EFFECT OF ENZYMATIC PRE-TREATMENT ON AEROBIC AND ANAEROBIC DIGESTION OF WASTEWATER SLUDGES

by

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ABSTRACT

Aerobic and anaerobic digestion processes aim to degrade the organic matter content of the sludge, remove pathogenic microorganisms from sludge, and produce a stabilized sludge. In the last years, pre-treatment of sludge prior to the digestion process gained more importance due to the potential of increasing sludge solubilization and minimization. This study investigates the effect of enzymatic pre-treatment application prior to the aerobic and anaerobic digestion processes of wastewater sludges. For this purpose, sludge samples were obtained from four different urban and domestic wastewater treatment plants. Digestion tests were carried by operating one control reactor and one enzymatically pre-treated reactor for each sludge sample obtained. An enzyme mixture consisted of alpha-amylase, beta glucanase, lipase, protease, and celllulase was added to the sludge samples 0,5% by volume.

The results of the study showed that the enzymatic pre-treatment plays an important role on the performances of the digestion processes. In aerobic digesters, minimization of sludge production was enhanced by the application of enzymatic pre-treatment. Enzyme added reactors showed better TS, VS, MLSS, and MLVSS removals by 43%, 51%, 44%, and 46% reduction in average, compared to the removals of control reactors by 24%, 31%, 28%, and 32% respectively, in average. Enzymes had great impact on the solubilization of large molecular compounds into smaller molecules by the destruction of cell structure. Compared to the control reactors, enzymatically pre-treated reactors showed better performances on the solubilization, which is determined by increased sCOD and DOC concentrations. Enzymatic pre-treatment also affected the release of extracellular polymeric substances from sludge flocs and enzyme addition led to an increase in the protein and carbohydrate contents of sludge. During the digestion process, COD, sCOD, and EPS contents of the sludge samples decreased, where pre-treated reactors showed better removal efficiencies. The dewaterability of sludge samples was deteriorated when they were enzymatically pre-treated at the initial stage. Then, by the aerobic digestion and the degradation of EPS, dewaterability was improved. Similar results were obtained for the anaerobic digesters in terms of sludge minimization and

solubilization. In addition, enzymatic pre-treatment prior to the anaerobic digestion resulted in improved biogas production and increased methane potential. In both digestion processes, removal of pathogens was successfully achieved. At the end of the aerobic digestion, the reductions in TC, FC, and FS were about 99,78%, 99,84%, and 99,82%, respectively. At the end of the anaerobic digestion, the reductions in TC, FC, FS, and salmonella were found to be about 99,82%, 99,96%, <99,99%, and 99,98%, respectively. During the study, both urban and domestic wastewater sludge samples were tested but no relationship was found between the source of the sludge and the digestion process.

ÖZET

Aerobik ve anaerobik bozulma çalışmaları çamurun organik madde içeriğinin giderilmesini, çamurdan patojenik mikroorganizmaların giderilmesini ve stabil bir çamur üretmeyi hedeflerler. Son yıllarda, bozulma çalışmaları öncesi ön-işlem uygulamaları çamurun çözünebilirliğini ve minimizasyonunu arttırdıkları için daha fazla önem kazanmışlardır. Bu çalışma, aerobik ve anaerobik bozulma prosesi öncesi atıksu arıtma çamurlarına enzim ön-işlemi uygulanmasının etkilerini araştırmıştır. Bu amaçla, dört farklı evsel ve kentsel atıksu arıtma tesisinden çamur numuneleri alınmıştır. Bozulma çalışmaları, her farklı tesisten alınan çamur numunesi ile bir kontrol reaktörü ve bir de enzim ön-işlemi uygulanmış reaktör kurularak sürdürülmüştür. Alfa-amilaz, beta glukanaz, lipaz, proteaz ve sellülazdan oluşan bir enzim karışımı, hacimsel olarak 0,5% olmak üzere çamur numunelerine eklenmiştir.

Çalışmanın sonuçları, enzim ön-arıtımın bozulma işlemlerinin performansında önemli bir rol oynadığını göstermiştir.Aerobik reaktörlerde, çamur minimizasyonu enzim ön-işlemi uygulanarak arttırılmıştır. Enzim eklenen reaktörler TKM, UKM, AKM ve UAKM parametleri için sırasıyla ortalama %43, %51, %44 ve %46 giderim elde ederken aynı giderimler kontrol reaktörlerinde %24, %31, %28 ve %32 olarak belirlenmiştir. Enzimler, hücre yapısını parçalayarak büyük moleküler bileşenlerin küçük moleküler bileşenlere çözünebilirliğini arttırılmasında büyük etki göstermiştir. Kontrol reaktörlerine kıyasla, enzim ön-işlemi görmüş çamurların çKOİ ve ÇOK değerleri artarak daha iyi çözünebilirlik gösterdiklerini ortaya koymuşlardır. Enzim ön-işlemi ayrıca hücredışı polimerik maddelerin çamur floklarından serbest bırakılmasını ve çamurun protein ve karbonhidrat içeriğinin artmasını sağlamıştır. Artan zamanla, KOİ, çKOİ ve hücre dışı polimerik maddeler azalmış olup, enzim ön-işlemi uygulanan çamur numuneleri daha iyi giderim verimliliği göstermişlerdir. Çamurun susuzlaştırılabilirliği, enzim eklenmesi ile kötüleşmiş olup, daha sonra bozulma işlemi ve hücre dışı polimerik maddelerin giderimi ile iyileşmiştir. Anaerobik reaktörlerde de minimizasyon ve çözünebilirlik anlamında benzer sonuçlar elde edilmiştir. Ek olarak, toplam biyogaz üretimi ve üretilen biyogazın metan içeriği enzim eklenen reaktörlerde

daha iyi sonuçlar vermiştir. İki bozulma işleminde de, patojenlerin giderimi başarıyla sağlanmıştır. Aerobik bozulma sonunda TK, FK ve FS giderimleri sırasıyla yaklaşık %99,78, %99,84 ve %99,82 iken anaerobik bozulma sonunda TK, FK, FS ve salmonella giderimleri sırasıyla yaklaşık %99,82, %99,96, <%99,99, and %99,98 olarak belirlenmiştir. Çalışmada evsel ve kentsel atıksu arıtma tesislerine ait çamur numuneleri kullanılmış olup, çamurun kaynağı ve de bouzlma işlemi arasında bir bağlantı bulunanamamıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

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)
WWTP	Wastewater Treatment Plant	
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)

1. INTRODUCTION

Biological treatment processes are the most important and commonly used parts of wastewater treatment plants that treat biodegradable wastes of incoming wastewaters. However, application of these processes leads to generation of considerable amount of excess sludge. Gradual increases in population and increased stringency of environmental quality requirements of sludge by legislations are also causing an enormous increase in sludge production.

Sludge minimization is the reduction of the mass of sludge that is produced at the wastewater treatment plants. It aims to minimize the sludge production, reduce the investment and operational costs, and to enhance the performance of the subsequent treatment and ultimate disposal process. Application of pre-treatment (disintegration) techniques is one of the methods of sludge minimization.

Sludge disintegration methods are developed as pre-treatment processes in order to achieve sludge minimization, accelerate the hydrolysis of sludge before the digestion, and to increase the stabilization degree (Bougrier et al., 2005; Weemaes et al., 2001; Erdinçler and Vesilind, 2000). Among the other pre-treatment methods, enzymatic disintegration is one of the most promising, environmentally friendly, and cost-saving methods.

In recent years, new technologies have been introduced to advance the biological decomposition of sludge by aerobic and anaerobic digestion processes. Aerobic and anaerobic digestion processes are the most commonly applied sludge stabilization processes. Biological hydrolysis of sludge with hydrolytic enzymes is one of the methods for the disintegration purpose and appears to be an efficient alternative in this field. It aims cell destruction by using enzymes. Enzymes catalyze the degradation of organic substances in sludge as a function of the substrate. The result of the enzymatic pre-treatment prior to the biological sludge stabilization process is enhanced degradation of macro molecular compounds, EPS, and the

other biological slimes and gels and improved releasing capacity of water (Barjenbruch & Kopplow, 2003; Dey et al., 2006; Roman et al., 2006). Researchers report that the biological hydrolysis of sludges by enzymatic pre-treatment seems to be an effective and cost reducing process for the treatment plants in many respects.

In this study, the effect of enzymatic pre-treatment on aerobic and anaerobic digestion processes was investigated. Improvement of the stabilization processes by enzyme addition was the main purpose. Since single enzymes had limited hydrolytic activity, an enzyme mixture consisting of a variety of hydrolytic enzymes was used during the pre-treatment. Moreover, this study aimed to improve sludge degradation, investigate the effects of enzyme additions on the hydrolysis of particulate organic matters, determine the effects of enzymes on different operation parameters and sludge characteristics, and find a relationship between enzyme addition and extracellular polymeric substances solubilization/degradation. In addition, sludge samples that were taken from both urban and domestic wastewater treatment plants were used in order to find out if there was a relationship between the type of the wastewater treatment plant and the stabilization process.

2. LITERATURE REVIEW

Biological treatment processes are the most important and commonly used parts of wastewater treatment plants that treat biodegradable wastes of incoming wastewaters. However, application of these processes leads to generation of considerable amount of excess sludge. Gradual increases in population and increased stringency of environmental quality requirements of sludge by legislations are also causing an enormous increase in sludge production.

2.1. Sludge Minimization

Sludge minimization is the reduction of the mass of sludge that is produced at the wastewater treatment plants. It aims to minimize the sludge production, reduce the investment and operational costs, and to enhance the performance of the subsequent treatment and ultimate disposal process. Application of pre-treatment (disintegration) techniques is one of the methods of sludge minimization. Sludge minimization technologies can be categorized into two groups based on the stage of the treatment process that minimization takes place. Those main technologies are identified by Perez-Elvira (2006) as given in Table 2.1 (Perez-Elvira, 2006).

Processes in the water line aims to minimize the sludge production before it is produced at the source, and during the biological wastewater treatment rather than the post-treatment of sludge after generation. On the other hand, processes in the sludge line aims to minimize the organic matter in the already produced sludge by enhanced treatment of sludge. Application of pre-treatment techniques prior to stabilization enhances the digestion of sludge by eliminating the effects of rate limiting steps.

Table 2.1. Sludge minimization processes (Perez-Elvira, 2006).

Processes In The Water Line	Processes that reduce the yield coefficient	Lysis cryptic growth Maintenance metabolism Uncoupling metabolism Predation on bacteria
	Processes with low yield coefficient	Aerobic/anaerobic systems
Processes In The Sludge Line	Pre-treatment processes	Physical pre-treatments Chemical pre-treatments Biological pre-treatments Combined pre-treatments
	Modified anaerobic digestion processes	Two-stage AD Temperature phased AD Anoxic gas flotation

Early attempts at sludge minimization focused on long solids retention times within the biological sludge process, and the reduced sludge production was seen as a benefit of extended aeration plants. All of the used technologies utilize one or more of three basic approaches to minimize the amount of waste sludge produced: cell lysis, cyclic oxic environments, and long solids retention time (Roxburgh et al., 2006). Sludge minimization has advantages as minimizing the sludge mass that is produced, optimizing energy use, reducing costs, and reducing carbon footprint.

Sludge reduction in the water line can be achieved by reducing the yield coefficient and by using methods such as extended aeration, cannibal process, lysis cryptic growth, and ozonation. Application of these methods during the wastewater treatment process at the facility leads to a decrease in the sludge production. After the fact approaches are focused on increasing the sludge biodegradability. In the last decade, different pre-treatment technologies have been proposed and tested for primary and secondary sludges generated mostly by

municipal treatment plants (Saha et al., 2011). Sludge contains microbial cells and extracellular polymeric substances (EPS), which are resistant to digestion due to slow and incomplete hydrolysis. To overcome this limitation, recent studies are focused on the disruption the sludge floc structures. Ultrasonication, ozonation, microwave, alkali treatment, enzyme addition are the sludge disintegration techniques that are applied as pre-treatment techniques to the sludge in order to increase biodegradability and thus a decrease in the mass of sludge takes place.

The ideal approach for the sludge minimization is to reduce sludge production during the wastewater treatment process rather than application of a post-treatment (Liu and Tay, 2001; Liu, 2003; Wei et al., 2003). This study focuses on the process that take place in the sludge line, especially biological pre-treatment processes.

2.2. Sludge Disintegration

Sludge disintegration methods are developed as pre-treatment processes in order to achieve sludge minimization, eliminate the rate-limiting step (hydrolysis) before the digestion, and to increase the stabilization degree. (Erdinçler and Vesilind, 2000; Weemaes et al., 2001; Bougrier et al., 2005;).

2.2.1. Sludge Disintegration Mechanisms

Sewage sludge disintegration can be described as the disruption of the sewage sludge's cell structure by the effects of external stresses. Application of a sludge disintegration method prior to the digestion process makes sludge more easily accessible for the degradation by microorganisms in the preceding digestion process.

The goal of pre-treatment application as disintegration of waste sludge is to release the cell content of microorganisms contained in the sludge through disruption of their cell walls for the intensification of the digestion performance (Figure 2.1). When microbial cells undergo

lysis, microbial cell contents are released into the medium and they provide an autochthonous substrate to other cells which they can use in microbial metabolism. This microbial growth due to microbial substrate is called as 'lysis-crypric growth' by Mason and Hamer (1987) and it results in the reduction of the overall biomass production. The released cell content of microorganisms is known as cell lysate, can accelerate digestion. Its benefit lies in faster and better degradation of organic substrates and the equivalent enhancement of biogas production for the anaerobic digestion. By the application of pre-treatment, the bioavailability of sludge can be increased particularly for aerobic and anaerobic bacteria.

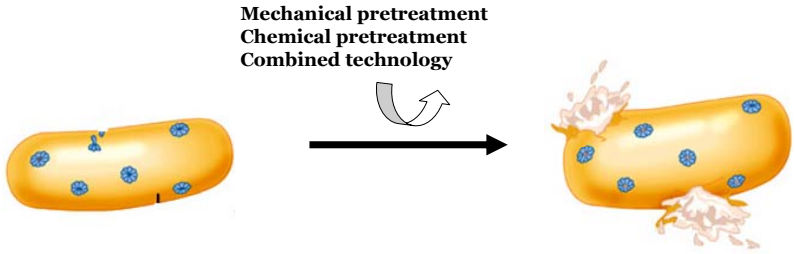


Figure 2.1. Principle of sludge disintegration technology (New World Encyclopedia, 2006).

Disintegration processes change many of the characteristic properties of the sludge (Müller et al., 2004). In a disintegration process, sludge floc structure is deteriorated, cell walls of microorganisms are destroyed, and organic components inside the cell are passed through the liquid phase (Vranitzky et al., 2005). Depending on the degree of stabilization, if a pre-treatment technique was applied prior to the stabilization process, less sludge is produced compared to the conventional digestion processes by decreasing the sludge volume, solids retention time is reduced, more stable sludge is obtained, biogas production and the methane content of the biogas is improved in the anaerobic digestion, and overall economics are reduced (Wang et al., 2005). Depending on the applied pre-treatment, improvement of sludge dewatering, reduction of pathogens, or suppression of foaming can be observed as other benefits of disintegration.

The organic content of a sludge usually measured by using chemical oxygen demand (COD) method. The method basically measures the amount of oxygen needed to oxidize the organic matter in a sludge sample and the results are given as mg O_2/L. The efficiency of a pre-treatment method and the comparison of different pre-treatment techniques can be calculated and defined by determining the disintegration degree. Müller and Pelletier, 1998, initially defined the degree of disintegration and it is expressed as the increase of organic material in the dissolved phase of sludge due to treatment, in relation to overall organic material in the sludge. It is expressed in terms of percentage and calculated by Müller and Pelletier's formula (1998) as given below:

$$DD\ [\%] = (sCODt\text{-}sCODr)/(sCODmax\text{-}sCODr)*100 \quad (1)$$

Where;

DD = Disintegration degree, %

$sCOD_t$ = Soluble COD of the pre-treatment applied sludge, mg O_2/L

$sCOD_r$ = Soluble COD of the raw sludge, mg O_2/L

$sCOD_{max}$ = Maximum soluble COD of the sludge that can be obtained by treating sludge with 1 M NaOH for 24 hours, mg O_2/L

Disintegration degree of sludge is increased by the application of a pre-treatment. Since large organic molecules are destroyed into soluble form and transferred into the medium as a result of pre-treatment, sCOD concentration of sludge is increased. sCOD concentration determines the disintegration degree and as a result, an increase in the sCOD results in increased disintegration degree. The increase in the disintegration degree leads to an increase in the volatile solids reduction efficiency, which is expected after the stabilization process.

