30 mL deionized water was added to the remaining pellets and the mixture was vortexed in DragonLab MX-S Vortex to mix. Centrifugation was repeated for 15 minutes at 12.000 xg, at +4^oC.

Supernatant of the sample was discarded, 25 mL PBS was added to the remaining biomass pellets and vortexed to re-suspend. Biomass that was re-suspended in PBS was homogenized in Heidolph Silent Crusher M Homogenizer for 4 minutes, at 800 rpm. The sample was kept in an ice bucket while homogenizing.

Homogenized biomass was poured into the extraction flask, which contained 35 g DOWEX and a magnetic stirrer. PBS was added to the flask to complete the final volume to 100 mL. The flask was kept in an ice bucket and it was covered with alu-folio. The mixing speed was adjusted to 800 rpm and the sample was mixed in the dark for 4 hours, at +4^oC. At the end of 4 hours, sample was centrifuged for 1 minute, at 12.000 xg, +4^oC. Then, centrifugation was repeated for 15 minutes, at 12.000 xg, +4^oC. Supernatant of the centrifuged sample was collected and stored at -20^oC for further analysis. These samples were used to estimate the bound EPS of the sludge.

Proteins. Total protein concentrations of the sludge samples were detrmined by Lowry Method (Lowry et al., 1951) using Bovine Serum Albumin (BSA) as the standard. Supernatants of the samples that were obtained at the end of the EPS extraction process were used during the analysis. 0,5 mL sample and 0,7 mL Lowry Solution were vortexed in a COD tube and incubated for 20 minutes in the dark at room temperature. After 20 minutes, 0,1 mL diluted Folin Reagent was added to the tubes, vortexed to mix, and the tubes incubated again for 30 minutes in the dark, again at room temperature. After 30 minutes, samples were transferred to semi-micro disposable cuvettes. For the calibration curve, solutions with differing concentrations of BSA were prepared from stock solution. Absorbance values of the samples were measured with 750 nm wavelength by using Shimadzu UV-160A UV-Visible Recording Spectrophotometer. Total amount of proteins of the samples were calculated from the calibration curve.

Carbohydrates. The amount of total carbohydrates in sludge samples was determined by Anthron Method (Gaudy, 1962), which is basically a colorimetric method. Supernatants of the samples that were obtained at the end of the EPS extraction process were used during the analysis. 1 mL sample, 2 mL 75% H_2SO_4 solution, and 4 mL anthron solution were added to the COD vials. The bottles were vortexed to mix, placed into the HACH digester and boiled for 15 minutes at 100^oC, then left to cool down. For the calibration curve, solutions with differing concentrations of glucose were prepared from the stock solution. Absorbance values of the samples were measured at 578 nm wavelength by using the Hach DR/2010 spectrophotometer. Total carbohydrates of the samples were calculated from the calibration curve.

4. RESULTS AND DISCUSSIONS

The effects of ultrasonic and microwave pretreatment on aerobic and anaerobic stabilization of sludge were investigated in this study. In addition, the EPS structure of the disintegrated sludge was analyzed.

4.1. Aerobic Digestion

Four different sludge samples, taken from the recycle line of the treatment plants, were aerobically digested for 21 days. Sludge samples were taken from İzmit Kullar wastewater treatment plant (WWTP), İstanbul Bahçeşehir WWTP, Düzce Akçakoca WWTP and Samsun Bafra WWTP. The initial characteristics of the sludge samples used for aerobic digestion are given in the Table 4.2.

In this section, some abbreviations were used to describe each reactor. These abbreviations are given in Table 4.1.

Table 4.1 Abbreviations used to describe the aerobic reactors.

Reactor	İzmit Kullar WWTP	İstanbul Bahçeşehir WWTP	Samsun Bafra WWTP	Düzce Akçakoca WWTP
Control	R1-C	R2-C	R3-C	R4-C
Pretreated by Ultrasound	R1-U	R2-U	R3-U	R4-U
Pretreated by Microwave	R1-M	R2-M	R3-M	R4-M

For ultrasonic pretreatment application, an optimization study was conducted with the aim of determining the optimum ultrasonic specific energy and ultrasonic power.

Table 4.2 Characteristics of the waste activated sludge used in the reactors.

Parameter	Unit	İzmit Kullar WWTP	İstanbul Bahçesehir WWTP	Samsun Bafra WWTP	Düzce Akçakoca WWTP
TS	mg/L	12343	5963	6463	5659
VS	mg/L	8550	4360	4060	3960
MLSS	mg/L	11660	5670	6060	5360
MLVSS	mg/L	7989	3943	3707	3699
pH	-	7.23	7.06	7.06	7
Alkalinity	mg $CaCO_3$/L	735	900	1600	500
Conductivity	mS/cm	2.51	1.74	2.86	2.34
Salinity	%o	1.2	0.9	1.5	1.1
COD	mg/L	6340	9062	6579	7773
sCOD	mg/L	120	145	203	343
TOC	mg/L	418	486	625	732
DOC	mg/L	22	18	20	25
TKN	mg/L	620	590	630	680
NH_4^+-N	mg/L	8	9	6	52.5
NO_3^-N	mg/L	0.9	0.2	0	5.25
NO_2^-N	mg/L	1	0	0	0
TP	mg/L	1600	1320	1110	1040
PO_4^{3-}-P	mg/L	4920	4060	3380	3180
P_2O_5-P	mg/L	3680	3040	2520	2380
SO_4^{2-}	mg/L	170	115	150	45
Cl^-	mg/L	212	212	318	212
CST	s	22.3	17	22.6	86.5
Total Coliform	[CFU/100mL]	1x108	1.4x108	1.6x108	2.6x108
Fecal Coliform	[CFU/100mL]	4.9x107	7.9x107	5.7x107	1x108
Fecal Streptococci	[CFU/100mL]	1.6x107	1.9x107	3.1x107	5.5x107

Optimization of Ultrasonic Specific Energy

In order to determine the optimum specific energy for the disintegration, four sludge samples, used in this study, were sonicated with different specific energies and disintegration degree (DD), soluble Chemical Oxygen Demand (sCOD), Capillary Suction

Time (CST), viscosity and particle size of the sludge samples were analyzed. Results of the optimization studies are given in Table 4.3, Table 4.4 and Table 4.5.

Table 4.3 sCOD and DD values of four different sonicated sludge samples with different specific energies.

Specific Energy (kJ/kg TS)	İzmit Kullar WWTP		İstanbul Bahçeşehir WWTP		Samsun Bafra WWTP		Düzce Akçakoca WWTP	
	sCOD (mg/L) (initially 122 mg/L)	**DD (%)**	**sCOD (mg/L)** (initially 210 mg/L)	**DD (%)**	**sCOD (mg/L)** (initially 194 mg/L)	**DD (%)**	**sCOD (mg/L)** (initially 561 mg/L)	**DD (%)**
5000	1601	15.3	1545	14.2	325	2.3	772	4.1
10000	1720	16.6	1671	15.6	435	4.2	810	4.9
15000	**1987**	**19.3**	**1963**	**18.7**	**791**	**10.3**	**939**	**7.4**
25000	3048	30.3	2764	27.2	1058	9.1	1267	13.8
50000	8187	83.7	6815	70.3	1929	30	1609	20.5

Table 4.4 CST and viscosity values of four different sonicated sludge samples with different specific energies.

Sludge Sample	CST (sec)			Viscosity (Mpas)		
	SE= 5000 kJ/kg TS	**SE= 15000 kJ/kg TS**	**SE= 50000 kJ/kg TS**	**SE= 5000 kJ/kg TS**	**SE= 15000 kJ/kg TS**	**SE= 50000 kJ/kg TS**
İzmit Kullar WWTP	1295	**1450**	1652	28	**23**	18
İstanbul Bahçeşehir WWTP	1190	**1708**	>2000	22	**21**	17
Samsun Bafra WWTP	276	**530**	787	16	**15**	15
Düzce Akçakoca WWTP	176	**216**	335	13	**13**	12

Table 4.5 Particle size values of four different sonicated sludge samples with different specific energies.

	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.1) µm	d (0.5) µm	d (0.9) µm
	İzmit Kullar WWTP				
Raw Sludge	36.400	81.931	18.763	63.037	145.369
SE=5.000 kJ/kg TS	22.100	42.821	18.360	39.676	73.045
SE=15.000 kJ/kg TS	**6.133**	**32.407**	**2.657**	**19.303**	**59.722**
SE=50.000 kJ/kg TS	3.419	38.744	1.176	11.677	109.639
	İstanbul Bahçeşehir WWTP				
Raw Sludge	48.086	151.427	24.857	83.805	269.358
SE=5.000 kJ/kg TS	12.959	40.106	7.918	29.309	68.244
SE=15.000 kJ/kg TS	**7.555**	**50.180**	**3.354**	**23.481**	**117.830**
SE=50.000 kJ/kg TS	7.995	78.565	3.473	38.788	189.127
	Samsun Bafra WWTP				
Raw Sludge	40.941	86.411	21.075	72.627	156.196
SE=5.000 kJ/kg TS	35.130	65.855	23.719	59.951	116.816
SE=15.000 kJ/kg TS	**28.004**	**67.047**	**22.744**	**57.107**	**115.866**
SE=50.000 kJ/kg TS	8.038	52.220	3.434	33.254	108.086
	Düzce Akçakoca WWTP				
Raw Sludge	28.578	81.168	14.367	46.629	145.607
SE=5.000 kJ/kg TS	21.198	61.082	12.603	37.907	107.836
SE=15.000 kJ/kg TS	**23.999**	**58.560**	**16.201**	**44.774**	**96.507**
SE=50.000 kJ/kg TS	24.640	63.900	18.514	51.606	109.909

Analyses showed that there is not a significant difference between the specific energies of 15000 and 25000 kJ/kg TS. Therefore, considering the energy consumption of the system, 15000 kJ/kg TS was chosen to be applied for the sludge disintegration. Then, different ultrasonic power values were applied with 15000 kJ/kg TS specific energy, with the aim of determining the optimum power. Results are given in Table 4.6.

Table 4.6 sCOD and DD values with 15000 kJ/kg TS specific energy and different ultrasonication powers.

Specific Energy (kJ/kgTS)	Ultrasonication Values	Ultrasonik Güç (Watt)	Kocaeli Kullar AAT (Kentsel)	İstanbul Bahçeşehir AAT (Evsel)	Samsun Bafra AAT (Evsel)	Düzce Akçakoca AAT (Kentsel)
15000	Disintegration Degree (%)	60	5.3	6.6	0.7	4.8
		100	14.3	19	4	6.3
		200	**27.6**	**18**	**8**	**9.6**
		300	25.9	30	7.3	8
	Soluble COD (mg/L)	60	634	829	231	804
		100	1494	1977	417	881
		200	**2782**	**1879**	**648**	**1051**
		300	2614	2963	614	971

Outcomes of the optimization studies showed that the specific energy of 15000 kJ/kg TS with the dose adjusted to 200 W and 70% amplitude is the optimum value for the sludge samples used in this study. Therefore, for ultrasonic pretreatment, sludge samples were subjected to these application conditions. Details of the applied ultrasonic pretreatment are given in Table 4.7.

Table 4.7 Specifications of the ultrasonicated sludge samples.

Pretreated Sludge	Initial Temperature (^{0}C)	Final Temperature (^{0}C)	Applied Ultrasonic Energy (kJ)	Specific Energy (kJ/kg TS)
İzmit Kullar WWTP	23	25.8	102.358	15000
Bahçeşehir WWTP	26.6	24	101.340	15000
Samsun Bafra WWTP	21.2	29.8	109.384	15000
Düzce Akçakoca WWTP	25	24.5	92.419	15000

For microwave irradiation Berghof MWS-3 Speedwave Microwave System was used. An optimization study was conducted with the aim of determining the optimum temperature and contact time.

Optimization of Microwave Irradiation

In order to determine the temperature and the contact time of the microwave irradiation, 400 ml of sludge was disintegrated with different temperatures (100,150, 175, 190 ^{o}C) and contact times (10, 20 and 30 minutes).

Change in sCOD values of the sludge samples with different temperatures and contact times are given in Table 4.8.

Table 4.8 sCOD values of sludge samples with different application conditions.

Irradiation Conditions	**İzmit Kullar WWTP**	**İstanbul Bahçeşehir WWTP**	**Samsun Bafra WWTP**	**Düzce Akçakoca WWTP**
	sCOD (mg/L)			
----	422	210	194	561
100°C 10'	1125	133	116	589
100°C 20'	2219	518	345	717
100°C 30'	2240	540	356	721
150°C 10'	3248	880	339	740
150°C 20'	3498	760	284	887
150°C 30'	3520	648	243	1863
175°C 10'	**4100**	**768**	**758**	**943**
175°C 20'	3810	853	684	1391
175°C 30'	3735	874	702	1406
190°C 10'	3415	524	396	687
190°C 20'	2562	487	447	906
190°C 30'	2590	421	456	987

Change in DD values of the sludge samples with different temperatures and contact times are given in Table 4.9.

Table 4.9 DD values (%) of sludge samples with different application conditions.

Irradiation Conditions	İzmit Kullar WWTP	İstanbul Bahçeşehir WWTP	Samsun Bafra WWTP	Düzce Akçakoca WWTP
	DD (%)			
100°C 10'	10.40	<0	<0	0,2
100°C 20'	21.8	3.3	2.6	3
100°C 30'	22	3.5	2.6	3
150°C 10'	32.4	7.1	2.5	3.5
150°C 20'	35	5.9	1.6	5.3
150°C 30'	35.1	4.7	0.9	25.5
175°C 10'	**41.3**	**5.9**	**9.8**	**10.4**
175°C 20'	38.3	6.8	8.5	16.3
175°C 30'	37.5	7	8.6	16.4
190°C 10'	34.2	3.3	3.1	2.6
190°C 20'	25.3	3	3.3	10.2
190°C 30'	25.4	2.8	3.3	10.5

Physical changes occurred in the sludge with different microwave irradiation conditions were analyzed with CST, viscosity and particle size parameters. Results of the analyses are given in Table 4.10, Table 4.11 and Table 4.12.

Table 4.10 CST of sludge samples with different application conditions.

Sludge Samples	100 °C 10 min	100°C 20 min	150 °C 10 min	150 °C 20 min	175 °C 10 min	175 °C 20 min	175 °C 30 min
	CST (sec)						
İzmit Kullar WWTP	520	530	516	460	**436**	424	480
İstanbul Bahçeşehir WWTP	17	26	31	24	**17**	18	21
Samsun Bafra WWTP	50	62	67	32	**38**	56	48
Düzce Akçakoca WWTP	40	47	50	163	**152**	148	55

Table 4.11 Viscosity of sludge samples with different application conditions.

Sludge Samples	100 °C 10 min	100°C 20 min	150 °C 10 min	150 °C 20 min	175 °C 10 min	175 °C 20 min	175 °C 30 min
	Viscosity (Mpas)						
İzmit Kullar WWTP	40.5	38.7	35.8	34	**31.2**	31	29.2
İstanbul Bahçeşehir WWTP	12.4	12.1	15.2	15	**14.4**	11.6	12.8
Samsun Bafra WWTP	12	11.8	11.2	16	**14.4**	12.4	12.9
Düzce Akçakoca WWTP	14	11.6	12.8	10.4	**13.6**	13.4	11.2

Table 4.12 Particle size of microwave applied sludge samples with the temperature of 175 °C and different different contact times.

Slugde Samples	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.1) µm	d (0.5) µm	d (0.9) µm
İzmit Kullar WWTP					
Raw Sludge	36.400	81.931	18.763	63.037	145.369
10 min of irradiation	86.094	173.504	50.102	147.851	331.816
20 min of irradiation	67.027	145.140	38.547	112.010	261.114
İstanbul Bahçeşehir WWTP					
Raw Sludge	48.086	151.427	24.857	83.805	269.358
10 min of irradiation	116.235	258.007	65.359	214.090	517.681
20 min of irradiation	145.195	431.406	71.195	313.989	980.080
Samsun Bafra WWTP					
Raw Sludge	40.941	86.411	21.075	72.627	156.196
10 min of irradiation	141.876	442.387	69.951	330.954	988.931
20 min of irradiation	176.335	572.458	80.867	468.250	1243.838
Düzce Akçakoca WWTP					
Raw Sludge	28.578	81.168	14.367	46.629	145.607
10 min of irradiation	33.376	130.970	21.851	74.286	284.268
20 min of irradiation	36.745	254.974	42.865	114.347	321.764

At the end of the optimization studies, temperature of 175°C for 10 minutes was determined to be the optimum condition when considered the solubility of the sludge and energy consumption of the process. Therefore, for microwave disintegration, sludge samples were subjected to 175°C for 10 minutes. Disintegration degrees of the pretreated sludge samples are given in Table 4.13.

Table 4.13 Disintegration degrees of the pretreated sludge samples.

Treatment Plant	**Reactor**	**DD (%)**
İzmit Kullar WWTP	Pretreated by Ultrasound	19
	Pretreated by Microwave	46
İstanbul Bahçeşehir WWTP	Pretreated by Ultrasound	25
	Pretreated by Microwave	41
Samsun Bafra WWTP	Pretreated by Ultrasound	14
	Pretreated by Microwave	60
Düzce Akçakoca WWTP	Pretreated by Ultrasound	13
	Pretreated by Microwave	32

Table 4.14 Analysis frequency for the aerobic digestion reactors.

Parameter	**Analysis Frequency**
TS,VS	2 Times in a Week
MLSS,MLVSS	2 Times in a Week
Alkalinity	2 Times in a Week
DO,ORP	Every day
Conductivity	Every day
pH	Every day
Temperature	Every day
Salinity	Every day
COD	2 Times in a Week
sCOD	2 Times in a Week
TOC	Once in a Week
DOC	Once in a Week
TN	Once in a Week
TP	Once in a Week
CST	Once in a Week
NH_4^+-N, NO_3^-, NO_2^-, $SO_4^=$, Cl-	In the 1st and 30st days
Particle Size Distribution	Once in a Week
Microbiologic Analyses	In the 1st and 30st days

4.1.1. Temperature

Temperature is one of the most important environmental factors affecting microbial growth and thus the biological systems. Temperature can affect the biological reactions by influencing either enzymes for enzymatic reactions or substrate diffusion rates. It is known that most of the microorganisms can be active between specific temperature ranges (Zavala et al. 2004). For this study, temperatures of the reactors were kept generally between 20 ^{o}C to 25 ^{o}C as seen in the Figure 4.1.

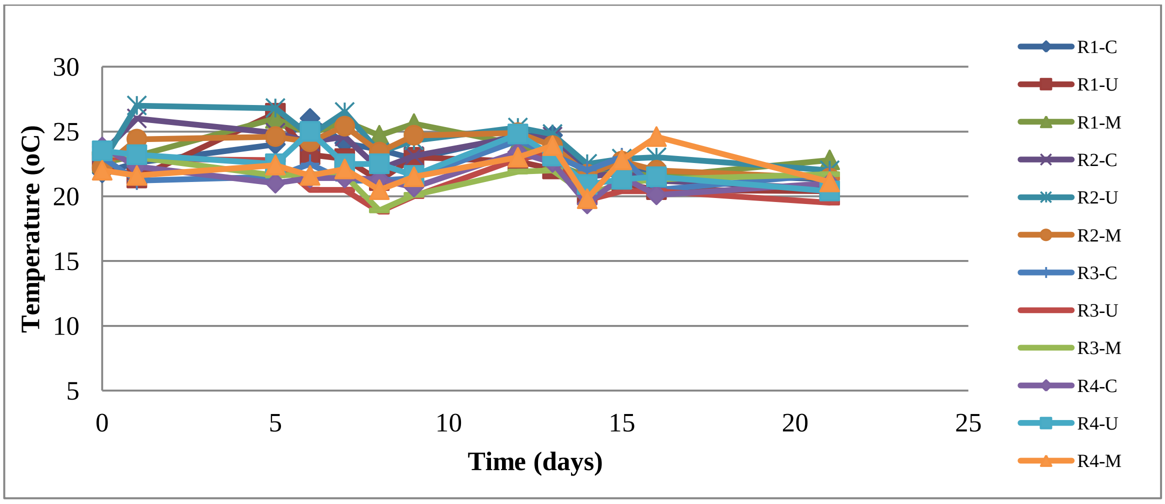


Figure 4.1 Temperature changes in the aerobic digestion reactors.

4.1.2. pH

pH is an important parameter for the stabilization process of a material. Generally, for aerobic stabilization, pH level ranges between the 5.5 and 8.5, which might be considered as neutral. Throughout this study, pH level of each reactor was measured on a daily basis and the results are given the **Figure 4.2**. It is seen that for the first few days, pH levels are more or less the same for every sample and disintegration method and varied from 7.0 to 8.5. However, as the stabilization progressed, some little changes have occurred. Although it did not prevent the biological process to take place, pH levels of R4 reactors decreased to pH level of 5.3, after Day 5. This may be caused by hydrolytic metabolism, and consequently volatile fatty acids production, or by nitrification reactions which take place during the stabilization (Oveido, 2003).

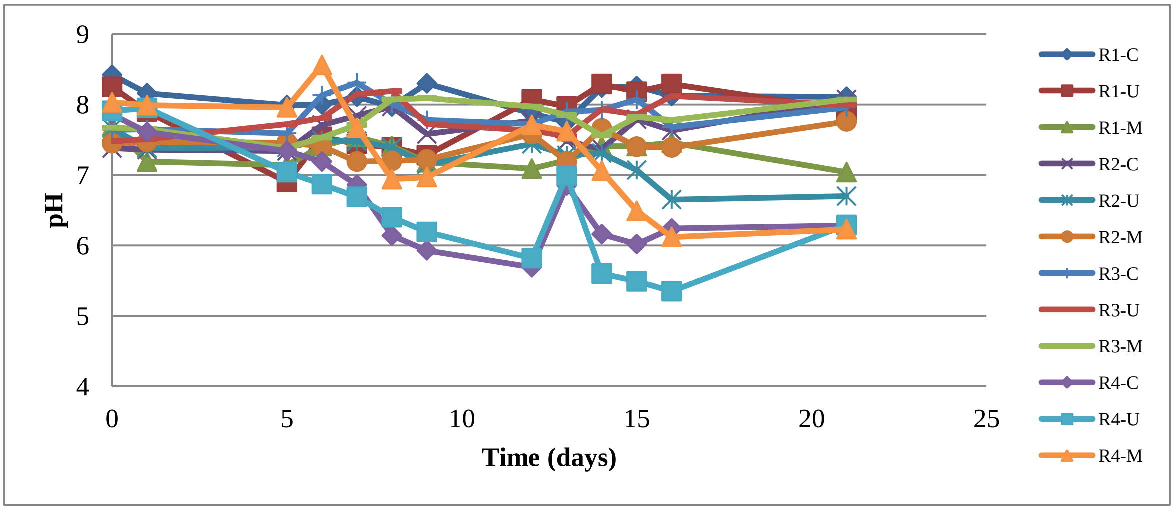


Figure 4.2 pH changes in the aerobic digestion reactors.

4.1.3. Alkalinity

Alkalinity is naturally found in water and acts as a buffer to the acids that are generated by microorganisms. If sufficient alkalinity is available, the pH remains within the desired range for aerobic bacteria to grow, in other words, digest the sludge. Although pH levels are easier to measure, alkalinity is a more reliable indicator for the acid production in the liquid medium. In Figure 4.3, alkalinity concentrations of the reactors are shown. Initial alkalinity concentrations ranged between 800 and 1200 mg $CaCO_3$/L whereas final concentrations were about 300 mg $CaCO_3$/L, except R3 reactors. Similar to pH levels, alkalinity concentrations of the R3 reactors were a little higher than the other reactors. The decrease of the alkalinity values after Day 15 may have been caused by nitrifying bacteria which convert ammonia nitrogen (NH_3) in the influent to nitrate (NO_3) in the aeration tank. During this conversion of ammonia to nitrate, the nitrifying bacteria generate acids. Nevertheless, alkalinity concentrations were sufficient throughout the operation for buffering the acid formation in the media.

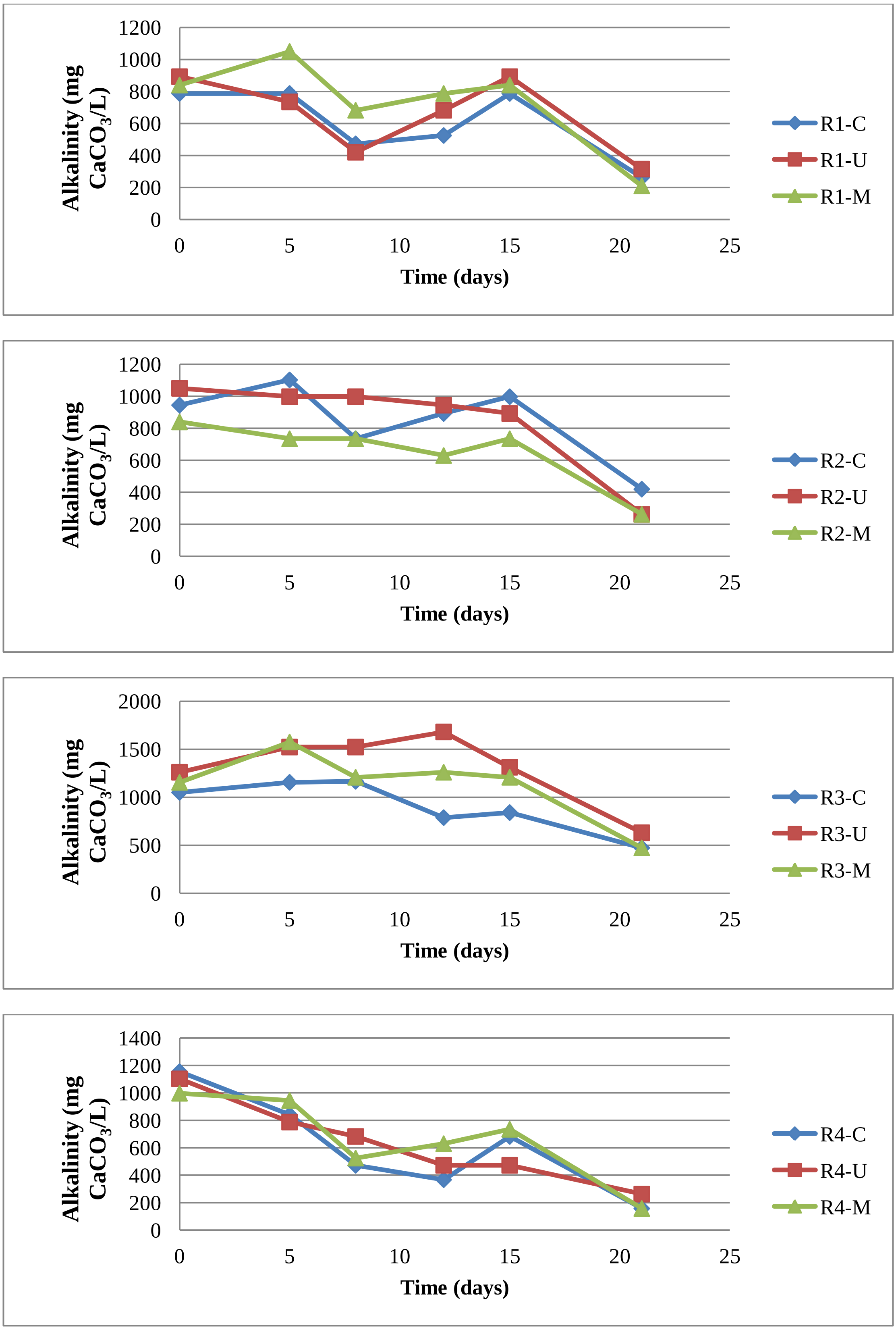


Figure 4.3 Alkalinity concentrations in the aerobic digestion reactors.

4.1.4. Dissolved Oxygen

In an aerobic digestion system, it is recommended that dissolved oxygen (DO) concentration should not be less than 1 mg/L (Tchobanoglous et al., 2003). During the aerobic stabilization, DO levels were monitored daily and DO concentrations of the reactors were kept above 2 mg/L by the proper adjustment of airflow into the reactors. Dissolved oxygen data are given in Figure 4.4. In the middle of the operation period, a decrease in DO levels was observed. It was assumed be caused by an overnight problem in the aeration device. The problem was solved by fixing the aeration device before the oxygen levels dropped to a degree that might damage the aerobic activity.

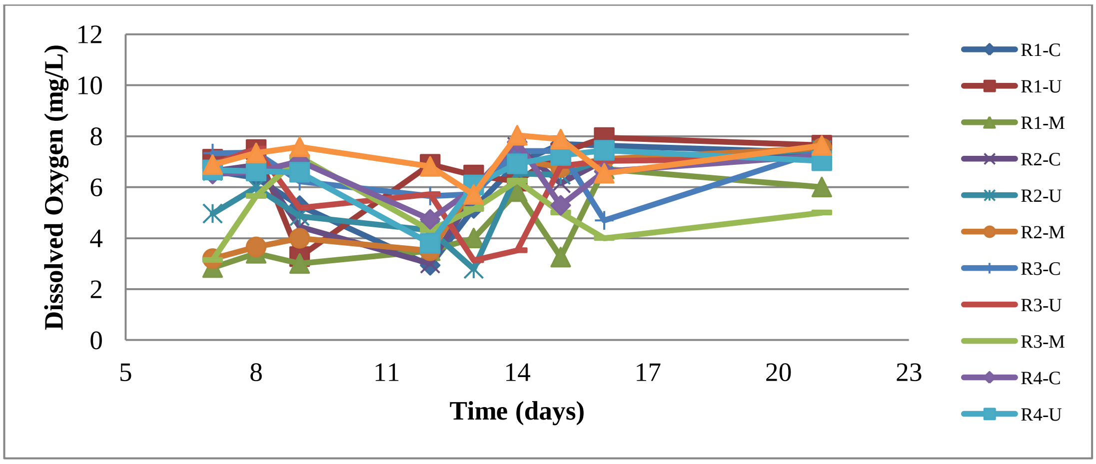


Figure 4.4 DO changes in the aerobic digestion reactors.

4.1.5. Oxidation Reduction Potential

Oxidation reduction potential (ORP) is an important factor for the activity of aerobic microorganisms since the term indicates the occurrence of biologic reactions in the presence of oxygen. ORP measurements were conducted daily throughout the process and the data are given in Figure 4.5. ORP values of the reactors in this study ranged between 40 mV to 200 mV. It is seen from the Figure 4.3 and Figure 4.4 that ORPs of the reactors showed parallelism with the dissolved oxygen values.

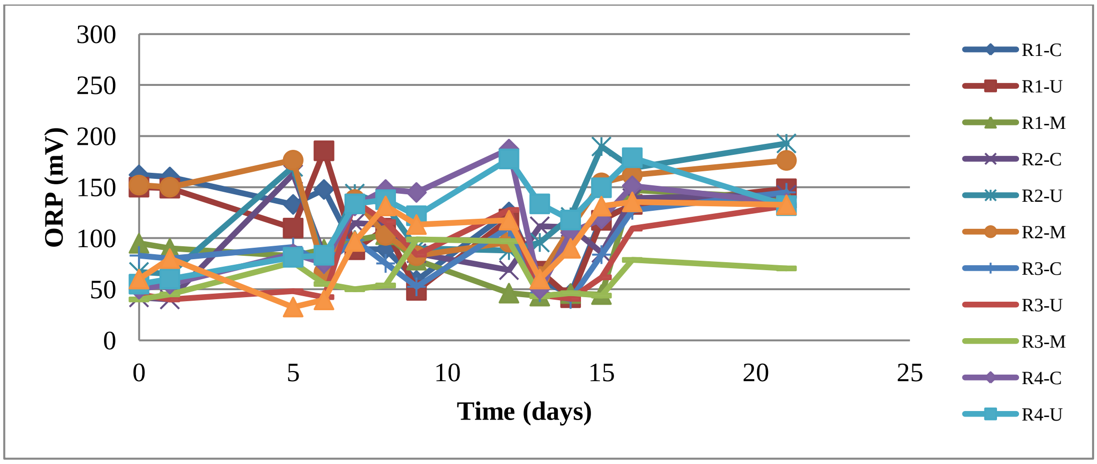


Figure 4.5 ORP changes in the aerobic digestion reactors.

4.1.6. Electrical Conductivity

Electrical conductivity is generally used as an indicator for the quantity of dissolved ions in the media. Besides, EC is also measured to determine the salinity concentrations in sludge. Therefore, a strong parallelism is expected between EC and salinity. The EC values in the control and pretreated reactors were measured throughout the aerobic digestion process. The EC in the reactors were almost constant. The results are given in Figure 4.6.

Electrical conductivity levels of the reactors range between 2 – 3.5 mS/cm, except R4 reactors. During the second half of the operation period, EC values of R4 reactors increased up to 5.5 mS/cm. It can be concluded from the results that this increase was not caused by the applied pretreatment methods but the sludge sample itself. Activities of nitrifying bacteria may be also the cause of this increase.

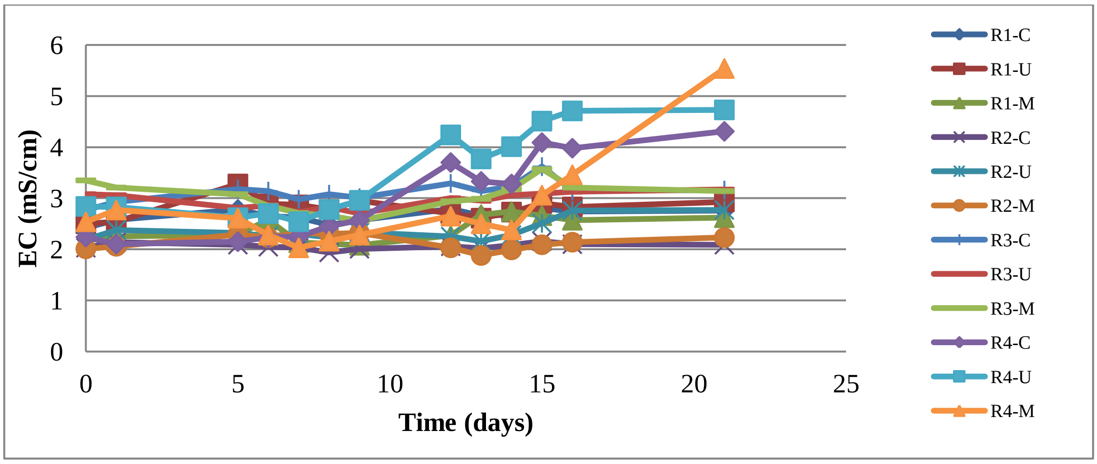


Figure 4.6 EC changes in the aerobic digestion reactors.

4.1.7. Salinity

Salinity levels of the reactors were analyzed daily and the data are given in Figure 4.7. Salinity values ranged between 0,8 – 1,7 ‰ for the reactors, except R4 reactors. Similar to EC values, salinity levels of those reactors increased after Day 10. Salinity measurements of R4 reactors were above all others, increasing up to 2.8 ‰ at the end of the operation time.

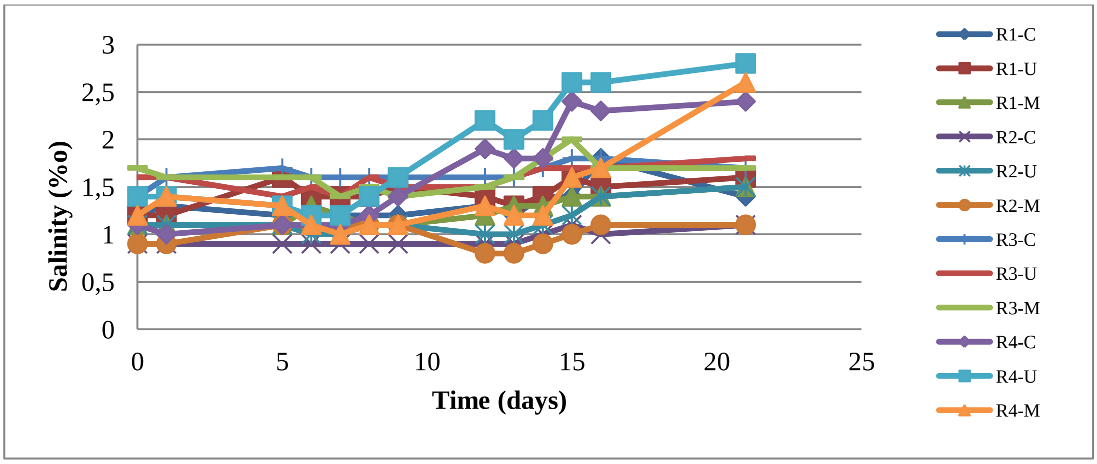


Figure 4.7 Salinity changes in the aerobic digestion reactors.

4.1.8. Total Solids and Volatile Solids

Total solids (TS) and volatile solids (VS) concentrations of the reactors analyzed every 3rd- 4th day of the operation, during the aerobic stabilization. TS removal means the mass reduction of the sludge at the end of the stabilization process and therefore these parameters are of vital importance for a successful stabilization of the sludge. **Figure 4.8** and Figure 4.10 show the results of these analyses. Besides that, overall TS and VS removal efficiencies of the aerobic stabilization are represented in Figure 4.9 and Figure 4.11, respectively.