2.2.2. Sludge Disintegration Methods

In the recent years, several different techniques are developed for the disintegration purposes. Disintegration of a sludge sample can be achieved by applying physical, chemical

and biological processes to the sludge. Main sludge disintegration (pre-treatment) techniques can be grouped as in Table 2.2.

Table 2.2. Pre-treatment methods.

Mechanical	Chemical	Biological
• Microwave • Freeze and thaw • Ultrasonication	• Fenton Addition • Ozonation • Alkali	• Enzyme treatment

Many minimization methods such as given in Table 2.2 have been reported in previous research studies including acidic or alkali chemical treatment, freeze-thawing, mechanical disintegration using ultrasonic devices, advanced oxidation processes like ozonation, fenton, and biological hydrolysis with enzymatic treatment (Liu and Tay, 2001; Chu et al., 2001; Wei et al., 2003). Those methods are investigated by many researchers in both laboratory and pilot scale with the purpose of sludge disintegration and sludge disintegration methods have been shown to enhance the performance of the digesters (Odegaard, 2004). In addition to the given methods, combination of different pre-treatment techniques with each other can also be applied to the sludge (Müller 2001).

2.2.2.1. Ultrasonication. In an ultrasonic pre-treatment process, sludge structure is disrupted by the generated high shear forces depending on the ultrasonication parameters such as sonication frequency, power, and time. This pre-treatment should be applied by using an ultrasonicator that the ultrasonication time and power can be adjusted. There are two important mechanisms in an ultrasonic pre-treatment: cavitation which benefits at low frequencies and chemical reactions at high frequencies results from $OH^{\cdot}$, $HO_{2}^{\cdot}$, and H radicals (Kim et al., 2010). In contrast to chemical digestion of sewage sludge, application of ultrasonic pre-treatment to the sludge does not require addition or removal of chemicals. However, ultrasonic treatment is an energy-intensive process, which causes great concerns on its practical application (Scheminski et al., 1999; Zhang et al., 2008).

2.2.2.2. Microwave. The application of microwave irradiation for sludge pre-treatment purposes either causes the acceleration of irons and then collide with other molecules or causes dipoles to rotate and line up rapidly with an alternating electric field resulting in a change in secondary and tertiary structure of proteins of microorganisms (Banik et al., 2003). Materials capable of absorbing microwave energy are heated by the microwave irradiation because of the apparent changes in vibrant energy of the molecules. In other words, the alternating electric field caused by microwave irradiation causes continuous repetition of a rapid alignment and realignment of dipoles in a polar solvent generates friction resulting in heat generation. Application of microwave pre-treatment leads to a significant reduction of sludge volume, time, and energy (Yu et al., 2010).

2.2.2.3. Alkali. Addition of strong acids or alkali can also be used for the disintegration purposes. The use of acid or alkali chemicals allows macromolecules such as lipids, hydrocarbons and proteins to hydrolyze into smaller soluble compounds such as aliphatic acids, polysaccharides and amino acids. Hydrochloric acid and sulfuric acid can be used as acidic reagents however alkaline pre-treatment with lime and sodium hydroxide addition is more commonly used. Sodium hydroxide is the most effective chemical in alkali sludge solubilization, followed by potassium hydroxide, magnesium oxide, calcium hydroxide (Carrere et al., 2010). In alkaline process, pH is increased to 12 by alkaline addition and kept under that condition approximately 24 hours.

2.2.2.4. Freeze and Thaw. Freeze and thaw pre-treatment is an effective method in the reduction of pathogens. In nature, freeze and thaw naturally takes place in soil and change its characteristics. In a same way, the characteristics of wastewater sludges can change due to being left out in cold weather that results in freezing. As a result, freeze and thaw method can be used in municipal wastewater treatment plants. The mechanism behind this process involves the separation of the solid and liquid phase in the sludge, when ice crystals are formed; this formation leads destruction in the floc structure (Montusiewicz et al., 2010). Freeze and thaw operations require high energy and time. Therefore, cost analysis and freezing time are important parameters in the large-scale applications. Because of the high

operation costs of mechanical freezing, this promising technology is limited in the widely application (Chu et al., 2001).

2.2.2.5. Ozonation. It is well known that ozone is the strongest oxidizing agent in sludge treatment and it can oxidize a wide range of compounds. Ozone-oxidation with direct and indirect reactions enables the destruction of cell walls of microorganisms in sludge and elutes cytoplasm into bulk solution. The mechanisms of ozonation are described as two achievements: cell disintegration and mineralization (Park et al., 2002). Cell disintegration refers to the effects of ozone on sludge are explained by the destruction of the bacteria cell membrane whereas mineralization refers to the conversion of cell walls into the mineralized materials by subsequent oxidation of soluble organic matter according to carbon dioxide. Sludge particulates are transformed into soluble composition regarding proteins, lipids, and polysaccharides. Therefore, it has been used for sludge pre-treatment to increase biodegradation of sludge (Weemaes, 2000).

2.2.2.6. Fenton Addition. Fenton process is an advanced oxidation process, which takes place with the catalyzer effect of Iron (II) salt and oxidation effect of hydrogen peroxide. It results in the degradation of organic pollutants. In addition to the usage in wastewater treatment, Fenton process started to being used within the purpose of sludge digestion. With the addition of Fenton, it is known that, the dewaterability characteristic of the sludge is improved (Dewil et al., 2005).

2.2.2.7. Biological Disintegration by Enzyme Addition. The aim of biological disintegration is to increase the degradability of organic matters by the destruction of the cell wall (Liu and Tay, 2001; Müller, 2001). The cells can't directly take proteins and carbohydrates that are present in the structure of organic matters. As a result, microorganisms convert those macromolecules into small molecules such as sugars, amino acids and amino acids. In order to achieve this conversion, microorganisms excrete enzymes such as cellulose, protease and lipase. Pre-treatment with enzymatic disintegration can take place by spontaneously in ambient temperature, or by enzyme addition to the system. A significant increase in the disintegration degree can be obtained by the application of enzymatic disintegration. Within

this purpose, there are many researches conducted recently intended to use enzymes (Thomas, et al., 1993, Goel, et al., 1998, Lai et al., 2001). Researchers report that the biological hydrolysis of sludges with enzyme additions seems to be an effective and cost reducing process for the treatment plants in many respects.

In this study, enzymatic pre-treatment (sludge disintegration) will be applied to the sludge samples prior to the aerobic and anaerobic digestion processes. Pre-treatment is used for the sludge minimization purposes. Moreover, detailed information about enzymatic disintegration is given in Section 2.4.

2.3. Sludge Stabilization

The ultimate destiny of a produced sludge in a wastewater treatment plant is to be disposed of. In order to dispose the produced sludge, there are different methods such as; composting, landfilling, and incineration. But disposal of the sludge with those methods is expensive, difficult and has environmental impacts, which are major concern (Vesilind et al., 2001; Liu, 2003; Wei et al., 2003; Pérez-Elvira et al., 2006; Tokumura et al., 2007;). As a result, treatment, reuse and disposal of sludges gained a special importance all over the world.

Sludge stabilization before the ultimate disposal is mandatory due to many different reasons. Some of those reasons are:

- There are social and environmental regulations related to the sludge use on land, so the management of the excess sludge is one of the challenges for these plants.
- The cost of sludge disposal is calculated per unit volume, so reduction in the sludge production leads to cost saving (Roman et al., 2006).
- The cost of disposal and the negative environmental impacts of the latter disposal methods have motivated a search for methods that reduce the overall solid content of sewage sludge requiring disposal (Parmar et al., 2001).

- Legislations concerning land application of sludge is being tightened in order to prevent health risks to human and livestock due to toxic element potential of sewage sludge (Perez-Elvira et al., 2006).

The excess sludge that is coming from a biological sludge process has three undesirable aspects:

- Biological instability: the excess sludge is putrescible due to the high fraction of biodegradable organic matter and enters into decomposition within hours after the interruption of aeration
- The hygienic quality of the excess sludge is very poor: a very large variety of viruses, bacteria and other pathogens are present
- The suspended solids concentration in the excess sludge is low: 3 to 50 g/L depending on the origin of the sludge (Van Haandel and Van Der Lubbe, 2012).

Sludge stabilization is the process to make the ultimate sludge disposal acceptable. Raw sludge involves pathogens, it has a bad odor and its water content is too high. Therefore, the main objectives of a sludge stabilization process are pathogen reduction (disinfection), odor control, decomposition of biodegradable matter and volume reduction. At the end of a stabilization process, a stable sludge is obtained and it can be defined as a sludge, which does not cause disease, nuisances, and damage the ecosystem.

Table 2.3. Sludge stabilization processes.

Biological Stabilization	**Chemical Stabilization**	**Thermal Stabilization**
Aerobic Digestion Anaerobic Digestion Lagoons Composting	Lime Chlorine Oxygen	Heat

In order to achieve sludge stabilization, there are different processes that can be applied to the sludge. These processes can be listed as in Table 2.3.

Most commonly applied sludge stabilization processes are aerobic and aerobic digestion processes. Both processes will have a positive influence on the hygienic quality of the sludge. In this study, both digestion processes were used and their effects on sludge quality were investigated.

2.3.1. Aerobic Digestion

In an aerobic digestion process, sludge is kept in an aerobic environment by continually aerating it for long periods of time. During this aeration process, microorganisms extend into the endogenous respiration phase. In an aerobic digestion system, biodegradable organic matters are hydrolyzed into biodegradable soluble organic matters and ammonia nitrogen and phosphates are released. Then, produced soluble organic matters are converted into water, carbon dioxide, and active biomass by the action of heterotrophic bacteria. The biomass then decays and generates more carbon dioxide, water, and debris (Figure 2.2). The presence of heterogeneous population in an aerobic digester makes it a complex system. Bacterial cells use their own protoplasm for energy and some cells die and can serve as a food source for members of the population. Thus, degradable organic matter is reduced which leads a decrease in volatile solids content.

Performance of an aerobic digestion system is affected by some parameters and these parameters are:

- Rate of sludge oxidation,
- Sludge loading rate,
- Sludge age,
- Mixing, and
- Sludge solids characteristics.

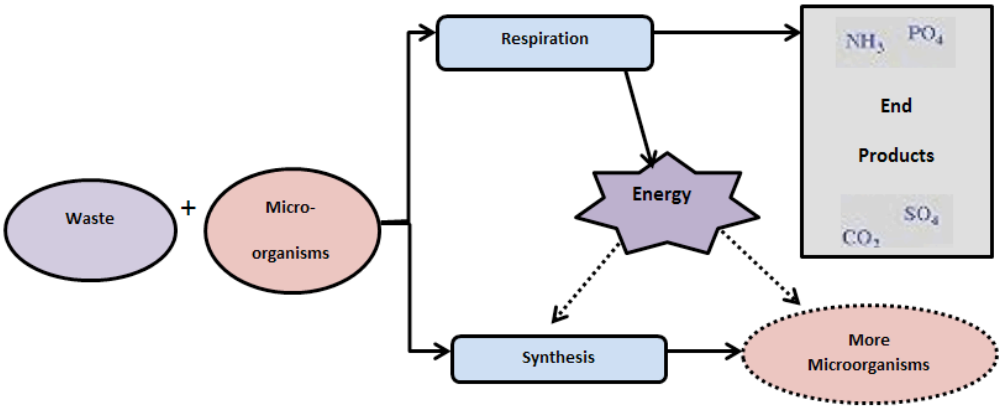


Figure 2.2. Path of an aerobic digestion process.

The performance of an aerobic digestion system can be quantified by volatile suspended solids reduction efficiency and the specific oxygen uptake rate of the digested solids (Grady et al., 1999).

Even though aerobic digestion is feasible for small-scale treatment plants, it might not be economically advantageous in large-scale plants. The major advantages and disadvantages of the process can be seen in Table 2.4.

Table 2.4. Advantages and disadvantages of an aerobic digestion process.

Advantages of Aerobic Digestion	**Disadvantages of Aerobic Digestion**
• Stable and high quality supernatant • Low construction costs • Good dewatering characteristics • Good volatile solids reduction • Easy to operate • No odors	• High power costs (for aeration) • High operating costs • Bad settling characteristics • Lack of methane production • Process affected by temperature changes

2.3.2. Anaerobic Digestion

In an anaerobic digestion process, sludge is kept in oxygen-free conditions. Under these conditions, specialized bacteria develop and use sludge as a source of organic matter. Destruction of organic constituents by anaerobic microorganisms and conversion them into methane and carbon dioxide is called as anaerobic digestion. Almost any organic material can be processed with anaerobic digestion, including waste paper and cardboard, leftover food, industrial effluents, sewage and animal waste (Friends of Earth, 2007).

The main purpose of an anaerobic digestion system is to reduce sludge volume and decompose highly putrescible organic matter. At the end of the process, methane is also gained, which is a valuable by-product and it can be used as an energy and electric source.

A wide range of microorganisms including primarily prokaryotic, mainly bacteria and methanogens are involved in anaerobic digestion. During digestion, many complex biochemical reactions take place. The decomposition of organic constituents mainly occurs in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Vesilind, 1979; Parkin et al., 1986; Speece, 1996; Tchobanoglous et al., 2003; Vesilind and Spinosa, 2001). The stages of an anaerobic digestion process are shown in Figure 2.3.

Hydrolysis is the first stage of the anaerobic digestion process in which both insoluble organic material and high molecular weight compounds (lipids, polysaccharides, proteins and nucleic acids) are converted into soluble organics which are monomers like sugar, fatty acids and amino acids. The hydrolysis reactions are catalyzed by extracellular enzyme (cellulases, proteases). Temperature, biodegradable organic matter concentration, biomass nature, pH and particle size play an important role on the hydrolysis rate (Elefsiniotis et al., 1996).

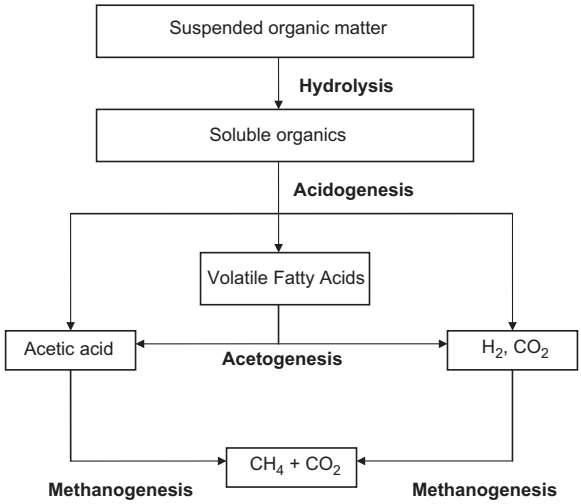


Figure 2.3. Subsequent steps in the anaerobic digestion process (Appels et al., 2008).

The components formed during hydrolysis step are further split in the second stage, acidogenesis. During acidogenesis, volatile fatty acids (VFA) are produced by acidogenic (fermentative) bacteria along with ammonia, carbon dioxide, hydrogen sulfide and other by-products. In an anaerobic digestion system, acidogenic bacteria are the most dominant group, covering 90% of the total population (Zeikus, 1980).

In the acetogenesis stage, molecules produced during acitogenesis are digested by acetogens to produce mainly acetic acid, as well as carbon dioxide and hydrogen. This conversion is controlled to a large extent by the partial pressure of hydrogen in the mixture.

In the last step of the digestion, methanogenesis, the intermediate products formed during the entire anaerobic digestion are converted into methane, water, and carbon dioxide (Appels et al., 2008). Methanogens use acetate, CO_2 and H_2O in order to produce methane gas. Methanogens are divided into two groups. Aceticlastic methanogens that convert H_2 and CO_2 into CH_4, and H_2-oxidizers that convert H_2 and CO_2 into CH_4. In an anaerobic digestion process, among the substrates that methanogens use, two-thirds of the methane produced is derived from acetic acid while only one-third is from H_2 and CO_2 (Gujer and Zehnder, 1983).

In an anaerobic digestion system, hydrolysis step determines the rate of the decomposition of organic matters depending on the characteristics of the sludge and it is defined as the 'rate-limiting step' in the literature (Eastman and Ferguson, 1981). Mostly, pre-treatment techniques are applied to the sludge prior to the anaerobic digestion in order to eliminate the rate-limiting step and speed up the reactions.

During the operation of an anaerobic digestion system, proper monitoring and control are needed in order to prevent operational problems. The parameters should be monitored during the digestion process are:

- Temperature,
- pH and alkalinity,
- Organic loading rate,
- Mixed liquor suspended solids and mixed liquor volatile suspended solids concentrations,
- Food to microorganism (F/M) ratio,
- Hydraulic retention time and solid retention time (SRT),
- Gas flow,
- Methane and carbon dioxide contents of the biogas.

The temperature of an anaerobic digestion system should be kept in the optimum ranges and the changes should not more than $1^{o}C$ in a day. The operation temperature of the anaerobic digester influences the metabolic activities of the microbial population, including the rates of hydrolysis and methane formation. Anaerobic digester systems are more traditionally designed to operate at mesophilic temperatures between 30°C and 38°C (Tchobanoglous et al., 2003). Optimum pH for an anaerobic digestion system is between 6,8 and 7,4 and the system should be operated under those pH ranges. Otherwise, methanogens can be inhibited, or ammonia release can be increased resulting in toxicity. If methanogenic activity is inhibited in the reactor, organic acids accumulate in the reactors, leading a pH drop and failing the system (Grady et al., 1999; Lay et al., 1997). Even though pH is stated as the

major control parameter of an anaerobic digester, volatile fatty acids, alkalinity and biogas production rate are better parameters as indicators of inhibitions (Grady et al., 1999).

The performance of an anaerobic digestion system can be measured by the destruction of organic matters (volatile solids), volume and composition of gases produced, pH, volatile acids, and alkalinity.

Anaerobic digestion can be improved by the breakdown of large organic particulate matters into smaller organic matters in order to increase the surface area available for contact with bacteria responsible for degradation in order to improve the efficiency of digested process (Sangave and Pandit, 2006).

Typical hydraulic retention time (HRT) for an anaerobic digestion process is more than 20 days and the degree of the organic matter degradation is in the range of 25% to 60% during this period of time (Nickel et al., 1999). Even though the organic matter degradation is high in an anaerobic digestion system, there are some disadvantages that should be considered. The major advantages and disadvantages of the process can be seen in Table 2.3.

Table 2.5. Advantages and disadvantages of an anaerobic digestion process.

Advantages of Anaerobic Digestion	Disadvantages of Anaerobic Digestion
• Lower energy requirements • Higher process stability • Lower operating costs • Methane production • Odors/vectors are removed • Less sludge is produced	• Requires large volumes of digesters • Formation of foam • Susceptible to upsets due to toxic substances • Require alkalinity addition • Sensitive to temperature changes

2.4. Enzymes

Enzymes are biologic catalysts that catalyze chemical reactions of other substances. Without the presence of enzymes, these reactions take place at a rate too slow while their presence accelerates them. An enzyme may add atoms to a molecule, remove atoms from a molecule, split a large molecule into two smaller molecules or join together smaller molecules to form a larger molecule. But the important point is that enzymes always change molecules.

2.4.1. Main Characteristics of Enzymes

Enzymes are proteins with large complex molecules, with the only exception of ribozymes. They are made up from chains of amino acids linked together by peptide bonds. The enzymes are not alive themselves, but they are synthesized by all living organisms. One of the properties of enzymes that makes them so important is the specificity they exhibit relative to the reactions they catalyze. In general, there are four distinct types of specificity and they are absolute, group, linkage and stereo chemical specificity.

2.4.1.1. Lock and Key Model. The actions of enzymes depend on their particular molecular shape. Enzymes have unique shapes and reactive sites that allow them to bind with specific molecules. The active site of an enzyme has the right shape and functional groups to bind to one of the reacting molecules, called as substrate. Enzymes are highly specific to substrates. As can be seen in Figure 2.4, the active site of the enzyme has a shape that fits with its specific substrate molecule. Substrate binds to the enzyme at the active site and an enzyme-substrate complex forms. The interactions between the substrates and the enzyme stresses or weakens some of the chemical bonds in the substrates. These stresses encourage substrate leading to the formation of a different molecule. As a result of the chemical interactions within the active site, a new product is formed. The product is released from the active site, the enzyme turns back into its original shape and is free to work again. This fit between an enzyme and its substrate is described by the ‘Lock and Key Model’. The enzyme is the lock and the substrate is the key – only a specific substrate will fit the enzyme’s active site. In the reactions that enzymes catalyze, they don’t undergo any permanent chemical change. They are neither used

up in the reactor nor do they appear as reaction products. Another model of enzyme-substrate interaction is called the 'Induced Fit Model'. This model recognizes that there is much flexibility in an enzyme's structure. It proposes that once the enzyme binds the substrate to the active site, the enzyme is able to change its shape slightly to bind the substrate even more firmly.

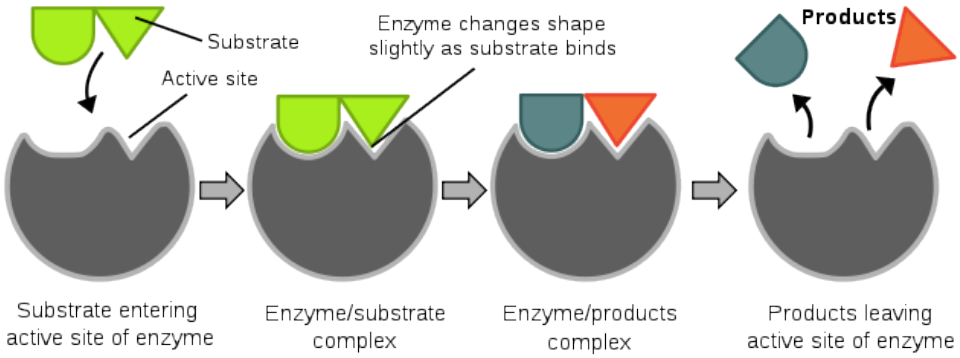


Figure 2.4. Formation of an enzyme – substrate complex (New World Encyclopedia, 2006).

2.4.1.2. Activation Energy. Enzymes work by weakening bonds, which lowers activation energy. For a chemical reaction to occur, there is a need for an initial input of energy (activation energy) level to exceed. Increasing the temperature could provide this energy but the heat would subsequently destroy many molecules in cells. Enzymes lower this activation energy level, so that the reactions can go faster in a given period of time. For example, for the hydrolysis of sucrose 26.000 calories/mole activation is energy is required, but when sucrase enzyme is used, the required energy is reduced to 9.000 calories/mole. Hence, given a total amount of available energy, more molecules of substrate would be converted when enzymes are present. In the Figure 2.5, the illustration of the concept is seen. Activation energy that is required without the presence of enzyme is much more than the activation energy required with the presence of an enzyme.

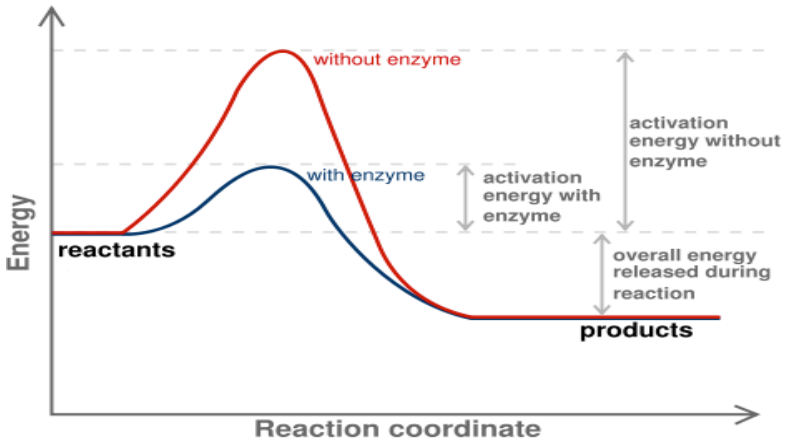


Figure 2.5. Representation of the required activation energy with or without the presence of an enzyme (New World Encyclopedia, 2006).

2.4.2. Factors Affecting Enzymatic Activities

Knowledge of basic enzymatic kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate (Worthington, 1972). Several factors affect the rate at which enzymatic reactions proceed and those factors are listed below:

2.4.2.1.Environmental Conditions. Enzymes are affected from the changes in the temperature, pH, and ionic concentrations. Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. At low temperatures, enzyme action is low because of the low movement of molecules. Increasing the temperature speeds up the movement of molecules, therefore it results in the increased enzyme action. Every enzyme-catalyzed reaction has an optimum temperature at which enzyme activity is the highest, and it ranges between 25-40^{o}C. Above the optimum temperature, the enzyme structure begins to denature. pH is also another factor that is important for the enzyme activity. When pH changes, it changes hydrogen bonds in enzyme's structure and breaks intra and intermolecular bonds, thus the active site of the enzyme changes. Therefore substrates can't fit into the active

site of the enzyme and as a result, enzyme effectiveness decreases. Each enzyme has an optimum pH at which its activity is the best but in general optimum pH ranges between 6,0-8,0.

2.4.2.2. Cofactors and Coenzymes. Many enzymes require the presence of other compounds and cofactors, before their catalytic activity can be exerted. A holoenzyme is an active and complex enzyme formed by combination of a cofactor and an apoenzyme. A cofactor can be a coenzyme, a prosthetic group or a metal ion activator (e.g. K^{+}, Fe^{2+}, Cu^{+}, Mg^{2+} Zn^{2+}, Ca^{2+}). An apoenzyme is the protein component of an enzyme. A coenzyme is a non-protein organic substance that is attached to the protein portion. A prosthetic group is an organic substance that is attached to the protein portion. The relationship between an apoenzyme and a cofactor can be seen in Figure 2.6. The cofactor binds to the apoenzyme so that it is activated to connect with its substrate.

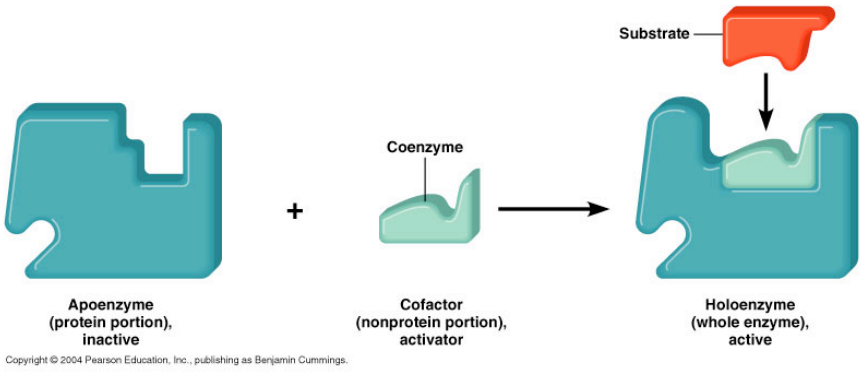


Figure 2.6. Formation of a holoenzyme (Midlandstech Lecture Notes, 2012).

2.4.2.3. Enzyme Inhibitors. Enzyme inhibitors are divided into three groups and these are competitive, noncompetitive, and uncompetitive inhibitors. In enzymology, it is common for enzymes to be inhibited by their products, or to be stimulated by the presence of their substrates (Burgess and Pletschke, 2008). A competitive inhibitor binds to the active site of the enzyme and prevents substrate molecule from entering. A noncompetitive inhibitor binds to an enzyme at a place other than the active site called as the allosteric site. Upon binding, the enzyme changes shape and modifies its active site such that it cannot accept the substrate

molecules. In an uncompetitive inhibition, the inhibitor can't bind to the enzyme directly, but it binds to the enzyme-substrate complex. Both inhibitors decrease the rate of an enzyme-catalyzed reaction (Sadava et al., 2009).

2.4.3. Enzyme Classification

Enzymes are divided into six categories in the Enzyme Nomenclature by International Union of Biochemistry. Nomenclature of enzymes embodies three general principles. Initially, enzyme names, especially those ending in –ase, should be used only for single enzymes. Second, an enzyme is named and classified according to the reaction it catalyzes. Third, enzymes are named and classified according to the type of the reaction they catalyze (Aehle, 2007). Enzyme classifications and the reactions they catalyze are listed in Table 2.6.

Table 2.6. Enzyme classifications (Enzyme Nomenclature, 1992).

Enzyme Code	Enzyme Name	Reaction Catalyzed
EC 1	Oxidoreductases	Oxidation-reduction reactions (Transfer of electrons)
EC 2	Transferases	Transfer of groups from one compound to other
EC 3	Hydrolases	Hydrolytic reactions
EC 4	Lyases	Non-hydrolytic addition/removal of groups from substrates
EC 5	Isomerases	Geometric or structural changes within one molecule
EC 6	Ligases	Joining two molecules together by synthesis of new bonds

Enzymes play a diversified role in many aspects of everyday life. In the 21st century, usage of enzymes for different purposes has extended. Many enzymes have practical applications in food and textile industry, agriculture, clinical purposes and biotechnology. They are used as ingredients of detergents, reagents for analysis of drugs or blood components, soil fertilizers, replacements of chemicals in textile and leather industry, food

additives, fiber processing in the paper industry, and environmental purification. Enzymes are also used in the wastewater treatment processes.

2.5. Extracellular Polymeric Substances in Sludge

Extracellular polymeric substances (EPS) are organic macromolecules that are formed by polymerization of similar or identical building blocks, which may be arranged as repeating units within the polymer molecules (Windenger et al., 1999). Major compounds of EPS are usually carbohydrates and proteins. Humic substances may also be a key component of the EPS in sludge in biological wastewater treatment reactors, accounting for approximately 20% of the total amount (Frolund et al., 1995). In addition to carbohydrates, proteins, and humic substances; lipids, nucleic acids, uronic acids acids), and some inorganic components may also be present in the EPS matrix. Figure 2.7 shows the composition of organic matter in sludge, with emphasis on EPS.

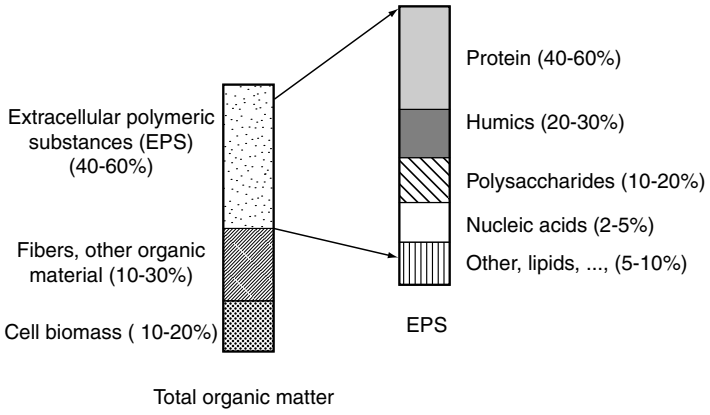


Figure 2.7. Composition of the organic part of sludge (Bitton, 2011).

The contents of EPS crucially affect the properties of microbial aggregates, such as adsorption ability, surface characteristics, mass transfer stability, flocculation ability, settle ability, dewatering ability, stability, adhesion ability and the formation of microbial aggregates (Neu and Lawrence, 2010).

EPS play a key role in binding floc components together. They act together with multivalent ions to aid the formation and settling of sludge flocs in both aerobic and anaerobic treatment systems (Wawrzynczyk, 2007). The main forces involved in these binding functions are Van der Waals, hydrophobic, and electrostatic interactions (Parmar et al., 2001). On the other hand, an excess EPS may hinder dewatering of sludge, bio-flocculation and settling of sludge (Forster and Lewin, 1972; Liu and Fang, 2003; Pere et al., 1993; Urbain et al., 1993).The adsorption of organic pollutants by microbial aggregates may be attributed to the fact that there are some hydrophobic regions in EPS (Spath et al., 1998). EPS with negative charges is capable of binding with positively charged organic pollutants via electrostatic interaction (Neu and Lawrance, 2010; Sheng et al., 2010).

EPS can be used by bacteria as sources of carbon and energy. In biological wastewater treatments reactors, the enzymes for the degradation of EPS are available. Dey et al. (2006) stated that enzymes were useful both for releasing EPS. The bacteria in sludge can utilize EPS that are excreted by other bacteria for metabolic activity. The small molecular substances that are produced as a result of EPS degradation can be used as carbon and energy sources for cell growth in conditions of nutrient shortage. EPS degradation can also result in the deflocculation of sludge flocs. The non-degradable portion of EPS may flow with the effluent from reactors and deteriorate the quality of the effluent (Sheng et al., 2010).