TS contents of the four sludge samples were different at the beginning of the study since they were obtained from different WWTPs of different regions. In order to have a better observation of the effects of different disintegration methods on different types of sludge samples and to eliminate the possible influence of differing initial TS concentrations for the process, TS concentrations were adjusted to approximately 1% for the set-up of each reactor.

Changes in TS concentrations did not exhibit a very different course for control and disintegrated reactors throughout the study. However, final TS values of the pretreated samples were lower than the control reactors for each reactor sets.

In order to evaluate the enhancement of the pretreatment methods in TS removal, during the aerobic digestion, the increase rate of the TS removal efficiencies of the control reactors and the reactors containing the pretreated samples were calculated. According to that, ultrasonication enhanced the TS removal efficiencies by 64%, 35%, 19% and 25% for R1, R2, R3 and R4 reactors, respectively whereas the values for microwave pretreatment with the same order were; 76%, 68%, 54% and 26%. Here, it was observed that the rate of solids removal efficiency enhancement depends not only on the disintegration method but also the sludge type since the increase in the same reactor sets was similar for both pretreatments. However, when compared two different pretreatments, microwave showed a better performance for TS removal during aerobic digestion process.

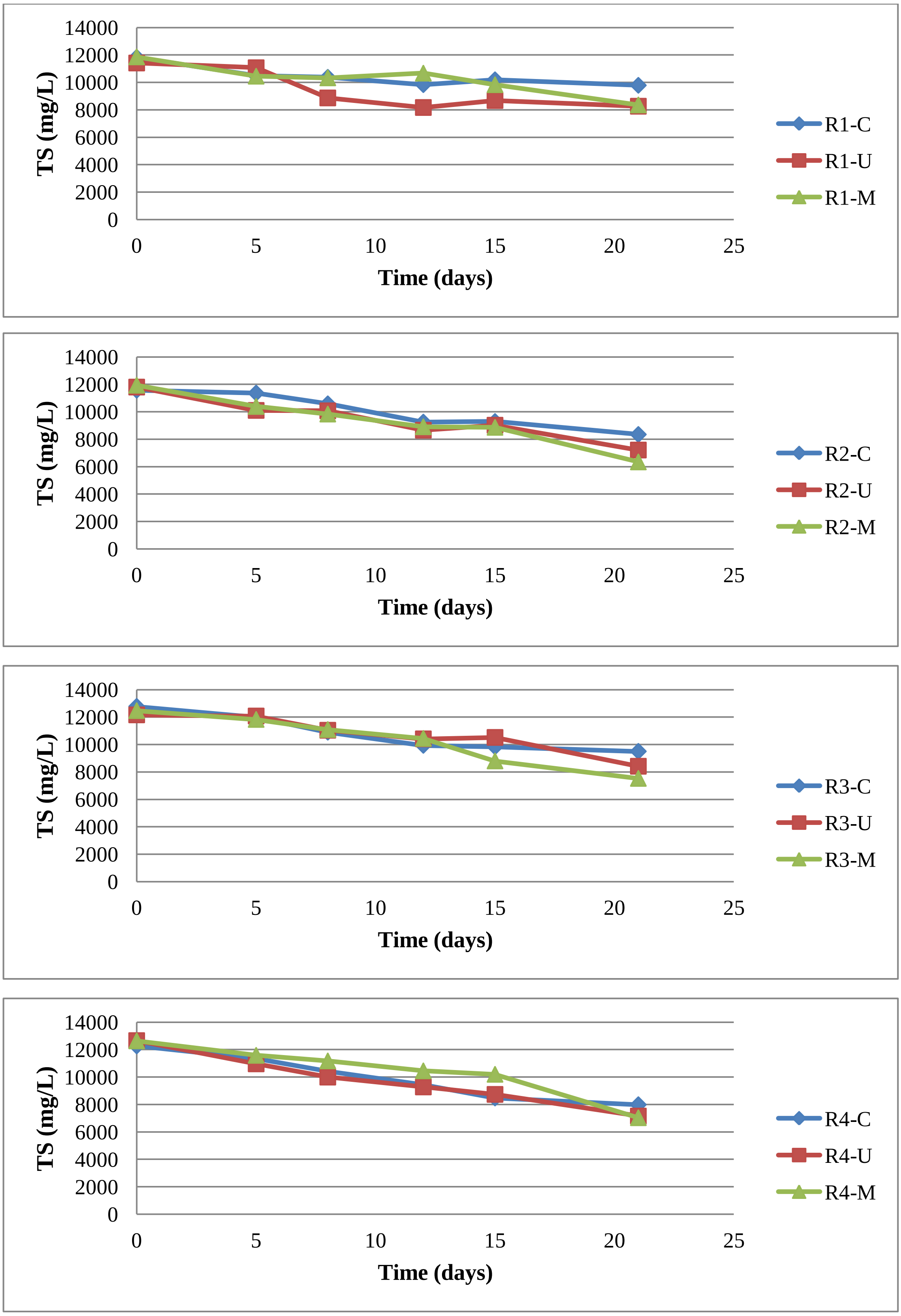


Figure 4.8 TS change in the aerobic digestion reactors.

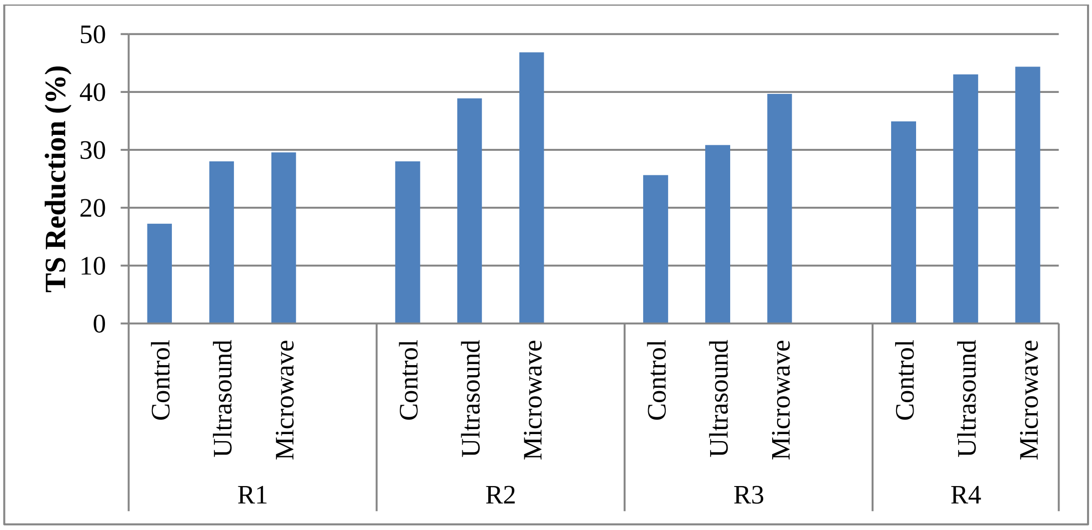


Figure 4.9 TS reduction efficiencies in the aerobic digestion reactors.

During aerobic stabilization, ultrasonication enhanced the total organic removal efficiencies by 43%, 46%, 17% and 23% for R1, R2, R3 and R4 reactors, respectively whereas the values for microwave pretreatment with the same order were; 43%, 85%, 46% and 19%. Here, it was observed that the rate of solids removal efficiency enhancement depends not only on the disintegration method but also the sludge type since the increase in the same reactor sets was similar for both pretreatments. However, when compared two different pretreatments, microwave showed a better performance for TS removal during aerobic digestion process.

As the values suggested, VS analyses showed a similar trend with TS values of the reactors except R4 set. In R4 reactors, TS removal efficiencies and therefore the TS removal efficiency enhancement of the two pretreatments were similar. On the other hand, ultrasonication resulted in a slightly better VS removal in R4 reactors. This implies that higher portion of the removed TS was organic in this set of reactors. This result came along with the fact that during aerobic stabilization process, TS/VS ratios of the sludge samples might change a little.

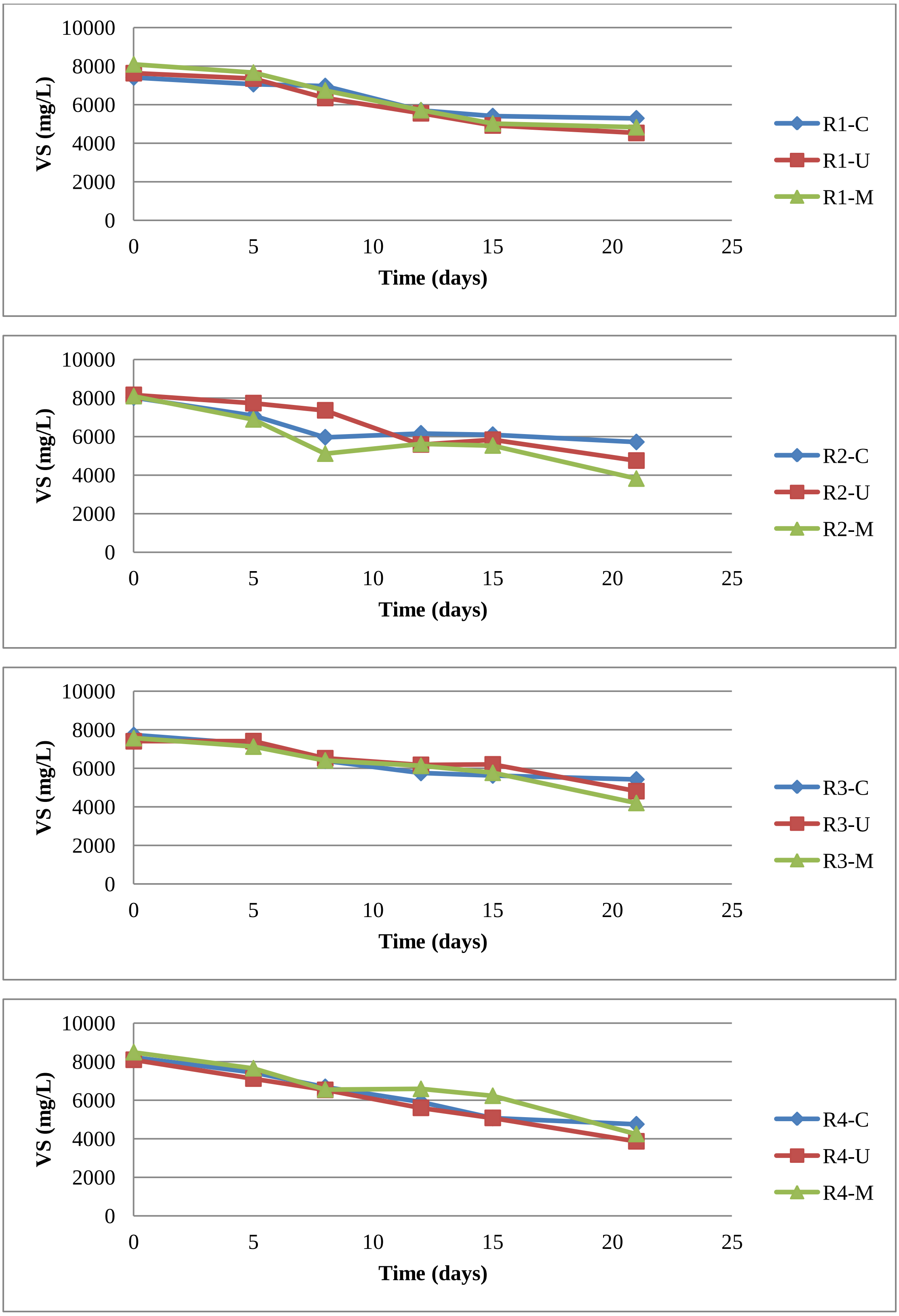


Figure 4.10 VS change in the aerobic digestion reactors

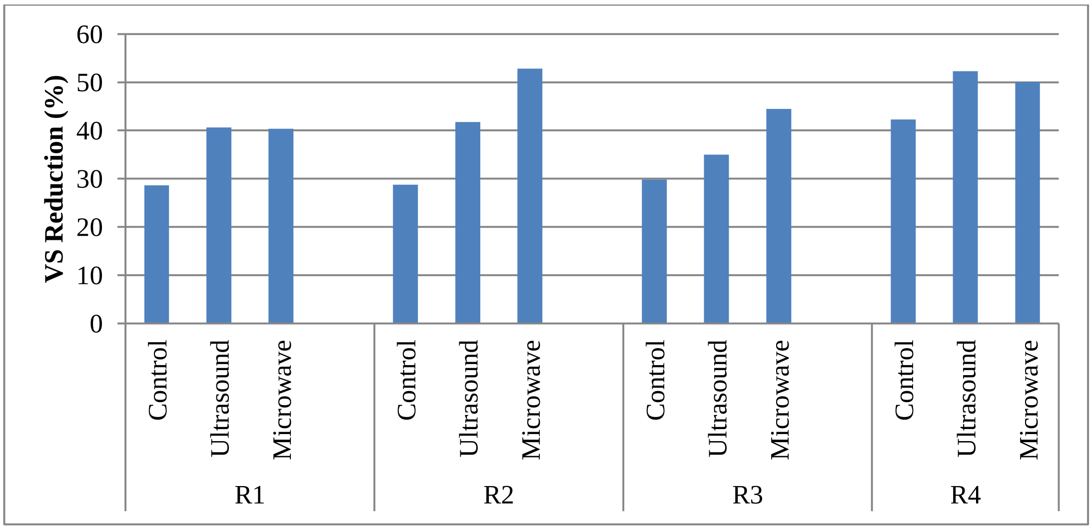


Figure 4.11 VS reduction efficiencies in the aerobic digestion reactors.

4.1.9. Mixed Liquor Suspended Solids, Mixed Liquor Volatile Suspended Solids

Mixed liquor suspended solids (MLSS) of a sludge sample consist of organic (mostly microorganisms), inorganic (non-biodegradable) and other inert suspended matters. It is the indirect measure of the total biomass and therefore very important factor during the operation of a biological reactor. MLSS analyses were conducted during the aerobic stabilization process and the results are given in Figure 4.12. The MLSS removal efficiencies are given in Figure 4.13.

Initial MLSS concentrations of the all reactors are around 10000 mg/L±1000. R4 reactors were have the highest MLSS removals same as TS and VS values in overall. Particularly R4-U and R4-M reactors seemed to digest nearly half of its biomass with 49% MLSS reduction. At the end of the aerobic digestion process, MLSS reductions in the control reactors were 27%, 22%, 28%, and 40% for R1-C, R2-C, R3-C, and R4-C, respectively. In the reactors containing the ultrasonicated samples, MLSS reductions were 29%, 40%, 34%, and 49% for R1-U, R2-U, R3-U, and R4-U respectively and in the reactors containing microwave applied samples, MLSS reductions were 34%, 41%, 44% and 49% for R1-M, R2-M, R3-M and R4-M, respectively. The increase in the rate of MLSS reduction efficiencies obtained by pretreatments was very similar to the TS parameter.

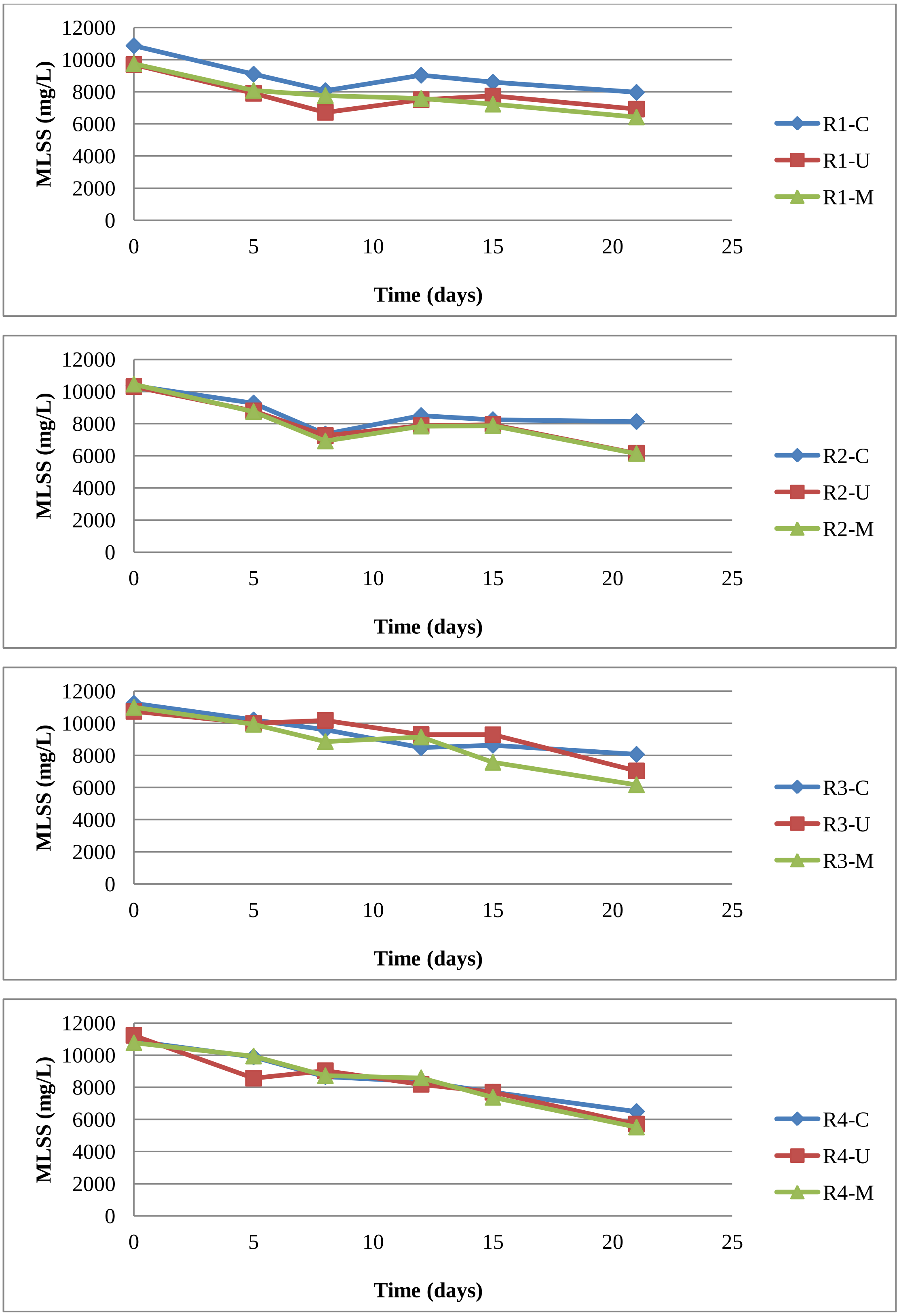


Figure 4.12 MLSS changes in the aerobic digestion reactors.

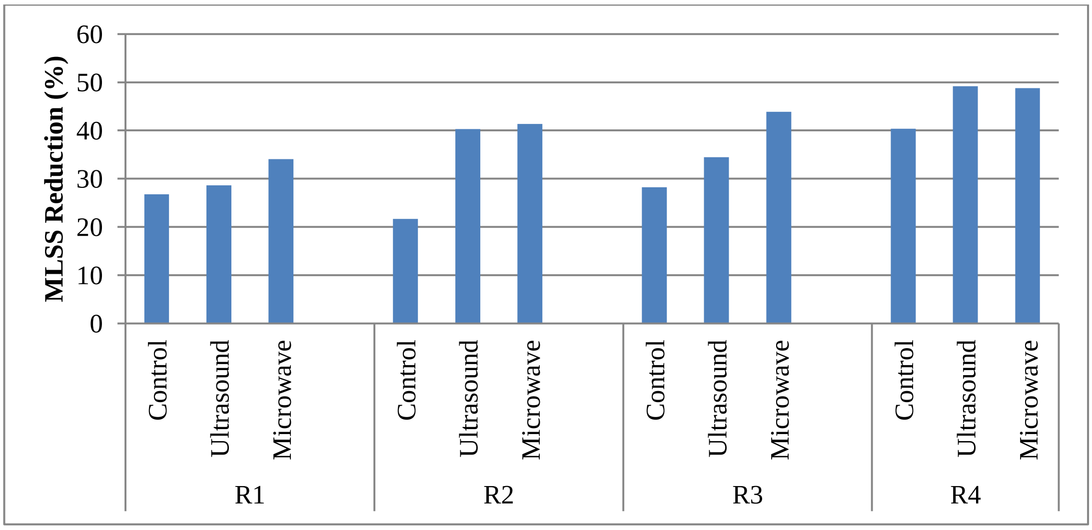


Figure 4.13 MLSS reductions in the aerobic digestion reactors.

Mixed Liquor Volatile Suspended Solids (MLVSS) is the portion of MLSS which consists mostly of microorganisms and organic matter. This volatile portion is used as the indicator of the active biomass that actually performs the digestion. The changes in the MLVSS concentrations during the aerobic digestion and MLVSS reduction efficiencies are given in Figure 4.14 and Figure 4.15, respectively.

A decrease was observed in all reactors of R2 set on Day8 of the aerobic digestion. It was believed to be an abnormal condition since it is unexpected for the the following MLVSS concentrations to be higher during a stabilization process. This might be caused by the flaw in providing the homogeneity of the reactor after adding the compensatory tap water. MLVSS reductions in the reactors showed very similar trends with MLSS reductions. In all reactor sets, pretreatment methods proved to have a positive effect on solids and organic removal.

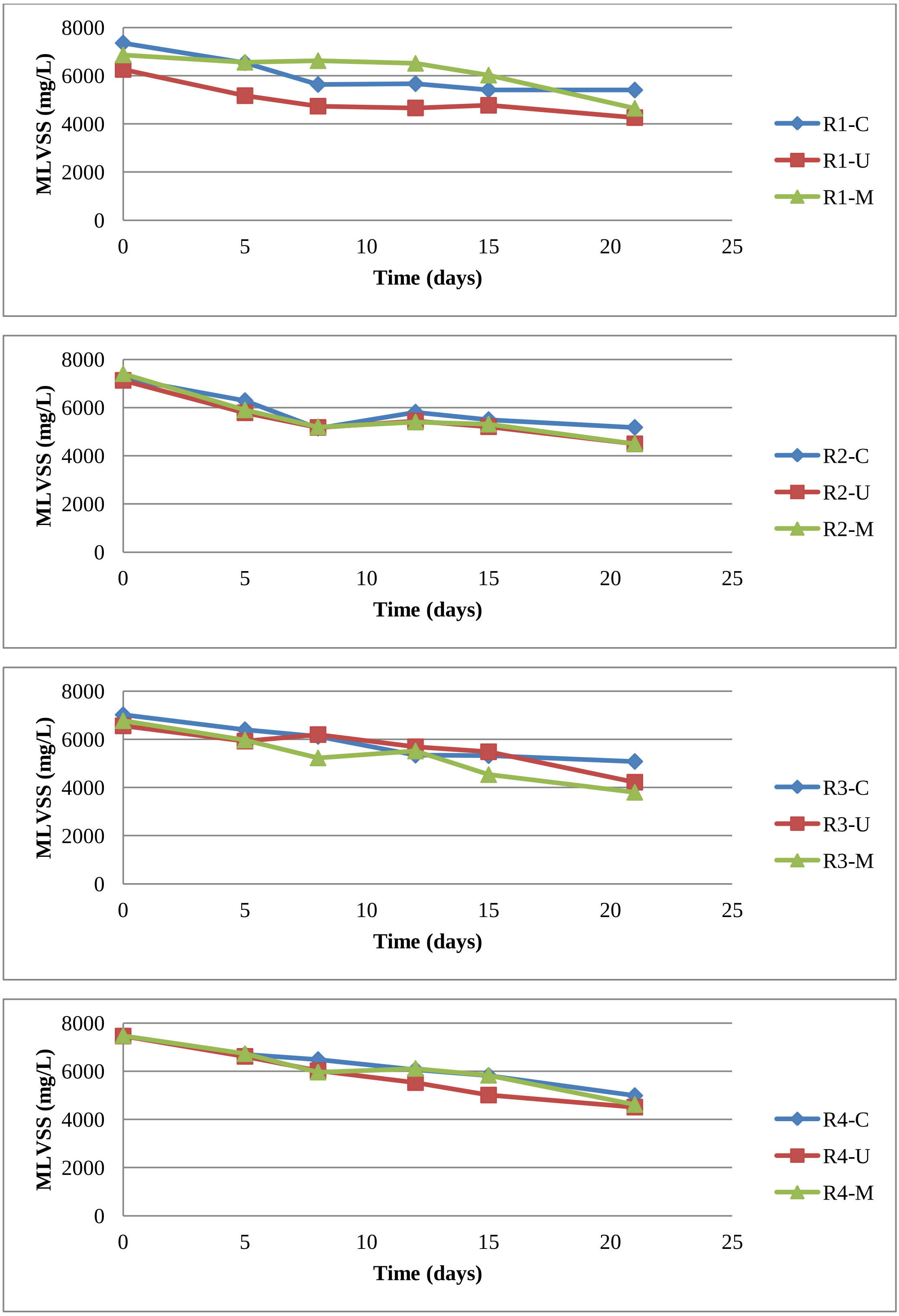


Figure 4.14 MLVSS change in the aerobic digestion reactors.

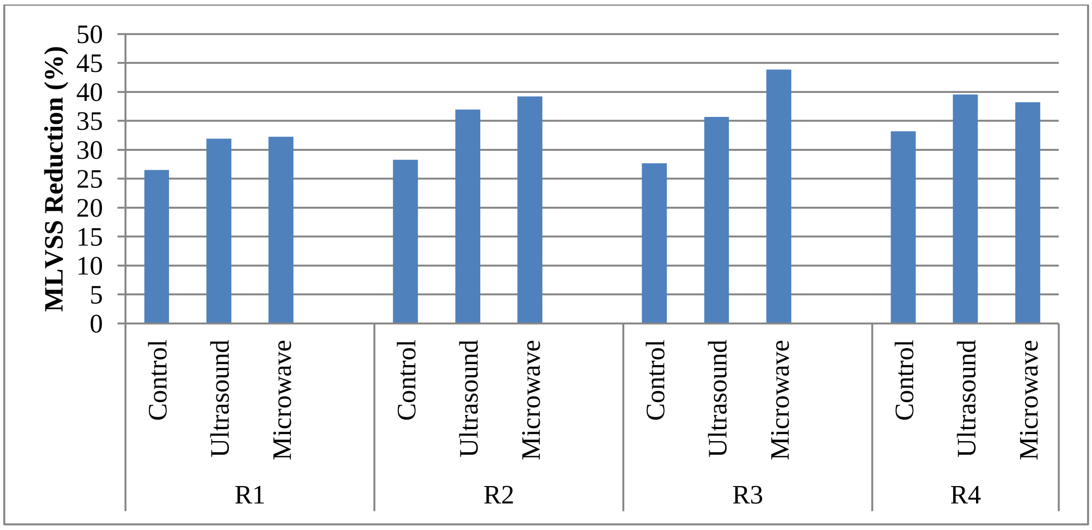


Figure 4.15 MLVSS reductions in the aerobic digestion reactors.

In a continuous aerobic stabilization system, it is important to keep the MLSS at a certain level which optimizes the food to microorganism ratio (F/M). By this ratio, loading and recycle rates are determined so that there is sufficient quantity of active biomass to digest the organic load coming from the wastewater. However, in a batch reactor system, like the one operated in this study, as well as organic portion, suspended material is also expected to be reduced. In the stabilization process, microbial community is left to starve and consume itself, which is called as digestion.

Variations in the ratio of MLVSS/MLSS indicate a change in amount of biomass share. Typically MLVSS/MLSS ratio should be within the range 0.65-0.85 for an efficient microbial stabilization. MLVSS/MLSS ratios of the control and pretreated reactors are shown in Figure 4.16.

MLVSS/MLSS ratio was almost constant throughout the process and there was no direct relation with a pretreatment method. In aerobic digestion reactors, the MLVSS/MLSS ratios ranged between 0.59 and 0.84. These results also showed that no significant changes in the amount of viable sludge were occurred during the stabilization process.

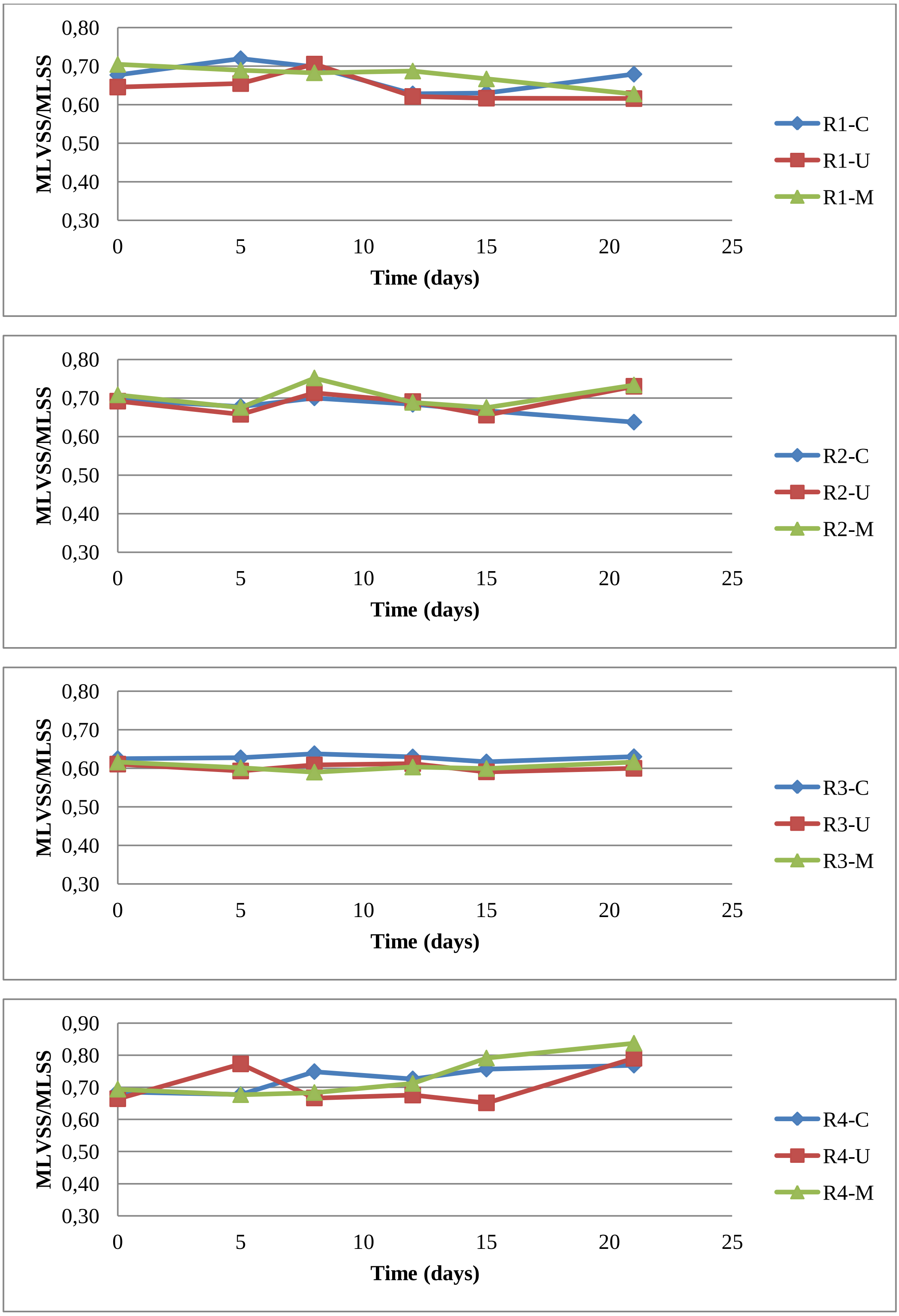


Figure 4.16 MLVSS/MLSS ratio changes during the aerobic digestion process.

4.1.10. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

By definition, chemical oxygen demand (COD) is a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. Because oxygen demand is not a pollutant, it poses no direct threat to any living organisms, it can, however, pose an indirect threat to living organisms by reducing the level of dissolved oxygen. Therefore, by stabilization process of the sludge, it is expected that the COD values of the sludge samples are reduced to some level. COD analyses were done twice a week and the results are shown in Figure 4.17.

At the beginning of the study, no certain distinctness in the COD concentrations for the control and pretreatment applied reactors were observed, since the total organic content of the sludge did not change much after the disintegration methods, but the form of the organics were expected to be differed.

As seen in the **Figure 4.17**, COD reduction was mostly completed in the first half of the operation. After Day12, only slight changes in COD concentrations were observed. At the end of the stabilization period, COD concentrations of the reactors were mostly between 4000 to 6000 mg/L, except the R2-C. The initial COD concentrations in R2 reactors were the highest of all and therefore, the final COD concentration in the control reactor of this set was slightly higher than others; 6256 mg/L. Particulate organic matter, which are highly resistant for biodegradation and oxidizable inorganic compounds, were mainly responsible for this remaining COD content of the sludge.

Overall COD reductions are shown in **Figure 4.18**. Similar to previous parameters explained, microwave pretreatment method proved to be the more efficient in the COD reduction. Considering the R1 and R2 reactors, microwave irradiation seemed to be successful with 47% and 32% increase in COD reduction efficiencies, respectively whereas the ultasonication enhanced the COD removal efficiencies by 29% and 16% for the same sludge type. On the other hand, when considered the R3 and R4 reactors, no remarkable increase in COD removal was observed after both pretreatment methods. The reason behind this is the higher solubilisation of COD achieved by pretreatments in R1 and R2 sets.

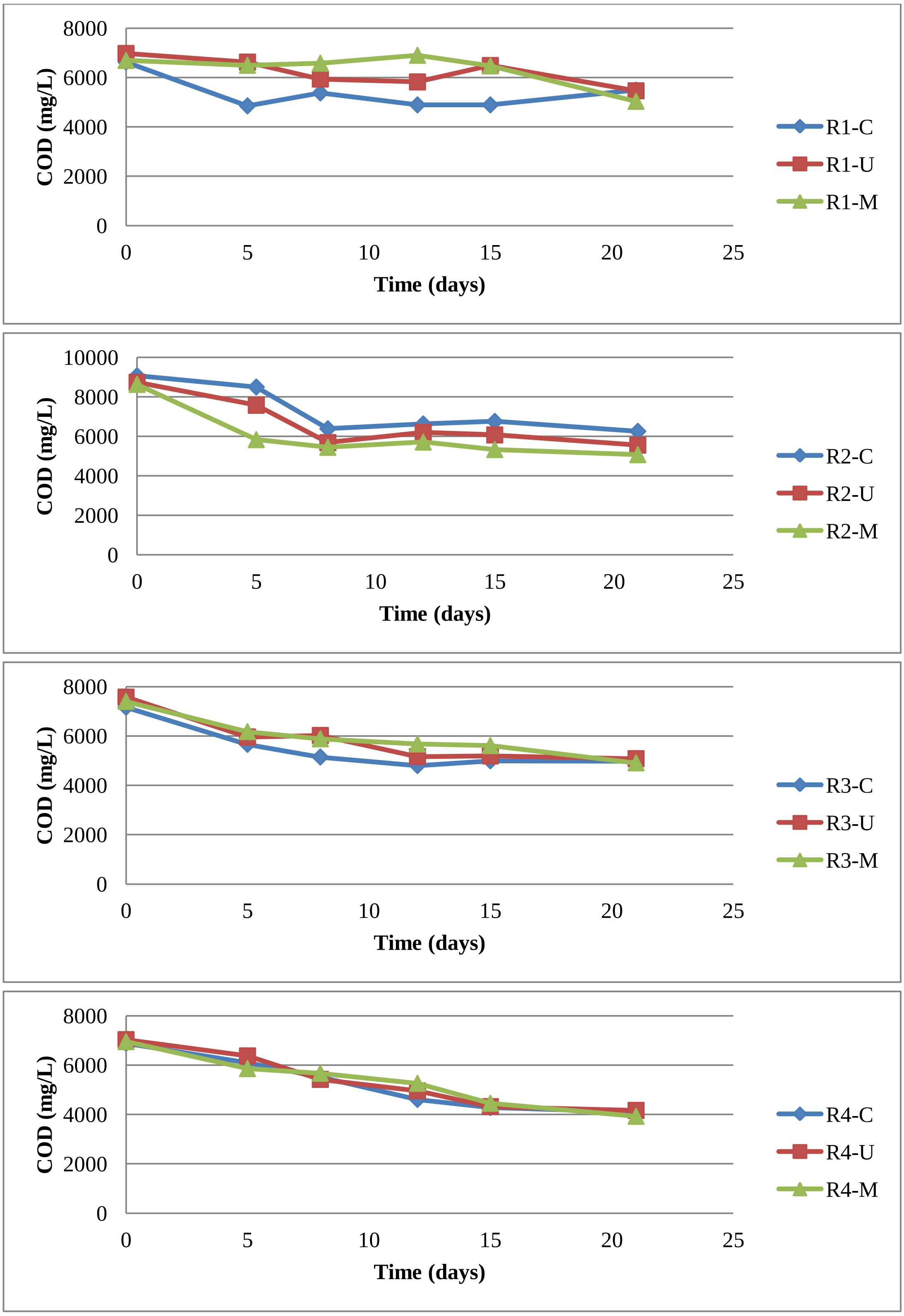


Figure 4.17 COD changes in the aerobic digestion reactors.

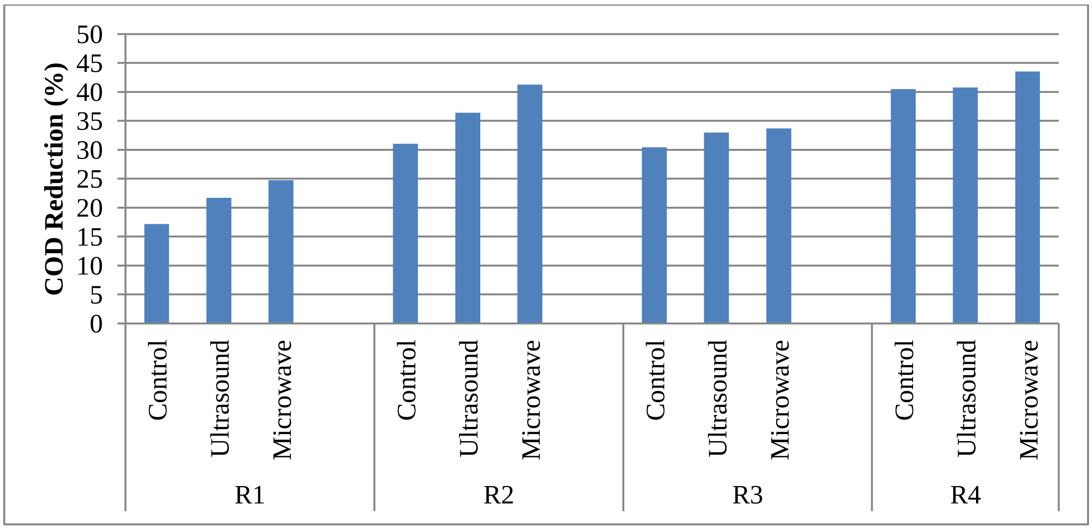


Figure 4.18 COD reductions in the aerobic digestion reactors.

Soluble COD is the part of the COD found in the supernatant which represents the organic matter in the non-particulate form. Any procedure which can cause cell disruption, and therefore lead to solubilization of the sludge, is expected to increase the sCOD values. sCOD changes in the reactors were monitored twice a week and the results are given in Figure 4.19. sCOD removal efficiencies are also shown in Figure 4.20.

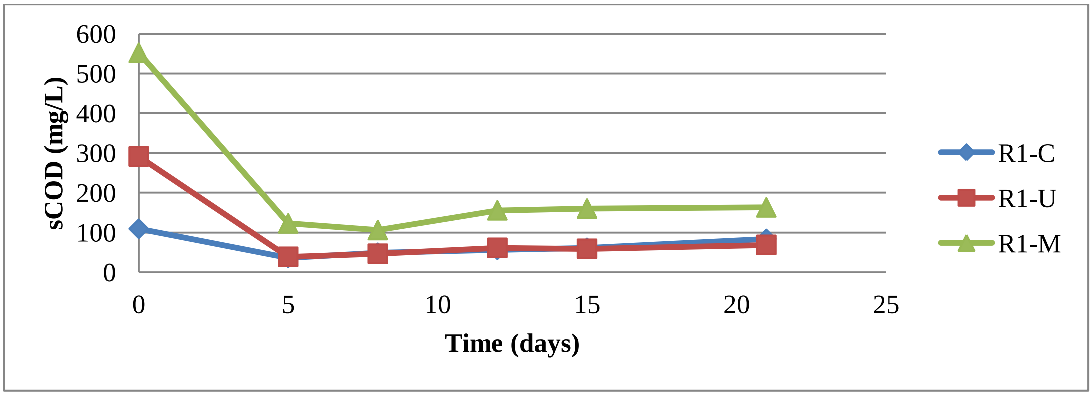
According to Bourgier et al., 2006, the term "solubilization" represents the portion of transferred particulate matter (of COD or solids) to the soluble portion of the sludge (supernatant after centrifugation).

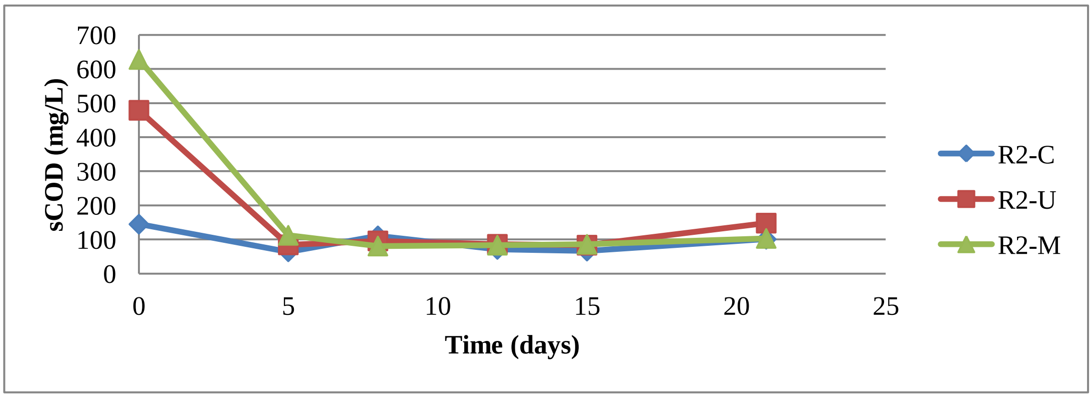
As seen in Figure 4.19, sCOD concentrations of pretreated reactors were increased significantly when compared to control reactors. This result showed that the cell disruption was achieved with both disintegration methods, for each reactor. The smallest difference between control and pretreated reactors occurred in R4 reactors, where sCOD concentration in the R4-C was also relatively high. This indicated that solubilization with disintegration also has it limits; cell lysis of the sludge can take place up to a specific degree.

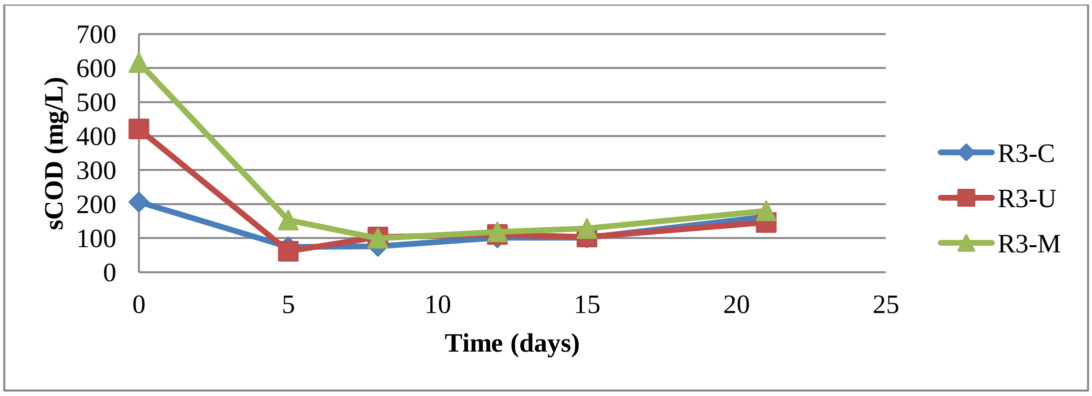
In all reactors, microwave irradiation led to a higher solubilization. The solubilization rates of the COD, obtained by microwave irradiation in R1, R2, R3 and R4 reactors were 406%, 333%, 199% and 37%, respectively. Solubilization rates achieved by ultrasonication pretreatment in the reactor sets with the same order were 166%, 230%, 104% and 15%. The relatively lower solubilizations in R4 set was also reflected in COD removals as described above. Bourgier et al., 2006 also compared three disintegration methods (ultrasonication, ozonation and thermal treatment) and stated that "Solubilization is much more higher with thermal treatment than with sonication or ozonation".

Increased soluble portion of the sludge samples was removed within the earlier days of the operation. After Day5, sCOD concentrations were almost the same. In an aerobic system, it is expected for readily biodegradable soluble organics to be removed in a rather short period.

In Figure 4.20, sCOD reduction efficiencies are given. Since the final sCOD values of the reactors were in the same range for each reactor set, there was a significant difference between the sCOD removal efficiencies in control reactors and pretreated samples.







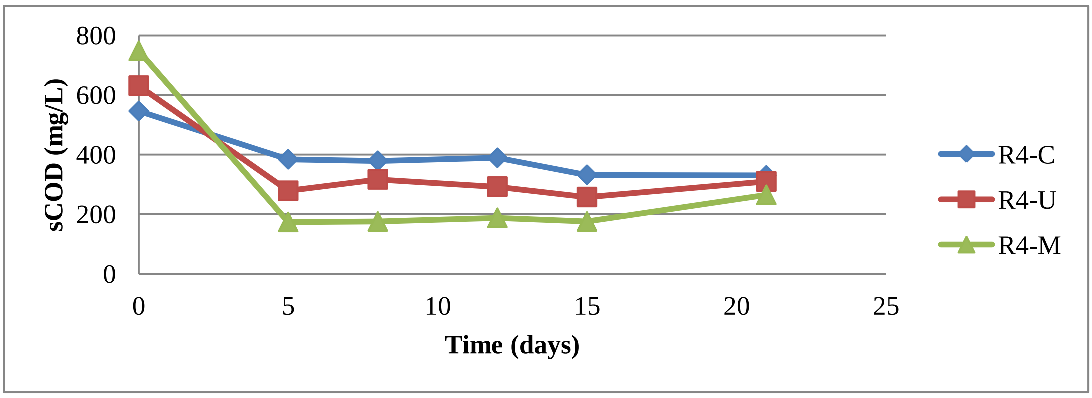


Figure 4.19 sCOD changes in the aerobic digestion reactors.

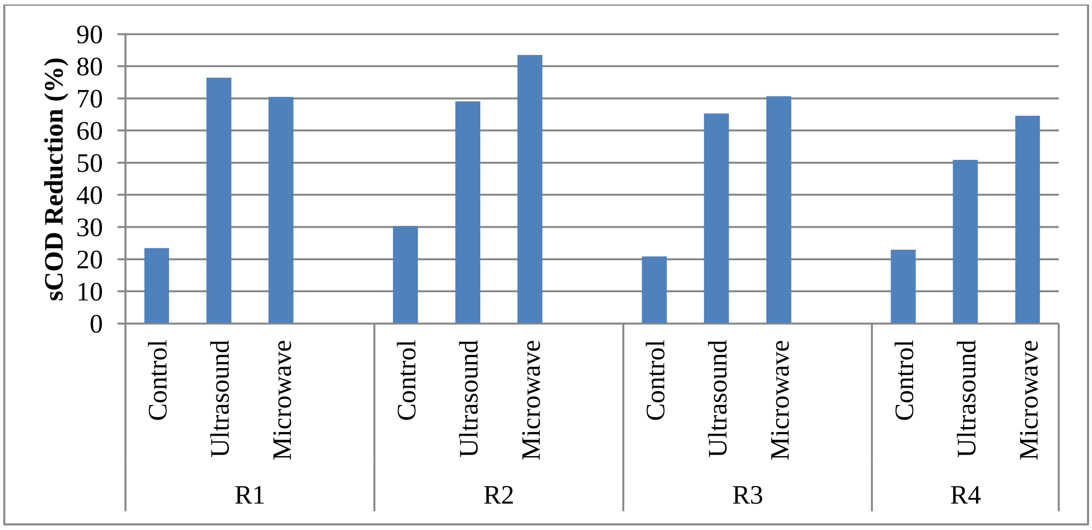


Figure 4.20 sCOD reductions in the aerobic digestion reactors.

4.1.11. Total Organic Carbon and Dissolved Organic Carbon

Total organic carbon (TOC) measurement principally gives the amount of organic compound in a water sample. TOC analyses were conducted once a week throughout the operation and the results are shown in Figure 4.21. TOC reduction efficiencies are also shown in Figure 4.22.

At the beginning of the operation TOC concentrations were almost the same for each reactor set (between 425 to 675 mg/L TOC). The higher TOC concentrations were observed in the R4 reactors (higher than 1000 mg/L TOC), unlike COD values. Since COD measurement indicates all organic content of the sample while TOC only gives the carbon content, we can conclude that high organic carbon does not necessarily mean a high COD value for a sample.

As seen in Figure 4.21, TOC content of the reactors continuously decreased during the aerobic digestion. However, an exceptional situation occurred in R3-C and R3-U after the second week. A slight increase in the TOC concentrations was observed in those reactors. This was possibly resulted from the dissolved carbon dioxide and carbonic acid salts present in the reactors since it is unlikely for organic carbon to increase during a stabilization process. This interference also affected the TOC reduction efficiency of the R3-U reactor. TOC removal seemed to be enhanced in all other pretreatment applied reactors, as seen in Figure 4.22. The highest TOC removal efficiencies were attained in R1 reactors (67%, 69% and 77% reduction efficiencies for control, sonication and microwave reactors, respectively.) while the lowest efficiencies were observed in R4 reactors (44%, 53% and 64% reduction efficiencies for control, sonication and microwave reactors, respectively). All in all, microwave irradiation seemed to be the most advantageous disintegration method for organic carbon removal.

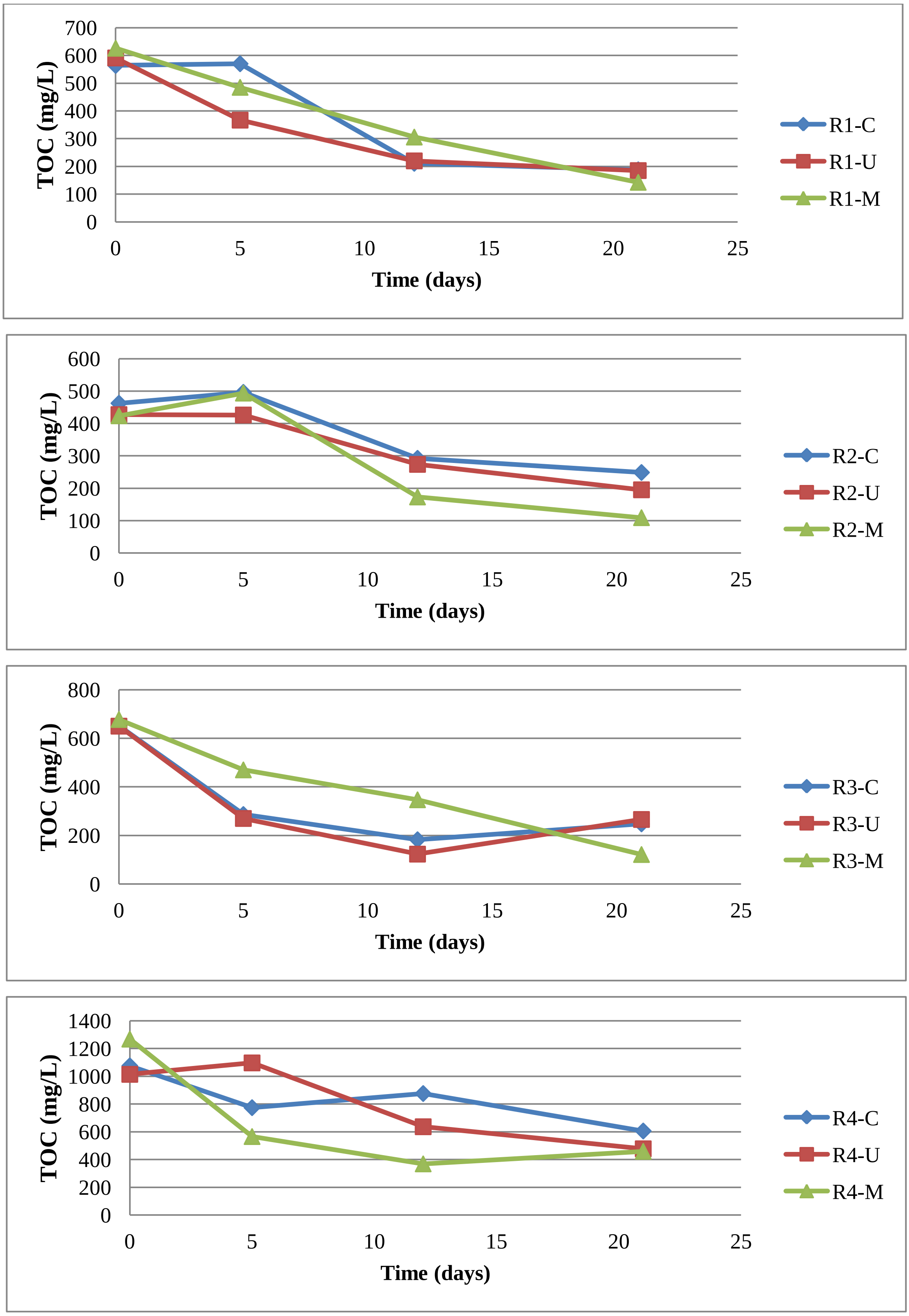


Figure 4.21 TOC changes in the aerobic digestion reactors.

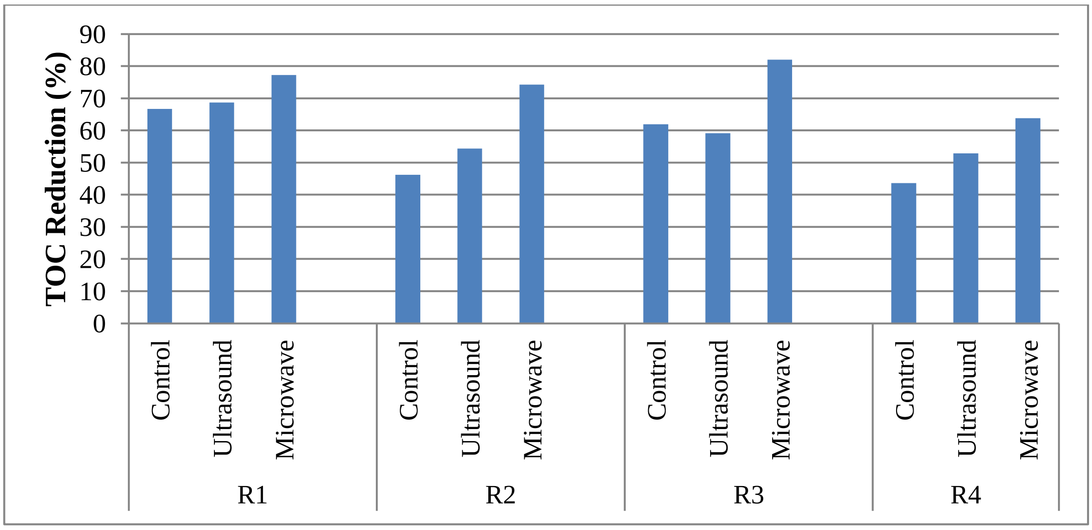


Figure 4.22 TOC reductions in the aerobic digestion reactors.

Dissolved organic carbon (DOC) value is the amount of organic carbon present in the supernatant of a sample after centrifugation. DOC analyses were conducted once a week and the results are shown in

Figure 4.23. DOC contents in the reactors showed parallelism with TOC concentrations and ranged between 14 mg/l and 30 mg/L at the beginning of the aerobic digestion process.

Since DOC values were in small amounts when compared to previous parameters explained above, a stable trend does not seem to be followed throughout the process. However, it is certain that DOC values of the supernatants increased as the aerobic digestion took place. This was also the case in Oveido's studies. The situation explained with the release into the medium of the organic material contained in the cellular protoplasm resulting from the death of the higher microorganisms (Oveido, 2003; Tas, 2010).

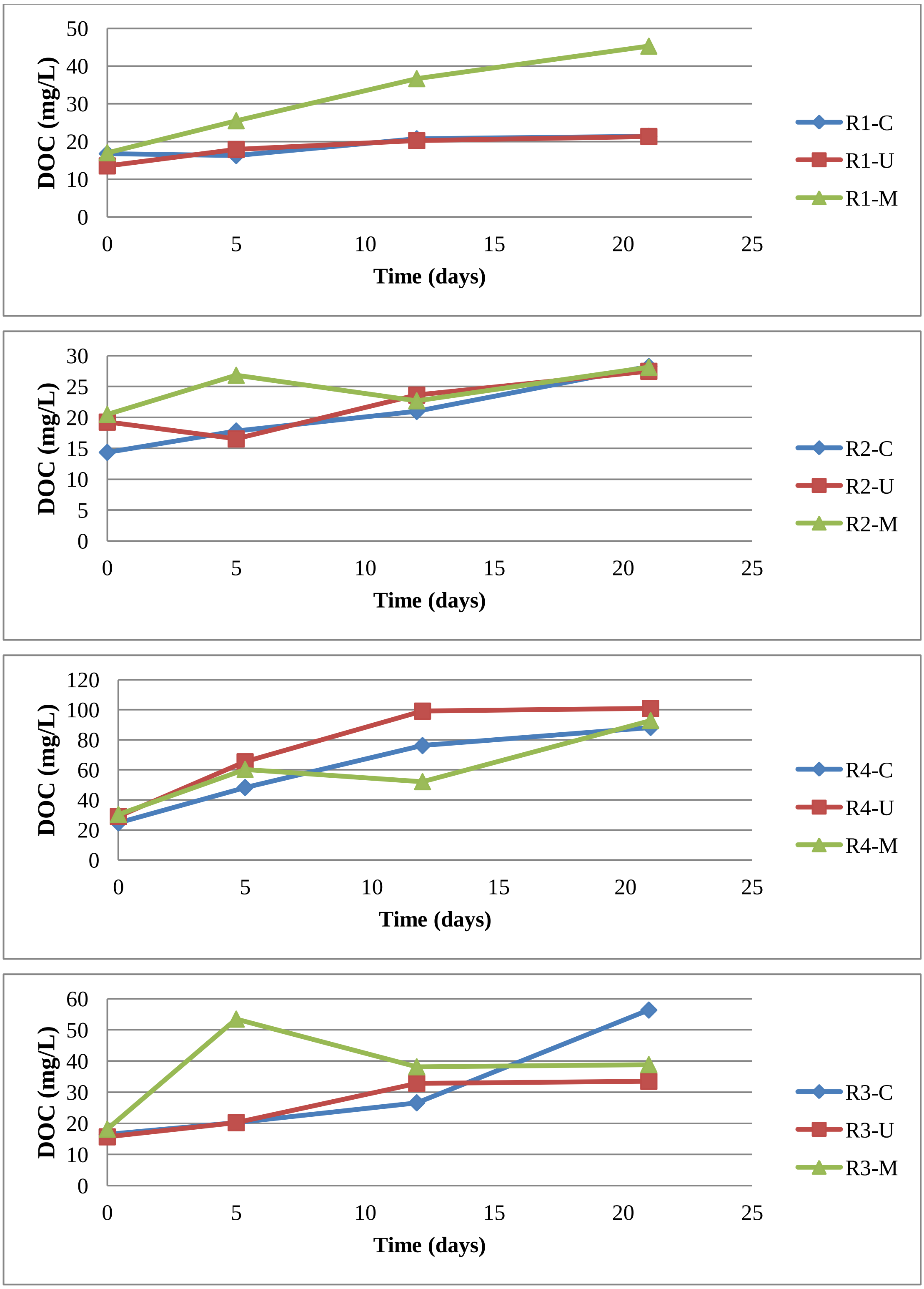


Figure 4.23 DOC changes in the aerobic digestion reactors.

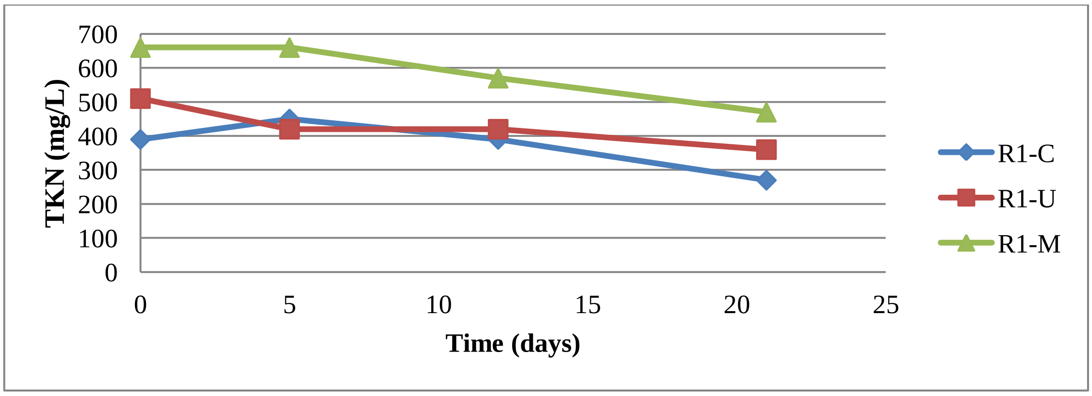
4.1.12. Total Kjeldahl Nitgoren, Nitrite, Nitrate and Ammonium

Total Kjeldahl nitrogen (TKN) consists of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in a sample. TKN measurements were done every week and the results are shown in Figure 4.24.

In an aerobic stabilization process, nitrification ought to take place with the presence of some specific microorganisms. Nitrification occurs in two steps:

1. $NH_3 \rightarrow NO_2$ (by *Nitrosomonas*)
2. $NO_2 \rightarrow NO_3$ (by *Nitrobacter*)

Therefore, during the aerobic digestion process, TKN values are expected to decrease by the nitrification process as it is the case also in this study. At the beginning of the stabilization, TKN concentrations of the reactors ranged between 400 mg/L and 800 mg/L TKN values show that in all reactors, some degree of organic nitrogen removal and nitrification process took place, independent of the pretreatment application. When considered the initial and final concentrations, the TKN removal efficiencies are within the range of 30-60%.



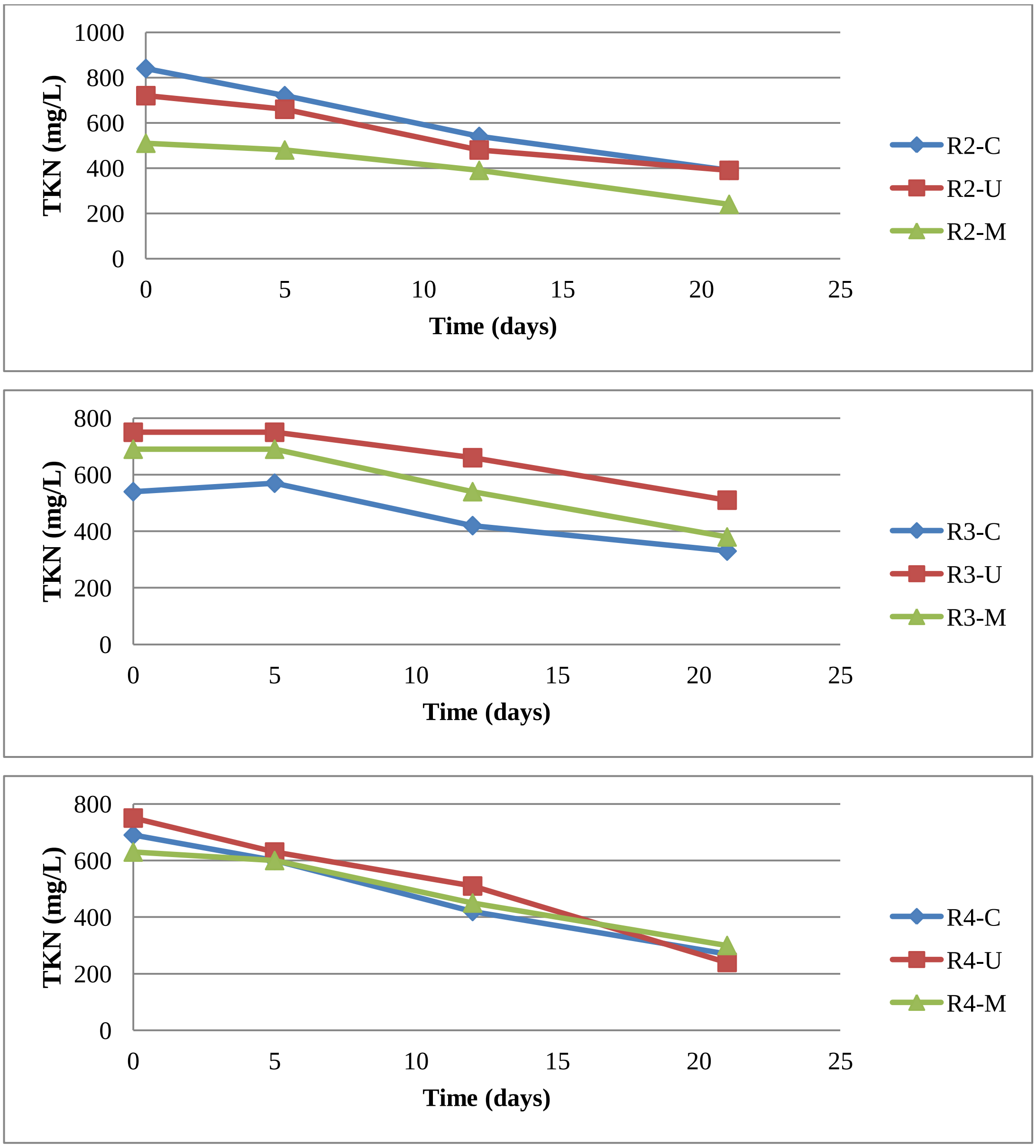


Figure 4.24 TKN change in aerobic digestion reactors.

Initial and final nitrite, nitrate and ammonium concentrations are shown in Table 4.15. As seen in the table, ammonium and nitrite concentrations decreased where the nitrate concentrations increased as a result of nitrification process.

Before the digestion process, nitrite concentrations in the reactors were lower than 20 mg/L. Furthermore, no nitrite content was measured for R2-C and R2-U reactors. That was probably a result of sampling, since the values were very low. However, after the stabilization, nitrite concentrations of all reactors were decreased to 8 mg/L at maximum. In all microwave disintegration reactors, nitrite seemed to be converted to nitrate completely. This was also confirmed by the increase of nitrate concentraions with a few exceptions, which also may have been caused by some experimental errors of measurement or sampling.

Table 4.15 Nitrite, nitrate and ammonium change in aerobic digestion reactors.

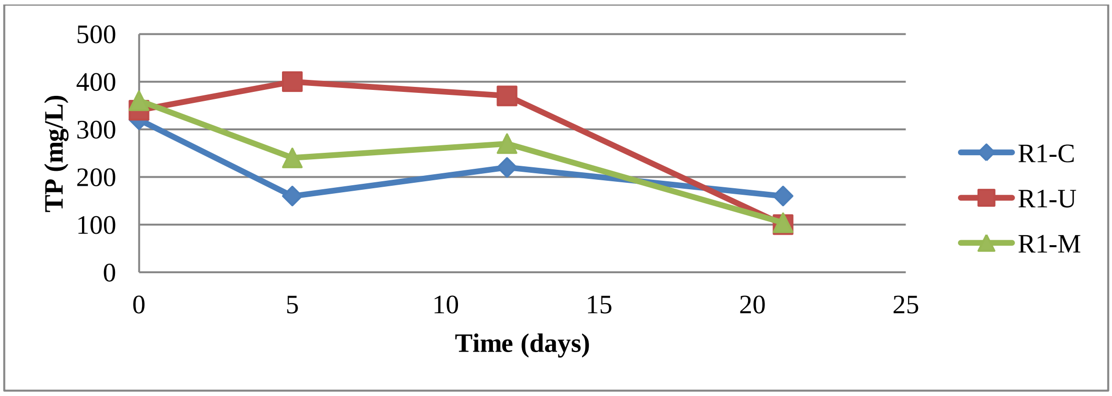
Day	İzmit Kullar WWTP (R1)			İstanbul Bahçeşehir WWTP (R2)		
	Control	Ultrasound	Microwave	Control	Ultrasound	Microwave
			NO_2^- (mg/L)			
0	10	16	27	0	0	17
21	4	2	0	0	0	0
			NO_3^- (mg/L)			
0	14.3	26.2	6.6	2.9	3.7	6.2
21	17.9	26.1	16.9	4.2	79.8	12.3
			NH_4^+-N (mg/L)			
0	7	19.5	16.7	19.8	30.1	21.3
21	2.7	8	2.5	13.3	8.5	0.3
	Samsun Bafra WWTP (R3)			**Düzce Akçakoca WWTP (R4)**		
Day	**Control**	**Ultrasound**	**Microwave**	**Control**	**Ultrasound**	**Microwave**
			NO_2^- (mg/L)			
0	11	3	21	13	2	3
21	2	1	0	8	0	0
			NO_3^- (mg/L)			
0	33	7.1	7.5	0.1	4.6	10.1
21	9.3	2.3	2.5	81.5	125.4	87.4
			NH_4^+-N (mg/L)			
0	11.3	24.6	41.6	61.7	105.2	101.5
21	4.1	5.8	5.2	36.8	108	123.5

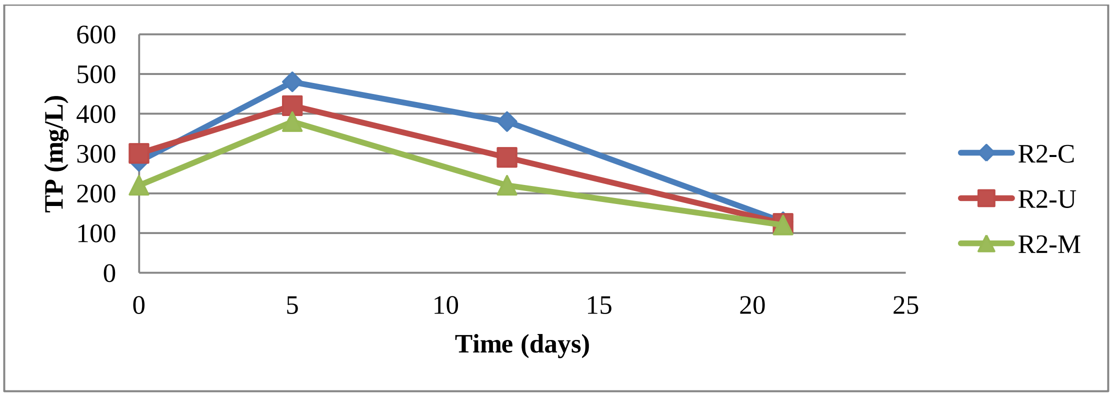
4.1.13. Total Phosphorus

Phosphorus is an important nutrient to be removed since it is the growth limiting factor for most of the microorganisms which results in eutrification problem in the water bodies. Total phosphorus (TP) analyese were conducted once in a week and the results are shown in Figure 4.25.

In the first week of the operation, an increase in the TP concentrations was observed. That was mainly due to the release of phosphprus content of the sludge cells, which hydrolyzed during the digestion. R1-C and R1-M reactors were the exceptions of this case. A rapid hydrolysis may have been taken place in R1-M reactor and the release of phosphorus may have been occurred in first few days and already started to decrase on Day5 since the final TP content was the lowest. For R1-C reactor, however, it was not expected for control reactor to hydrolyse that fast; therefore, it can be interpreted that hydrolysis in R1-C initiated later than other reactors.

Final TP concentrations were close to each other in all reactor sets. Still, TP contents of the pretreated reactors seemed to be higher. However, the method of pretreatment differed for different sludge types. For R1 set, TP removal efficiencies of R1-U and R1-M were equal and 71%. R2-U reactor had the best TP removal efficiency, which is 59%. Ultrasonication reactor also had the highest removal efficiency (45%) in R4 set. On the contrary, R3-M reactor had a higher removal of TP than ultrasonication. Its removal efficiency was 62%.





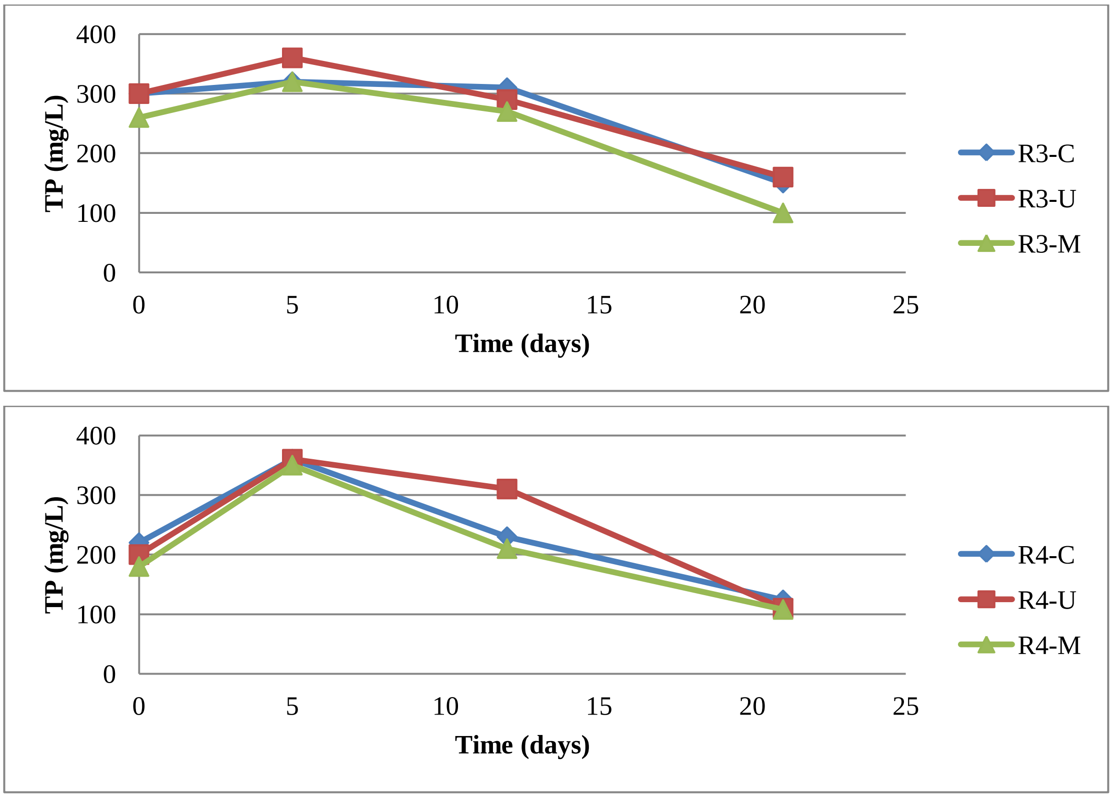


Figure 4.25 TP change in aerobic digestion reactors.