Surface-active compounds like EPS are solubilized by enzymatic action and boost the digestion (Dey et al., 2006). Hydrolytic enzymes break down EPS through multi-step processes summarized as follows (Foladori et al., 2010; Wawrzynczyk et al., 2007a):

- Hydrolytic enzymes adsorb to the sludge-substrate and attack the polymeric substances forming enzyme-substrate complexes,
- Small polymers loosely bound to the surface are hydrolyzed,
- Cell lysis transfers cell content into the medium,
- Solubilization of sludge solids occurs,

- The end products are biodegraded by microbial metabolism,
- The more compact part of sludge flocs is hydrolyzed at a lower rate because enzymes diffuse with difficulties inside the floc matrix.

The hydrolysis of complex organic structures such as EPS, heavily depends on the hydrolytic enzymes, like glucosidases, lipases, and proteases. It was proved that a combination of protease, lipase and endo-glycanases could accelerate solubilization of municipal sludge (Yang et al., 2010; Roman et al., 2006; Wawrzynczyk et al., 2003)The presence of EPS is the reason that especially sludge is difficult to digest and to dewater. The hydrolysis of EPS by the addition of enzymes is therefore the first step to make sludge more available to aerobic and anaerobic degradation (Recktenwald et al., 2008).

2.6. Enzyme Disintegration as Pre-Treatment

In recent years, new technologies have been introduced to improve the biological decomposition of sludge by aerobic and anaerobic digestion processes. Biological hydrolysis of sludge with hydrolytic enzymes is the one of the applied methods for this purpose and it appears to be an efficient alternative in this field. It aims cell destruction by using enzymes extracted from microorganisms. Enzymes catalyze the degradation of organic substances in sludge as a function of the substrate. The results of enzyme additions during biological sludge stabilization process are the increased soluble matter concentrations, enhanced degradation of EPS, and improved the releasing capacity of water (Barjenbruch & Kopplow, 2003; Dey et al., 2006; Roman et al., 2006).

Sludge solubilization, cell lysis, and cryptic growth are considered as the main mechanisms, which occur during the enzymatic disintegration process. In an enzymatic disintegration process, by the addition of enzymes, cell wall components are ruptured by the enzyme canalization. Selected hydrolytic enzymes act on a specific substrate-biopolymer present in the sludge, cleave it and release products with lower molecular weight into the solution. This process can take place at ambient temperature with the enzyme addition from

outside, or spontaneously by itself. Consequently the floc structure is disrupted and elevated amounts of proteins, peptides, and carbohydrates are released (Recktenwald et al., 2008; Dey et al., 2006; Watson, et al., 2004). The released organic matter becomes more available for the metabolism and as a result, the addition of the specific hydrolytic enzymes induces a higher turnover in the digestion chamber (Recktenwald et al., 2008). With the implementation of enzymes inside to the intracellular fluid, the disintegration degree is increased (Goel et al., 1998, Lai et al., 2001).

Enzymes are able to catalyze their specific macromolecules and degrade them to smaller molecules. In the literature, mostly hydrolytic enzymes are used during enzymatic disintegration. For instance, protease and pronase E, are common for protein disintegration, lipidase is used for lipids destruction and cellulase, alpha-amylase, and cabohydrase are widely used for carbohydrate disintegration (Barjenbruch and Kopplow, 2003; Roman et al., 2006; Wawrzynczyk et al., 2007b). In addition, combination of stated enzymes are also applied for the sludge (Parmar et al., 2001; Roman et al., 2006; Rashed et al, 2010). In the literature, there were both studies that were concerned about the effects of both single and mixed enzyme additions. The results show that single enzyme has limited hydrolytic activity whereas an enzyme mixture is greater than the sum of the hydrolytic activity of single enzymes (Roman et al., 2006; Yang et al., 2010). The reason of this is that a specific type of enzyme can only be effective to one definite substrate (Zhou et al., 2009; Yang et al., 2010). Due to the heterogeneous characteristics of a sludge sample, it is very likely that a wide range of enzymes is essential for the hydrolysis of different substrates. Thus, a mixture of hydrolytic enzymes is used during this study.

Hydrolytic enzymes can break down polymeric substances through multi-step processes, during which compounds can be transformed from a recalcitrant state to one that is more biodegradable (Gianfreda and Rao, 2004). Enzymes are able to increase the degradation rate of biodegradable substances, such as activated sludge, allowing for more efficient treatment processes (Whiteley et al., 2006).

During an enzymatic disintegration process, enzymes destroy cell walls by catalyzing reactions. Even though cells are able to produce enzymes at ambient temperature, the production is not enough to make it as a pre-treatment process. So, addition of enzymes externally is needed. While an enzyme destroys the cell wall, it is captured into the sludge cell, and then the cell lysis and cryptic growth take place. As a result, sludge cell is disrupted which results in the improved biodegradability. The enzymatic disintegration of sludge also improves the degradation of organic matter by an increase of the specific surface area available for more enzymatic hydrolysis, leading to higher methane production, better dewaterability, and less volume followed by reduced transport costs and easier handling of the sludge after the dewatering step.

Using enzyme is an effective method in the cell disintegration. Previously conducted studies show that; enzyme addition can decrease the anaerobic digestion hydraulic retention time and increase the sludge digestion efficiency (Wawrzynczyk et al., 2003), and reduce the cost of final disposal of sludge (Ronja, 2008). The information in the literature is clear to say that enzymes are effective in the release of extracellular polymeric substances (EPS) (Dey et al., 2006). In this regard, one of the studies investigated the effect of bacterial hydrolysis of polymeric substances in the degradation of the activated sludge and it is found out that with the application of the pre-treatment, volatile suspended solids decreased and the soluble chemical oxygen demand increased (Del Borghi et al., 1999).

Enzymatic pre-treatment has several advantages compared to the other disintegration techniques. The advantages of enzymatic pre-treatment include: higher yields; applicability at high or low contaminant concentrations; toleration of shock loading rates; reduction in the sludge volume; operation over a wide range of pH, temperature, and salinity; low energy requirements; minimal byproduct formation; low economical requirement of the process in the application to the large scale; mild operational conditions and the simplicity of the process (Karam and Nicell, 1997).

Even though the main mechanisms of enzymatic pre-treatment on sludge are not clear yet, there are some studies related to this topic. In the literature, previous studies reported that

enzyme addition prior to aerobic and anaerobic digestion process could lower digesting time, improve sludge digestibility, reduce disposal costs, be easily controlled and its products were harmless to the environment (Yang et al., 2010; Wawrzynczyk et al., 2007a; Ronja, 2008; Roman et al., 2006; Parmar et al., 2001; Ahuja et al., 2004). In other words, enzymatic pre-treatment showed that the enzymatically pre-treated sewage sludge solubilized the particulate organic matter more effectively. Below, studies related to enzymatic pre-treatment are discussed in detail.

The effects of enzymes in reducing the solid content of the sludge were studied by Parmar et al. (2001). Cellulase, protease, lipase enzymes, and mixture of them were used during the study. For enzymatic treatment, enzyme added sludge samples were incubated at 40^{o}C for 96 hours. The results showed that a dramatic reduction in the solids content was observed in the first 6 hours, after that time reduction continued at a slower rate. Lipase, cellulase, alkaline protease, and enzyme mixture reduced TSS 21,4%, 25%, 32,1%, and 29%, respectively. The authors state that enzymes have potential to reduce 50% TSS content of the sewage sludge solids according to the results. It is also indicated that enzymes had great effect in improving sludge settleability, with protease being the greatest. The reducing volumes of settled solids indicate that the treatment improved the settleability of sludge solids (Parmar et al., 2001).

The effect of enzyme addition in sludge solids reduction was also studied by Roman et al., (2006). Sludge was obtained from a municipal wastewater treatment plant. The authors investigated the effects of enzymes to anaerobic digesters in solubilizing the organic components of digested solids. The enzymes that were used during the study were cellulase, pronase E, and combination of both. The results showed that with the addition of enzymes, TSS and COD removals were enhanced and operational conditions were improved. The best TSS removal rate was 80% and it was obtained in the reactor that contained enzyme mixtures, whereas control reactor only had 20% reduction. On the other hand, pronase E and cellulase added reactors reduced TSS only %36 and %39, respectively. Also, enzyme mixture added reactor had the highest removal rate for the particulate chemical oxygen demand removal with 93%.

In the overview on pre-treatment process of sludge, Rashed et al., 2010 used six different enzymes and investigated their effects on anaerobic digestion. The enzymes that were used during the study were protease, alcalase (proteases), carezyme (cellulases), celluclast, lipolase (lipases), termamyl, viscosyme, and mixture of them. In addition, the effect of the solid content and the optimum enzyme dosage was also investigated. The experimental study shows that, an enzyme's performance hugely depends on the characteristics of the sludge. Different enzymes had different VS reduction rates on the same sludge. Increasing enzyme dosage from 0.1% to 0.5% does not increase the VS reduction so that 0.1% dosage is more preferable. The authors indicate that enzymatic pre-treatment can be successfully used in the treatment plants.

The study conducted by Rudolfs (1932) contradicts with the literature. Enzymes pepsin, trypsin, and lipase were added to sludge samples and incubated at 28^{o}C for 35 days under anaerobic conditions. In addition, semi-plant scale experiments were also conducted. The results of the study showed that the addition of enzymes does not change the digestion time, but increases gas production a little. The author claims that bacterial groups can produce sufficient enzymes during the digestion process, so that further enzyme addition is not required.

Yang et al.(2010) investigated the effects of pH, temperature and addition of different enzyme types on the sludge hydrolysis. Excess sludge was obtained from a municipal wastewater treatment plant. Two batches of hydrolysis tests with single enzyme and mixed-enzyme treatment were conducted. When enzymes amylase and protease were added to the sludge samples separately, amylase had the highest VSS reduction and sCOD/tCOD increase compared to protease. The highest VSS reduction was obtained in the enzyme mixture with 1:3 – protease:amylase ratio. The study shows that, enzyme addition can improve the biological sludge hydrolysis, enhance sludge solubilization, and increase VSS reduction. The study also shows that, temperature increase has a positive effect on the enzymatic hydrolysis.

Barjenbruch and Kooplow (2003) applied enzymatic, mechanical and thermal pre-treatments to a surplus sludge in order to reduce foaming that occurred in the sludge. Enzyme

pre-treatment was carried out by using carbohydrase enzyme. Even though enzymatic pre-treatment was not effective in reducing foam formation, it increased the VSS reduction. In addition, enzymatic pre-treatment was not as effective as the other pre-treatment methods in increasing biogas yield, but the authors indicated that it showed relatively high increase in degradation degree. As a result, more studies focused on the enzymatic pre-treatment on the anaerobic degradation were suggested.

Since sewage sludge contains a variety of pathogens, it's crucial to minimize the pathogen substances in sludge. Parmar et al. (2001) investigated the effect of enzyme addition on the removal of pathogens from sludge. Alkaline protease (alcalase) was added to the sludge samples and the mixture was incubated on a rotary shaker, at 40^{o}C, for 96 hours. Coliform and salmonella were used as pathogen indicators. The effects of different pH values and temperatures were also investigated. When alcalase was added to the sewage sludge at 40^{o}C and varying pH values, complete kill of salmonellae was not observed, while all coliforms were killed at pH 10 and 12. Running the same experiment with increasing the temperature to 50^{o}C made a significant impact on destroying salmonellae. The results indicate that alcalase accelerates removal of pathogens from sewage sludge.

Industrial-scale application of anaerobic digestion by enzyme addition was studied by Recktenwald et al. (2008). The sludge that was used during the study was a mixture of primary and waste activated sludge. A mixture of two glycosidic enzymes were used and dosed to the digestion chambers during a 6-month period. The experiments showed that glycosidic enzymes caused a better solubilisation of the sludge than the tested proteases and lipases. The anaerobic reactor that was treated with enzyme addition showed better results. Increase in the methane production and dewaterability, and decreased amount of polymer consumption were the indicators that enzymes improved the hydrolysis step prior to acidogenesis. In addition, the authors investigated the effect of enzyme addition in terms of economical aspects. As a result, it was seen that the running costs ranged below those of many other chemical sludge treatment processes due to the low dosage of enzymes.

Luo et al. (2012) focused on the development of thermodynamics model and the analysis of dynamic behaviors of substances conversion during waste activated sludge hydrolysis process enhanced by alpha amylase. Different enzyme dosage added sludge samples were agitated at 100 rpm and 50^{o}C for 4 hours. Also, enzyme added sludge samples were incubated at 40, 50, 60, and 70^{o}C for 8 hours in order to investigate the effect of temperature. As a result, it was concluded that alpha amylase strongly enhances the waste activated sludge hydrolysis according to the increased VSS reduction rates, and it was stated that 0.06 g alpha amylase/g dissolved solids was found to be the optimum dosage. It was also clear that the hydrolysis efficiency was increased with the increasing temperature. The VSS reduction for 40, 50, 60 and 70^{o}C was 14.5, 32.7, 51.5, and 68.4%, respectively.

The effects of enzymatic autohydrolysis on the secondary sludge under thermophilic aerobic conditions were studied by Pena et al. (2008). The results of the study showed that under those conditions, secondary sludge was hydrolyzed at shorter solids retention time. The optimum conditions for the hydrolysis were found to be 55^{o}C, 0.15 vvm air flow, and 1 day solids retention time and 48% of VSS was hydrolyzed under such conditions. The authors also indicated that the initial solids concentration is an important parameter that should be considered but an optimum value was not found.

Comparison of three pre-treatment methods were studied by Davidsson and Jansen, 2006. Hygienisation, ultrasonication, and enzyme addition methods were applied to sludge samples prior to the anaerobic digestion. The pre-treatment methods were used separately or in combination on biosludge and mixed primary and biosludge. 2 different enzyme mixtues were used during the study. Highest methane productions were observed in enzyme added and ultrasonicated and enzyme added hygienised sludges. On the other hand, only enzyme addition had very little effect on methane production compared to the other combinations. The authors note that, from the previous study of their co-workers, the sCOD increase on enzyme pre-treatment was very high. But in terms of methane production, it is not effective. As a result, high COD solubilization from a pre-treatment method doesn’t always successfully lead to methane production.

Addition of substrates to the anaerobic digestion process is a method which is getting more popular in the last decades. Romano et al. (2009) combined Jose Tall Wheat Grass substrate with enzyme mixture and investigated the effects on anaerobic digestion. During the study, two different enzyme mixtures were used. The first enzyme set contained cellulase and hemicellulose, and the second enzyme set contained cellulase and beta-glucosidase. Initially, wheat grass was treated with the enzymes alone. Based on those results, enzymes were then added to anaerobic digesters at different stages: enzyme pre-treatment prior to anaerobic digestion, direct enzyme addition to a one-stage digester, and enzyme addition to the hydrolysis stage of a two-stage digester system. Treatment 1 involved second enzyme mixture while Treatment 2 included first mixture. When enzymes were directly added, it was seen that sCOD increased 94% and %31 for Treatment 1 and 2, respectively. First enzyme set was used while adding prior to one-stage digestion and the results didn't show a significant difference when compared to control reactor, in terms of biogas and methane yields and VSS reduction. Enzyme pre-treatment prior to anaerobic digestion and to the first stage of a two-stage digester system also didn't show a significant difference.

Wawrzynczyk et al. (2007b) investigated the effects of chelating and ion-binding agents alone or in combination with enzymes on solubilisation of bio-sludge and digested sludge. Combination of cellulase, endo- and exocellulase, and alpha-amylase enzymes were used separately and in combination during the experiments. When sludge samples were treated with cation-binding agents only, high release of organic matter and high changes in suspended matter were observed. Among the other agents, 50 mmol/L citric acid had the highest COD release with 8 g COD/L from bio-sludge and 3g/L from digested sludge and the highest suspended matter reduction was observed at 50 mmol/L STPP with 40% reduction for bio-sludge and 20% for digested sludge. Since highest dissolution and reduction rates were observed at low agent concentrations, 5 mmol/L cation-binding agents were used on enzymatic sludge solubilization. At the end of the experiments, it was observed that enzymatic pre-treatment was effective on sludge solubilization and the most effective treatment was achieved with enzyme mixture. Also, addition of small dose of cation-binding agents significantly improved sludge solubilization. The most effective agent was citric acid because it had the highest organic matter release.

In the following research of Wawrzynczyk et al. (2007c) possible mechanisms of enzyme activation due to the sludge matrix was investigated. It is stated that the added enzymes were partially adsorbed to, entrapped by or bound to the sludge structure. During the study, sludge samples were pre-treated with STPP, EDTA or citric acid prior to enzyme addition in order to understand whether the agent addition prevents enzymes from being attached to the sludge surface has an effect on the stability and activity of added enzymes and improves the enzymatic hydrolysis of sludge. The study showed that in the presence of cation-binding agents sludge solubilisation was enhanced by about 30-40%, enzymatic hydrolysis of sludge was improved by 50-85% and the adsorption of the added enzymes onto solids was significantly reduced.