4.1.14. Sulfate and Chloride

Sulfate and chloride analyses were conducted before and after the digestion of the reactors and the results are given in Table 4.16.

On general, chloride concentrations decreased with pretreatment method at the beginning of the operation. After the stabilization, all chloride values increased. This also explains the increase in the electrical conductivity and salinity values after the first week of the digestion. One of the reasons of this increase may be due to the tap water addition to the reactors to compensate the evaporated water during the operation. Though, chloride concentrations were in the optimum range and therefore, an inhibitory effect did not occur.

When sulfate concentrations of the reactors are examined, it is hard to establish a statement since no obvious trend was observed for this data. For R1 and R3 reactor sets, microwave irradiation caused a decrease in the sulfate concentration of the sample, while in R2 and R4 sets, sulfate concentrations were slightly changed by disintegration.

Moreover, neither of the reactors seemed to be affected in a certain way by the stabilization in terms of the sulfate concentrations. Although there was an increase in the sulfate contents of each reactor of the R3 and R4 sets, this was not confirmed by R1 and R2 sets.

Table 4.16 Sulfate and Chloride change in aerobic digestion reactors.

	İzmit Kullar WWTP (R1)			**İstanbul Bahçeşehir WWTP (R2)**		
Day	**Control**	**Ultrasound**	**Microwave**	**Control**	**Ultrasound**	**Microwave**
			Chloride (mg/L)			
0	104	138	69	104	69	35
21	138	173	119	169	207	138
			SO_4^{2-} (mg/L)			
0	225	255	45	145	185	160
21	205	280	35	105	112	175
	Samsun Bafra WWTP (R3)			**Düzce Akçakoca WWTP (R4)**		
Day	**Control**	**Ultrasound**	**Microwave**	**Control**	**Ultrasound**	**Microwave**
			Chloride (mg/L)			
0	173	173	138	69	69	69
21	207	242	277	173	113	138
			SO_4^{2-} (mg/L)			
0	235	175	150	190	220	190
21	255	315	225	270	320	220

4.1.15. Capillary Suction Time

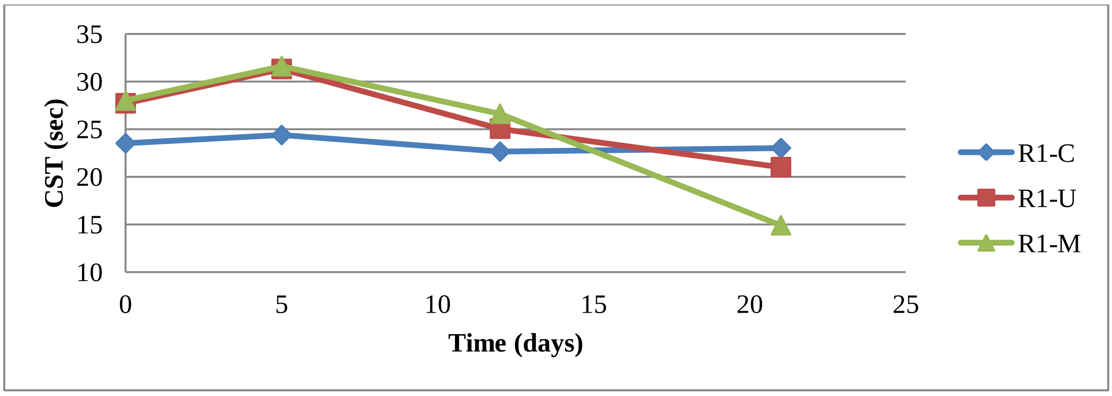
Capillary suction time (CST) is a measure of filterability of the sludge, which is also an indicator for sludge dewaterability. Filterability is a physical characteristic of the sludge and it strongly dependent on the type of the treatment and also the type of wastewater. CST measurements were conducted once a week to observe the effects of the disintegration and the stabilization itself on the filterability of the sludge and the results are given in Figure 4.26.

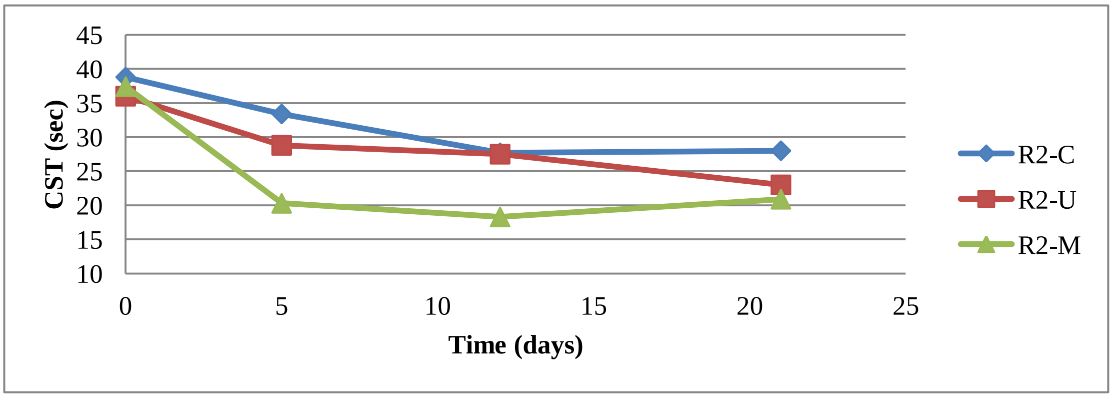
Since all reactor sets represent the sludge samples of a different WWTP, CST values were varying. For instance, CST values in the R1, R2 and R3 sets were similar and ranged between 23 and 38 seconds; however, in R4 set (which is Düzce Akçakoca WWTP) much higher CST values were observed, beyond 300 seconds. Except R2 reactors, it seems that both disintegration methods worsened the filterability of the sludge. According to Bourgier et al., disintegration, by decreasing particle size, led to the damage of filterability

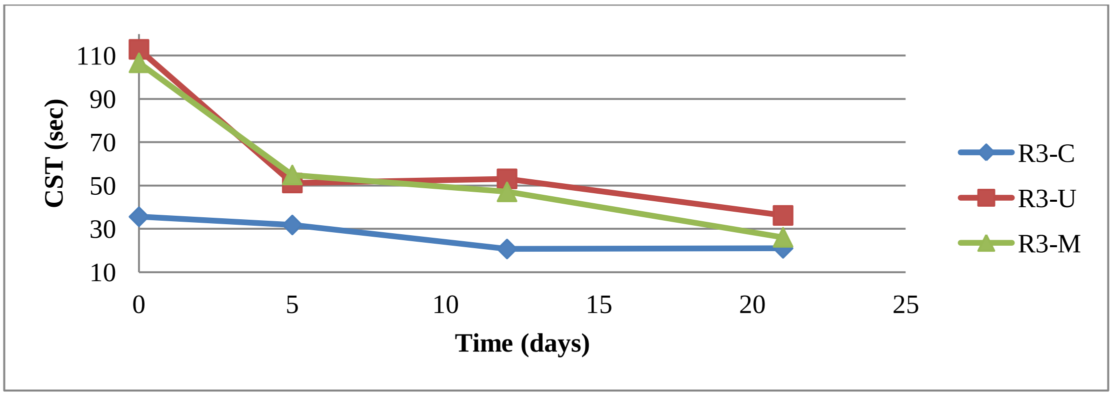
(Bourgier et al., 2006). Braguglia et al. (2012), also confirmed this case by their study and stated that "the floc disintegration caused a significant worsening of sludge dewaterability due to increase of fine particles and bound water" (Braguglia et al., 2012).

It was expected for the CST value of unpretreated sludge samples to increase by the digestion because of the release of colloidal particles in hydrolysis step. However, pretreatment of sludge may enhance the consumption of this released organic matter and attenuate the negative effects of pretreatment (Braguglia et al., 2012). In this study this was the case for microwave irradiation method for R1 and R2 sets. Ultrasonication, on the other hand, worsen the filterability of the sludge as the stabilization progressed.

According to these results, the selected disintegration methods did not affect the filterability of the sludge samples, significantly. Haug et al (1978)., Braguglia et al. (2012) and Bourgier et al. (2006) also observed different disintegration methods have different effects on sludge samples. The type of the stabilization process itself also affects the dewaterability of the sludge samples.







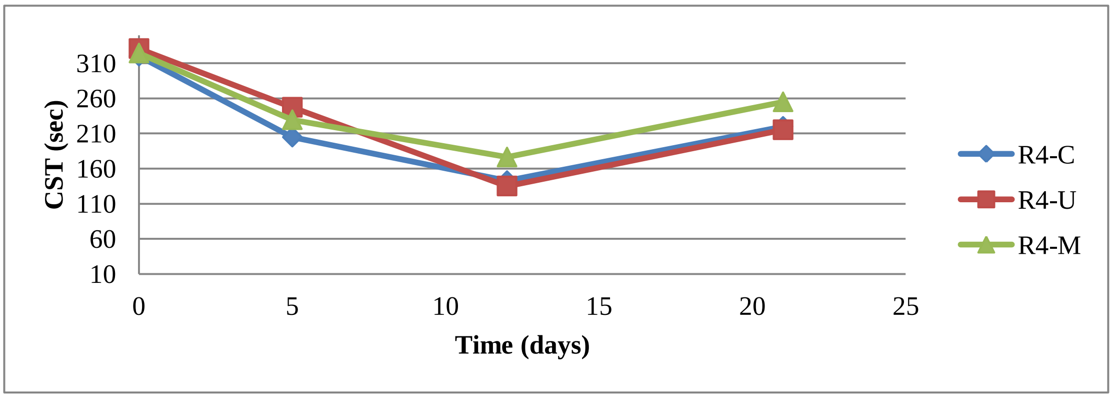


Figure 4.26 CST change in aerobic digestion reactors.

4.1.16. Particle Size Distribution

Particle size distribution analyses were conducted once a week throughout the operation of aerobic stabilization and the results are shown in Table 4.17.

Kim et al. (2003) and Tiehm et al. (1997) confirmed that ultrasonication and thermal disintegrations reduce the particle size of the sludge by causing aggregate deagglomeration and microbial cell disruption. They examined the disintegrated sample and compared it with the unpretreated sludge.

Since the pretreated sludge volume of the R-U and R-M reactors only one fourth of the whole reactor, no big differences between the control and pretreatment applied reactors were observed regarding the particle size. Nevertheless, according to d (0.5) parameter, particle sizes of all pretreated reactors decreased at the beginning of the study. Afterwards, with the stabilization took place, particle size distributions varied with sludge type, microbial activity and content of the reactor.

Table 4.17 Particle size distributions of the aerobic digestion reactors.

İzmit Kullar WWTP (R1)	**Surface Weighted Mean D[3.2]**	**Volume Weighted Mean D[4.3]**	**d (0.9) µm**	**d (0.5) µm**	**d (0.1) µm**
Control					
Day 0	16.009	52.230	142.582	31.169	83.197
Day 5	12.888	35.598	98.905	22.902	58.669
Day 12	14.289	40.994	111.234	26.467	66.812
Day 21	13.755	37.404	101.076	25.173	51.545
Ultrasound					
Day 0	16.115	48.740	131.678	30.226	74.924
Day 5	14.159	41.186	109.993	25.881	56.953
Day 12	14.920	44.370	121.535	27.88	63.088
Day 21	15.003	42.989	155.943	28.119	75.136
Microwave					
Day 0	17.646	57.803	178.575	30.365	96.921
Day 5	15.043	44.556	121.974	28.094	97.716
Day 12	15.557	46.111	118.730	27.961	66.383
Day 21	14.868	44.334	112.017	25.537	60.349

İstanbul Bahçeşehir WWTP (R2)	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
Control					
Day 0	20.965	58.634	186.703	41.061	95.101
Day 5	21.921	58.805	179.522	40.101	88.289
Day 12	22.830	61.026	168.616	41.352	88.253
Day 21	21.843	57.520	184.979	39.597	104.587
Ultrasound					
Day 0	21.731	59.576	201.93	40.264	93.426
Day 5	22.994	62.151	182.76	42.019	96.088
Day 12	22.014	57.483	157.053	38.748	85.98
Day 21	16.048	48.108	148.726	29.641	75.138
Microwave					
Day 0	21.842	60.615	176.818	40.199	85.242
Day 5	24.175	68.707	219.836	44.966	101.229
Day 12	23.914	65.158	181.696	43.274	93.27
Day 21	21.681	59.747	194.426	39.341	108.923

Samsun Bafra WWTP (R3)	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
Control					
Day 0	13.125	42.654	102.067	26.175	51.636
Day 5	13.426	41.646	98.219	25.807	52.708
Day 12	13.551	98.073	86.100	24.312	48.58
Day 21	12.349	33.360	79.848	21.712	45.004
Ultrasound					
Day 0	13.618	37.904	93.719	24.252	50.423
Day 5	14.380	41.419	104.598	27.021	56.647
Day 12	14.330	41.616	100.885	26.437	54.518
Day 21	13.235	36.621	94.866	23.890	60.737
Microwave					
Day 0	13.672	41.679	112.618	25.620	60.416
Day 5	16.567	54.076	127.809	32.736	70.121
Day 12	14.330	41.616	100.885	26.437	54.518
Day 21	12.675	35.763	82.851	22.624	46.567

Düzce Akçakoca WWTP (R4)	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
Control					
Day 0	7.634	23.911	82.561	15.215	48.594
Day 5	7.444	23.583	113.942	13.795	52.941
Day 12	6.535	24.971	72.663	13.290	39.734
Day 21	5.231	21.305	98.583	11.355	45.444
Ultrasound					
Day 0	8.874	27.146	77.685	14.798	47.803
Day 5	7.805	23.912	71.472	13.763	39.744
Day 12	8.684	27.710	87.917	15.280	49.851
Day 21	6.638	26.943	164.073	14.303	73.088
Microwave					
Day 0	7.990	25.011	109.233	14.454	66.899
Day 5	9.667	27.805	74.26	18.986	43.165
Day 12	7.567	23.613	76.468	13.581	40.658
Day 21	7.066	26.553	123.372	13.657	53.322

4.1.17. Microbiology

Pathogen reduction of the sewage sludge is one of the key points of the stabilization process. Depending on the further use and disposal site of the treated sludge, it is important to achieve a certain degree of pathogen removal.

In this study, Total Coliform (TC), Fecal Coliform (FC) and Fecal Streptococci (FS) measurements were completed in order to represent the stabilization efficiency and the results are shown in the Table 4.18.

Since the microbial concentrations of the samples were too high and it was hard to count that much concentrated samples, microbial analyses were conducted with dilution rates. Therefore, results may have been affected with the high dilution factors.

According to the results shown below, in all reactors, pathogen removal achieved with the order of 10^3-10^4 CFU/ mL. This also means the stabilization process was successfully achieved regarding the pathogen removal.

At the beginning of the operation, disintegration reactors did not seem to have a significant effect on the microbial content of the reactors. It is hard to tell whether the concentrations increased or decreased with disintegration since they both proved to be occurred for all three parameters. Besides, after stabilization process, pathogens were proved to be reduced in all reactors, whether pretreated or unpretreated. However, if microbial analyses have been conducted in the middle of the operation or once a week, we may have seen the difference between the control reactor and the other two. Since the stabilization period kept high (21 days), it was expected for pathogen removal to reach the highest efficiencies even with the unpretreated sludge.

Table 4.18 Microbial analyses of the reactors before and after the aerobic digestion.

Reactor	**Total Coliform [CFU/100mL]**		**Fecal Coliform [CFU/100mL]**		**Fecal Streptococci [CFU/100mL]**	
	Day 0	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**
R1-C	$4.1x10^7$	$1.1x10^5$	$3.7x10^7$	$7x10^4$	$3x10^6$	$3x10^4$
R1-U	$6.3x10^7$	$3.3x10^5$	$3.1x10^7$	$1.2x10^5$	$1x10^7$	$2x10^4$
R1-M	$3.9x10^7$	$1.8x10^5$	$1x10^7$	$4x10^4$	$1.7x10^7$	$3x10^3$
R2-C	$1x10^8$	$2.3x10^5$	$5.9x10^7$	$1.4x10^5$	$3x10^7$	$1.5x10^4$
R2-U	$1x10^8$	$9x10^4$	$4.6x10^7$	$4x10^4$	$3.7x10^7$	$3x10^4$
R2-M	$1.8x10^8$	$4x10^4$	$8.3x10^7$	$2x10^4$	$1.5x10^7$	$3x10^4$
R3-C	$1.5x10^8$	$8x10^4$	$5.5x10^7$	$4x10^4$	$1.8x10^7$	$1.2x10^3$
R3-U	$1.3x10^8$	$3x10^4$	$6.1x10^7$	$2x10^4$	$2x10^7$	$3x10^4$
R3-M	$9x10^7$	$1.5x10^5$	$7.3x10^7$	$3x10^4$	$1.7x10^7$	$1.2x10^4$
R4-C	$1.7x10^8$	$3.1x10^5$	$1x10^8$	$6x10^4$	$3.8x10^7$	$3.8x10^3$
R4-U	$1.6x10^8$	$1x10^4$	$7.9x10^7$	$5x10^4$	$3.1x10^7$	$3.1x10^4$
R4-M	$1.3x10^8$	$1.5x10^6$	$7x10^7$	$5.5x10^5$	$2.5x10^7$	$1x10^4$

4.2. Anaerobic Digestion

Four different sludge samples, taken from the recycle line of the treatment plants, were used for the operation of anaerobic digestion for 21 days. Sludge samples were again obtained from İzmit Kullar wastewater treatment plant (WWTP), İstanbul Bahçeşehir WWTP, Düzce Akçakoca WWTP and Samsun Bafra WWTP. Additionally, seed sludge was added to provide the anaerobic microorganisms and this sludge was obtained from the full-scale anaerobic digesters of the wastewater treatment plant of a potato chip factory. The initial characteristics of the sludge samples used for anaerobic digestion are given in the Table 4.19.

For anaerobic digestion process, as in the previous chapter, two different disintegration methods were applied to 25% of the recycled sludge volume and the results were compared with unpretreated sludge samples which are control reactors.

During the discussion of the anaerobic digestion results, same abbreviations in Chapter 4.1 will be used as described in Table 4.20.

The specific energy of ultrasonication was 15000 kJ/kg TS with the dose adjusted to 200 W and 70% amplitude as in aerobic digestion. Details of the ultrasonic pretreatment are given in Table 4.20.

Table 4.19 Characteristic of the sludge samples used in anaerobic digestion process.

Parameter	**Unit**	**Seed Sludge**	**İzmit Kullar WWTP**	**İstanbul Bahçeşehir WWTP**	**Samsun Bafra WWTP**	**Düzce Akçakoca WWTP**
TS	mg/L	38331	13121	6163	6720	3547
VS	mg/L	25360	8900	4184	3850	2360
MLSS	mg/L	37250	14380	5450	2910	3320
MLVSS	mg/L	24000	8300	4050	1980	2150
COD	mg/L	38470	14327	8337	7486	5299
sCOD	mg/L	5154	327	142	70	94
TKN	mg/L	870	780	390	270	330
NH_4^+	mg/L	294	60	32.25	26.63	55.75
NH_3-N	mg/L	312	46.75	30	20.63	43.75
NH_3	mg/L	278	56.75	30.5	25.13	52.5
TP	mg/L	430	540	350	300	270
PO_4^{-3}-P	mg/L	1310	1640	1080	910	830
SO_4^{-2}	mg/L	110	-	50	55	25
Cl^-	mg/L	602	126	106	142	71
CST	s	>1000	22.7	22.7	11.4	34.7
Alkalinity	mg $CaCO_3$/L	5302.5	1470	997.5	840	1155
pH	-	7.3	6.9	7.1	7.4	7.1
Conductivity	mS/cm	20.1	2.95	2.35	3.31	2.22
Salinity	‰	14	1.8	1.1	1.7	1.8
Total Coliform	CFU/100mL	$1x10^6$	$8x10^7$	$5.5x10^7$	$6x10^7$	$9x10^7$
Fecal Coliform	CFU/100mL	$5x10^5$	$5.1x10^6$	$6.5x10^6$	$1.8x10^6$	$1x10^7$
Fecal Streptococci	CFU/100mL	$1x10^5$	$3x10^6$	$2.5x10^6$	$5.4x10^6$	$5x10^6$
Salmonella	CFU/100ml	$8x10^4$	$1.2x10^5$	$1.9x10^5$	$5.3x10^5$	$5x10^5$

Table 4.20 Specifications of the ultrasonicated sludge samples.

Pretreated Sludge	**Initial Temperature (^{0}C)**	**Final Temperature (^{0}C)**	**Applied Ultrasonic Energy (kJ)**
İzmit Kullar WWTP	23.3	26.6	102.410
Bahçeşehir WWTP	26.6	24.6	101.161
Samsun Bafra WWTP	24.6	25.6	109.361
Düzce Akçakoca WWTP	25.6	24.1	92.509

For microwave disintegration, sludge samples were subjected to temperature of 175°C for 10 minutes using Berghoff MWS-3+ device. Disintegration degrees of the pretreated sludge samples are given in Table 4.21.

Table 4.21 Disintegration degrees of the sludge samples in anaerobic digestion.

Treatment Plant	**Reactor**	**DD (%)**
İzmit Kullar WWTP	Pretreated by Ultrasound	34
	Pretreated by Microwave	42
İstanbul Bahçeşehir WWTP	Pretreated by Ultrasound	26
	Pretreated by Microwave	35
Samsun Bafra WWTP	Pretreated by Ultrasound	30
	Pretreated by Microwave	38
Düzce Akçakoca WWTP	Pretreated by Ultrasound	33
	Pretreated by Microwave	42

The performances of the anaerobic digestion reactors and the effects of ultrasonic and microwave pretreatment on aerobic digestion were investigated in terms of pH, oxidation reduction potential, electrical conductivity, salinity, temperature, total and volatile solids, mixed liquor suspended solids and mixed liquor volatile suspended solids, chemical oxygen demand, soluble chemical oxygen demand, total organic carbon, dissolved organic carbon, alkalinity, total Kjeldahl nitrogen, nitrite, nitrate, ammonium, total phosphorous, sulfate, chloride, capillary suction time, volatile fatty acids, particle size, gas chromatography (GC) and microbiology as described in Chapter 3. All analyses were conducted at the beginning and the end of the anaerobic digestion, except GC analyses, which were conducted twice a week.

4.2.1. Temperature and pH

Temperatures of the reactors were kept at 37°C, mesophilic temperature during the operation of anaerobic digestion in order to maintain the microbial activity.

pH is an important environmental factor for microorganisms. In an anaerobic digestor it is known that there are more than one microorganism group and each of them has a different range of optimum pH. In order to provide a fully anaerobic stabilization of the sludge including hydrolysis, acidogenesis, acetogenesis and methanogenesis stages, a pH value that is at the intersection of all types of microorgamisms should be maintained. Among the anaerobic microorganisms, methanogens are the most sensitive ones and known to be active at the pH values between 6.5 and 7.5 and therefore, this level must be sustained.

During this study, pH values of the reactors were analyzed initially and at the end of the operation and the results are given in Figure 4.27. Initial pH values in the reactors varied between 7.25 and 7.45 where, after stabilization process these values decreased and ranged between 7.2 and 7.3. Decrease of the pH values also indicated that all stages of the anaerobic digestion completed.

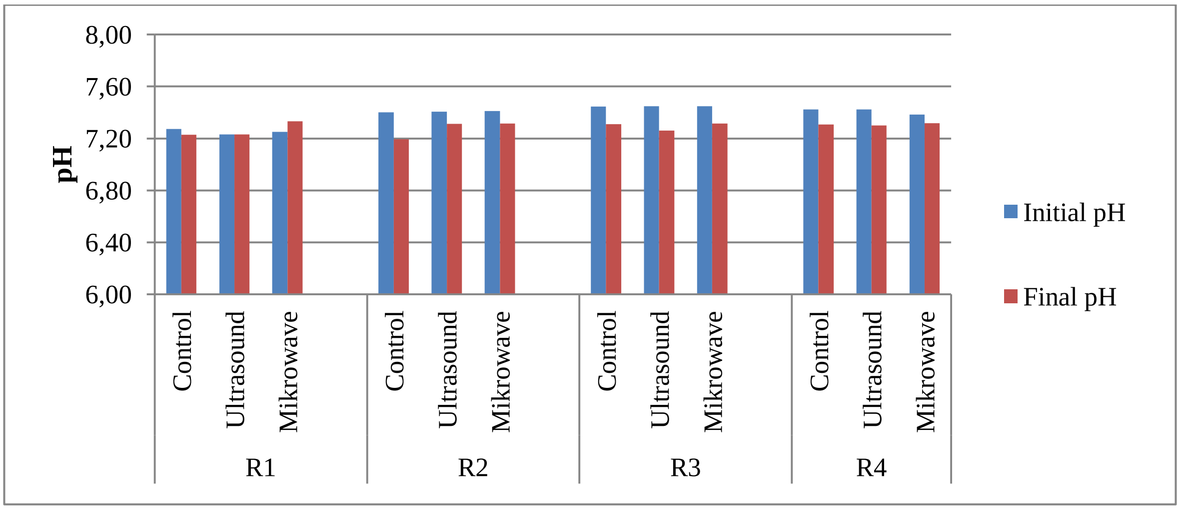


Figure 4.27 pH changes in anaerobic digestion reactors.

4.2.2. Alkalinity

Alkalinity of a water medium represents the buffering capacity of the sample to significant pH changes, acids accumulations as a result of the activity of acidogens. Since the organic matter found in the wastewater sludge can be easily converted to some acidic structure it is important to maintain the alkalinity of the system between 2000 to 3000 mg $CaCO_3$/L for an anaerobic digester.

Alkalinity concentrations of the reactors are presented in Figure 4.28. Initial alkalinity concentrations of the reactors were varying between 2050 and 2900 mg $CaCO_3$/L where they elevated by approximately 30 % with the stabilization process. In a completed anaerobic digestion, alkalinity is expected to increase since with the methanogenesis step, H^+ ions are consumed. This is also the reason of the pH decrease as seen in Figure 4.27.

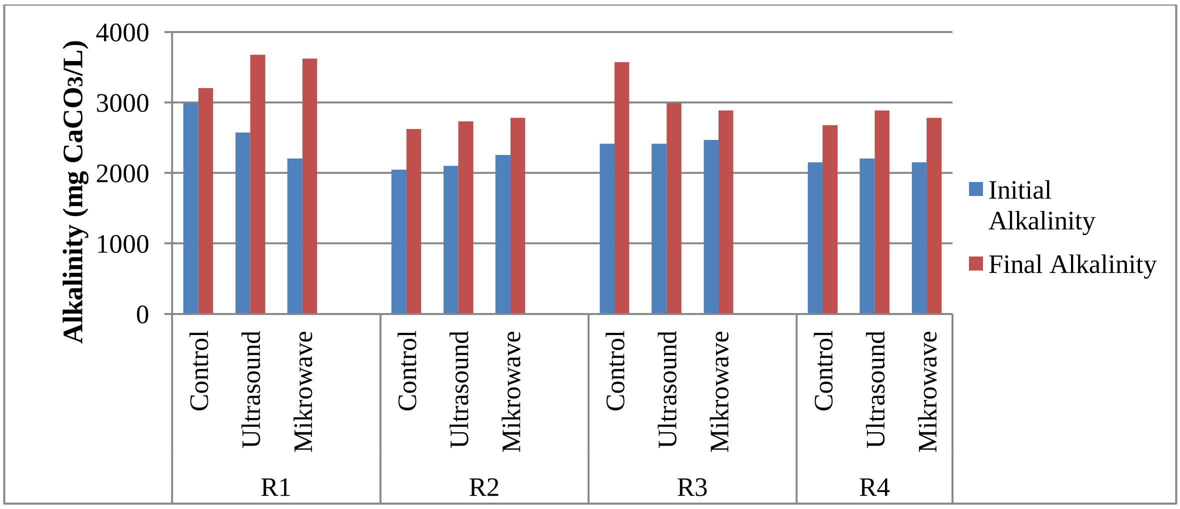


Figure 4.28 Alkalinity changes in anaerobic digestion reactors.

4.2.3. Oxidation Reduction Potential

Oxidation Reduction Potential (ORP) in the reactors at the beginning and the end of the anaerobic digestion is shown in Figure 4.29.

As the name implies, ORP is expected to be negative when there is no oxygen for oxidation-reduction reactions to take place. Even very low concentrations of oxygen can make the ORP increase. Since oxygen is toxic for most of the anaerobic microorganisms and suspend their activity ORP is a useful parameter to observe whether anaerobic conditions are sustained or not.

Initially, ORP values were below -200 mV and as the digestion proceeded, ORP values decreased to – 280 mV. System got more anaerobic during the operation without decreasing below 300 mV ORP, which is inhibiting for methanogens.

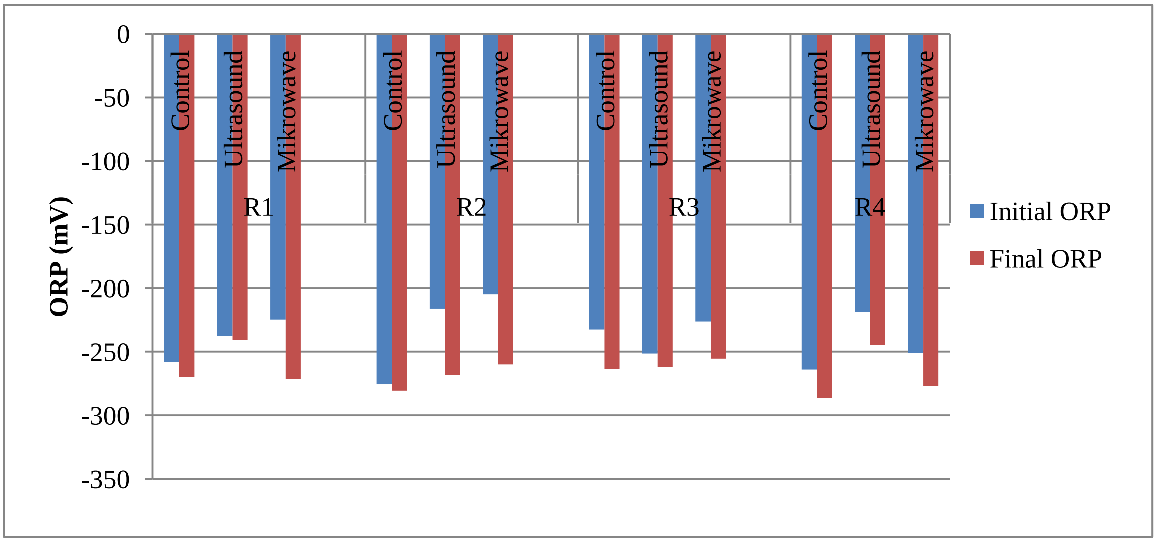


Figure 4.29 ORP changes in anaerobic digestion reactors.

4.2.4. Electrical Conductivity

EC of the reactors were measured before and after the anaerobic stabilization process and the results are shown in Figure 4.30. Initially, EC values in the reactors containing the pretreated samples were slightly higher than control reactors, possibly because of the release of some ions with the disintegration.

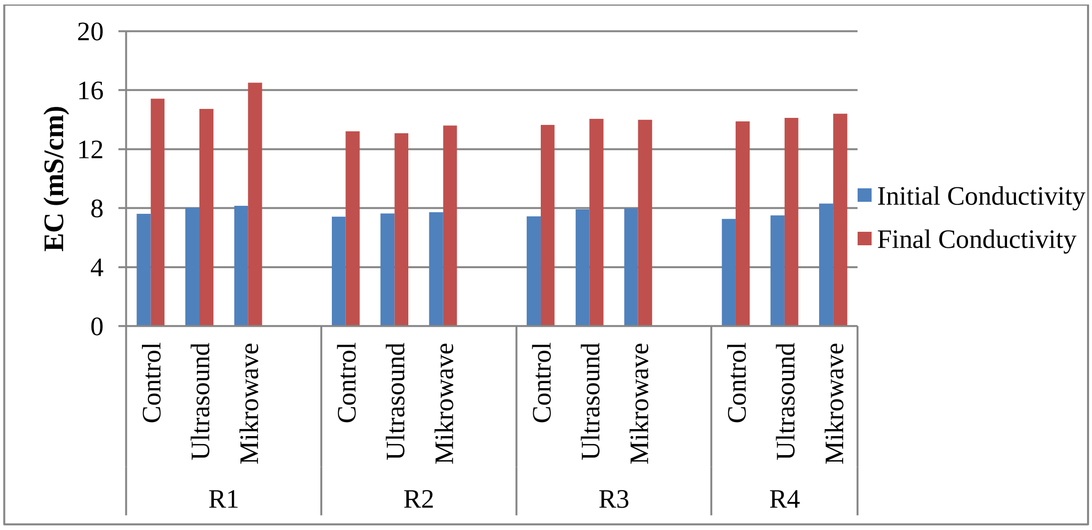


Figure 4.30 EC changes in anaerobic digestion reactors.

At the end of the digestion, EC of the reactors were almost doubled and increased from 7.5 to 14 mS/cm. Conversion of organic materials to ionic forms and increase of some ions such chloride (Cl^-) may be the reason of EC increase observed in the reactors.

4.2.5. Salinity

Results of salinity analyses are given in Figure 4.31 below. Salinity values were in accordance with EC values. Initially, salinity measurements of reactors were ranging between 4.9 to 5.5 ‰. Salinity values of R-M reactors increased after the microwave pretreatment. The higher increase in the salinity value was observed in R1 set. That means salinity change of the sludge not only depends on the treatment type but also the sludge characteristic itself. Final salinity values of the reactors ranged between 7.2 and 9 ‰.

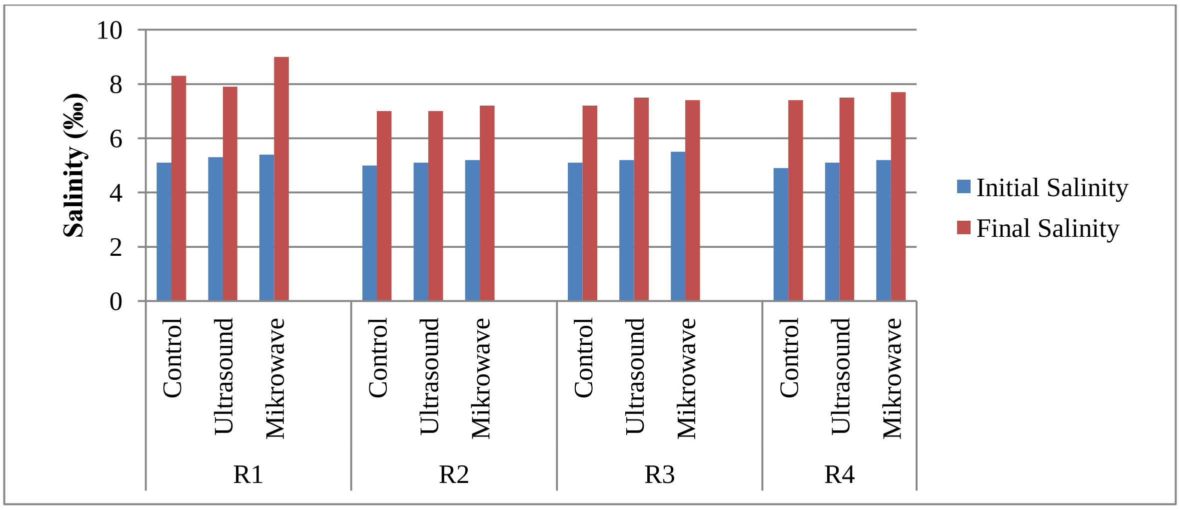


Figure 4.31 Salinity changes in anaerobic digestion reactors.

4.2.6. Total Solids and Volatile Solids

Solids content of the reactors were analyzed with TS and VS measurements and the data are given in Figure 4.32, Figure 4.33, Figure 4.34 and Figure 4.35.

Initially, TS values in the reactors were almost the same for each reactor set but there were differences between different sludge samples. TS value in the R1 set was about 2% whereas it was about 1.4% in the R2 and R4 sets and 1.8% in the R3 reactors. Therefore, as a result of higher initial solid content, higher reduction efficiency was observed in R1 reactors.

When control reactors were compared with the reactors containing the disintegrated samples, it was obvious that microwave disintegration enhanced the TS removal.

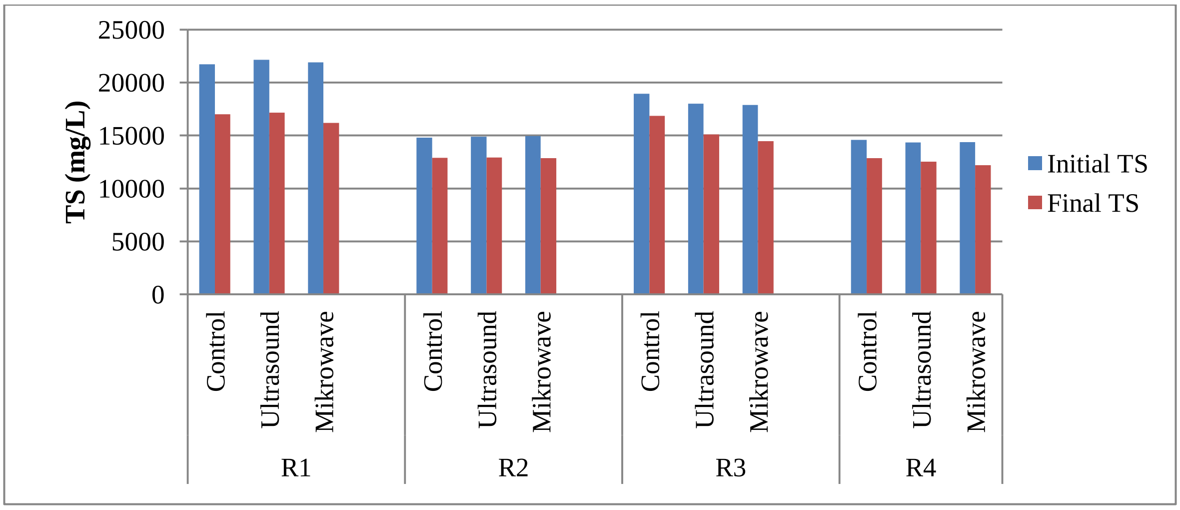


Figure 4.32 TS changes in anaerobic digestion reactors.

The highest TS removal occurred in R1-M reactor and 26% TS removal was achieved. However, TS removal efficiencies in the other reactors were about 15% which is quite low for mass reduction of an anaerobic stabilization system. Astal et al. (2013) applied anaerobic digestion to seven mixed sewage sludge from different WWTPs and at least 34% TS removal achieved without any disintegration methods. Although initial TS content of the sludge samples used in that study were generally higher than the samples in the R1,R2 , R3 and R4 sets, at least 35% TS removal was expected for a successful digestion process. Since higher TS removal rates were achieved with aerobic stabilization system, the lesser amount of active stabilization volume may be linked to lower solids removal efficiencies.

Volatile solids are expected to be converted into biogas and therefore VS reduction is an important parameter for the anaerobic digestion. However, as Raposo et al. (2011) states, not all VS are the same and they exhibit different rates and extents of biodegradation during the digestion. Initial and final VS contents of the reactors are given in Figure 4.34 and VS reduction efficiencies are given in Figure 4.35.

Similar to TS content, VS content was higher in R1 set since VS/TS value is similar and around 0.63 in all reactors. Besides, the initial VS content of the pretreated samples were lower in the bar chart. This may have been caused by the rapid conversion of ruptured organic materials into biogas.

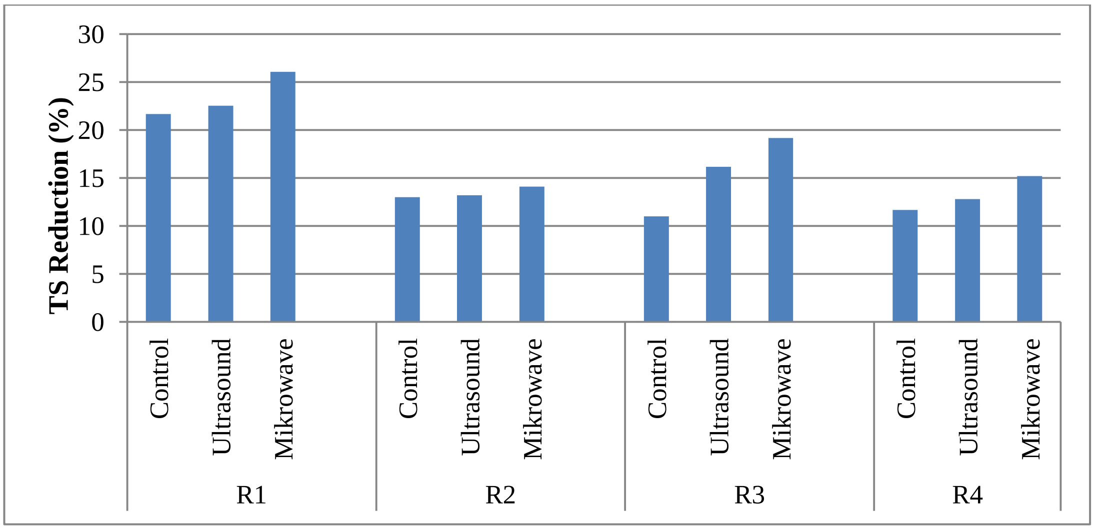


Figure 4.33 TS reductions in anaerobic digestion reactors.

VS reduction efficiencies were higher in pretreated reactors and except the R2-U reactor, microwave irradiation seemed to have a better effect regarding the VS removal. Lowest reduction of VS was observed in R3 control reactors, which is 19% while the VS removal in the reactor containing ultrasonicated sample was 26% and 31% after microwave irradiation.

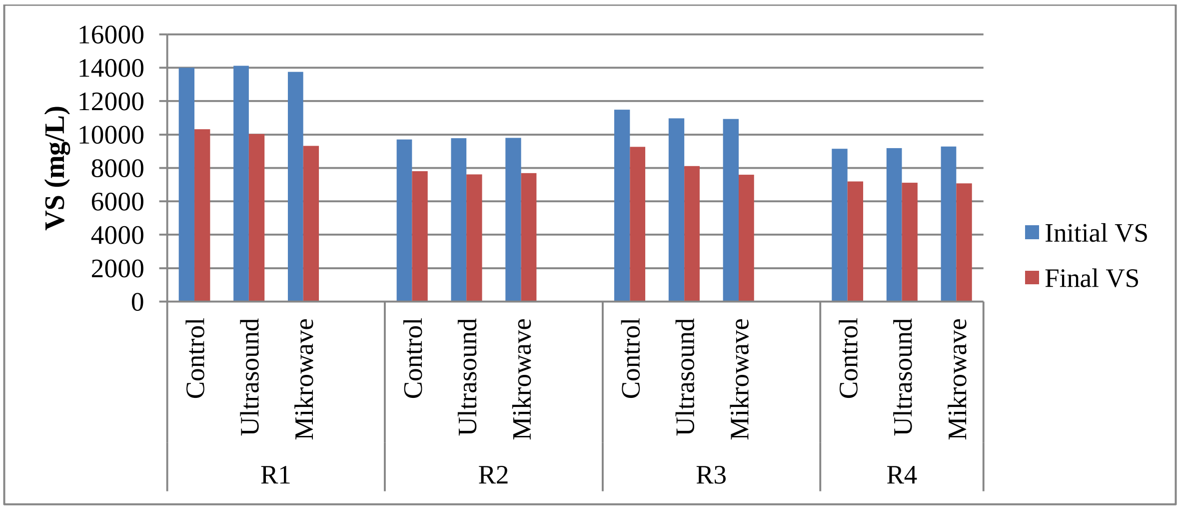


Figure 4.34 VS changes in anaerobic digestion reactors.

Highest VS reductions were achieved in R1 set; 26%, 29% and 32% removal efficiencies of unpretreated sample, ultrasonicated and microwave pretreated samples, respectively.

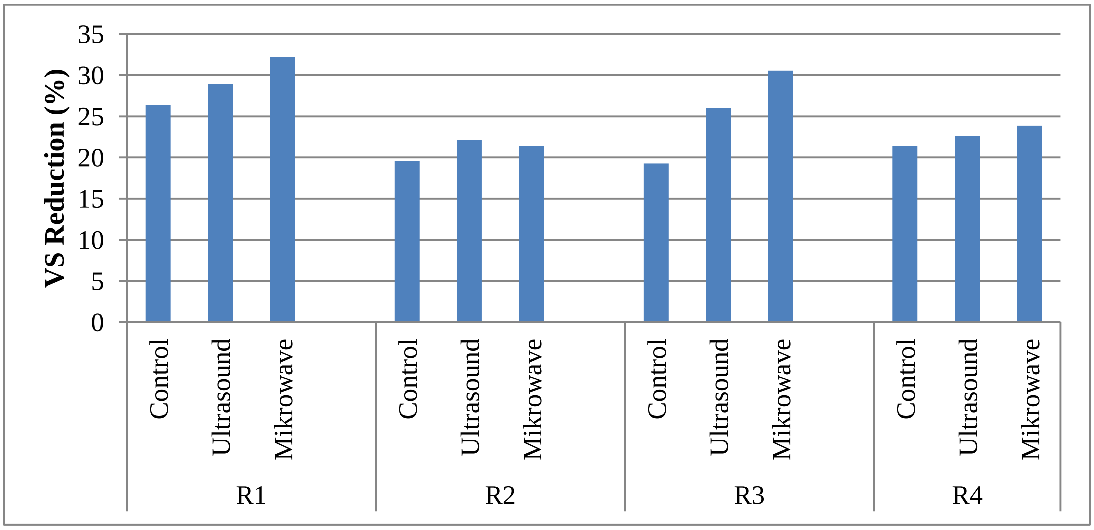


Figure 4.35 VS reductions in anaerobic digestion reactors.

Initial VS/TS values of the reactors were around 0.63 on average as stated above, and the final VS/TS values were about 0.55 on average. However, the differences were slightly higher in the reactors containing pretreated samples. This means particular fraction of sludge became more mineral with disintegration methods. This was also the case in the Bourgrier’s study (Bourgrier et al, 2006).

4.2.7. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

MLSS and MLVSS analyses were conducted at the initial and final stage of the anaerobic digestion and the results are shown in Figure 4.36 and Figure 4.37.

MLSS reductions showed parallelism with TS removals but decrease in TS content was higher, as expected. The highest MLSS removal achieved was 26%, in R1-M reactor. Possibly because the MLSS contents of the R1 set were the highest, removal efficiencies were also the highest ones. Very little MLSS removal with 5% reduction efficiency was observed in R4-C reactor.

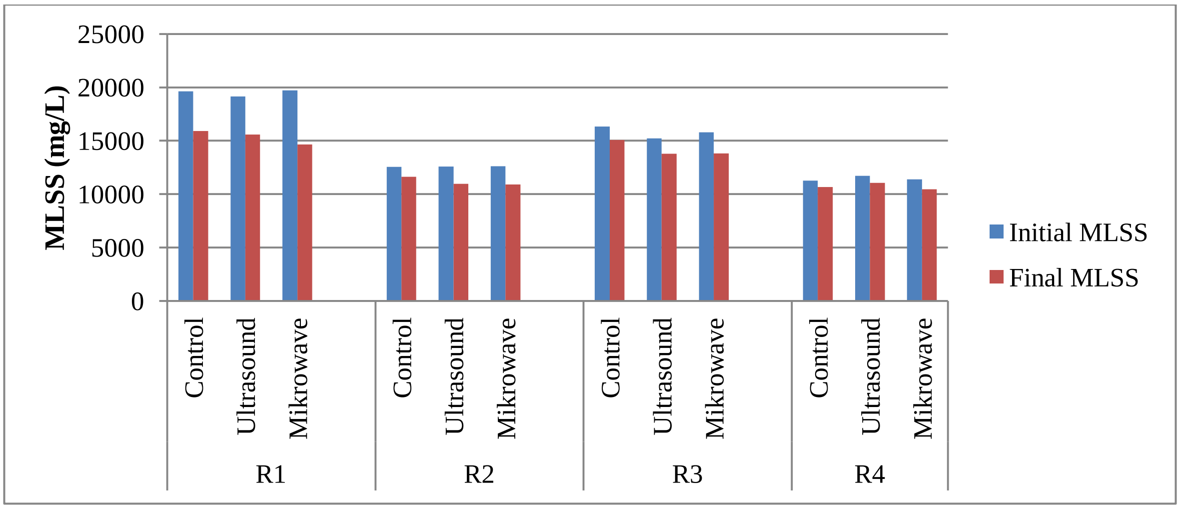


Figure 4.36 MLSS changes in anaerobic digestion reactors.

MLVSS analyses showed that almost all of the MLSS removal was achieved with the biodegradation of volatile suspended solids. Other than the VS reduction in R4-C, which is 9%, results were similar to literature data and were about 25% MLVSS reduction.

In all reactor sets, ultrasonication and microwave irradiation enhanced the VSS removal. Yu et al. (2010) also stated that VSS solubilization increased after microwave pretreatment and they concluded that the disintegration method is capable of disrupting the sludge cell and releasing the organic matter into soluble phase; lipids were hydrolyzed to palmitic acid, stearic acid and oleic acid; the protein was hydrolyzed into a series of saturated and unsaturated acids, ammonia, and some carbon dioxide, and the carbohydrates were hydrolyzed into polysaccharides with a smaller molecular weight and even into simple sugars. This conversion makes those compounds easily decomposed by microorganisms.

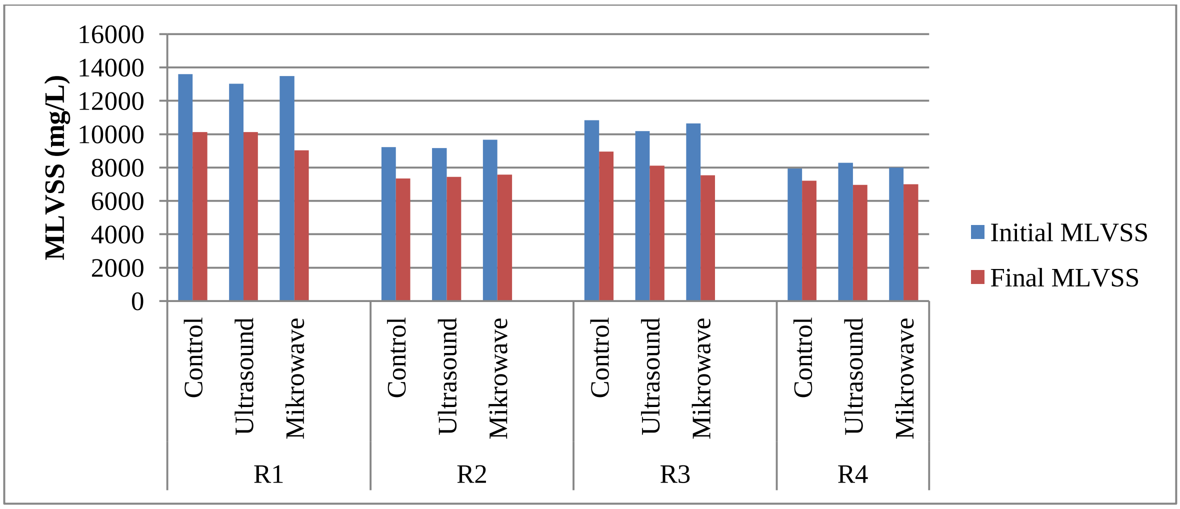


Figure 4.37 MLVSS changes in anaerobic digestion reactors.

4.2.8. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

COD concentrations of the reactors were analyzed before and after the anaerobic digestion and the results are shown in Figure 4.38 and Figure 4.39.

Since the solid content of the R1 reactors were higher, the COD concentrations of those reactors were also high. For the initial concentrations no certain trend for control or pretreatment applied reactors was observed; COD of R4-C was the lowest of the set while R3-C has the highest COD content of the R3 set. The highest COD removal efficiency of the set was observed in R3-M reactor which is 18% as shown in Figure 4.39.

For the COD data, the apparent result is the enhancement achieved by pretreatment methods for COD removal of the sludge. There is a particular difference between the control reactors and the disintegrated samples. For microwave irradiation, the difference is even more apparent in R1 and R3 sets.

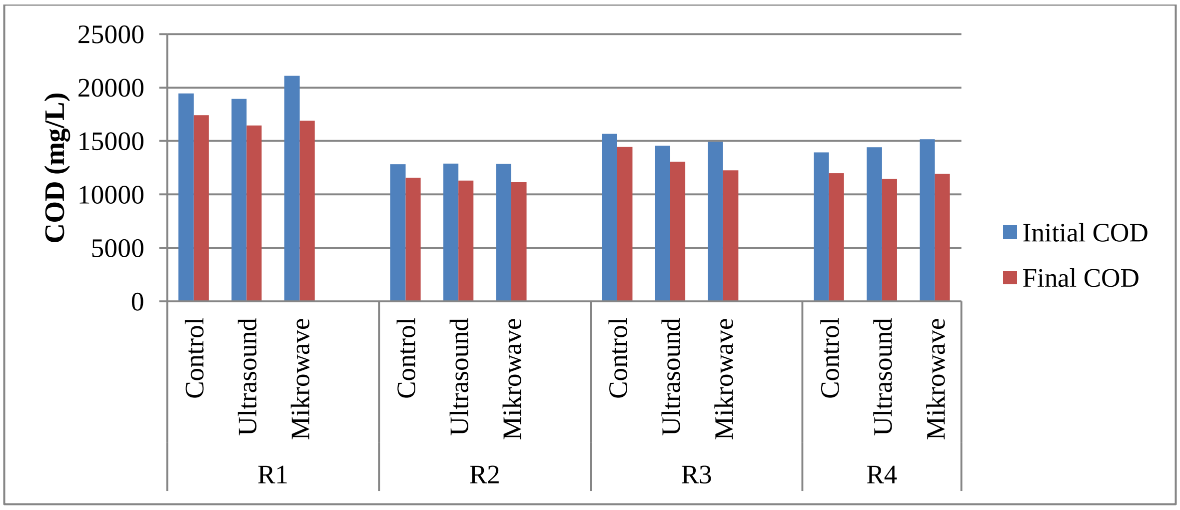


Figure 4.38 COD changes in anaerobic digestion reactors.

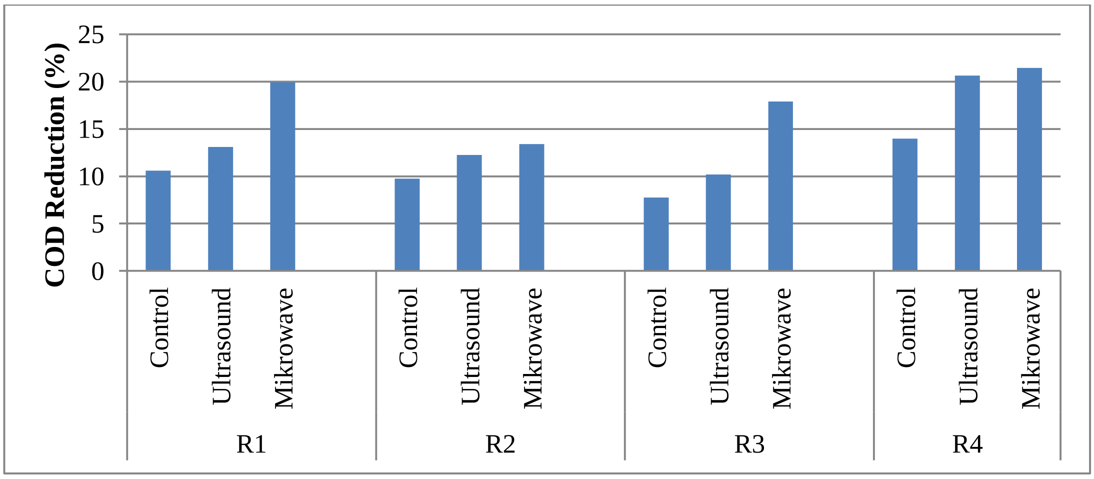


Figure 4.39 COD reductions in anaerobic digestion reactors.

Soluble COD value of the sludge indicates the sludge solubilization which has a major importance for pretreatment investigations of sludge since in almost all treatment methods it is linearly correlated with methane/biogas production (Bougrier et al, 2006). sCOD concentrations were measured and reduction efficiencies were calculated for 12 reactors. Results are given in Figure 4.40 and Figure 4.41.

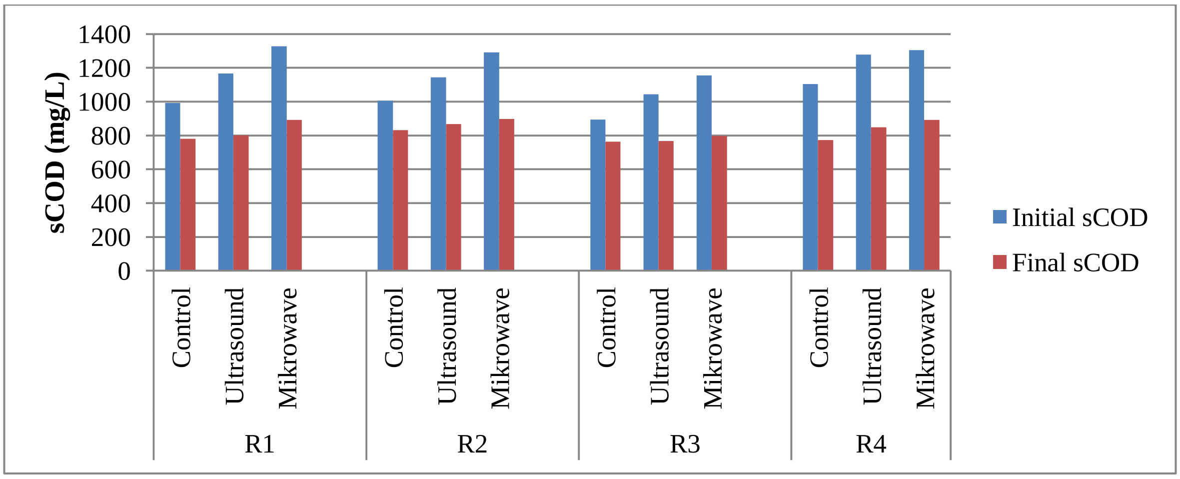


Figure 4.40 sCOD changes in anaerobic digestion reactors.

Initial sCOD values of the pretreated samples were higher as a result of disintegration, as expected. For each reactor set, microwave irradiation achieved a higher solubilization rate than ultrasonication. Yu et al. (2010) also proved that microwave irradiation quickly dissolves the COD and the transfer of materials from solid phase to liquid phase positively affects the further treatment. However, when compared to aerobic stabilization process, solubilization achieved by pretreatment was lower. In R1 set 17% and 38%, in R2 set 13% and 29%, in R3 17% and 29% and in R4 set 16% and 18% solubilization of COD was observed after ultrasonication and microwave pretreatment, respectively.

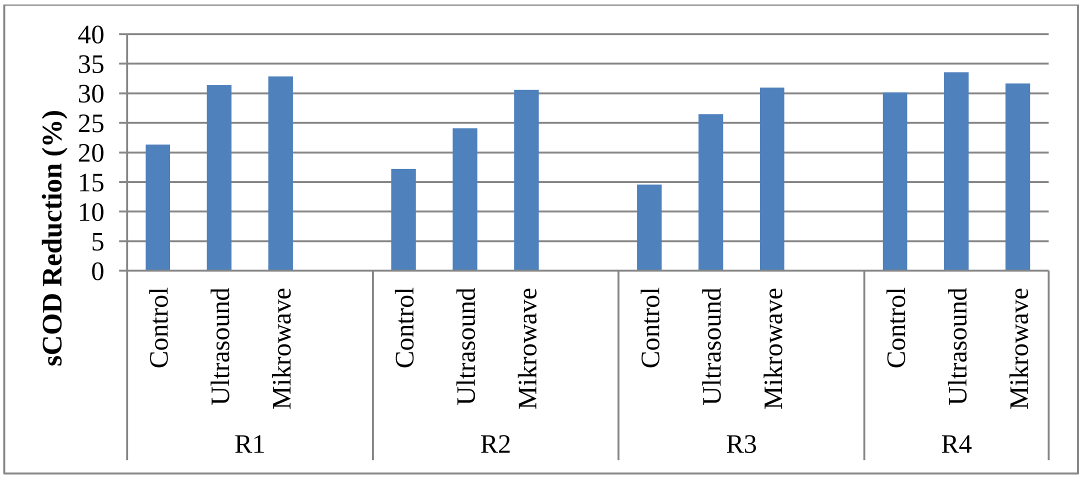


Figure 4.41 sCOD reductions in anaerobic digestion reactors.

4.2.9. Dissolved Organic Carbon

DOC parameter analyses of the reactors were conducted before and after anaerobic digestion and the results are given in Figure 4.42.

Similar to sCOD values, soluble carbon fraction also increased with the applied pretreatment methods except the R3-M reactor. Since significant sCOD increase of the same reactor proved that solubilization was achieved with the microwave irradiation, this situation may be explained as the hydrolyzed materials which cause the increase in sCOD concentrations were not the carbonaceous part of the sludge.

Overall, DOC concentrations increased with pretreatment applications and at the end of the stabilization process higher reductions were achieved in R-U and R-M reactors.

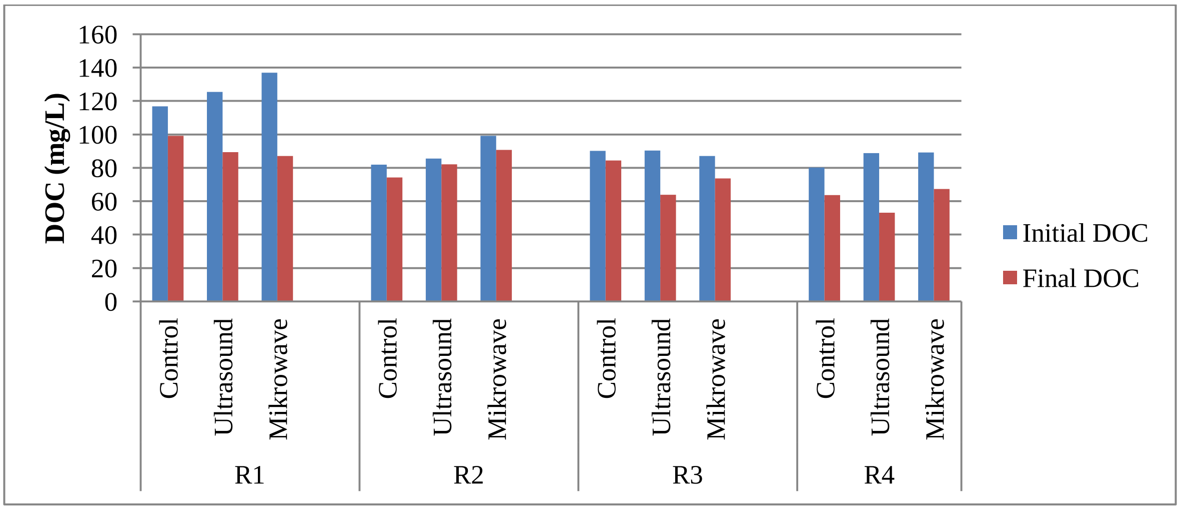


Figure 4.42 DOC changes in anaerobic digestion reactors.

4.2.10. Total Kjeldahl Nitrogen and Ammonium

TKN and ammonium of the sludge were analyzed initially and after the anaerobic digestion and the results are given in Figure 4.43 and Figure 4.44.

As described before in Chapter 4.1.12, TKN is the sum of organic nitrogen, ammonia and ammonium where the term, ‘organic nitrogen’ refers mostly to the proteins. In an anaerobic digestion system proteins are hydrolyzed to peptides and individual amino acids which are in turn oxidatively degraded to short-chain fatty acids. Additionally, ammonia is produced through the hydrolysis of proteinaceous material (Wilson and Novak, 2009). Therefore, high reductions of TKN concentrations are not expected in anaerobic digestion of sludge.

TKN removal, despite the significant ammonium increase, can be linked to the fact that conversion of proteins to fatty acids was greater than the ammonia production during the digestion.

When considering the pretreatment applications, no obvious effects of ultrasonication or microwave irradiation were observed on the TKN reduction. Even if the applied disintegration accelerated the sludge hydrolysis, ammonia increase may have been balanced the TKN content of the reactor.

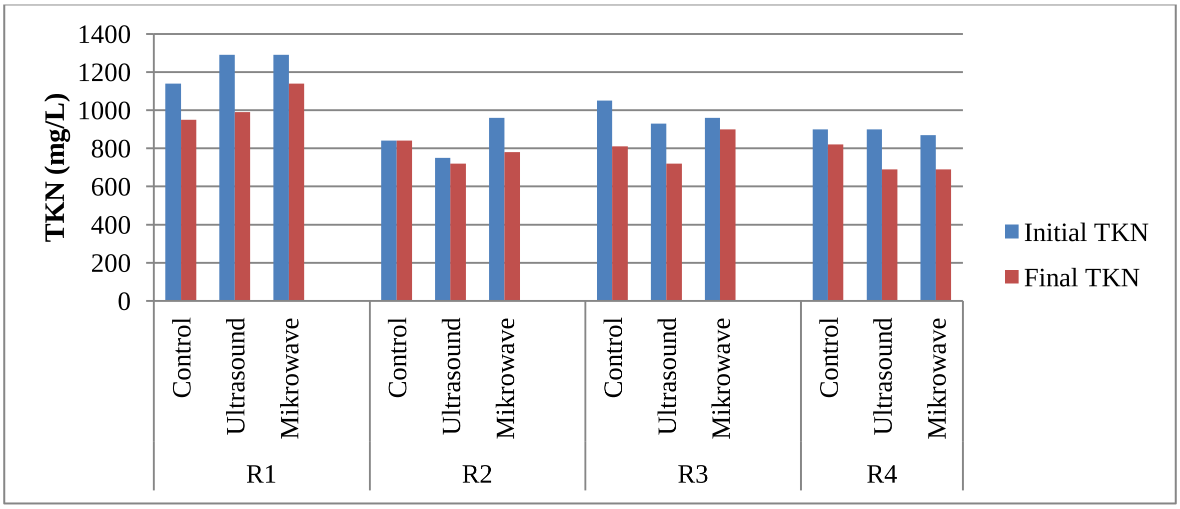


Figure 4.43 TKN changes in anaerobic digestion reactors.

Ammonium concentrations increased during the anaerobic digestion, as expected. Ammonium ion and free ammonia are two principal forms of inorganic ammonia nitrogen in aqueous solution. Ammonia is known to be inhibitor for methanogenesis; as ammonia concentrations increase in the range of 4051-5734 mg NH_3-N/L (Chen et al, 2008).

However, measured level of ammonia did not exceed 700 mg/L through the operation of anaerobic digestion process and therefore did not constitute an inhibitory effect on methanogenic microorganisms in this study.

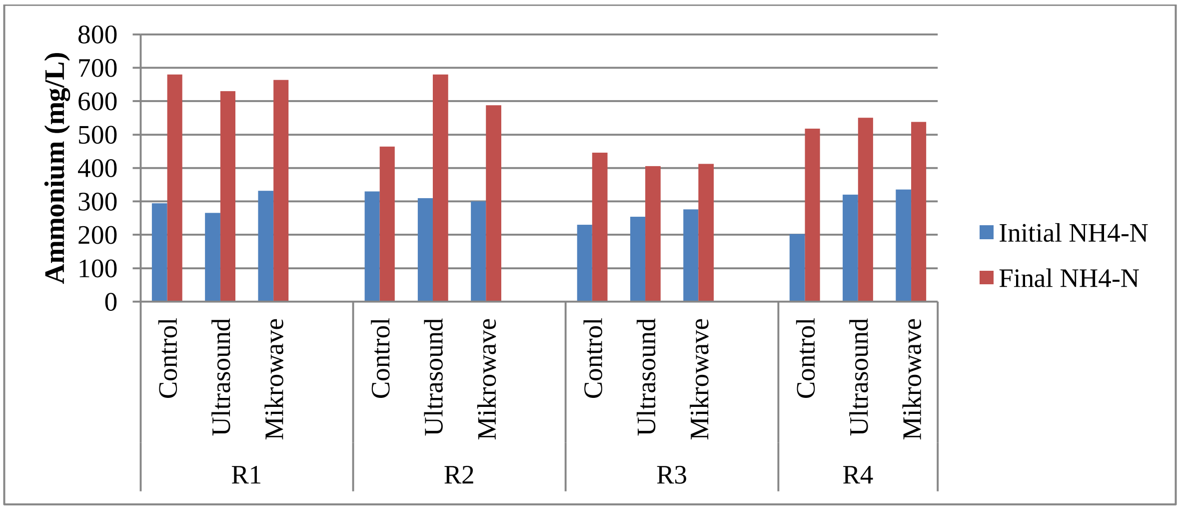


Figure 4.44 Ammonium changes in anaerobic digestion reactors.

4.2.11. Total Phosphorus

Total phosphorus analyses were conducted before and after the digestion process and the results are shown in Figure 4.45.

There was a significant increase in TP concentrations of the sludge samples, which is believed to be directly linked to increase in ortho-phosphate. This was the result of the hydrolysis stage of the sludge. Higher TP increase observed in the disintegration applied reactors also strengthened this theory since improved disruption of sludge cells speeds the hydrolysis of the sludge samples.

TP increase in R4 reactor set is lower than other three sets. This difference may be explained with the influent wastewater characteristic of the WWTP which the sludge sample obtained from.

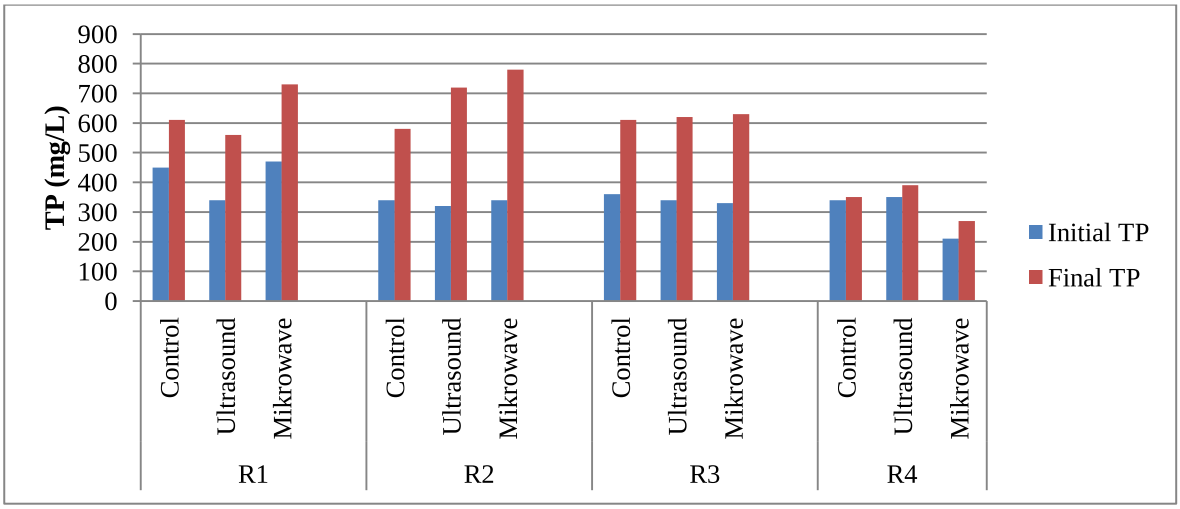


Figure 4.45 TP changes in anaerobic digestion reactors.

4.2.12. Sulfate and Chloride

Sulfate and chloride analyses of the anaerobic digestion reactors are given in Figure 4.46 and Figure 4.47.

During anaerobic digestion process sulfate ion was expected to be reduced to sulfide by sulfate reducing bacteria. This conversion also has inhibitory effects in an anaerobic system in two ways: Primary inhibition is caused by the competition for common substrates, which inhibits the methanogens, secondary inhibition is due to the toxicity of sulfide to various bacteria group (Chen et al., 2008). In the reactors, pH value ranged between 7.2 and 7.45 as previously shown in Figure 4.27. For this pH range, 50-125 mg H_2S/L is reported to be inhibitor (Chen et al., 2008). Since the decrease in sulfate concentrations was 90 mg/L at most, no inhibitory effect was expected to take place in this study. Sulfate concentrations in the R1 set was significantly lower than other reactor sets, which was directly related to WWTP sludge characteristics. Sulfate content in the R1-U reactor was initially so low that sulfate reduction did not seem to take place. In addition, disintegration techniques did not present any obvious effects on the sulfate reducing reaction.