3. MATERIALS AND METHODS

In this chapter, information about the materials and methods that were used during the experimental studies is given.

3.1. Materials

3.1.1. Sewage Sludge

In this study, sewage sludge samples were obtained from the recycle lines of four different wastewater treatment plants. The plants were chosen from two regions of Turkey, Marmara, and Black Sea Regions, and the treatment facilities are located in İzmit Kullar, İstanbul Bahçeşehir, Samsun Bafra, and Düzce Akçakoca. Among them, İzmit Kullar and Düzce Akçakoca are urban treatment plants, whereas İstanbul Bahçeşehir and Samsun Bafra are domestic.

3.1.2. Seed Sludge

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

3.1.3 Enzymes

An enzyme mixture that comprised of four hydrolytic and one cellulosic enzymes was used during the study. The classification of the enzymes are Alpha-amylase, Beta-glucanase (endo-(1,2(4)-), Lipase, Protease and Cellulase.

3.1.4. Aerobic Reactors

In the aerobic stabilization process, aerobic reactors were designed as batch reactors and they were operated for 21 days. The reactors were aerated by air blowers, the air was distributed by diffuser systems and the dissolved O_2 concentrations in the reactors were kept above 2 mg/L. Temperature of the reactors were kept constant at the room temperature (22± 2 ^oC). 8 aerobic batch reactors were used with 4L active working volume each. One control reactor, and one enzymatically pre-treated reactor were set for each treatment facility with their own sludge samples. For the enzymatic treatment of the sludge, the enzyme mixture was added 0,5% by volume to the reactors. In order to standardize the reactors, their total solid content was set to approximately 1%. The reactors were shaken and their evaporated portion was completed by tap water daily. The characteristics of the sludges that were used in the aerobic stabilization process are given in Table 3.1.

3.1.5. Anaerobic Reactors

Anaerobic reactors were also designed as batch reactors and they were operated for 22 days. The sewage sludge samples were put in amber colored glass bottles and the bottles were closed with specially designed bottle caps. The caps were designed as two pipes coming out of them, one was connected to the miligas counter, and the other one was used for the collection of gas samples. The impermeability of the reactors was provided by siliconising the caps of the reactors. In order to ensure an anaerobic environment inside the reactors, nitrogen gas was passed through the reactors for five minutes. The anaerobic reactors were placed in water baths and their temperature was set constant at 37^oC. 8 anaerobic reactors were used with 1,6 L active working volume. One control reactor, and one enzyme added reactor were set for each treatment facility. 400 mL seed sludge was mixed with 1200 mL sewage sludge, and 300 mL of the sewage sludge was pre-treated with enzymes. The enzyme mixture was added to the reactors 0,5% by volume. The reactors were shaken daily. The characteristics of the wastewater sludges and the seed sludge that were used in the anaerobic stabilization process are given in Table 3.2.

Table 3.1. Characteristics of the sludge samples that were used in the aerobic digestion.

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	‰	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-}	mg/L	4920	4060	3380	3180
P_2O_5	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]	$1x10^8$	$1,4x10^8$	$1,6x10^8$	$2,6x10^8$
Fecal Coliform	[CFU/100mL]	$4,9x10^7$	$7,9x10^7$	$5,7x10^7$	$1x10^8$
Fecal Streptococci	[CFU/100mL]	$1,6x10^7$	$1,9x10^7$	$3,1x10^7$	$5,5x10^7$

Table 3.2. Characteristics of the sludge samples that were used in the anaerobic digestion.

Parameter	Unit	Fritolay 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	$8x10^4$	$1,2x10^5$	$1,9x10^5$	$5,3x10^5$	$5x10^5$

3.2. Methods

For performance evaluations of the reactors, total solids (TS), volatile solids (VS), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), alkalinity, dissolved oxygen (DO), redox potential (ORP), conductivity, pH, temperature, salinity, total chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), total organic carbon (TOC), dissolved organic carbon (DOC), total Kjeldahl nitrogen (TKN), total phosphorous (TP), capillary suction time (CST), nitrite, nitrate, ammonia, sulfate, chloride, particle size distribution, microbiologic analyses, volatile fatty acids (VFA), methane concentration of the biogas, and EPS parameters were analyzed during the experimental studies.

Sludge samples were collected from the aerobic digestion reactors two times in a week in needed amounts. For the analyses of the anaerobic digestion reactors, samples were collected from the reactors only before and after the stabilization process. The analyses that were carried out for each stabilization process and their frequencies can be seen in Table 3.3.

All analyses that were carried out during this study were conducted according to the Standard Methods. For the analyses that didn't have a standard method, the most accepted methods in other relevant studies were chosen. All experiments were done in duplicates or triplicates.

3.2.1. 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.

Table 3.3. Analyses and their frequencies for aerobic and anaerobic reactors.

Parameter	Analysis Frequency of Aerobic Reactors	Analysis Frequency of Anaerobic Reactors
TS	2 Times in a Week	In the 1st and 30st days
VS	2 Times in a Week	In the 1st and 30st days
MLSS	2 Times in a Week	In the 1st and 30st days
MLVSS	2 Times in a Week	In the 1st and 30st days
Alkalinity	2 Times in a Week	In the 1st and 30st days
DO	Every day	-
ORP	Every day	In the 1st and 30st days
Conductivity	Every day	In the 1st and 30st days
pH	Every day	In the 1st and 30st days
Temperature	Every day	In the 1st and 30st days
Salinity	Every day	In the 1st and 30st days
COD	2 Times in a Week	In the 1st and 30st days
sCOD	2 Times in a Week	In the 1st and 30st days
TOC	Once in a Week	In the 1st and 30st days
DOC	Once in a Week	In the 1st and 30st days
TN	Once in a Week	In the 1st and 30st days
TP	Once in a Week	In the 1st and 30st days
CST	Once in a Week	-
NH_4+-N, NO3-, NO2-, SO4=, Cl-	In the 1st and 30st days	In the 1st and 30st days
Particle Size Distribution	Once in a Week	In the 1st and 30st days
Microbiologic Analyses	In the 1st and 30st days	In the 1st and 30st days
VFA	-	In the 1st and 30st days
GC	-	Two times in a week
EPS Extraction	Once in a Week	-

3.2.2. Conductivity and Salinity

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

3.2.3. Dissolved Oxygen

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

3.2.4. Total Solids and Volatile Solids

TS and VS analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater. Initially, evaporating dishes were brought to the constant weight by heating in muffle furnace (Protherm PLF 110/8) for 1 hour at 550^{o}C. For TS measurement, 30 mL of well-mixed sludge sample was put in evaporating dish, weighed, 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 for 1 hour at 550^{o}C, cooled in desiccator and weighed. TS and VS concentrations of the samples were found by calculations.

3.2.5. Mixed Liquor Suspended Solids and Mixed Liquor Suspended Solids

Filter papers and crucibles that were used in MLSS and MLVSS analyses were brought to the constant weight initially. For MLSS measurement, 10 mL sludge sample was filtered through a constant and 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.

3.2.6. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

COD and sCOD analyses were performed using dichromate closed reflux colorimetric method according to the Standard Methods for the Examination of Water and Wastewater. Samples were refluxed with sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$) in Velp Scientifica COD Digester (Eco 25 Thermoreactor) for 2 hours at 150^oC. Sludge samples were diluted if needed. For sCOD analyses, sludge samples were centrifuged in Hettick Rottina 380 centrifuge for 30 minutes at 9000 rpm and supernatant portion of the sample was used. 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.

3.2.7. Alkalinity

Alkalinity analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater. Sludge samples were diluted if needed, then added to an Erlenmeyer flask with a magnetic stirrer. The initial pH of the sample was recorded 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.

3.2.8. Total Organic Carbon and Dissolved Organic Carbon

TOC and DOC measurements were completed by using Shimadzu TOC-V CSH total organic carbon analyzer. Before analyses, eluates for samples were prepared according to their TS contents and left at shakers for 24 hours. 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).

3.2.9. Total Kjeldahl Nitrogen

In order to carry out Total Kjeldahl Nitrogen (TKN) analysis, peroxide digestion method was followed. Sludge samples were digested by concentrated hydrochloric acid at 440^{o}C with hydrogen peroxide addition. Digested sample was cooled and then diluted to 100 mL. Readings of the samples were done by using spectrophotometer and the procedure that was given in HACH handbook was followed.

3.2.10. Nitrite and Nitrate

For nitrite and nitrate analyses, sludge samples that were centrifuged at 9000 rpm were used. Centrifuged samples were diluted in needed proportions and spectrophotometer was used for the readings. The procedure that was given in HACH handbook 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 was read at 585 nm. 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 and wavelength was read at 500 nm.

3.2.11. Ammonia

For the ammonia analysis, Nessler Method that was given in HACH handbook was followed. Centrifuged sludge sample was diluted if necessary and completed to 25 mL by deionized water. 3 drops mineral stabilizer, 3 drops polyvinyl alcohol 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 concentration of it was read at 425 nm in the spectrometer.

3.2.12. Total Phosphorous

Sludge samples that were digested at 440^{o}C for TKN analyses were used during the total phosphorous analysis. PhosVer 3 Method that was given in HACH handbook was applied. Sample was diluted if necessary and it was poured into a 10 mL sample cell. PhosVer 3 phosphate pillow powder was added to the sample, and the sample was immediately stoppered and shaken vigorously for 30 seconds. Then, 2 minutes reaction period was started. Same steps were completed for the blank by using deionized water in order to zero the sample in the spectrophotometer. Test results were measured at 880 nm in the spectrometer.

3.2.13. Sulfate

SulfaVer 4 Method that was given in HACH handbook was followed for sulfate analysis. The contents of SulfaVer 4 Reagent powder pillow were added to the sample and the sample cell was swirled vigorously. Then, five-minute reaction period started and the cell left undisrupted during that time. Same steps were completed for the blank by using deionized water in order to zero the sample in the spectrophotometer. Test results were measured at 880 nm in the spectrometer.

3.2.14. Chloride

The amount of chloride in sludge samples was determined by silver nitrate method (Mohr Argentometric method). 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. Potassium chromate was added to the flask, the color of the solution turned into yellow and it was titrated with silver nitrate to the first appearance of red color. Standardization of the silver nitrate solution was made by titrating it with NaCl. Same steps were completed for the blank by using deionized water. The amount of titrant used was noted and the calculations were made to find the chloride concentration.

3.2.15. Particle Size Distribution

The sizes of the sludge solid particles were measured by using Malvern The Mastersizer 2000 (with Hydro2000MU wet dispersion unit). 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. The sample was fed into the analyzer unit in aliquots by using a pasteur pipette. Cleaning with deionized water was repeated three times after each measurement in order to clean the unit from the remaining particles.

3.2.16. 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. Since the samples had high bacterial concentration, high dilutions were necessary in order to be able to count.

3.2.17. Capillary Suction Time

CST analyses were conducted by using Triton Electronics Ltd. Type 304M capillary suction timer. CST paper, which was 7x9 centimeters, was used. 0,2 mL sample was taken and added to the CST sample cylinder, which had 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.

3.2.18. 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 after addition of phosphoric acid.

3.2.19. Biogas Production

Biogas productions in the anaerobic reactors were recorded daily by using Ritter MGC Milligas Counters. The counters were connected to the anaerobic reactors by a pipe. The inside of the counters were filled with silicone oil silox.

3.2.20. Biogas Composition

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.

3.2.21. Extracellular Polymeric Substances

Since there is not a standard method for the extraction of extracellular polymeric substances (EPS) in the literature, the most suitable technique for the extraction of EPS from sludge samples was chosen. The chosen 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^{o}C for further analysis.

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^{o}C.

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 until completing 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 dark for 4 hours, at +4^{o}C. At the end of 4 hours, sample was centrifuged for 1 minute, at 12.000 xg, +4^{o}C. Then, centrifugation was repeated for 15 minutes, at 12.000 xg, +4^{o}C. Supernatant of the centrifuged sample was collected and stored at -20^{o}C for further analysis.

3.2.21.1. Protein. The amount of total proteins in sludge samples was estimated by Lowry Method. 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 at room temperature. After 30 minutes, samples were transferred to semi-micro disposable cuvettes. For the standard curve, solutions with differing concentrations were prepared from BSA (Bovine Serum Albumin) stock solution. Absorbance values of the samples were read at 750 nm by using Shimadzu UV-160A UV-Visible Recording Spectrophotometer. Total proteins of the samples were calculated from the calibration curve.

3.2.21.2 Carbohydrate. The amount of total carbohydrates in sludge samples was estimated by Anthron Method, 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^{o}C, then left to cool down. For the standard curve, solutions with differing concentrations were prepared from the glucose stock solution. Absorbance values of the samples were measured at 578 nm by using the spectrophotometer. Total carbohydrates of the samples were calculated from the calibration curve.

4. RESULTS AND DISCUSSION

During this study, the effects of enzymatic pre-treatment on aerobic and anaerobic digestion processes were investigated. For this purpose, lab-scale aerobic and anaerobic digesters were operated. Results and the evaluations of the experiments are given in this chapter. The impacts of enzymatic pre-treatment on both digestion processes will be examined separately.

4.1. Aerobic Digestion

The aerobic digestion reactors were operated for 21 days as batch reactors. Sludge samples that were obtained from four different wastewater treatment plants (WWTP) were used. İzmit Kullar WWTP and Düzce Akçakoca WWTP are urban WWTPs, whereas İstanbul Bahçeşehir WWTP and Samsun Bafra WWTP are domestic WWTPs. The characteristics of the sludges that were used during the aerobic digestion process were given in Table 3.1. All analyses were conducted as previously described in Chapter 3. The performances of the aerobic digestion reactors and the effects of enzymatic pre-treatment on aerobic digestion will be investigated in terms of pH, oxidation reduction potential, dissolved oxygen, 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, particle size and microbiology.

In this section, some abbreviations were used to describe sludge samples that were taken from different wastewater treatment plants. These abbreviations are given in Table 4.1.

Table 4.1. Abbreviations that were used to describe the reactors.

İzmit Kullar WWTP		**İstanbul Bahçeşehir WWTP**		**Samsun Bafra WWTP**		**Düzce Akçakoca WWTP**	
Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
R1-İKC	R2-İK	R3-İBC	R4-İB	R5-SBC	R6-SB	R7-DAC	R8-DA

4.1.1. pH

pH is an important parameter while operating a biological system. Sudden increases or drops in pH levels can be indicators of a reactor's instability. pH of a sludge sample in an aerobic digestion system typically ranges between 5,5 and 8,5. During this study, changes in the pH levels of sludges were measured daily throughout the aerobic digestion and the results are given in Figure 4.1. The pH values of the reactors ranged between 5,69 and 8,42. According to the changes in the pH values, it can be considered that the system was stable throughout the process and it was working well. The small pH changes during the stabilization process can be explained with the CO_2 production, CO_2 removal or uptake of ammonium or nitrate for microbial growth as an electron acceptor (Burgess and Pletschke, 2008).

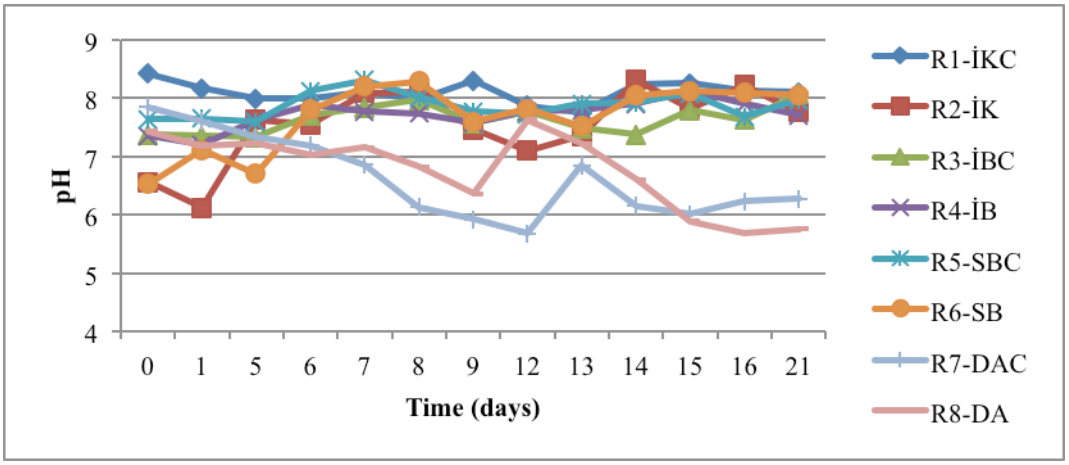


Figure 4.1.pH changes in the aerobic digestion reactors.