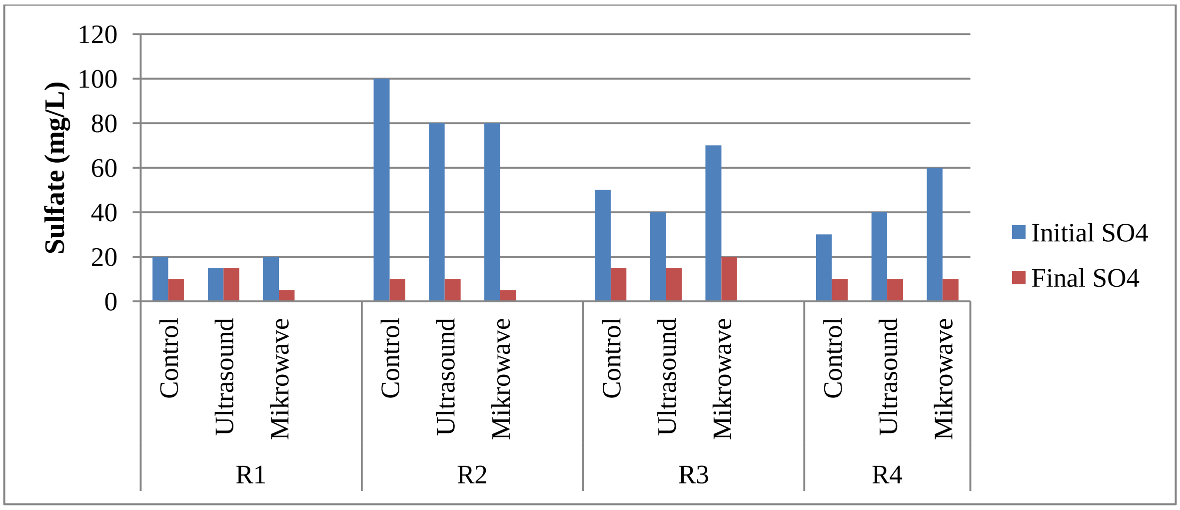


Figure 4.46 Sulfate changes in anaerobic digestion reactors.

Chloride concentrations of the reactors increased with the anaerobic digestion process in most of the reactors but the concentrations were in the optimum ranges. So, there was no inhibition in the reactors as a result of increased chloride concentrations. Besides, there was not a condition to link the level of increase to any disintegration method.

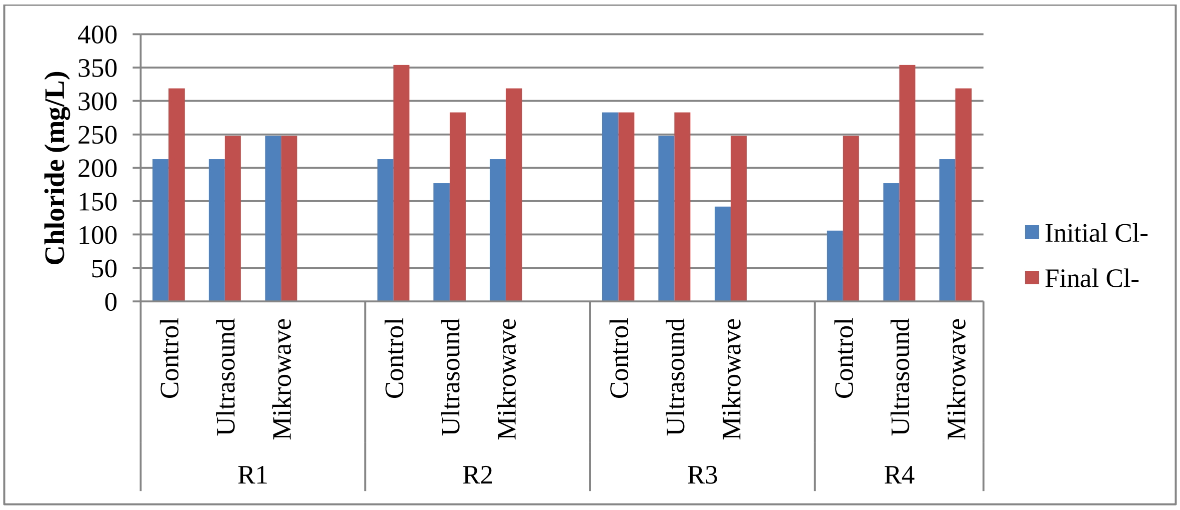


Figure 4.47 Chloride change in anaerobic digestion reactors.

4.2.13. Volatile Fatty Acids

Various types of fermentative bacteria are present in an anaerobic digestion system. These species ferments the primary substrates in the sludge, such as polysaccharides, proteins and lipids to fatty acids, H_2, CO_2 and perhaps ethanol. Later on, these products further degraded to CO_2 and CH_4 by methanogens (McInerney et al., 1979). Volatile fatty acids (VFA), or as recently called, short-chain fatty acids (SCFA) were measured before and after the digestion process and the results are shown in Table 4.22.

Initially, only little amounts of acetic acid and propionic acid were measured in the reactors. Acetic acid concentrations were higher since it is the primary form of SCFA. At the end of the digestion a decrease was observed in acetic acid concentrations which indicates the degradation of this products to methane and carbon dioxide has taken place successfully. Furthermore, the degradation efficiency was higher in the reactors containing the pretreated samples compared to the control reactors.

Propionic acid measurements showed that there was VFA production through the operation which was possibly converted to CH_4 and CO_2 but some level of produced propionic acid remained in the control reactors.

Table 4.22 VFA change in anaerobic digestion reactors.

Reactor		Volatile Fatty Acids			
		Acetic Acid (mg/L)		Propionic Acid (mg/L)	
		Initial	Final	Initial	Final
İzmit Kullar WWTP (R1)	Control	0.4039	0.2532	0.1461	-
	Ultrasound	0.4061	0.1658	0.1466	-
	Microwave	0.4478	0.2083	0.3651	-
İstanbul Bahçeşehir WWTP (R2)	Control	0.3512	0.3194	0.0429	0.0221
	Ultrasound	0.3904	0.2083	-	-
	Microwave	0.4025	0.2149	0.0297	-
Samsun Bafra WWTP (R3)	Control	0.4280	0.3226	-	0.0128
	Ultrasound	0.3968	0.3047	-	-
	Microwave	0.4415	0.1043	-	-
Düzce Akçakoca WWTP (R4)	Control	0.4469	0.2670	-	0.0239
	Ultrasound	0.2991	0.2031	-	-
	Microwave	0.4154	0.2361	-	-

4.2.14. Viscosity

Because of the higher solids content, filterability of the anaerobically digested sludges could not be measured. To observe the physical changes occurred in the sludge samples, viscosity analyses conducted before and after the digestion and the results are given in Figure 4.48.

Due to the solid reduction through the digestion process, viscosity of the sludge samples decreased and digested sludge became more viscous. Initially, R1 reactor set had higher levels of viscosity since the solid content of that set was the highest, however, at the end of the operation, viscosity of all samples were similar.

Before the stabilization, disintegration applications reduced the viscosity of R-U and R-M reactors when compared to control reactors. Still, no obvious enhancement of pretreatment applications for increasing the viscosity of sludge observed at the end of the digestion.

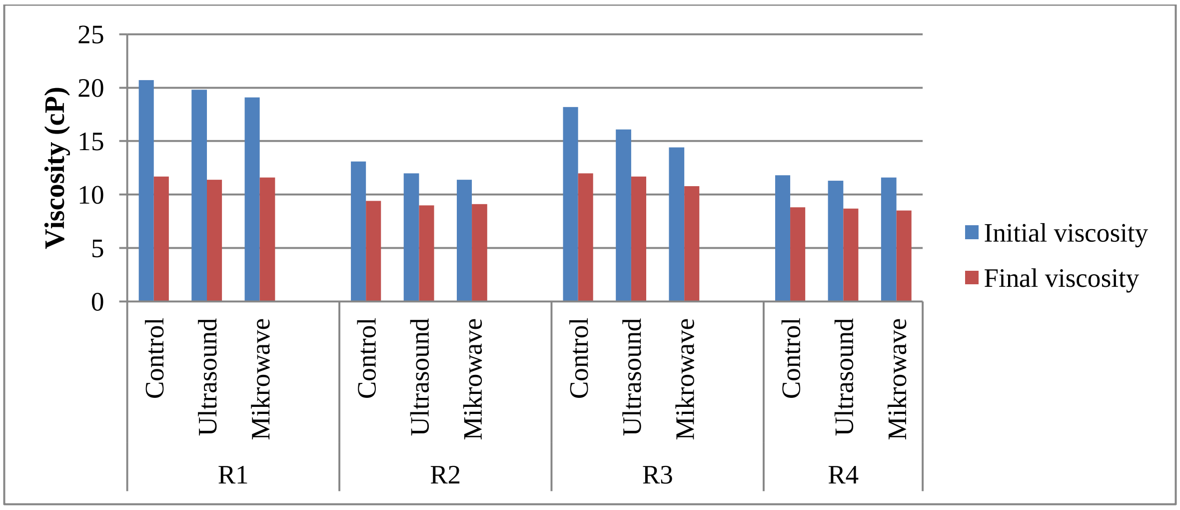


Figure 4.48 Viscosity change in anaerobic digestion reactors.

4.2.15. Microbiology

The microbiological analyses of the sludge samples were conducted before and after the anaerobic digestion process. Total coliform, fecal coliform, fecal streptococci, and salmonella measurements were detected in order to represent the anaerobic digestion efficiency on pathogen removal. The results of the analyses are given in Table 4.23.

Including the control reactors, a great reduction was observed in total count of microorganisms which is one of the most important indicators of a successful stabilization of sludge.

According to the results shown below, in all reactors pathogen removal was achieved with order of 10^3-10^4 CFU/ mL. At the beginning of the operation, disintegration reactors did not reflect a significant effect on the microbial content of the reactors. Also, at the end of the operation no significant difference was observed between the reactors containing pretreated and unpretreated samples. Again, as in Chapter 4.1.17, this was an expected result of high operation periods.

Table 4.23 Microbial analyses of anaerobic digestion reactors.

Reactor	Total Coliform [CFU/100mL]		Fecal Coliform [CFU/100mL]		Fecal Streptococci [CFU/100mL]		Salmonella [CFU/100mL]	
	Day 0	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**
R1-C	$1.1x10^7$	$5.4x10^4$	$4.9x10^6$	$2x10^3$	$1.3x10^5$	≤10	$1.8x10^4$	≤10
R1-U	$1.5x10^7$	$1x10^5$	$2.9x10^6$	$3x10^4$	$2.1x10^5$	≤10	$2.1x10^4$	≤10
R1-M	$1x10^7$	$1.6x10^3$	$5.3x10^6$	$3.2x10^2$	$4.2x10^5$	≤10	$4.2x10^4$	≤10
R2-C	$4.8x10^6$	$3x10^4$	$4.7x10^5$	$1.1x10^3$	$1.7x10^5$	≤10	$1.9x10^4$	≤10
R2-U	$7.3x10^6$	$1x10^3$	$8x10^5$	$1x10^2$	$1x10^5$	≤10	$1.1x10^4$	≤10
R2-M	$5.5x10^6$	$3x10^4$	$7x10^5$	$1.3x10^2$	$1x10^5$	≤10	$3.1x10^4$	≤10
R3-C	$5.3x10^7$	$5.5x10^3$	$4.2x10^6$	$1x10^2$	$3.2x10^5$	≤10	$3x10^4$	≤10
R3-U	$4.7x10^7$	$9x10^3$	$2.9x10^6$	$1x10^2$	$7.3x10^5$	≤10	$7.3x10^5$	≤10
R3-M	$3.6x10^7$	$3.1x10^5$	$6.9x10^6$	$7x10^3$	$1.1x10^6$	≤10	$1.7x10^5$	≤10
R4-C	$6.3x10^7$	$1x10^3$	$3.8x10^6$	$1.4x10^2$	$7.1x10^6$	≤10	$4.4x10^5$	≤10
R4-U	$7.7x10^7$	$7x10^4$	$4.1x10^6$	$2.3x10^2$	$3.7x10^5$	≤10	$1x10^4$	≤10
R4-M	$6.1x10^7$	$8x10^3$	$3.9x10^6$	$1x10^2$	$2.9x10^6$	≤10	$2.5x10^5$	≤10

4.2.16. Biogas Analysis

Main advantage of the anaerobic digestion operation is its ability of degradation and stabilization of complex organic matter by the activity of microorganisms leading to an energy-rich biogas which is a renewable energy. And so, the fundamental purpose of the pretreatment applications is to enhance the biogas production by accelerating the hydrolysis step in the anaerobic stabilization process. Therefore, biogas analyses conducted throughout the operation of reactors and the results are examined in the next three parts:

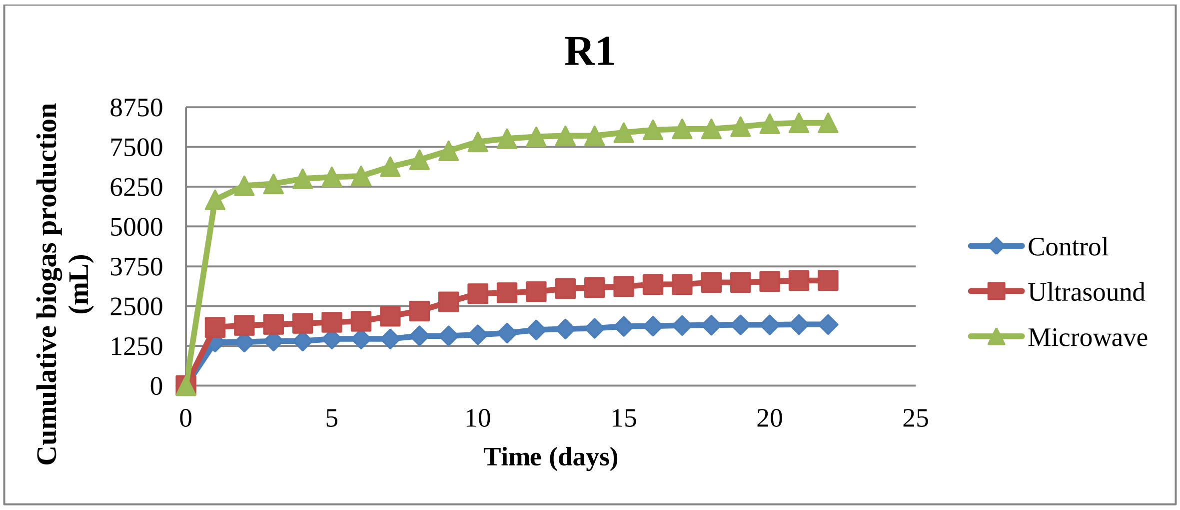
4.2.16.1. Cumulative Biogas Production

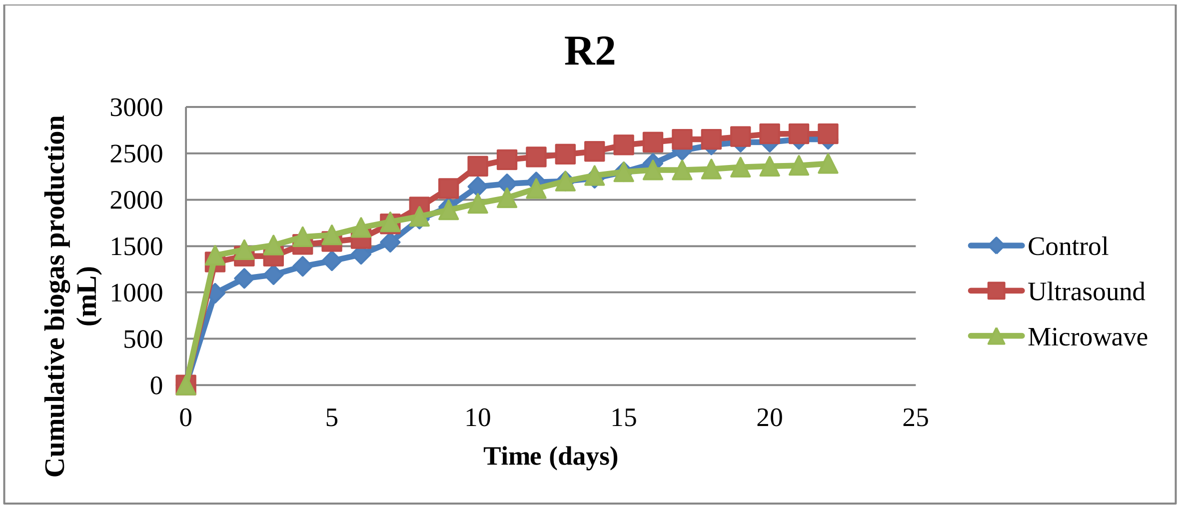
Biogas production of the anaerobic digestion reactors were measured daily and the results are shown in Figure 4.49.

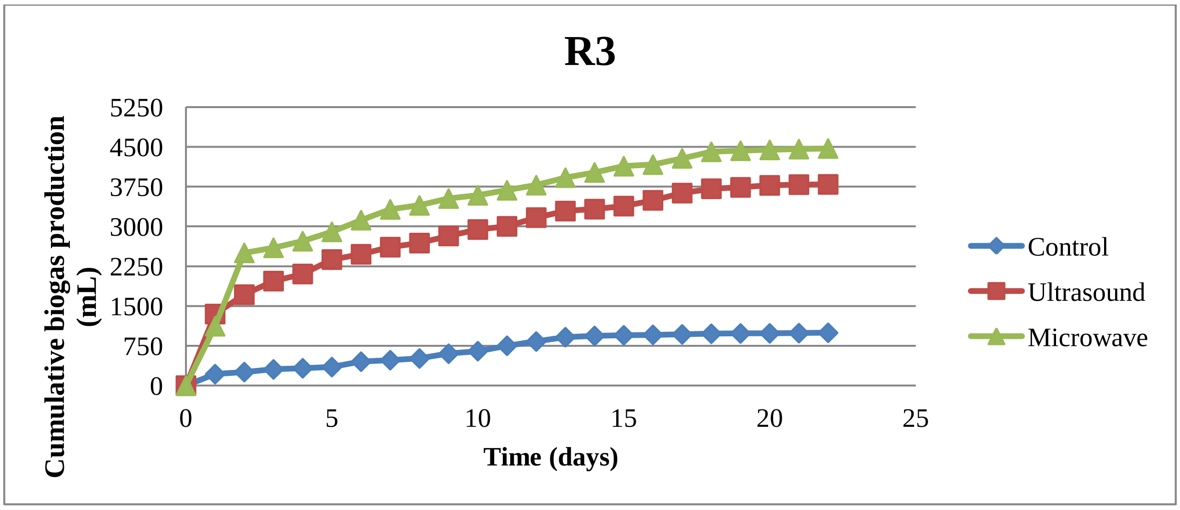
As seen in the Figure 4.49, biogas productions slowed down on the 20th day of the operation and the stabilization period was decided to be completed. It was observed that disintegration applications enhanced the biogas production significantly. The higher gas production occurred in R1 set. This is due to higher solid content of this set of reactors.

Microwave irradiation was the most effective disintegration application in terms of biogas production, other than R2 set. In that set of reactors, conversion of primary substrates to methane and carbon dioxide was achieved even in the control reactor. It may be suggested that sludge sample taken from Bahçeşehir WWTP contains readily biodegradable materials for methanogens to consume, without the help of the cell lysis achieved by disintegration applications. On the other hand, obtaining high biogas production is dependent on the many conditions and these related conditions may be reached an optimum degree for methanogenic microorganisms. One more explanation of this condition is that; pretreatment applications may have failed to satisfy the expected enhancement of biogas production in R2 set.

Cumulative biogas production trends of R3 and R4 sets are quite similar; sonication and microwave irradiation increased the gas conversion considerably. However, microwave irradiation reached a higher level of production. Eskicioglu et al.,(2006) also stated that there was an advantage provided to anaerobic digestion by thermal treatment of waste activated sludge since pretreated supernatants resulted in higher substrate stabilization per volume reactor per unit time, hence more efficient digestion. For the effects of sonication applications on biogas production Bougrier et al. (2006) reported that cumulative biogas production increased in the reactor fed with sonicated sludge in comparison with the reference reactor, and gain was approximately 26%.


R1
Cumulative biogas production (mL)
8750
7500
6250
5000
3750
2500
1250
0
0
5
10
15
20
25
Time (days)
Control
Ultrasound
Microwave


R2
Cumulative biogas production (mL)
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Time (days)
Control
Ultrasound
Microwave


R3
Cumulative biogas production (mL)
5250
4500
3750
3000
2250
1500
750
0
0
5
10
15
20
25
Time (days)
Control
Ultrasound
Microwave

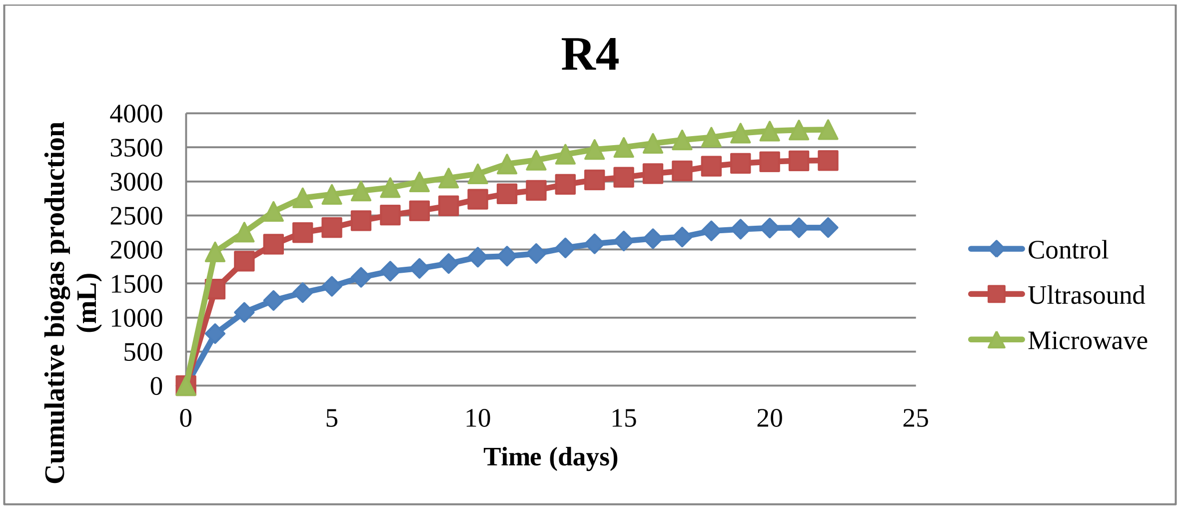
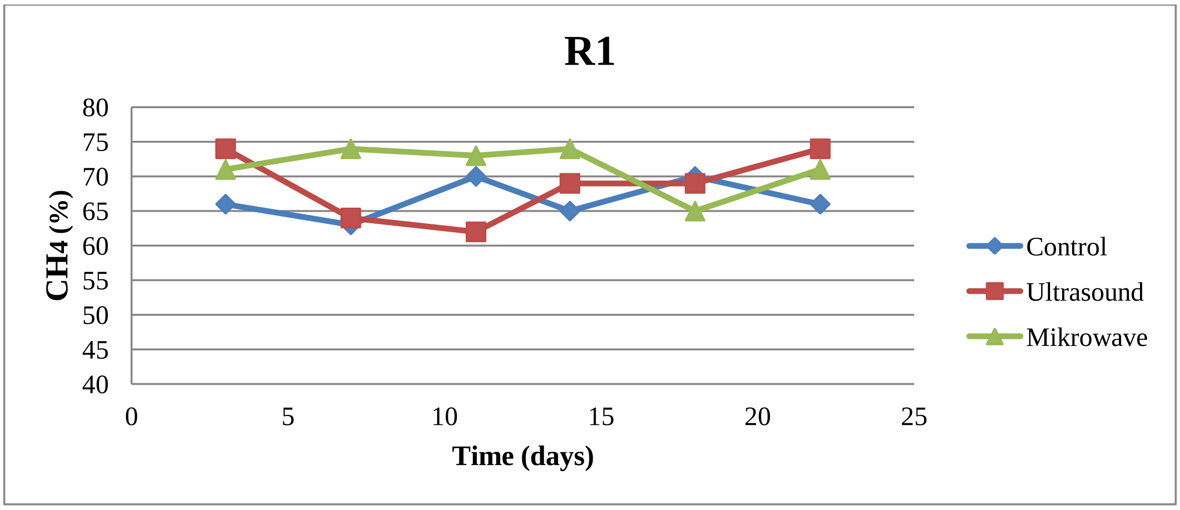


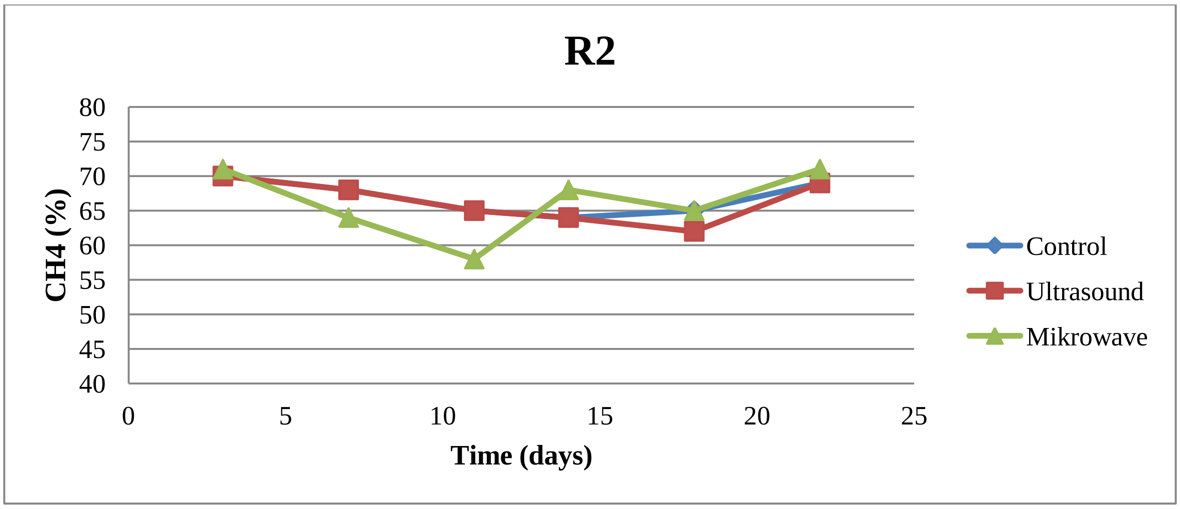
Figure 4.49 Cumulative biogas productions of anaerobic digestion reactors.

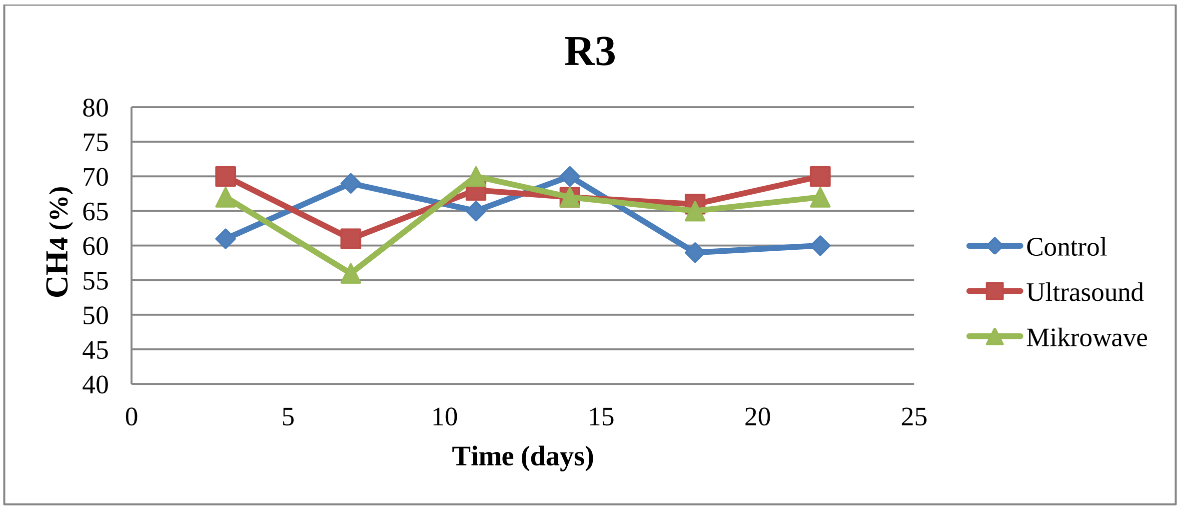
4.2.16.2. Methane Percentage

Produced biogas is mainly comprised of methane (CH_4) and carbon dioxide (CO_2). There are also some negligible amounts of nitrogen (N_2), hydrogen (H_2), hydrogen sulfide (H_2S) and oxygen (O_2). The most important one of these gases is methane since it can be replaced by fossil fuels to obtain energy. Typically in a successfully operated anaerobic system, 50-75% of biogas is expected to be methane. Therefore, to determine the quality of the system, methane content of the biogas analyzed twice a week and the results are shown in Figure 4.50.

Throughout the study, methane content of the biogas did not drop below 56% in any of the reactors. The higher methane percentage reached was 77% in R4-C near the end of the operation. The results show that in each reactor a successful anaerobic digestion took place. Since the total gas productions of disintegrated reactors are higher, consequently, obtained methane amounts are higher than control reactors. However, no difference in methane content of the produced biogas resulted from the pretreatment applications was observed.


R1
CH4 (%)
80
75
70
65
60
55
50
45
40
0
5
10
15
20
25
Time (days)
Control
Ultrasound
Mikrowave


R2
CH4 (%)
80
75
70
65
60
55
50
45
40
0
5
10
15
20
25
Time (days)
Control
Ultrasound
Mikrowave


R3
CH4 (%)
80
75
70
65
60
55
50
45
40
0
5
10
15
20
25
Time (days)
Control
Ultrasound
Mikrowave

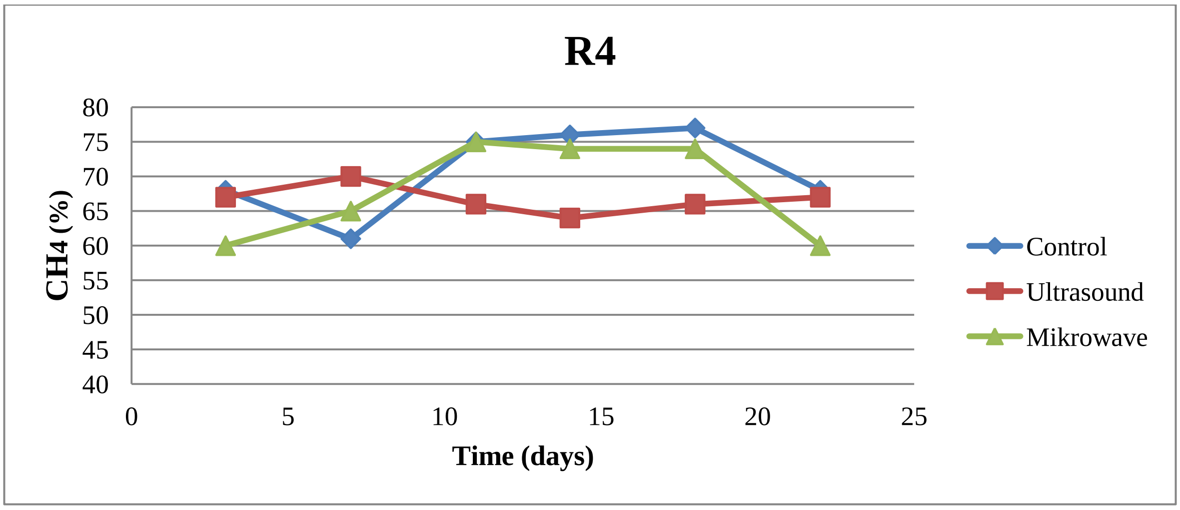


Figure 4.50 Methane percentages of produced biogas in anaerobic digestion reactors.

4.2.16.3. Biogas Yields

Biogas and methane yields obtained in the reactors are shown in Figure 4.51 as Lbiogas/ $VS_{removed}$, $LCH_4/VS_{removed}$ and $LCH_4/COD_{removed}$. Main purpose of this representation is to relate the produced biogas with the degraded organic substrate amount to have a clearer idea about the anaerobic digestion system. With biogas yields, it is possible to make comparisons with other anaerobic systems about the efficiency of the stabilization.

Since the biogas production of the R1-M was the highest of all, biogas yields were higher in that reactor. The difference between the COD and VS removal of that reactor and the other two was not that great and therefore biogas yields are significantly higher.

In R2 reactor set, COD and VS removals were enhanced with disintegration methods, as stated in Figure 4.35 and Figure 4.39. However, the amounts of produced biogas were quite similar for all three R3 reactors. Therefore, biogas yield was higher for the control reactor. This means the increased organic content removal did not correspond to biogas production in the system. This may be caused by suppressing effects of various contents present in the sludge matrix to methanogenic microorganisms in pretreated reactors.

In the general sense, disintegration of sludge proved to enhance the biogas production and the biogas yields in the reactors.

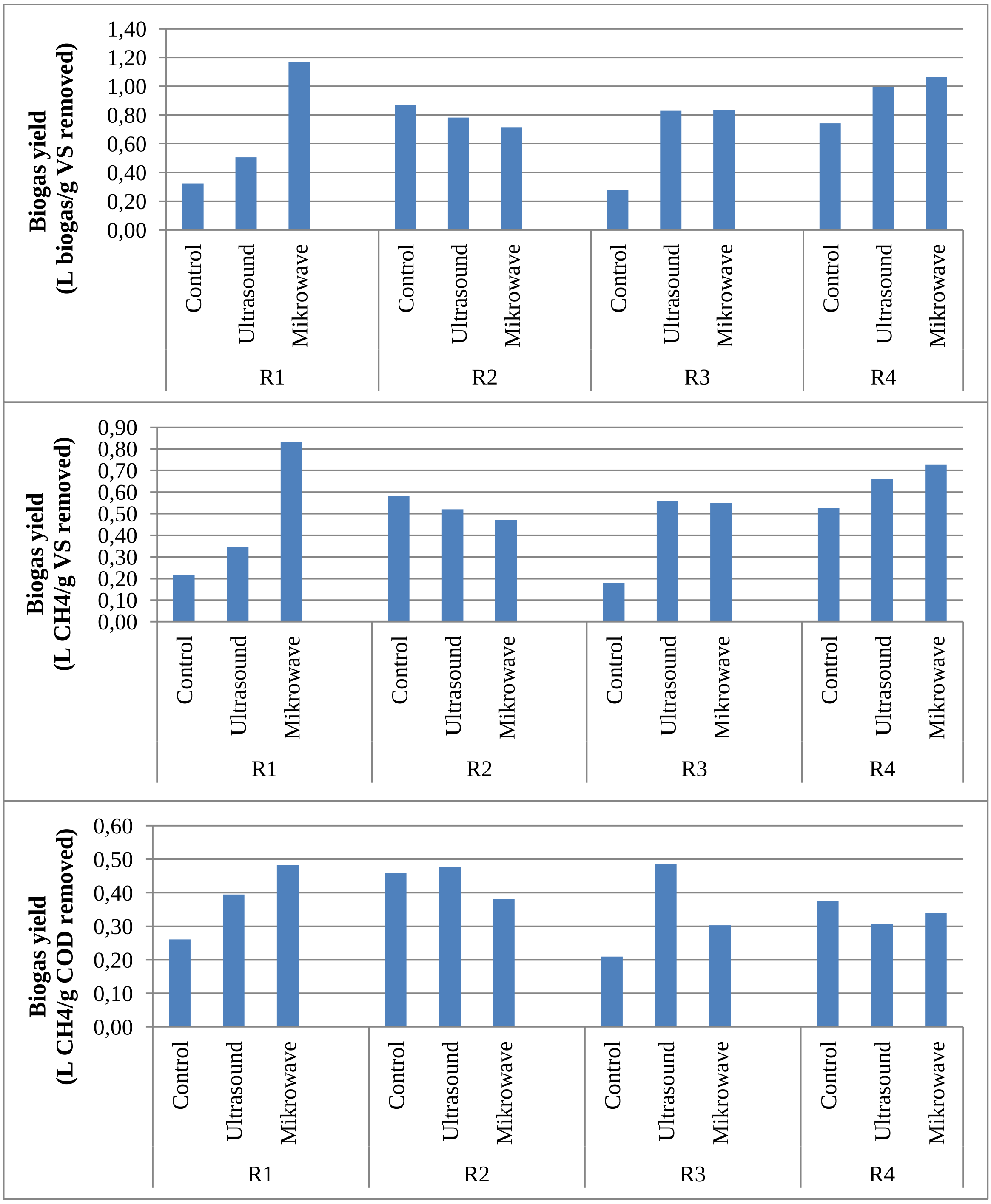


Figure 4.51 Biogas yields of anaerobic digestion reactors.

4.3. Extracellular Polymeric Structure of Sludge

Effects of pretreatment applications on Extracellular Polymeric Structure of the sludge are investigated in this part of the study. For the analyses, a different sludge sample which obtained from İstanbul Paşaköy advanced WWTP was used and the sludge stabilized both aerobically and anaerobically. Two pretreatment methods –ultrasonication and microwave irradiation- were applied to same sludge and three reactors including the control reactor were operated for aerobic digestion and also for anaerobic digestion. The characteristics of the digested sludge and the seed sludge used in anaerobic digestion are shown here in Table 4.24.