4.1.2. Oxidation Reduction Potential

Oxidation reduction potential (ORP) is an important parameter of aerobic activity. ORP measurements were conducted daily throughout the process and the data are given in Figure 4.2. ORP values in the reactors ranged between 40-190 mV. The small changes were observed in the ORP values based on the dissolved oxygen concentrations in the reactors. The positive ORP values verified that the aerobic conditions were maintained in the reactors throughout the aerobic digestion process.

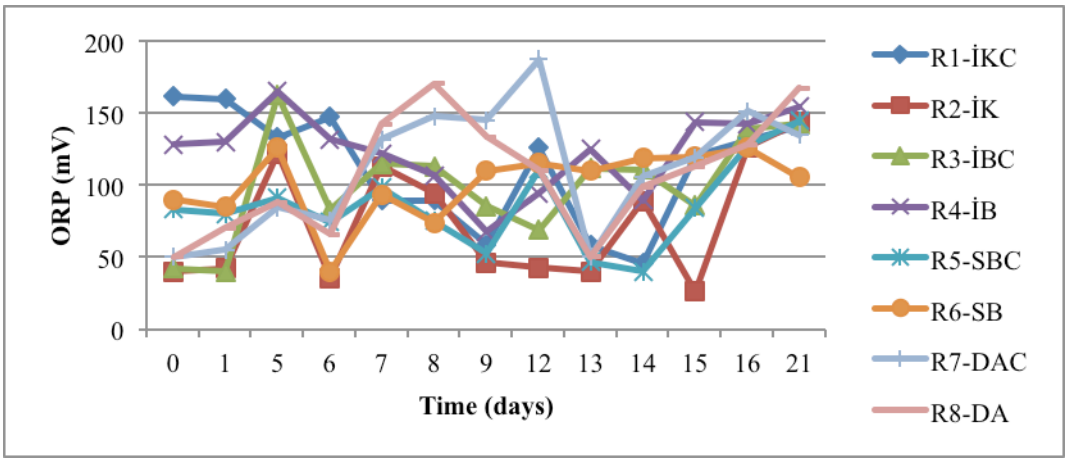


Figure 4.2. ORP changes in the aerobic digestion reactors.

4.1.3. Dissolved Oxygen

In an aerobic digestion system, it is recommended that dissolved oxygen (DO) concentration of sludge should not be less than 1 mg/L (Tchobanoglous et al., 2003). During the digestion process, DO levels were monitored daily and DO concentrations at the sludges were kept above 2 mg/L by the proper adjustment of airflow into the reactors. Dissolved oxygen data are given in Figure 4.3. At day 12, a decrease in DO levels was observed. It could be related to an overnight problem in the aeration device. The problem was solved by fixing the aeration device.

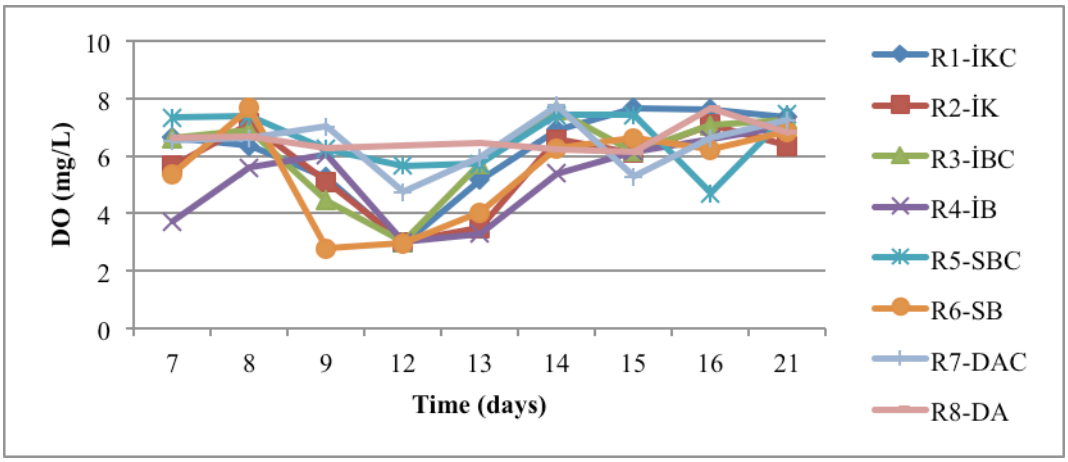


Figure 4.3. DO changes in the aerobic digestion reactors.

4.1.4. Electrical Conductivity

Electrical conductivity (EC) is used to quantify the dissolved ions in sludge samples. Sometimes, EC is measured to determine the salinity concentrations in sludges. So, there is a strong relationship between EC and salinity. The EC values in the control and pre-treated reactors were measured throughout the aerobic digestion process. The EC in the reactors were almost constant. The results are given in Figure 4.4.

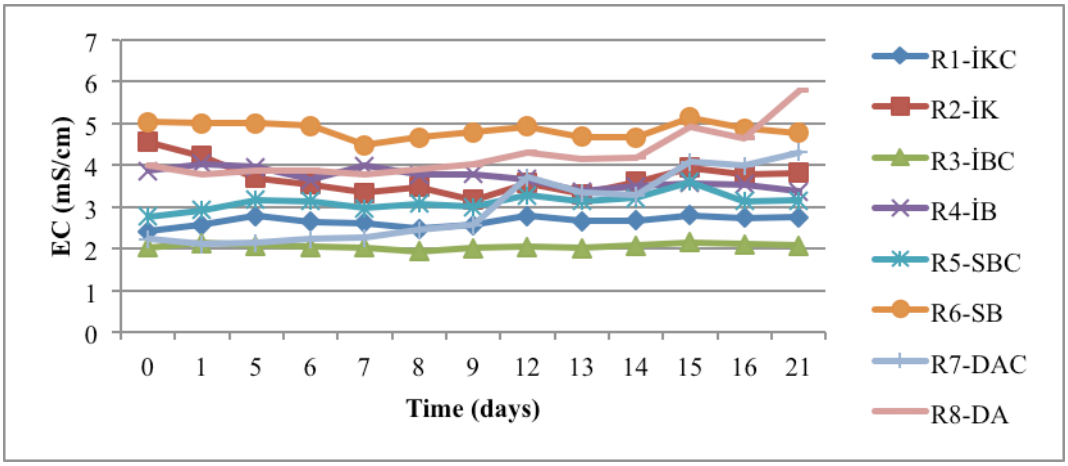


Figure 4.4. EC changes in the aerobic digestion reactors.

The EC values in the reactors ranged between 2 – 5,5 mS/cm. From the Figure 4.4, it can be seen that the EC in enzymatically pre-treated sludge samples are higher than the control reactors. As a result, it can be concluded that, enzyme addition increases the ability of a sludge sample to conduct electrical current through it.

4.1.5. Salinity

Salinity measurements in the reactors were conducted daily and the data are given in Figure 4.5. Salinity values ranged between 1,2 – 2,4 ‰ for İzmit Kullar WWTP sludge samples, 0,9 – 2,1 ‰ for İstanbul Bahçeşehir WWTP sludge samples, 1,4 – 2,8 ‰ for Samsun Bafra WWTP sludge samples and 1,1 – 2,7 ‰ for Düzce Akçakoca WWTP sludge samples. Salinity measurements were in accordance with the EC data. Same as in electrical conductivity results, enzyme added reactors' salinity measurements are higher than the control reactors.

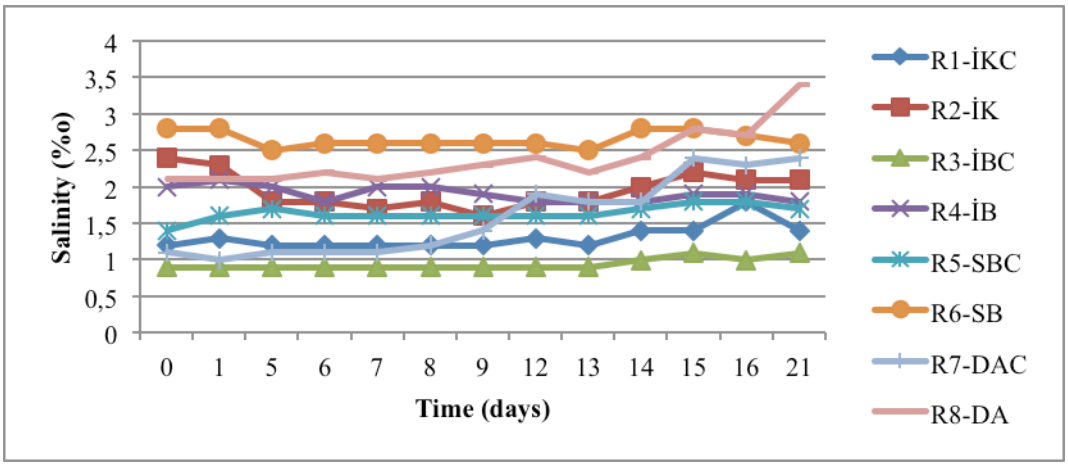


Figure 4.5. Salinity changes in the aerobic digestion reactors.

4.1.6. Temperature

Temperature influences almost all cellular reactions. In general, reactions proceed at faster rates under higher temperatures, but all enzymes have different optimum temperature ranges to work and below and above these ranges, substrate utilization rate of an enzyme is slowed (Burgess and Pletschke, 2008). The aerobic digestion experiments were conducted at room temperature. Depending on the room temperature, the temperature of the reactors ranges between 20 – 26 ^{o}C throughout the stabilization process. Changes in the temperature of the reactors can be seen in Figure 4.6.

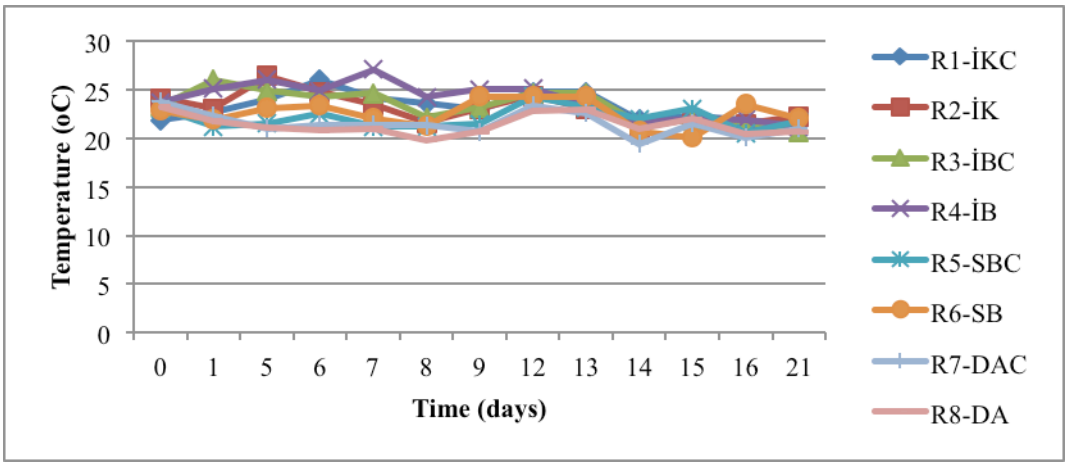


Figure 4.6. Temperature changes in the aerobic digestion reactors.

4.1.7. Total Solids and Volatile Solids

Total solids (TS) and volatile solids (VS) contents of the reactors were analyzed weekly and the changes in the concentrations throughout the process are given in Figure 4.7 and Figure 4.9, respectively. TS and VS reductions are important indicators of a successful stabilization. The reduction efficiencies of TS and VS are given in Figure 4.8 and Figure 4.10, respectively.

Initial TS contents of raw sludges that were taken from different WWTPs ranged between 5600 – 12000 mg/L. In order to eliminate the effect of differing TS concentrations on the digestion process, TS contents of the sludge samples were adjusted to approximately 1% by gravimetric settling methods. Then, TS concentrations in the control reactors were 10750, 10500, 11600, and 11555 mg/L for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively. Right after the enzyme addition into the sludge samples, a sudden and slight increase was observed in the TS concentrations of disintegrated sludge samples. This can be related with the increased microbial growth as a result of enzyme addition. In Table 4.11, it is seen that after the enzyme addition to the sludge samples, total coliform, fecal coliform, and fecal streptococci concentrations in the sludge samples increased. That increase in the microbial population due to the enzyme addition supports the increase in the TS content. Same as TS content, VS content of the sludges were slightly higher in the disintegrated reactors, compared to the control reactors.

Aerobic digestion process led to a decrease in the TS and VS contents of the sludge samples. TS reductions in the control reactors were 17%, 19%, 26%, and 35% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively. This means that, at the end of the aerobic digestion, mass of sludge solids was decreased by the given percentages. On the other hand, TS reductions in the enzymatically pre-treated reactors were 35%, 48%, 37%, and 50% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. Application of enzymatic pre-treatment prior to aerobic digestion resulted in increased TS reductions. Furthermore, VS reductions in the control reactors were 25%, 29%, 30%, and 42% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively. In the enzymatically pre-treated reactors, VS reductions were 43%, 56%, 43%, and 61% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. Enzymatic pre-treatment also enhanced the VS reductions in the sludge samples. Throughout the digestion process, TS and VS reductions showed a very similar trend.

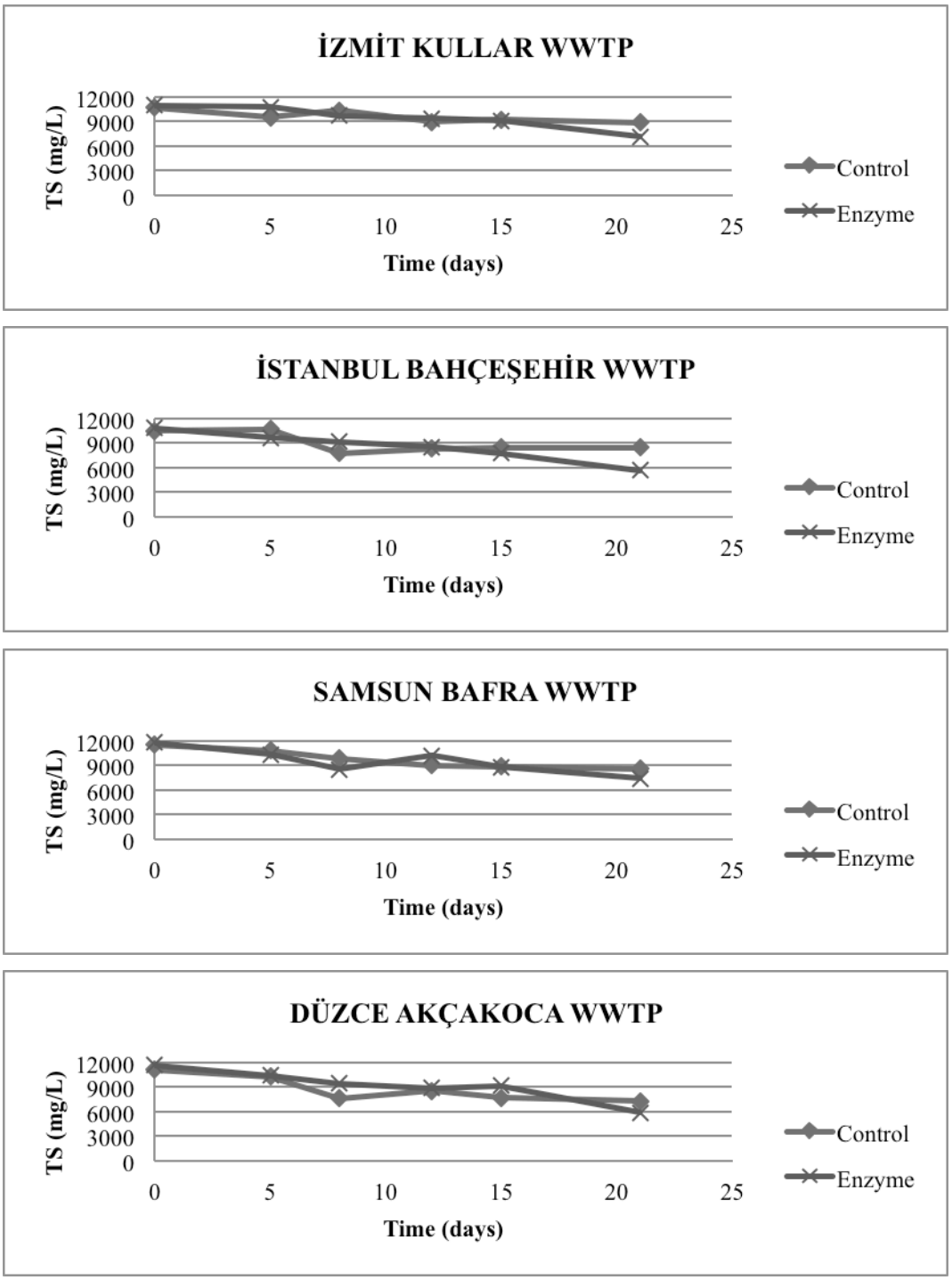


Figure 4.7. TS changes in the aerobic digestion reactors.