Table 4.24 Characteristics of sludge samples used in EPS studies.

Parameter	Unit	Seed Sludge	İstanbul Paşaköy WWTP
TS	mg/L	38331	15565
VS	mg/L	25360	9222
MLSS	mg/L	37250	18950
MLVSS	mg/L	24000	11900
COD	mg/L	38470	16750
sCOD	mg/L	5154	115
TKN	mg/L	870	2340
NH_4^+	mg/L	294	316.0
NH_3-N	mg/L	312	246.0
NH_3	mg/L	278	298.0
TP	mg/L	430	410
PO_4^{-3}-P	mg/L	1310	1260
SO_4^{-2}	mg/L	110	5
Cl^-	mg/L	602	142
CST	s	>1000	51.3
Alkalinity	mg $CaCO_3$/L	5302.5	1155
pH	-	7.3	7.2
Conductivity	mS/cm	20.1	32
Salinity	‰	14	1.8
Total Coliform	CFU/100mL	$1x10^6$	$7.5x10^7$
Fecal Coliform	CFU/100mL	$5x10^5$	$4.6x10^7$
Fecal Streptococci	CFU/100mL	$1x10^5$	$1.8x10^7$
Salmonella	CFU/100ml	$8x10^4$	$3.3x10^5$

Aerobic digestion of the sludge samples were performed in 5-L batch reactors, each having an active volume of 4 L. The sludge samples were stored in a refrigerator at $4^{0}C$ prior to use and their TS content were adjusted to 1% before experiments. Air was distributed using a diffuser and the dissolved oxygen concentrations in the reactors were kept above 2 mg/L at all times. Aerobic digestion was operated for 20 days.

The anaerobic digestion was performed in 2.5-L amber colored batch reactors each having an active volume of 1.6 L at mesophilic conditions. Inoculum/substrate ratio was adjusted to 1:3 for the digestion process and the reactors were operated for 17 days till the biogas productions were almost stopped. After loading and sealing of the reactors, nitrogen gas was purged for 1 minute in order to displace the oxygen from the system and all reactors were placed in water baths having a constant temperature of $37^{0}C$. Reactors were shaken daily by hand to prevent gravitational settling, hindering the microbial activity. Abbreviations used to describe the reactors are given in Table 4.25. Contents of the reactors are given in Table 4.26.

Table 4.25 Abbreviations used to describe the aerobic/anaerobic digestion reactors.

Reactor	Aerobic Digestion	Anaerobic Digestion
Control	AE-C	AN-C
Pretreated by Ultrasound	AE-U	AN-U
Pretreated by Microwave	AE-M	AN-M

Table 4.26 Operational details of the batch digestion reactors.

Reactor	İstanbul Paşaköy Raw (ml)	Pretreated with Ultrasound (ml)	Pretreated with Microwave (ml)	Inoculum (ml)
AE-C	4000	-	-	-
AE-U	3000	1000	-	-
AE-M	3000	-	1000	-
AN-C	1200	-	-	400
AN-U	900	300	-	400
AN-M	900	-	300	400

Disintegration methods were also applied with the same specific energy as in previously described. The specific energy of ultrasonication was 15000 kJ/kg TS with the dose adjusted to 200 W and 70% amplitude. Details of the ultrasonic pretreatment are given in Table 4.27.

Table 4.27 Details of ultrasonication application for aerobic/anaerobic digestion.

Pretreated Sludge	Initial Temperature (^{0}C)	Final Temperature (^{0}C)	Applied Ultrasonic Energy (kJ)	Applied Volume of Sapmle (ml)
AE-U	11.8	16.9	98.790	400
AE-U	13.2	16.2	121.750	500
AN-U	12.1	15.8	98.957	400
AN-U	12.9	15.9	98.790	400

For microwave disintegration, sludge samples were subjected to temperature of 175°C for 10 minutes using Berghoff MWS-3+ device. Disintegration degrees of the pretreated sludge samples are given in Table 4.28.

Table 4.28 Disintegration degrees of pretreated sludge samples.

Treatment Plant	Reactor	DD (%)
İstanbul Paşaköy WWTP Aerobic Digestion	Pretreated by Ultrasound	36
	Pretreated by Microwave	30
İstanbul Paşaköy WWTP Anaerobic Digestion	Pretreated by Ultrasound	25
	Pretreated by Microwave	31

In this part of the study, mainly the EPS content of the sludge is investigated and therefore other analyses were conducted in order to control the quality of the stabilization process. Frequency of the analyses is explained in Table 4.29.

Table 4.29 Analyses frequency of aerobic/anaerobic digestion operations.

Parameter	Analysis Frequency	
	Aerobic Digesiton	**Anaerobic Digestion**
Temperature	Every day	On the 1st and 17th days
pH	Every day	On the 1st and 17th days
ORP	Every day	On the 1st and 17th days
TS	Every 5 days	On the 1st and 17th days
VS	Every 5 days	On the 1st and 17th days
MLSS	Every 5 days	On the 1st and 17th days
MLVSS	Every 5 days	On the 1st and 17th days
COD	2 Times in a Week	On the 1st and 17th days
sCOD	2 Times in a Week	On the 1st and 17th days
CST	Once in a Week	-
Gas Chromotography	-	Once in a Week
EPS	Once in a Week	On the 1st and 17th days

4.3.1. Temperature and pH

Aerobic reactors were operated under room temperature whereas temperatures of the anaerobic digestion reactors were kept at 37°C, mesophilic temperature during the operation of anaerobic digestion in order to maintain the microbial activity. Temperature change in aerobic reactors is shown in Figure 4.52.

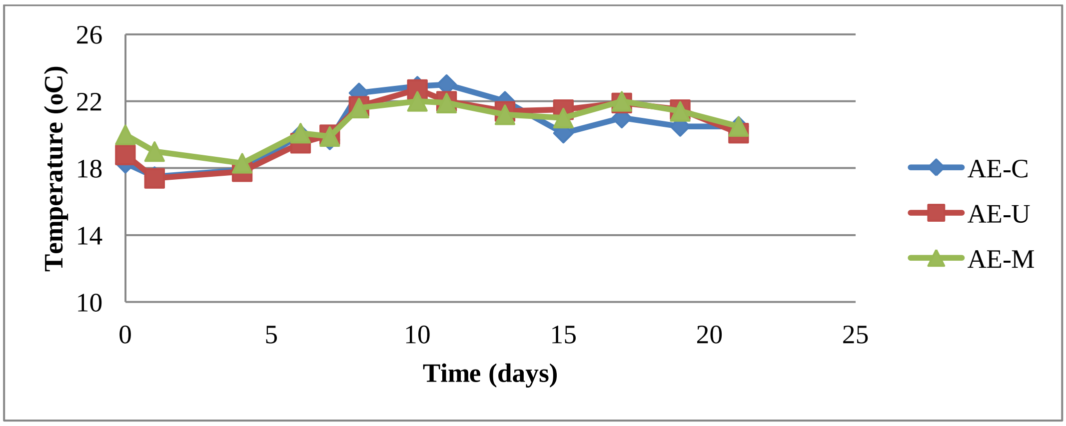


Figure 4.52 Temperature change in aerobic digestion reactors.

At the end of the first week of the aerobic stabilization period, temperature of the all three reactors elevated up to 23^{o}C and the final temperature of the reactors were about 20.5^{o}C. This increase in temperature after a few days of the operation was also observed in Chapter 4.1.1 and this condition is believed to be related with the maximum microbial activity.

pH measurements of the reactors are given in Figure 4.53. Aerobic stabilization systems have a wider pH spectrum than anaerobic systems since the aerobic microorganisms are not as sensitive as different group of anaerobic microorganisms. In other words, bigger pH variances occurred in aerobic systems is tolerable. In aerobic reactors, pH varied between 6.3 and 8.5 and the trend of pH changes was similar for all three reactors. In AN reactors, pH values of each reactor were almost the same. A little increase of acidity was observed after the digestion, which is expected for anaerobic systems.

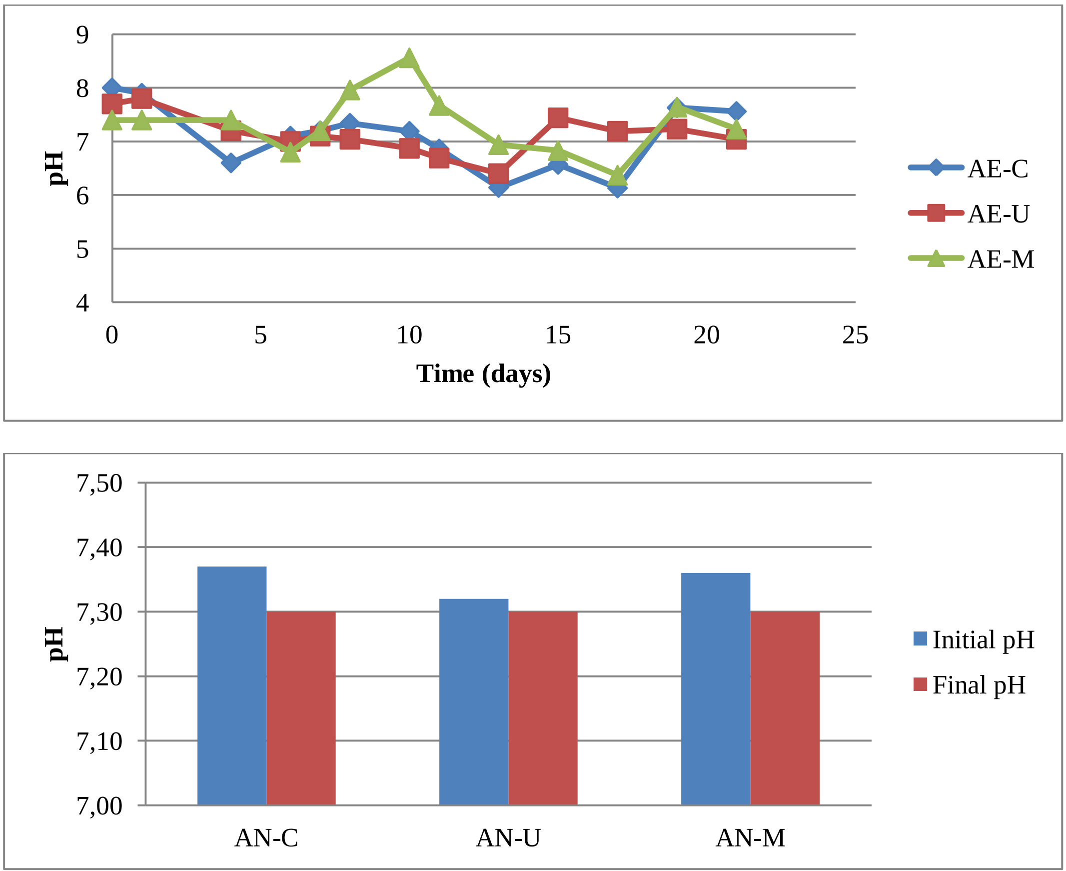


Figure 4.53 pH change in aerobic and anaerobic digestion reactors.

4.3.2. Oxidation Reduction Potential

ORP data was analyzed in order to check the sufficiency of the dissolved oxygen in the aerobic reactors and make sure that the anaerobic conditions remained in the anaerobic digestion process. ORP results of the reactors are shown in Figure 4.54.

An increase in the ORP value was observed during the first few days of the aerobic digestion with aeration of the reactors. A certain drop of ORP occurred in AE-M reactor on Day8 which was caused by a malfunction of aeration device. However, the problem fixed immediately and ORP rose again. Final ORP values of the aerobic reactors were around 150 mV.

In the anaerobic reactors, initial ORP values were between -230 and -250 mV whereas after the digestion ORPs were lower and ranged between -240 and -265 mV. That means the system was operated under fully anaerobic conditions.

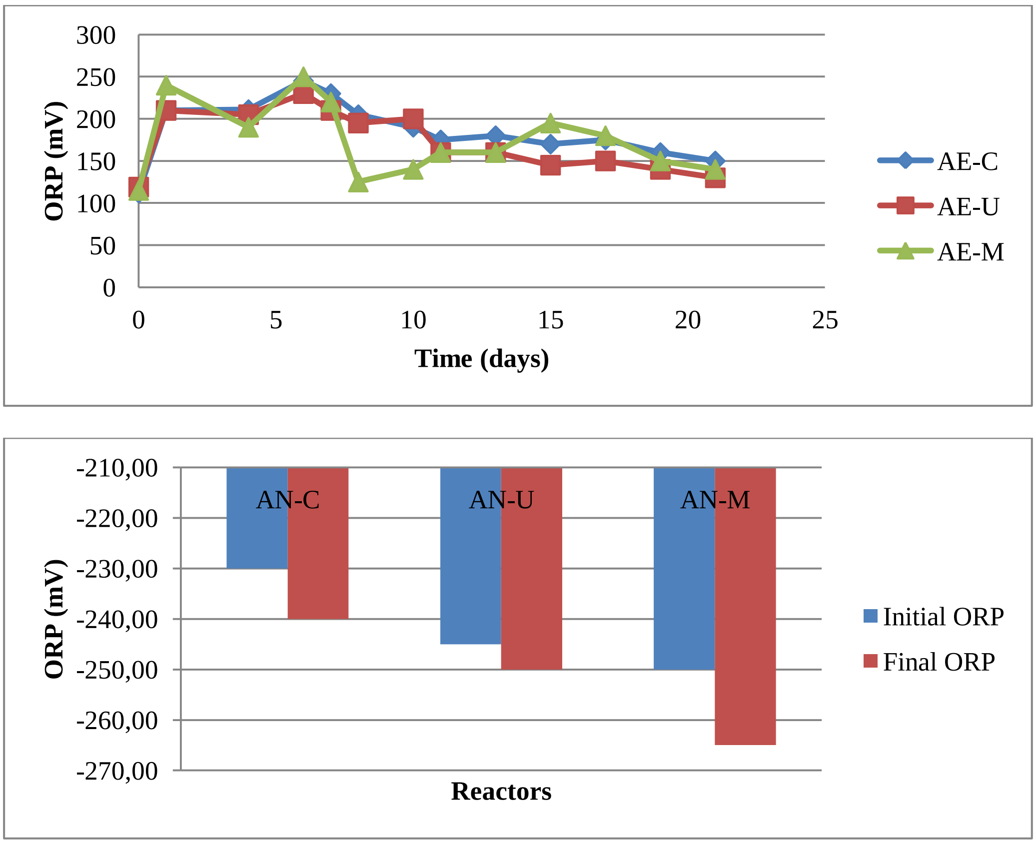


Figure 4.54 ORP change in aerobic and anaerobic digestion reactors.

4.3.3. Total Solids and Volatile Solids

The changes in the TS and VS components of the sludge samples are given in Figure 4.55 and**Figure 4.57 VS change in aerobic and anaerobic digestion reactors** Figure 4.57. TS and VS removal trends of the aerobic and anaerobic stabilization processes were similar for both digestion operations. It was observed that ultrasonication and microwave pretreatments enhanced the digestion efficiencies by solubilizing the sludge solids and thereby ease the removal of the TS and VS in all the reactors containing disintegrated sludge samples as given in Figure 4.56 and Figure 4.58. In the aerobic digestion process, TS reduction efficiency increased by 73% and 82% after ultrasonic and microwave pretreatments, respectively. VS removal efficiencies were slightly higher for the aerobically stabilized reactors containing pretreated sludge; 80% and 85% increase in VS reduction efficiency was observed for ultrasound and microwave applications, respectively.

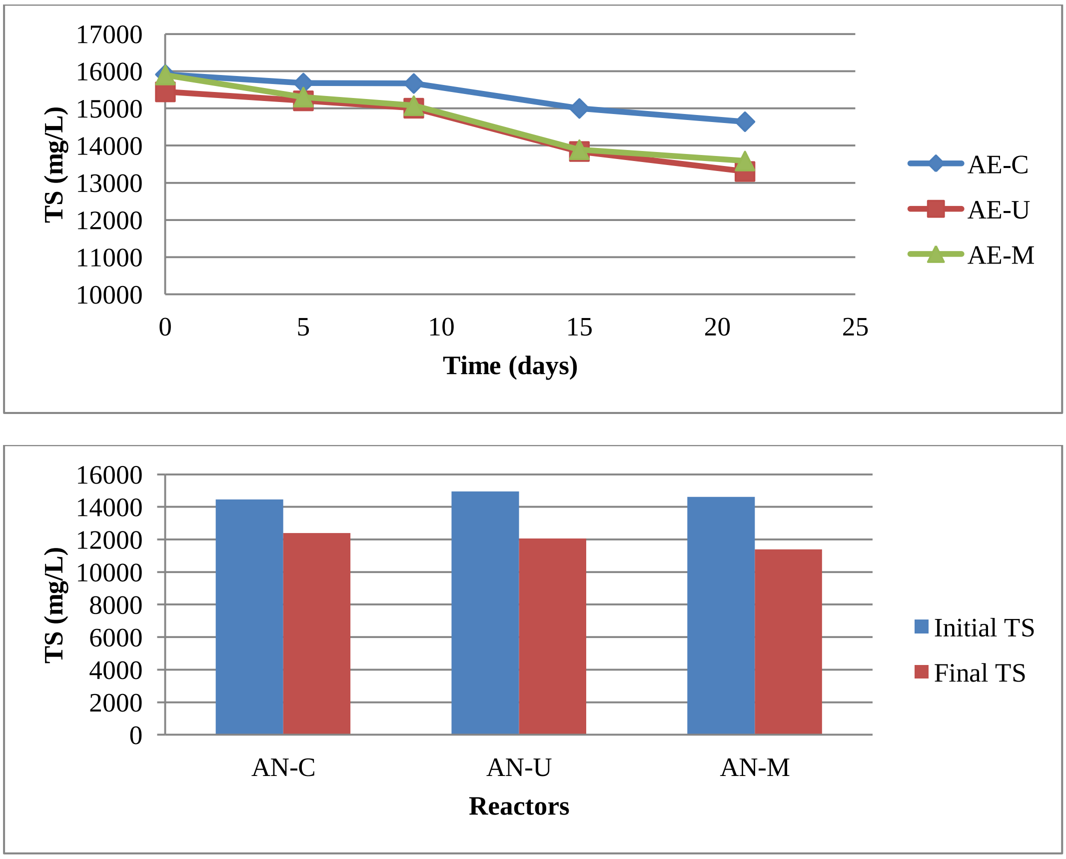


Figure 4.55 TS change in aerobic and anaerobic digestion reactors.

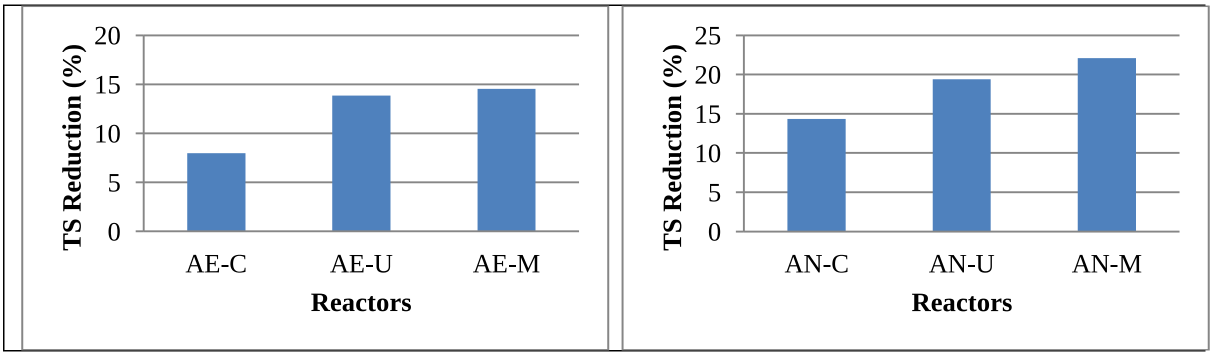


Figure 4.56 TS reductions in aerobic and anaerobic digestion reactors.

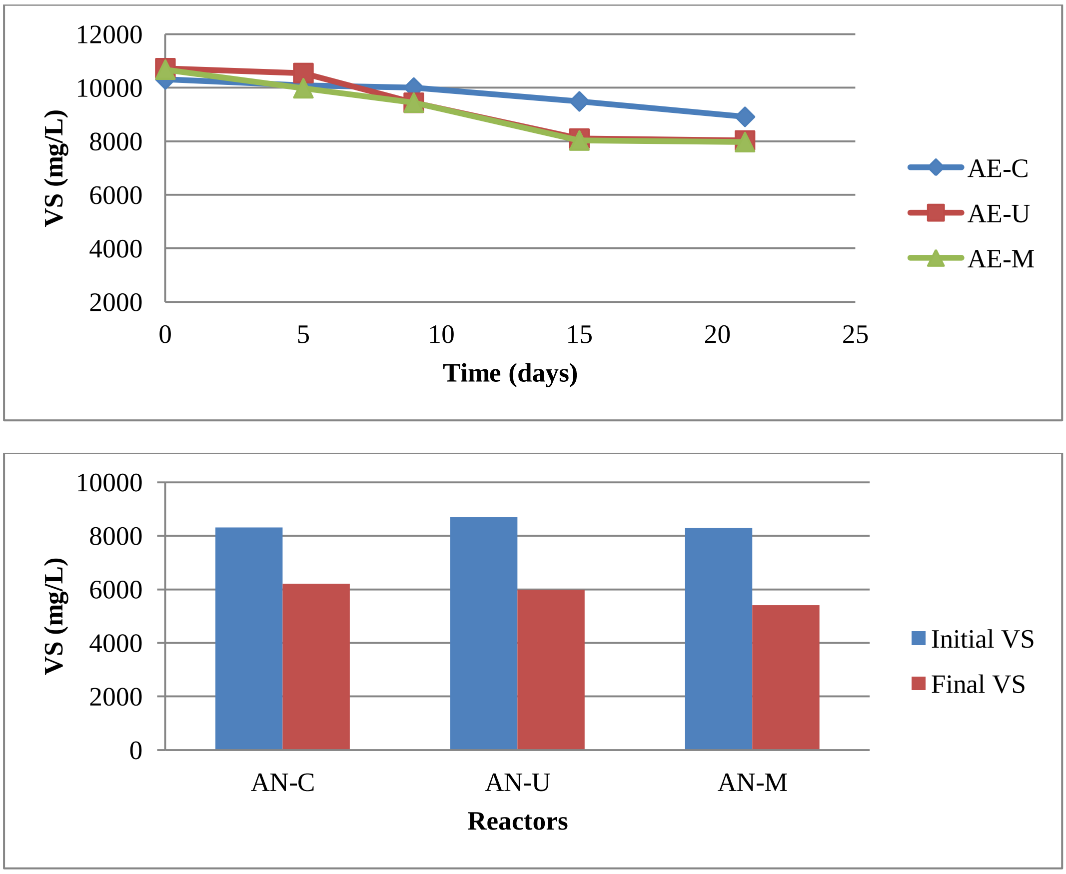


Figure 4.57 VS change in aerobic and anaerobic digestion reactors.

When compared the TS and VS reductions of the aerobically and anaerobically digested sludge samples, it was observed that the anaerobic digestion process had a better performance in solids and organic removal. Therefore, the positive effect of the disintegration applications for TS and VS removal during the anaerobic digestion process is not as apparent as for the aerobic digestion reactors. Still, TS removal efficiency increased by 35% and 54% after ultrasonic and microwave pretreatments, respectively; whereas, VS reduction efficiencies increased by 23% after ultrasonication and by 37% after microwave irradiation applications.

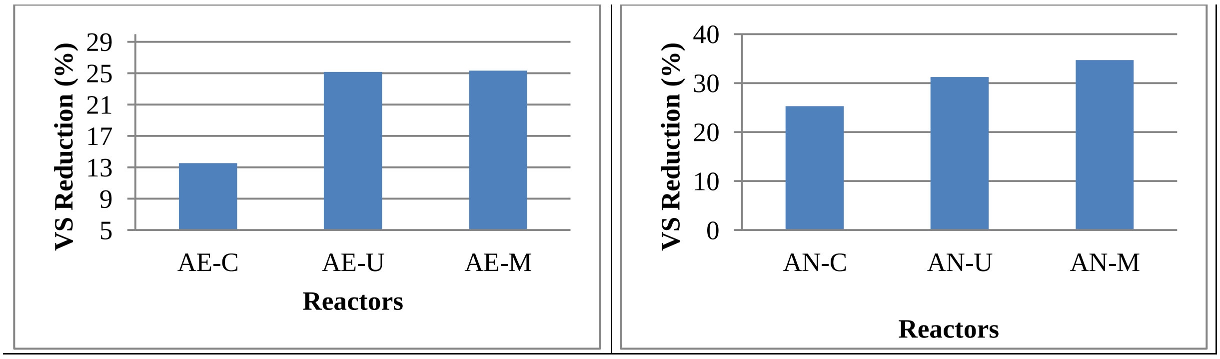
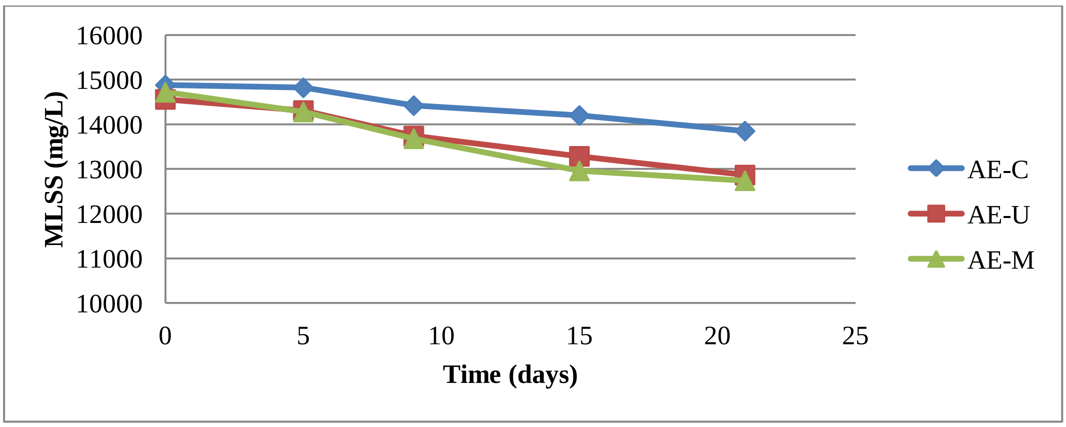


Figure 4.58 VS reductions in aerobic and anaerobic digestion reactors.

4.3.4. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

MLSS and MLVSS analyses were mainly conducted in order to be able to measure the EPS contents of the samples. The results of the analyses are shown in Figure 4.59, Figure 4.60, Figure 4.61 and Figure 4.62.



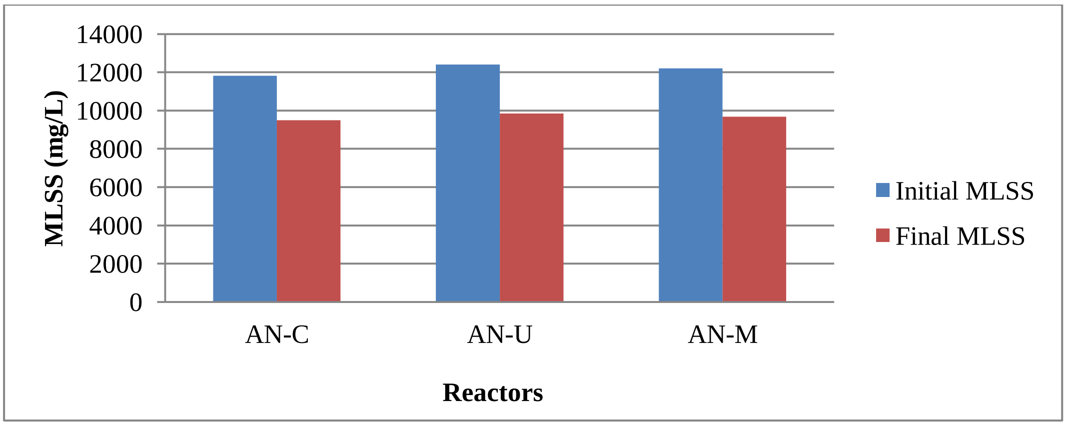


Figure 4.59 MLSS change in aerobic and anaerobic digestion reactors.

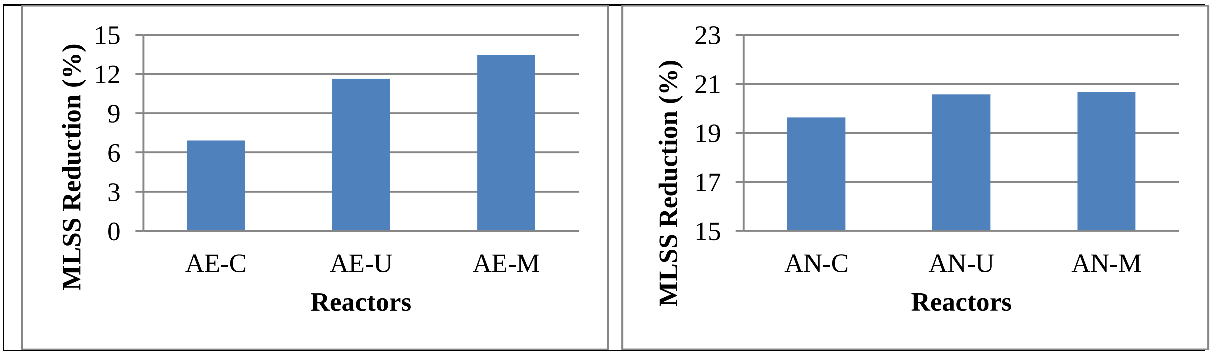


Figure 4.60 MLSS reductions in aerobic and anaerobic digestion reactors.

For these parameters, reduction trends are almost the same with TS and VS values. Only unexpected result was the MLSS reduction efficiency of the AN reactors, which have almost the same efficiencies with control reactor. However this was not the case for MLVSS parameter. Even the MLVSS reductions are also quite similar for all three anaerobic reactors; there was a slight improvement for pretreatment applications. When the values in Figure 4.59 and Figure 4.60 are compared, it was observed that almost all the MLSS removal was removed as MLVSS reduction in the anaerobic system.

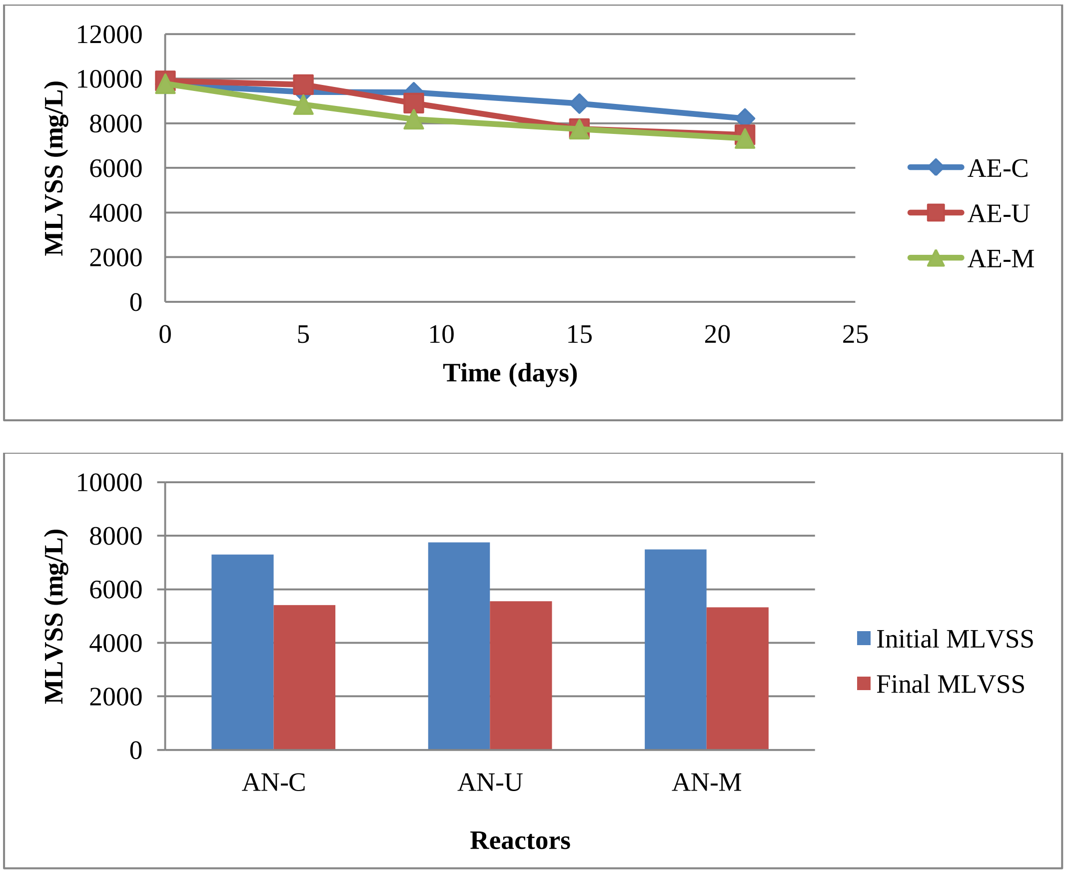


Figure 4.61 MLVSS change in aerobic and anaerobic digestion reactors.

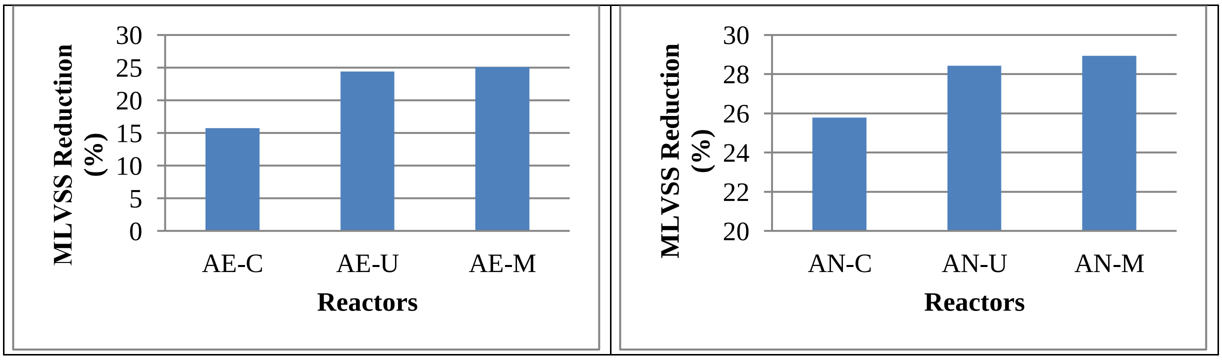


Figure 4.62 MLVSS reductions in aerobic and anaerobic digestion reactors.

4.3.5. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

The COD concentration of the aerobic digestion reactors were analyzed every 5 days. In anaerobic digestion reactors, only initial and final COD concentrations were measured. The COD removal/reduction data is given in Figure 4.63 and

Figure **4.64**.

As the results indicate, disintegration applications led to a higher increase in the COD removal of the anaerobic digestion reactors. When compared to the control reactor, the COD removal efficiencies increased by 35% and 50% in the anaerobic digestion reactors after ultrasonic and microwave pretreatments, respectively. On the other hand, there was a lower increase in the COD removal efficiency of the pretreated sludge samples for aerobic digestion. The difference between two stabilization processes is believed to be caused by the inoculum sludge added to the anaerobic digestion reactors.

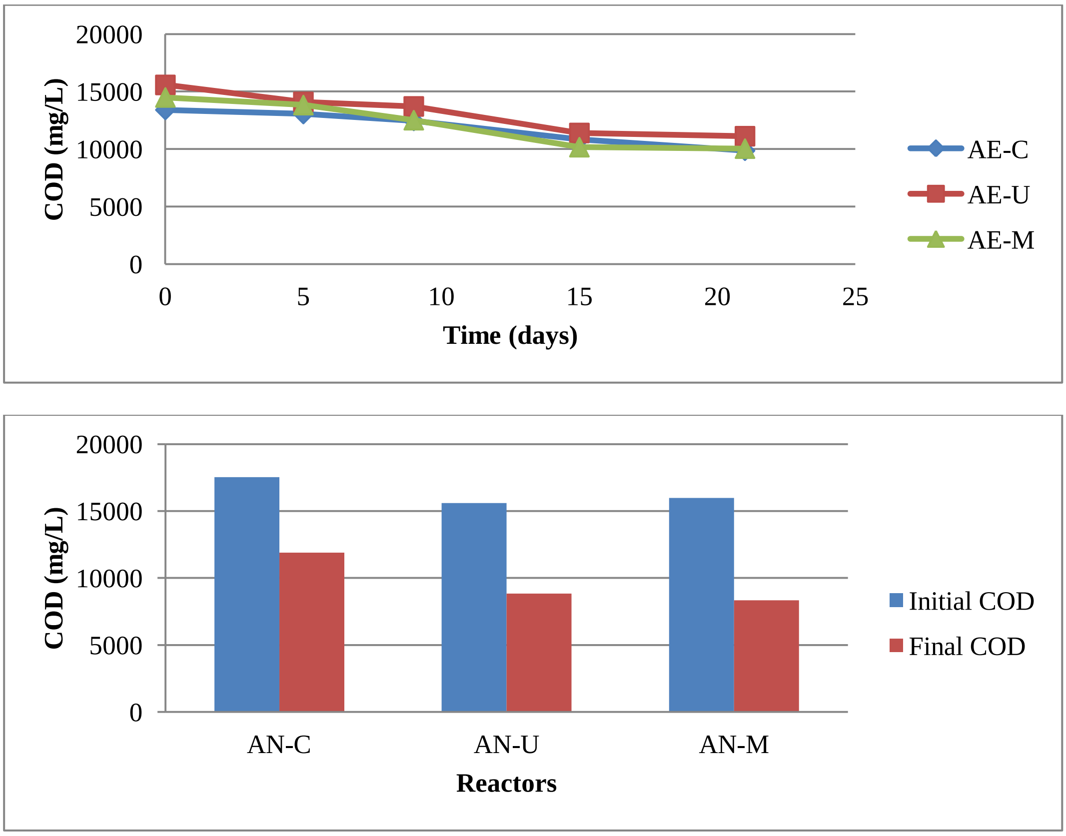


Figure 4.63 COD change in aerobic and anaerobic digestion reactors.

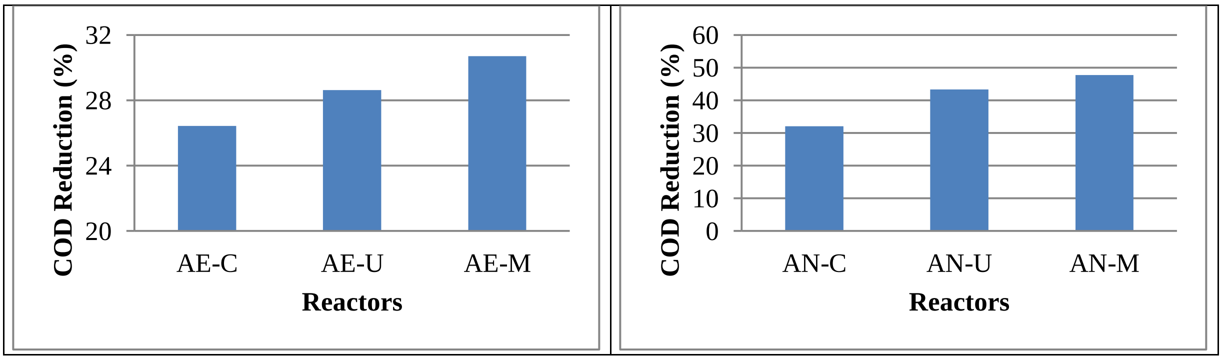
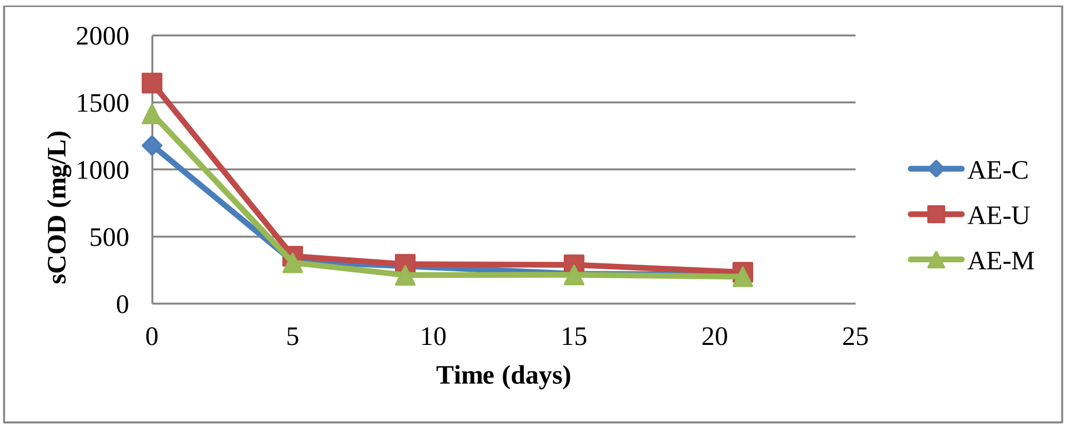


Figure 4.64 COD reductions in aerobic and anaerobic digestion reactors.

Since the initial COD values were higher in the anaerobic digestion reactors, higher solubilization rates were achieved with the applied pretreatments which led to a more apparent positive impact for COD parameter. Through the ultrasonic and microwave pretreatments of the sludge samples of the anaerobic digestion process, 50% and 95% COD solubilization was achieved, respectively. On the other hand, 20% and 39% COD solubilization was observed in the ultrasonicated and microwave applied sludge samples of the aerobic digestion process, respectively. These results indicate that the microwave pretreatment is particularly effective in solubilization of the COD of a sludge sample.



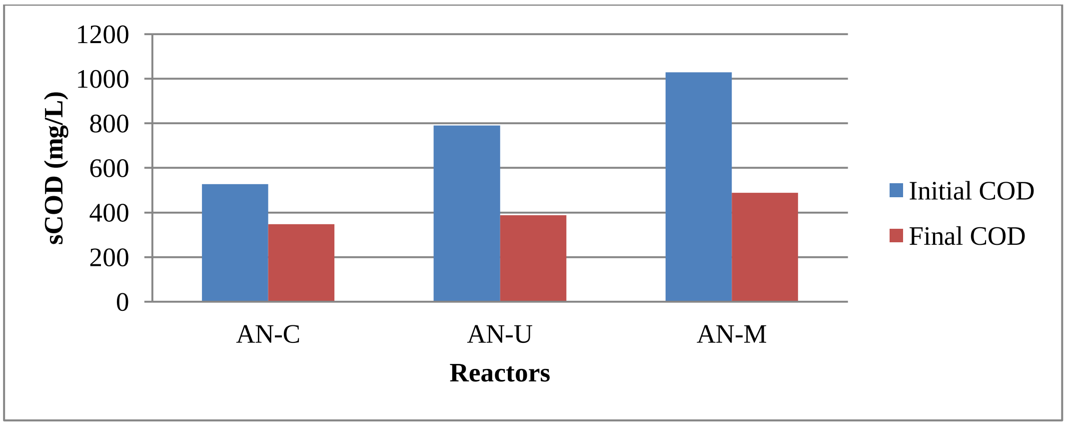


Figure 4.65 sCOD change in aerobic and anaerobic digestion reactors.

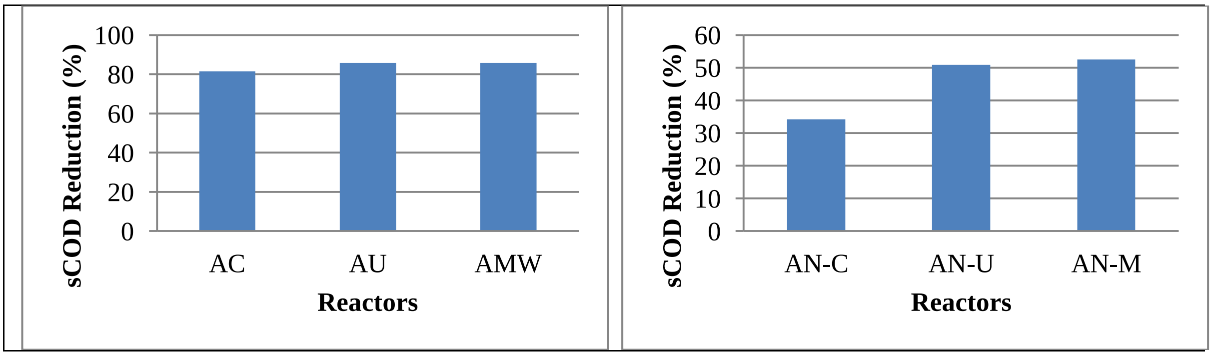


Figure 4.66 sCOD reductions in aerobic and anaerobic digestion reactors.

4.3.6. Capillary Suction Time

CST analyses were conducted only for aerobic reactors since inoculum addition makes the anaerobic sludge inappropriate for CST measurements. Results are given in Figure 4.67.

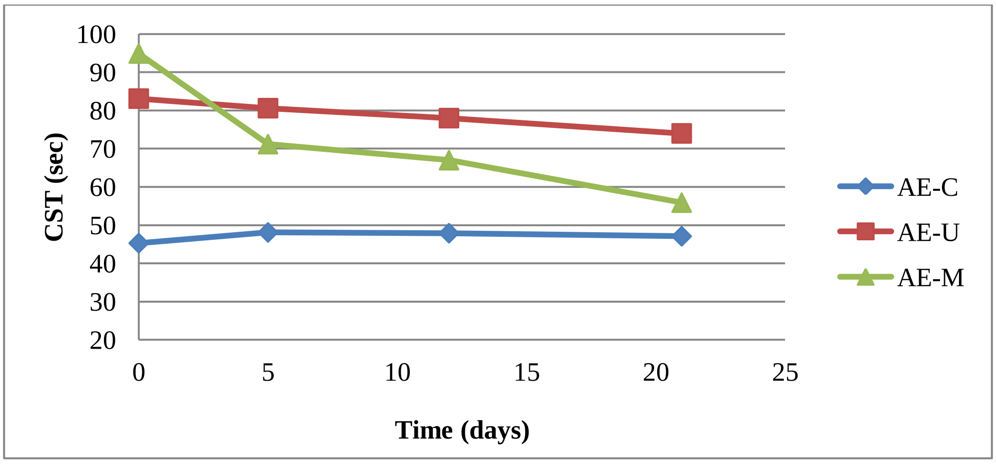


Figure 4.67 CST change in aerobic digestion reactors.

CST is a parameter of sludge filterability since it measures how quickly the sludge releases its water and therefore it is directly used to evaluate sludge dewaterability behavior. Initially, both disintegration techniques increased the CST value of the sludge significantly. However, at the end of the stabilization process CST of the samples decreased at some level. Previous studies also proved that cell lysis techniques worsen the sludge filterability and therefore dewaterability (Braguglia et al.,2012; Muller et al. 2004).

4.3.7. Biogas Analyses

Biogas analyses were conducted for anaerobic reactors and the results are given in the Figure 4.68, Figure 4.69 and Figure 4.70 as cumulative biogas production of the reactors, methane content of the produced biogas and biogas yields in terms of organic removal, respectively.

Daily biogas production of the anaerobic digestion reactors were monitored through wet gas meters. As the Figure 4.68 indicates, microwave treatment significantly improved the biogas production of the sludge sample. It is known that as volatile solids removal increases, biogas production of the system improves. Therefore, from Figure 4.57, this result was expected. However, even the VS removal was apparently enhanced with ultrasonication; this outcome was not sustained with the biogas production of AN-U reactor.

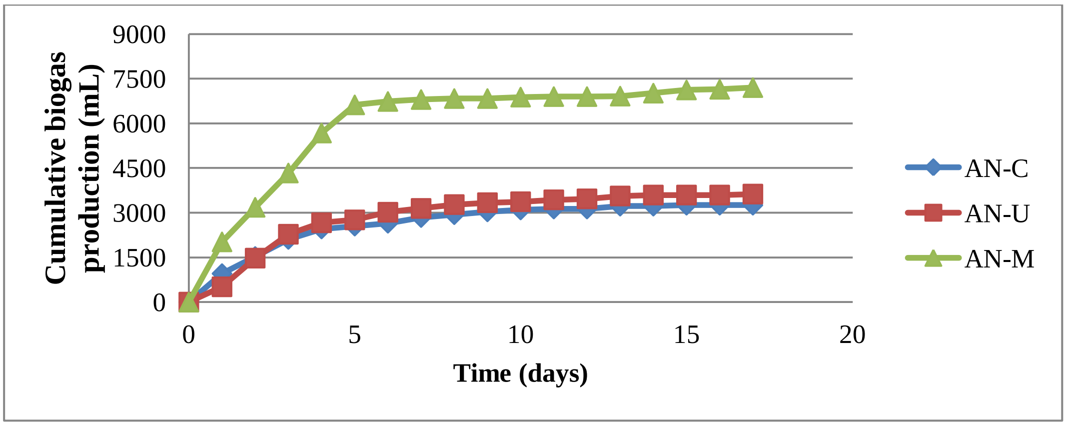


Figure 4.68 Cumulative biogas productions of anaerobic digestion reactors

Methane content of three anaerobic reactors were analyzed after a few days of the set-up, in the middle of the operation period and before terminating the digestion process. As the digestion proceeded, methane percentage of the biogas increased from 55% to 64% on average. This amount of methane in the biogas is referring biogas of a good quality, in other words, a healthy anaerobic digestion system. Pretreatment methods did not seem to have an additional positive effect on methane content of the system.

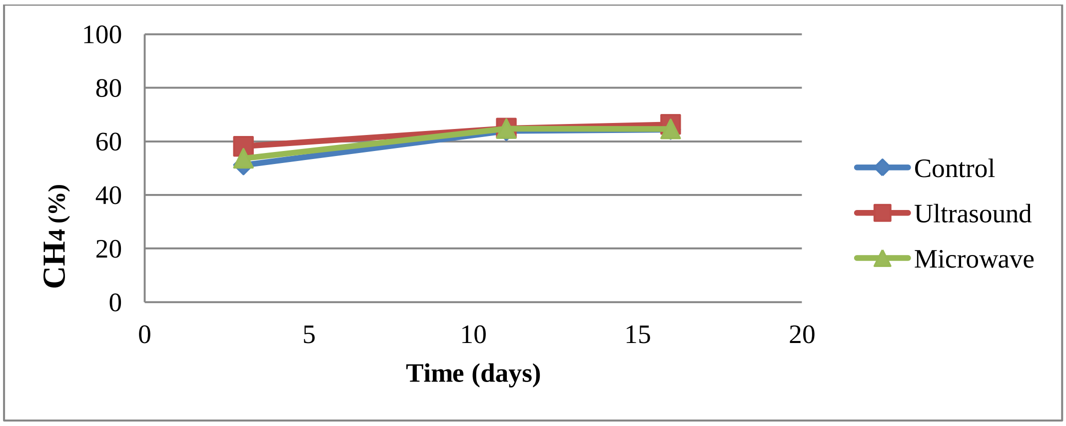


Figure 4.69 Methane percentages of anaerobic digestion reactors.

Figure 4.70 shows the biogas yields in the three reactors. Since the biogas production of AN-U reactor was below the estimations, biogas and methane yields were also lower than expected. Low biogas production counter to enhanced volatile solids and COD reduction of AN-U may have been caused by some inhibitory effect of ammonia, sulfate or chloride ions which may suppressed the methanogenic microorganisms in the reactor.

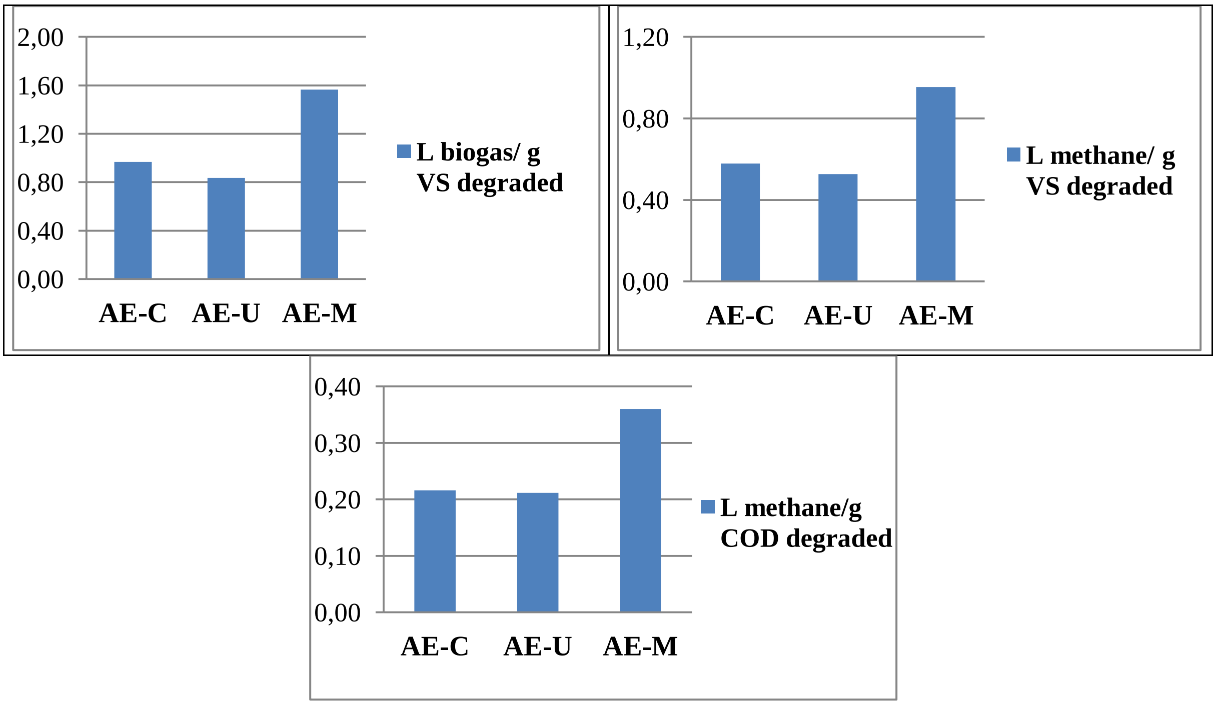


Figure 4.70 Biogas yields of the anaerobic digestion reactors.

4.3.8. Extracellular Polymeric Substances

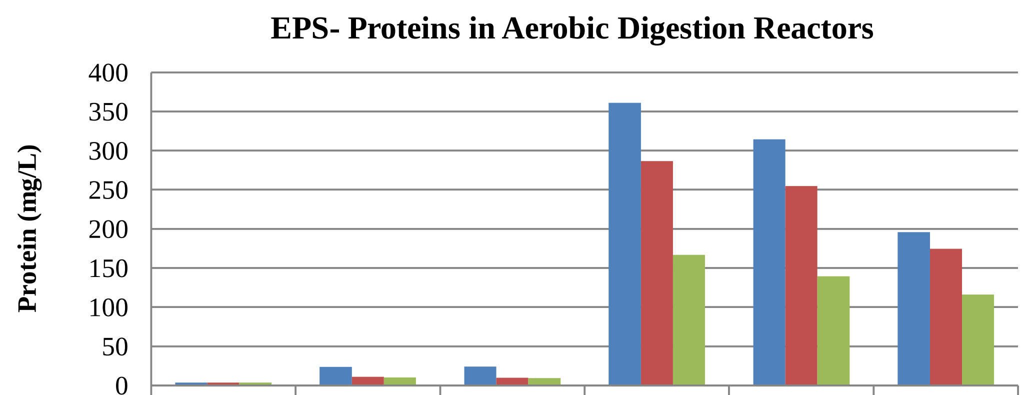
Recent studies in literature proved that sludge is a highly complex structure which comprised of varios groups of microorganisms, organic/inorganic matter agglomerated together in a polymeric network formed by microbial extracellular polymeric substances (EPS) and cations (Eskicioglu et al., 2006). It is believed that the sludge flocs are broken first and then EPS of the sludge flocs is released in to the bulk solution in the hydrolysis and acidification process (Shao et al., 2009). Therefore, accelerating the hydrolysis of EPS within the sludge flocs would enhance the biodegradation of sludge in a shorter period. In order to estimate the impacts of ultrasonication and microwave irradiation on EPS hydrolysis, EPS content of the reactors were analyzed and the results are shown in Figure 4.71 to Figure 4.74.

EPS in the sludge flocs is composed of soluble EPS (sEPS) and bound EPS (bEPS). bEPS can be further categorized as loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). In this study, EPS of the sludge samples were measured in terms of sEPS and bEPS only.

In order to extract the EPS, Cation Exchange Resin (CER) method, which is a modified form of the method described by Frølund et al. (1996), was applied. DOWEX was used as the strongly acidic CER in the study.

The composition of EPS was determined by performing carbohydrate and protein analyses in the extracted EPS samples. Protein and carbohydrate analyses of soluble EPS were conducted from the centrifuged samples at the initial stage of the extraction, and protein and carbohydrate analyses of bound EPS were conducted by using completely extracted samples. EPS content of the aerobic digestion reactors were analyzed once in a week whereas the analysis conducted at the initial and final stage of the anaerobic digestion process.

In Figure 4.71 the amount of total proteins present in the extracted EPS of aerobic digestion reactors is shown. As the results indicate, 530% and 551% solubilization of the proteinaceous part was observed in the reactors containing ultrasonicated and microwave pretreated sludge samples. The removal of soluble protein was also partly achieved in AU and AMW reactors within the first week of digestion, while the soluble protein concentration of the control reactor almost did not change. This suggests that the soluble EPS proteins are hard to be biodegraded below a certain level of concentration.

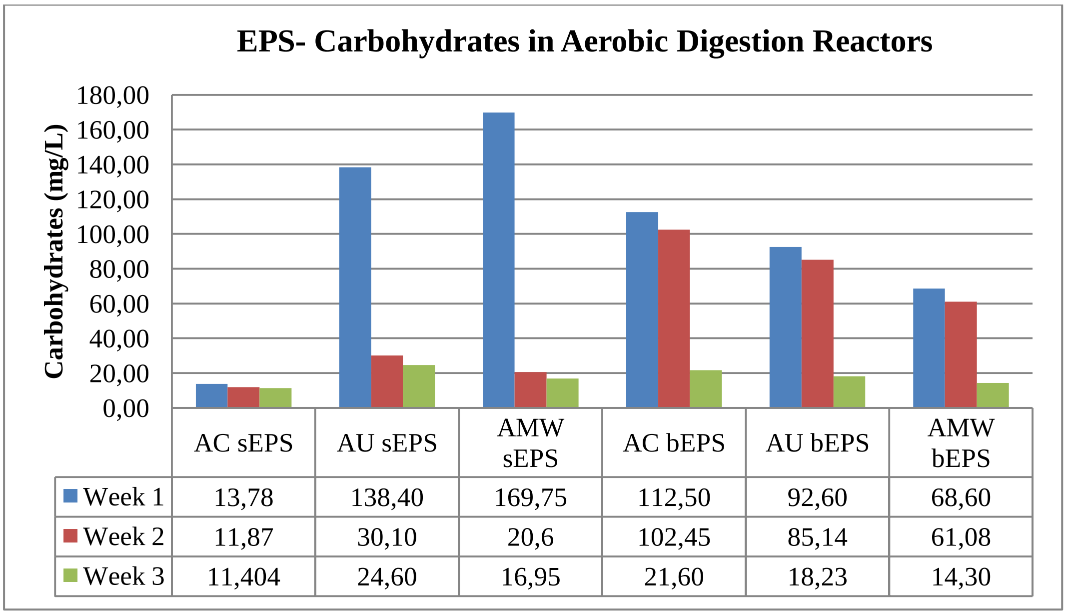


	AC sEPS	AU sEPS	AMW sEPS	AC bEPS	AU bEPS	AMW bEPS
Week 1	3,72	23,46	24,23	361,07	314,45	195,87
Week 2	3,54	10,86	9,87	286,4	254,7	174,6
Week 3	3,47	10,26	9,19	166,83	139,5	116,00

Figure 4.71 Protein concentrations in extracted EPS of aerobic reactors.

Carbohydrates present in the EPS of the sludge seem to be removed easier than proteins as Figure 4.72 indicates. This is possibly because proteins are the most dominant compounds in EPS and the protein concentrations are much higher than carbohydrates. It was also deduced from the results that carbonaceous part of the EPS has a higher tendency for solubilization and biodegradation when compared to proteinaceous part: with ultrasonication and microwave pretreatments 904% and 1131% solubilization of carbohydrates was achieved in the aerobic digestion reactors, respectively. The soluble carbohydrates were also mostly removed within the first days of the aerobic digestion process.

On the other hand, the change in bEPS concentrations implies that the insoluble carbonaceous part of EPS was mostly degraded after a certain period of aerobic digestion, the difference in the carbohydrates concentrations in the bEPS between the second and third weeks of the stabilization process was found to be much higher than the difference between the first two weeks.



	AC sEPS	AU sEPS	AMW sEPS	AC bEPS	AU bEPS	AMW bEPS
Week 1	13,78	138,40	169,75	112,50	92,60	68,60
Week 2	11,87	30,10	20,6	102,45	85,14	61,08
Week 3	11,404	24,60	16,95	21,60	18,23	14,30

Figure 4.72 Carbohydrate concentrations in extracted EPS of aerobic reactors.

As can be seen in the Figure 4.67, sludge dewaterability deteriorated both after ultrasonication and microwave irradiation pretreatments. This is believed to be mainly due to solubilization of EPS as a result of disintegration. Recent studies also showed that EPS structure plays a significant role in the deterioration of sludge dewaterability. Neyens and Baeyens (2003) suggest that there is a level of EPS at which the dewatering process of the sludge is easier. However, as the soluble EPS concentration increases sludge flocculation and consequently sludge dewaterability are reduced through water retention in the sludge matrix (Neyens and Baeyens, 2003).

In this study, specific energy (Es) of 15000 kj/kg TS was applied to the sludge samples for ultrasonic pretreatment, leading to an increase in soluble EPS protein and polysaccharide concentrations. As a result, the CST value of the sludge in the AU reactor increased, indicating the deteriorated dewaterability. Feng et al (2009) also reported similar results and stated that "Ultrasonic disintegration with E_s lower than 1000 kJ/kg TS has a conditioning effect on sludge by improving sludge settleability. However, with increasing Es, sludge settleability is deteriorated due to increase in the soluble EPS concentrations"

Long microwave irradiation periods deteriorate the dewatering of the sludge samples as well. In this study, sludge was irradiated by microwave at 175°C for a duration of 10 minutes. EPS analyses of the sludge samples showed that the proteins and polysaccharides were released into aqueous phase and this led to deterioration of sludge dewatering. Yu et al. (2010) reports that contact time over 120 seconds causes CST value to increase rapidly since complete disruption of the floc structure takes place and this causes the release of intracellular and extracellular materials which deteriorate the sludge dewaterability (Yu et al., 2009).

By both pretreatment applications, EPS of the sludge samples was solubilized prior to the stabilization process. As a result, the CST values of the pretreated sludge were higher than CST value of the unpretreated sample due to the released intra and extracellular materials. As the digestion proceeded, soluble EPS was removed so the CST values gradually decreased in accordance with the study of Shao et al. (2010), who also reported that CST of the sonicated sample have decreased while the CST of the unsonicated sample was gradually increasing (Shao et al., 2010).

Changes in the proteinaceous part of the EPS concentrations of the anaerobic digestion reactors are given in Figure 4.73. 41% and 59% of solubilization were observed in the EPS proteins in ultrasonication and microwave pretreatments, respectively. Soluble protein concentrations in the anaerobic digestion reactors were much higher than the ones in aerobic digestion reactors. This difference is believed to be caused by the characteristics of the seed sludge added to anaerobic reactors. On the other hand, it was observed that bEPS concentrations of the reactors containing the pretreated sludge samples were decreased. Although the bEPS removal efficiencies of the AnC, AnU and AnMW reactors were almost the same (57%, 61% and 60%, respectively), the overall protein removal was enhanced with pretreatment applications.

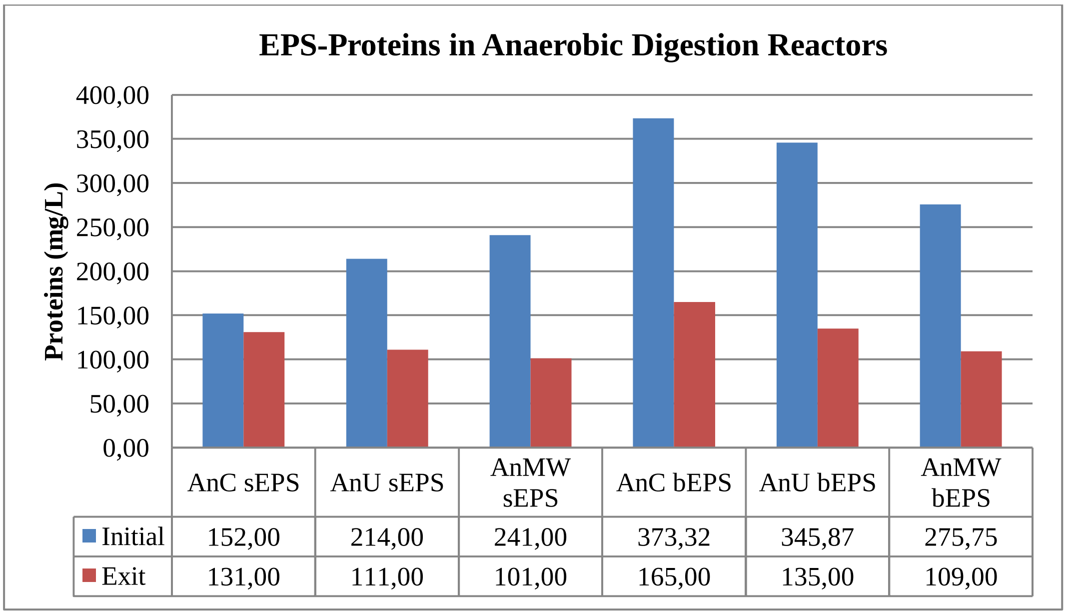


Figure 4.73 Protein concentrations in extracted EPS of anaerobic reactors.

EPS concentrations of the carbonaceous part of the sludge samples before and after the anaerobic digestion process are shown in Figure 4.74. Carbohydrate solubilization of the sludge samples increased by 117% and 149%, after ultrasonication and microwave pretreatments, respectively. When the final sEPS carbohydrate concentrations of the three anaerobic digestion reactors were compared, it was observed that the soluble carbohydrate uptake rate of the aerobically digested sludge samples were higher, irrespective of pretreatment application. That is believed to be caused by the characteristics of the anaerobic microbial community. On the other hand, removal efficiency of the total consumed soluble carbohydrates were highly enhanced by both disintegration applications.

bEPS concentrations of the reactors containing the pretreated sludge samples were decreased as the carbohydrates solubilized. As previously discussed, carbonaceous part of the bEPS was mostly removed after the first two weeks of the aerobic digestion process. The reason behind the lower bEPS carbohydrate removal efficiencies may be either the anaerobic sludge characteristics or the shorter retention time of the anaerobic digestion.

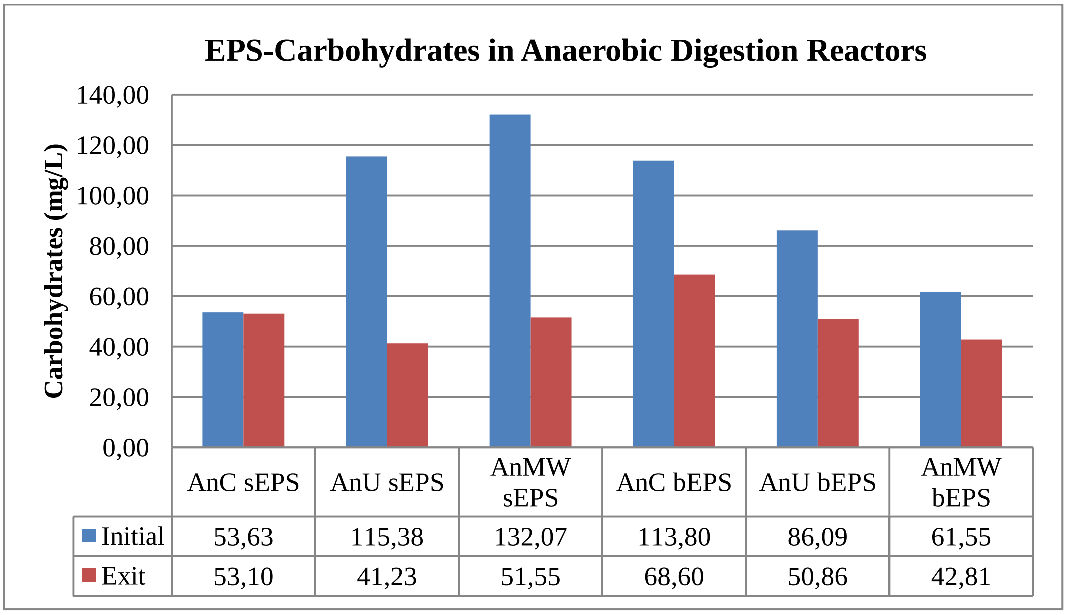


Figure 4.74 Carbohydrate concentrations in extracted EPS of anaerobic reactors.

5. CONCLUSION

This study investigated the effects of ultrasound and microwave pretreatment applications on the digestion efficiency and EPS structure of the sludge. Several conclusions are derived at the end of the work:

- Ultrasound and microwave applications increased the solubility of the COD in the sludge which resulted in the increased rate of hydrolysis and therefore the improved reduction of organic materials. In aerobic digestion, sCOD/COD ratios of the disintegrated sludge samples increased by 182% and 218% for ultrasound and microwave irradiation applications, respectively. In anaerobic digestion, ratios are increased in a lower amount because of the inoculum sludge: sCOD/COD increased by 17% and 23.5% in ultrasonicated and irradiated reactors, respectively.

- Organic content removal was enhanced with both pretreatment applications by the increased solubility of the sludge. At the end of the aerobic digestion, organic solids removal was increased by 10.25% and 13.25% on average for ultrasound and microwave applications respectively, when compared to unpretreated reactors. In anaerobic digestion, volatile solids removal was enhanced by 3.5% and 5.5% on average by ultrasound and microwave irradiation applications, respectively.

- Total sludge volume reduction corresponds to TS reduction analyses in the study. At the end of the aerobic stabilization process, ultrasonicated samples achieved 11.75% more total solid reduction on average, compared to control reactors. The value was higher for microwave irradiation application; on average, 16% more solid reduction was obtained. TS reduction was enhanced by 2.75% and 4% with ultrasound and microwave applications, respectively, when the sludge digested anaerobically.

- Both pretreatment techniques achieved cell lysis and therefore a decrease in particle size and increase in the surface area was observed.

- Right after the disintegration applications CST values of the samples were increased because of the floc destruction, in other words, ultrasonication and microwave applications worsened the sludge filterability, and therefore, sludge dewaterability. However, as the aerobic digestion proceeded and small particles were degraded, CST value decreased with time. At the end of the aerobic digestion, dewatering properties of the disintegrated samples increased compared to initial values. However, when compared to control reactors, it may be derived that sludge dewaterability was not affected much with the applied disintegration methods.

- After aerobic stabilization, the reductions in total coliform (TC), fecal coliform (FC), and fecal streptococci (FS) were about 99.8%, 99.86%, and 99.84%, respectively for microwave disintegration and 99.84%, 99.78%, and 99.92% for ultrasonication. At the end of the anaerobic digestion, the reductions in TC, FC, FS, and salmonella were found to be 99.82%, 99.94%, <99.96%, and 99.96%, respectively for microwave irradiation application and 99.86%, 99.98%, 99.78% and 99.84% for ultrasonic disintegration.

- COD reduction was enhanced in all pretreated reactors at the end of both stabilization process (aerobic and anaerobic digestion) compared to control reactors.

- During the anaerobic digestion process, biogas productions of the pretreated samples were higher. However, microwave irradiation increased the total biogas production significantly. Although the methane contents were not affected remarkably, total methane production was also increased because of the higher gas production.

- Both ultrasonication and microwave pretreatments seem very effective for EPS solubilization during the hydrolysis of the sludge for both aerobic and anaerobic digestion processes. This led to an increase in the total EPS removal efficiency in the reactors containing the pretreated sludge samples. Slightly higher removal efficiencies were obtained in the digestion reactors containing the microwave applied sludge samples.

- In aerobic digestion reactors, protein solubilization was increased by 530% and 551% by ultrasonication and microwave pretreatments, respectively while the results for carbohydrates were 904% and 1131% enhancement of carbohydrate solubilization. In anaerobic digestion reactors, ultrasound application achieved 41% increase in protein solubilization and 117% increase in carbohydrate solubilization when compared to unpretreated sample. The microwave irradiation improved protein solubilisation by 59% and carbohydrate solubilisation by 149% in anaerobic digestion reactors. At the end of both stabilization processes, overall EPS concentration of the sludge was reduced.

- Comparison of the two digestion processes revealed that the soluble EPS uptake in the aerobic digestion reactors was higher since both final soluble protein and carbohydrate concentrations were lower than the final concentrations of the anaerobically digested samples.

- Dewaterability of the aerobic digestion reactors containing the pretreated sludge samples deteriorated after the disintegration applications and this is mainly caused by the release of the intra and extracellular materials. As the digestion proceeded and the soluble materials were consumed by the microorganisms, CST values increased showing the improvement of the sludge dewaterability.

Ultrasonication and microwave irradiation applications are successful pretreatment technologies for sludge minimization and increasing the digestion efficiency. However, this study proved microwave application to be more effective on organic content reduction, biodegradability improvement, biogas production and EPS solubilization of the sludge. Even though it has higher operational costs due to energy need to provide higher temperatures, the system would compensate the elevated costs with the significantly enhanced biogas production for anaerobic digestion systems. For smaller WWTPs aerobic stabilization might be an option. However, a cost analysis seems to be essential to choose the proper disintegration application.

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