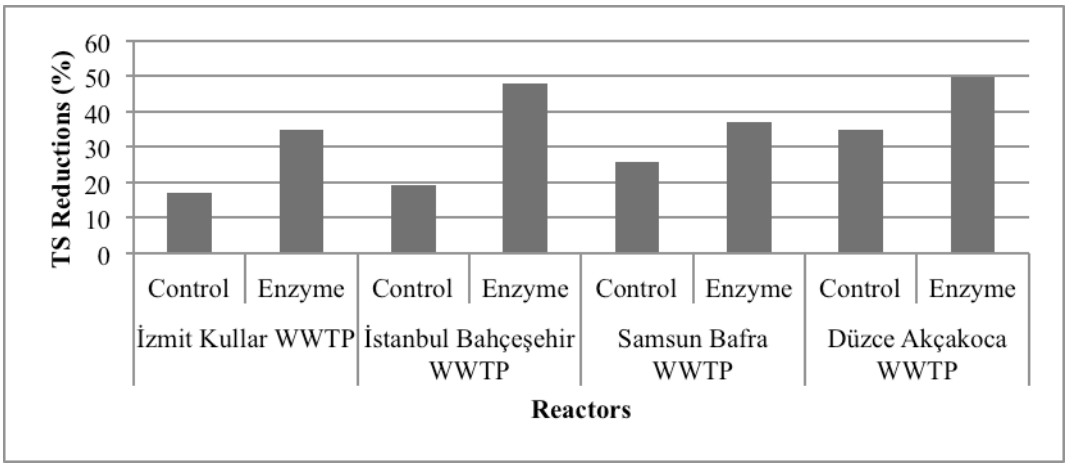


Figure 4.8. TS reductions in the aerobic digestion reactors.

Depending on the TS and VS reduction efficiencies, no relationship was found between the source of the sludge sample and the digestion process. The highest TS reduction by 50% and the highest VS reduction by 61% were achieved in the reactor containing enzyme added sludge sample of Düzce Akçakoca WWTP, which is treating urban wastewaters. The solids reduction efficiency was followed by sludge sample of İstanbul Bahçeşehir WWTP, with 48% and 56% reductions in TS and VS, respectively, which is treating domestic wastewaters.

One of the main aims of the stabilization was to improve volatile solids reductions in the reactors and obtain higher removal percentages. In an aerobic digestion system, degraded volatile solids are converted to CO_2. Typical VS reductions in aerobic digestion systems vary between 30-50% and since those digesters are not sized for complete oxidation, 30% VS reduction is often a satisfactory minimum. The results of TS and VS analyses show that application of enzymatic disintegration increases the biodegradability of the sludge and the efficiency of stabilization as well as it reduces the mass of sludge produced. Application of enzymatic disintegration enhanced the VS reduction performances of the reactors.

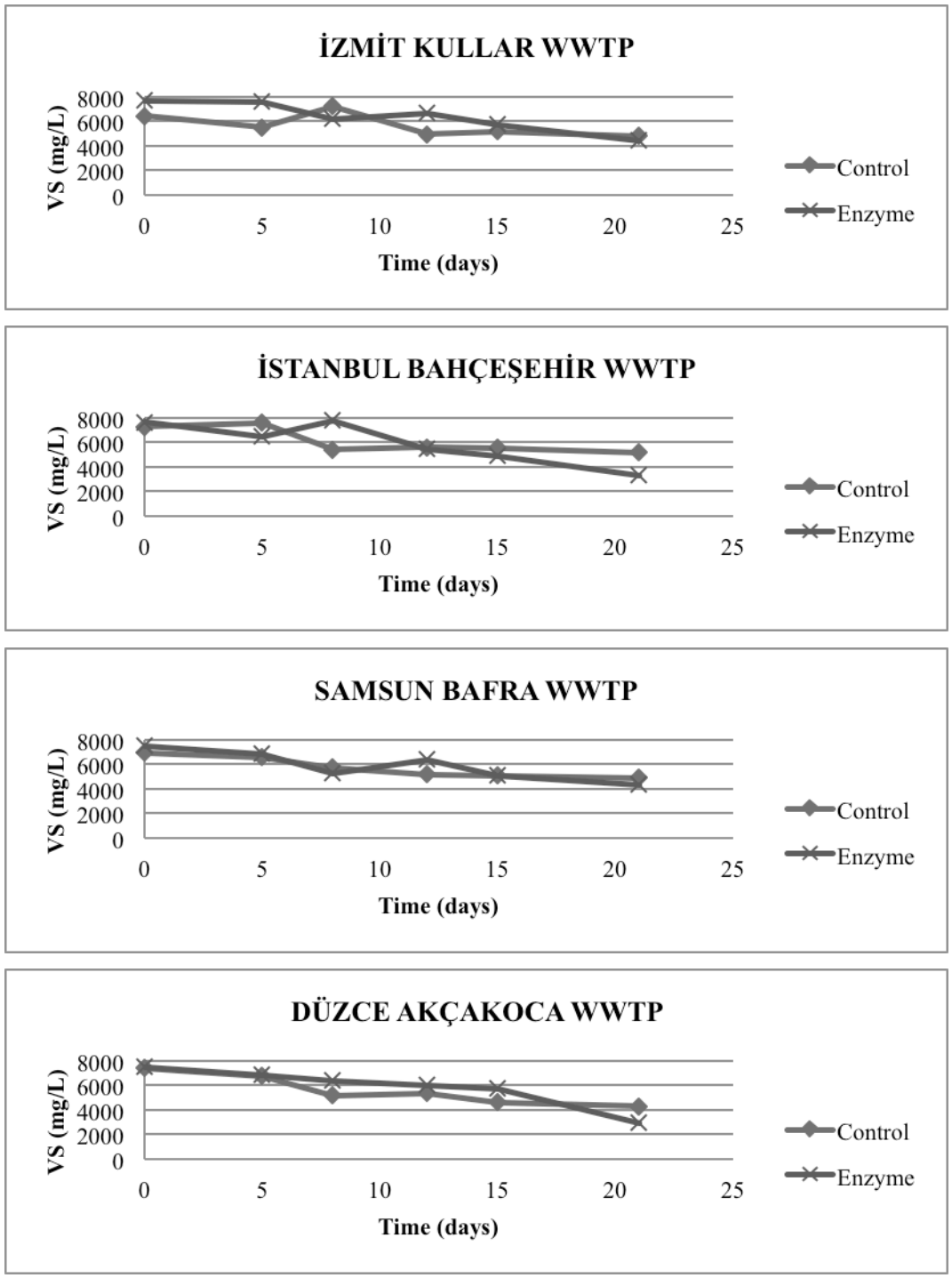


Figure 4.9. VS changes in the aerobic digestion reactors.

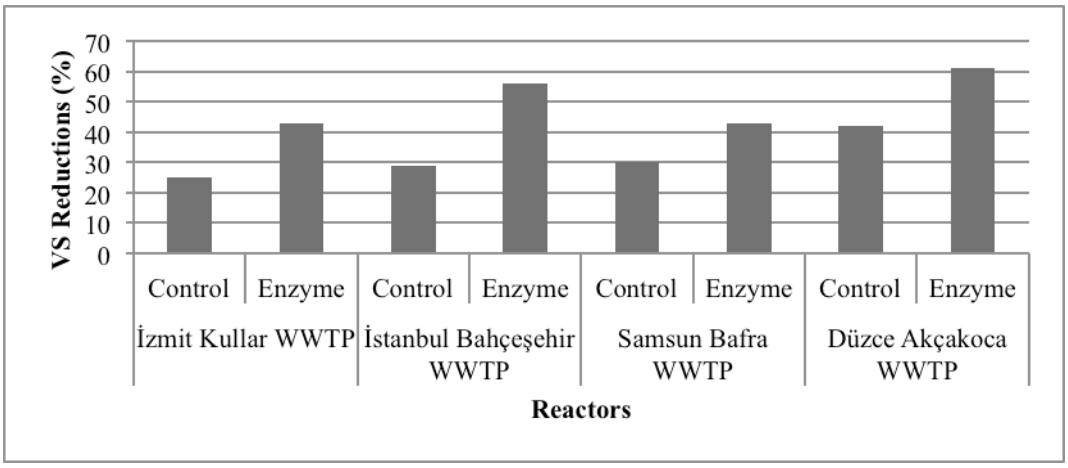


Figure 4.10. VS reductions in the aerobic digestion reactors.

4.1.8. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

Mixed liquor suspended solids (MLSS) of sludges consist of organic and inorganic matters, as well as microorganisms, and other inert suspended matters. Therefore, MLSS is the indirect measure of the total biomass. MLSS analyses were conducted during the aerobic digestion process and the results are given in Figure 4.11. The removal efficiency of MLSS is given in Figure 4.12.

The aerobic digestion process led to a decrease in the MLSS concentrations of the sludge samples. At the end of the aerobic digestion process, MLSS reductions in the control reactors were 18%, 22%, 28%, and 43% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively. In the disintegrated reactors, MLSS reductions were 32%, 49%, 36%, and 58% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. Enzymatically pre-treated reactors showed better performance on the removal of MLSS contents.

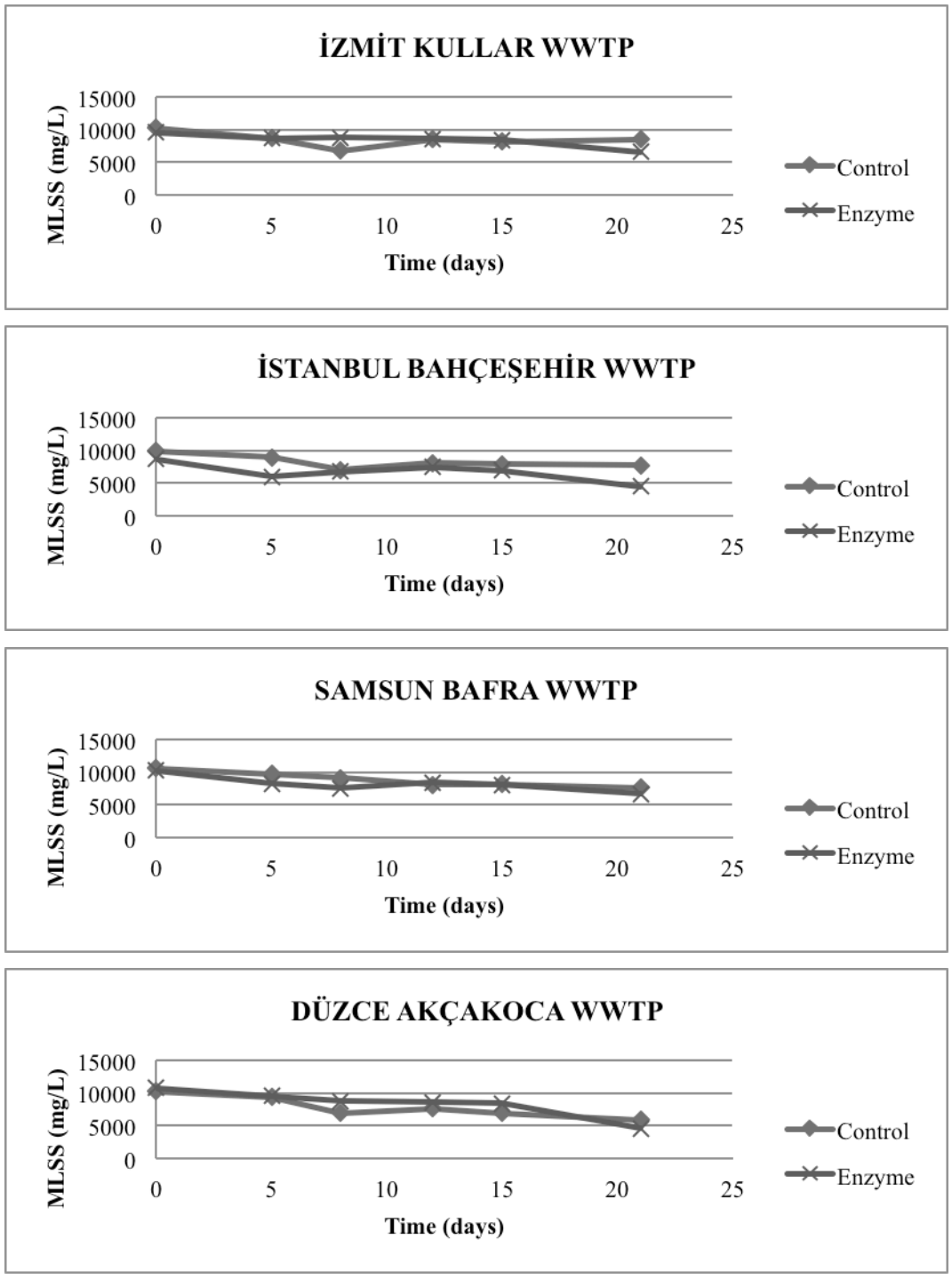


Figure 4.11. MLSS changes in the aerobic digestion reactors.

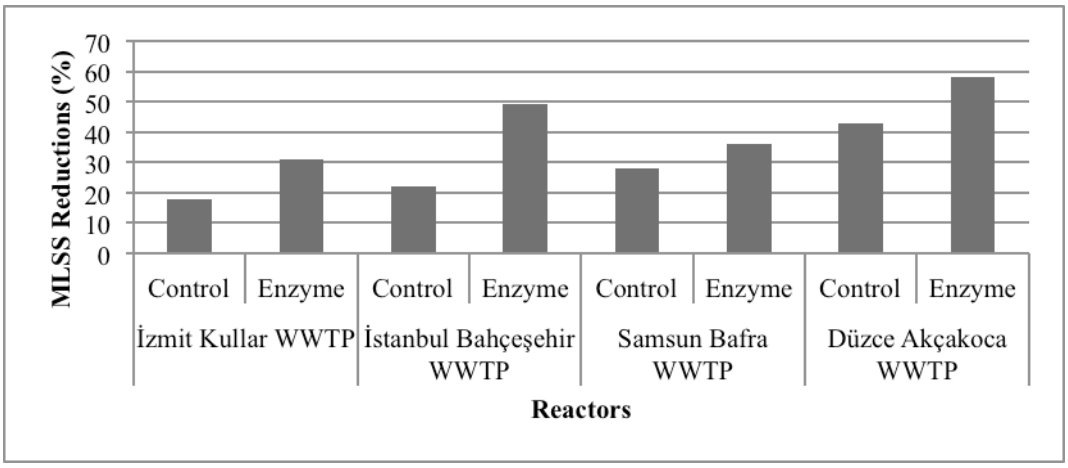


Figure 4.12. MLSS reductions in the aerobic digestion reactors.

MLVSS reduction is an important parameter, which is responsible for the sludge hydrolysis. MLVSS represents the amount of organic or volatile suspended solids in a sludge sample. This volatile portion is used as a measure or indicator of the presence of microorganisms. The changes in the MLVSS concentrations during the aerobic digestion and MLVSS reduction efficiencies are given in Figure 4.13 and Figure 4.14, respectively.

At the initial stage of the digestion, MLVSS concentrations of enzymatically pre-treated samples were slightly lower than the controls, except Düzce Akçakoca WWTP. This difference can be linked to the change of particulate organic matters into soluble organic matters after enzymatic hydrolysis.

A continuous decrease in the MLVSS contents of the sludge samples was observed for all reactors during the aerobic digestion. MLVSS reductions in the control and enzymatically pre-treated reactors were 24% and 32% in İzmit Kullar WWTP, 29% and 56% in İstanbul Bahçeşehir WWTP, 28% and 38% in Samsun Bafra WWTP, and 47% and 58% in Düzce Akçakoca WWTP, respectively. The results show that enzymatically pre-treated reactors are more effective on the degradation of MLVSS contents of the sludges.

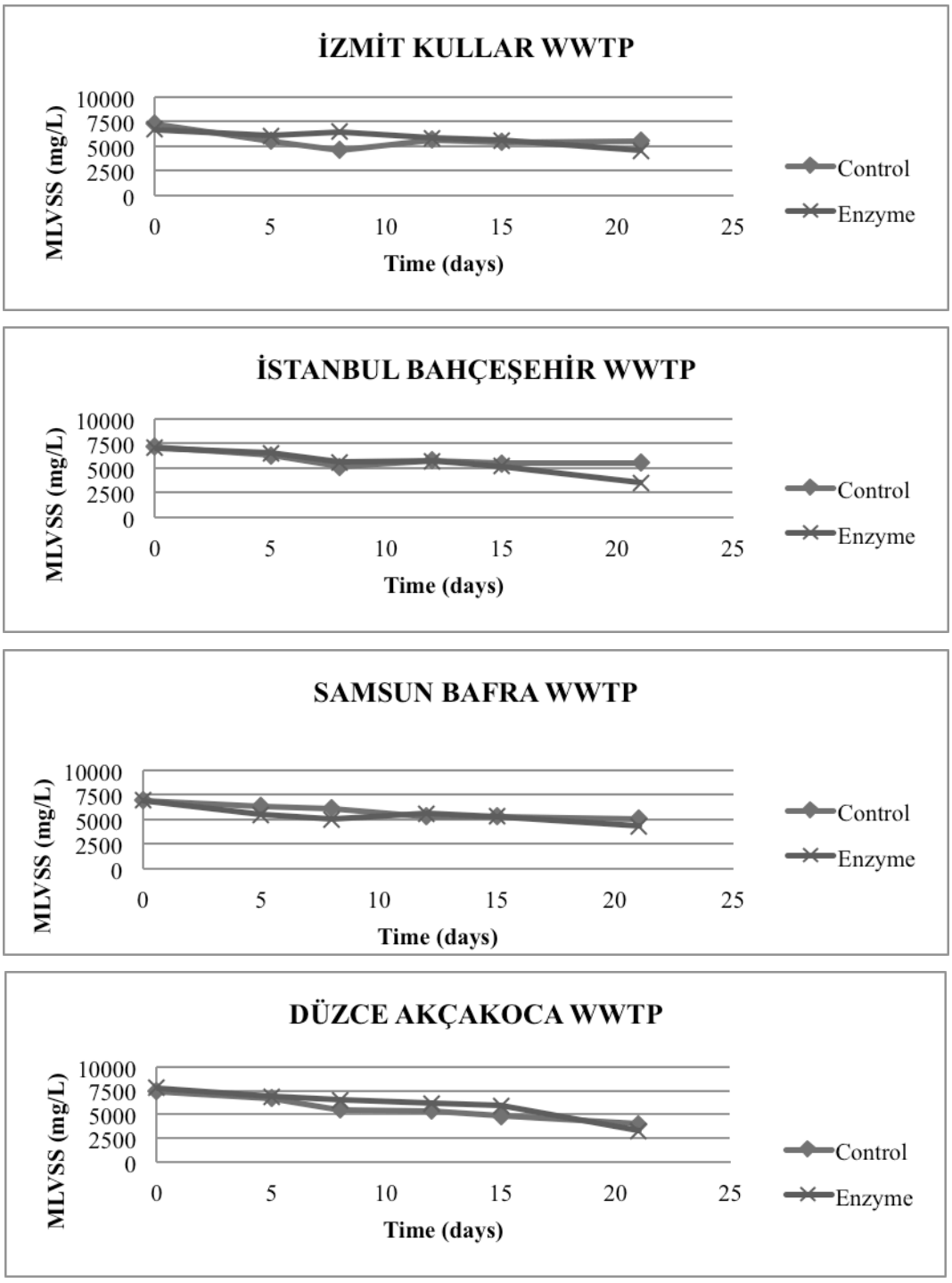


Figure 4.13. MLVSS changes in the aerobic digestion reactors.

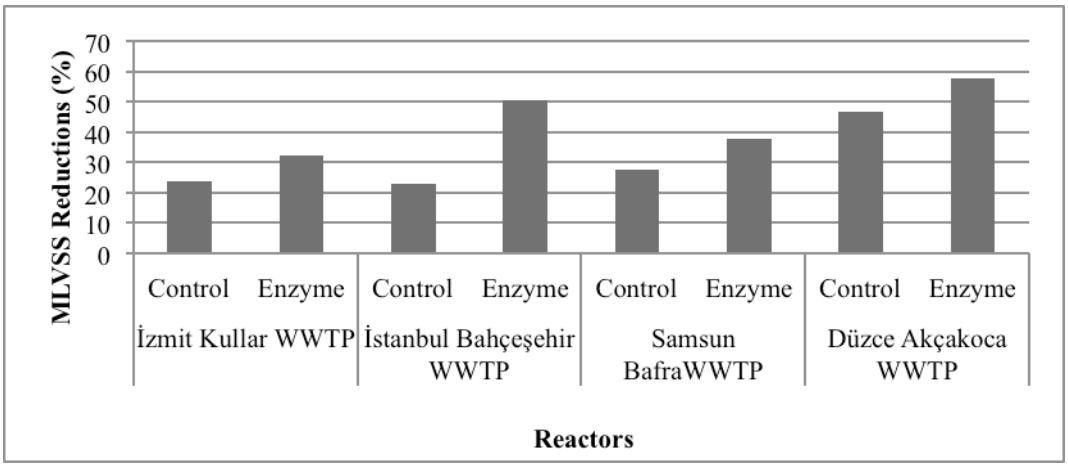


Figure 4.14. MLVSS reductions in the aerobic digestion reactors.

MLVSS/MLSS ratio is an important parameter while operating an aerobic reactor.The presence of microorganisms plays an important role in the process of biodegradation. Therefore, the ratio of MLVSS/MLSS can be used to determine whether there are enough microorganisms present to digest the sludge and biodegradation. Variations in the ratio of MLVSS/MLSS indicate a change in amount of biomass share. Typically MLVSS/MLSS ratio is within the range 0,65-0,85. MLVSS/MLSS ratios of the control and pre-treated reactors are shown in Table 4.2. The mean value of MLVSS/MLSS ratio for İzmit Kocaeli WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP are 0,67 - 0,72 - 0,66 and 0,72 in control reactors and 0,70 - 0,80 - 0,67 and 0,75 in disintegrated reactors, respectively. The ratio was a little bit higher in the disintegrated sludge samples since there was an increase in the microbial population as a result of the enzyme addition. These results also show that there were no significant changes in the amount of viable sludge during the aerobic digestion.

When we evaluate the performance of aerobic digestion reactors in terms of overall solids removals, observed reduction efficiencies are given in Table 4.2. The aerobic digestion was satisfactory in all reactors. On the other hand, no relationship between solids removals and the source of the sludge sample was found. In all reactors, initial TS, VS, MLSS, MLVSS concentrations were lowered at the end of the stabilization process, by the percentages given

in Table 4.3. In addition, TS, VS, and MLSS concentrations were slightly increased with the addition of enzymes, as a result of growth in the microbial population. As can be seen from Table 4.3, enzyme addition to the sludge samples improved the solids removal efficiencies. That means less sludge was produced to be sent to the final disposal in the disintegrated reactors at the end of the aerobic digestion. As a result, enzymatic pre-treatment achieved the minimization of sludge. The results are compatible with those in the literature (Bernard and Gray, 2000; Salsabil et al., 2009).

Table 4.2. MLVSS/MLSS ratio changes during the aerobic digestion process.

Days	**İzmit Kocaeli WWTP**		**İstanbul Bahçeşehir WWTP**		**Samsun Bafra WWTP**		**Düzce Akçakoca WWTP**	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
0	0,71	0,7	0,73	0,81	0,66	0,68	0,72	0,74
5	0,64	0,69	0,71	0,82	0,66	0,67	0,71	0,72
8	0,69	0,74	0,74	0,83	0,67	0,67	0,79	0,75
12	0,66	0,68	0,72	0,78	0,66	0,67	0,71	0,72
15	0,66	0,67	0,7	0,75	0,65	0,65	0,69	0,7
21	0,66	0,69	0,7	0,81	0,66	0,67	0,68	0,86

Table 4.3. Overall solids reductions in the aerobic reactors.

	İzmit Kocaeli WWTP		**İstanbul Bahçeşehir WWTP**		**Samsun Bafra WWTP**		**Düzce Akçakoca WWTP**	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
TS	17%	35%	19%	48%	26%	37%	35%	50%
VS	25%	43%	29%	56%	30%	43%	42%	61%
MLSS	18%	32%	22%	49%	28%	36%	43%	58%
MLVSS	24%	32%	29%	56%	28%	38%	47%	58%

4.1.9. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

Chemical oxygen demand (COD) is the measurement of organic pollutants present in the sludge. Removal of these organic pollutants in a treatment process is necessary prior to the ultimate disposal. Changes in COD concentrations can be used to measure the stability degree of sludge (Graczyk, 1984). During this study, changes in COD and soluble COD (sCOD) concentrations were monitored, the analyses were conducted twice a week, and the results are given in Figure 4.15 and Figure 4.17 respectively. Figure 4.16 and Figure 4.18 shows the COD and sCOD removal performances, respectively.

COD concentrations of raw wastewaters were 11307 mg/L, 9602 mg/L, 6579 mg/L, and 7773 mg/L in İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. In the first 5 days of the aerobic digestion process, COD concentrations decreased at a higher rate, and then the rate was slowed. After the 15^{th} day, COD values remained constant indicating that the system was stable. The results are compatible with those reported in the literature, the same constant COD concentrations after a period of time were also observed by other researchers (Coello Oviedo et al., 2005; Barragan Sanchez et al., 2006; Chang et al., 2011). The organic material that is highly resistant to the biodegradation or the non-biodegradable inorganic compounds that can't be degraded by microorganisms might cause the remaining and non-degraded COD.

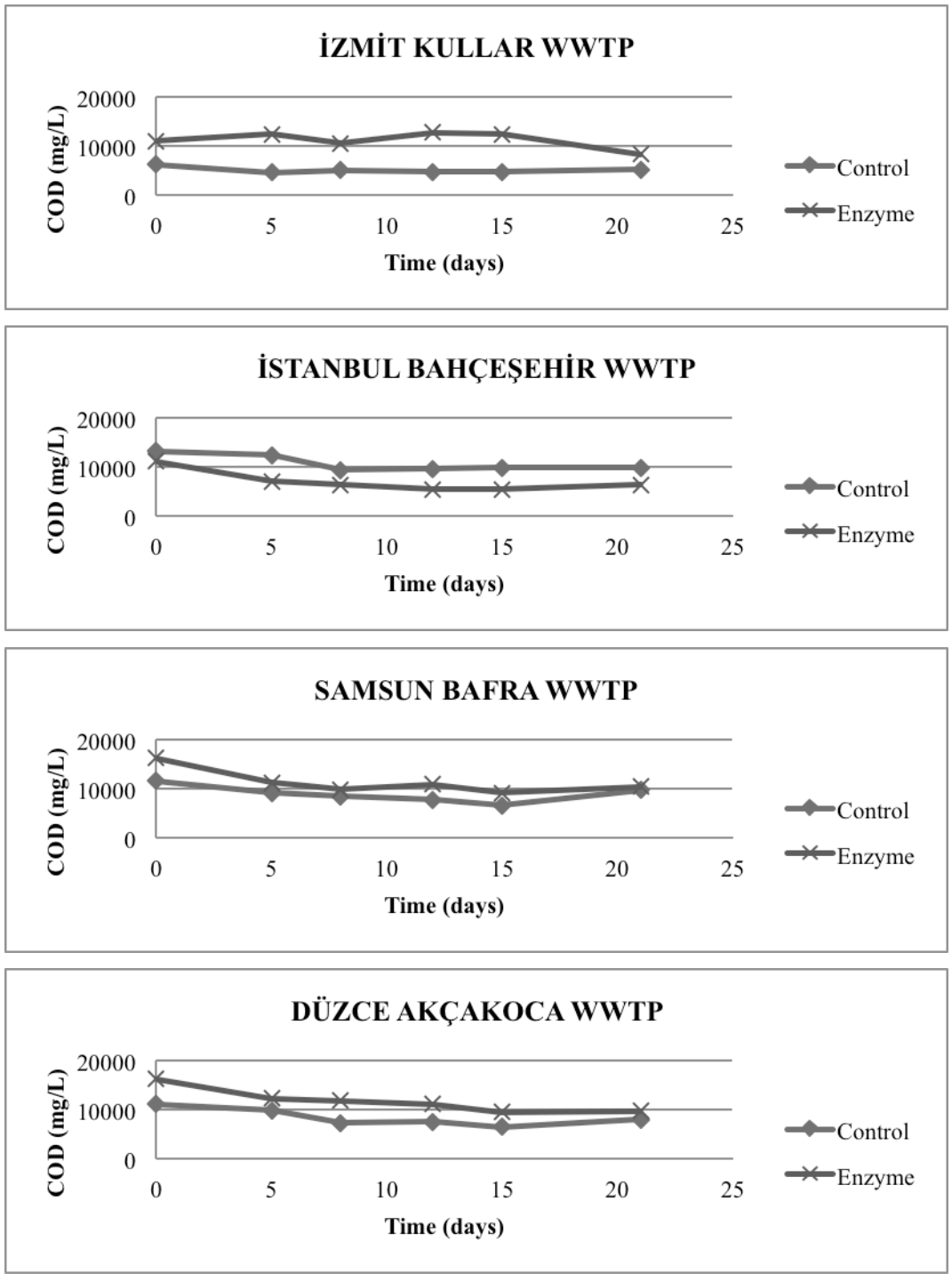


Figure 4.15. COD changes in the aerobic digestion reactors.

From the Figure 4.16, it can be seen that enzymatic disintegration played an important role on increasing the removal efficiency of the organic matter present in the sludge. The COD removal was 17%, 25%, 16%, and 29% in control reactors whereas it was 24%, 43%, 36%, and 41% in reactors containing disintegrated sludges of İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. As a result, enzymatically pre-treated reactors were more effective on the degradation of the organic matter. The highest COD reduction was achieved in R4-İB, from 11041 mg/L to 6250 mg/L, which leads to a reduction by 43%. It was followed by the reduction in R8-DA, by 41%. Since sludge samples of R4-İB and R8-DA comes from domestic and urban WWTPs, respectively, no relationship was found between the type of the WWTP and the organic matter degradation.

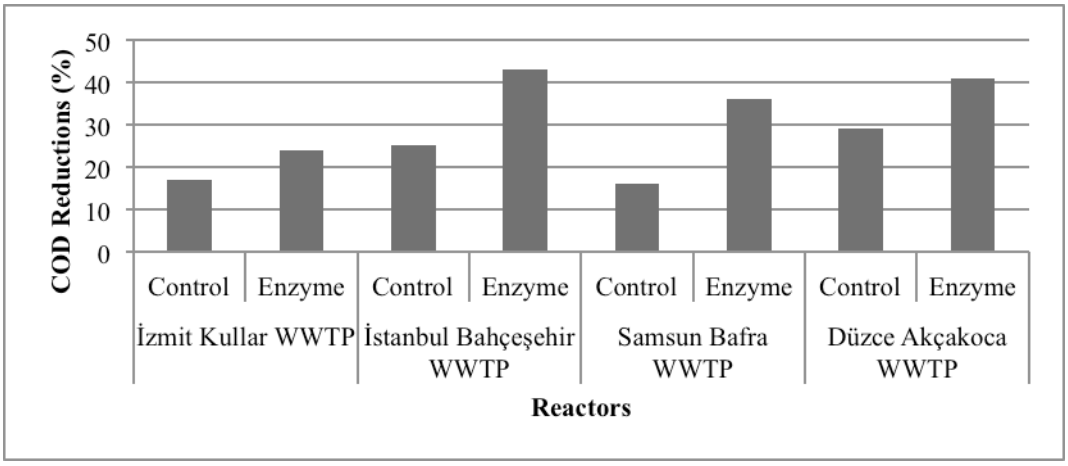


Figure 4.16. COD reductions in the aerobic digestion reactors.

In an aerobic digestion system, abundant organic matter can be released into the supernatant due to cell lysis, resulting in a rapid increase of sCOD. On the other hand, viable microbes of the aerobic digestion system could use the released substrate to achieve cryptic growth, possibly leading to a significant decline in sCOD (Haner et al., 1994; Loosdrecht and Henze, 1999; Liu et al., 2012).

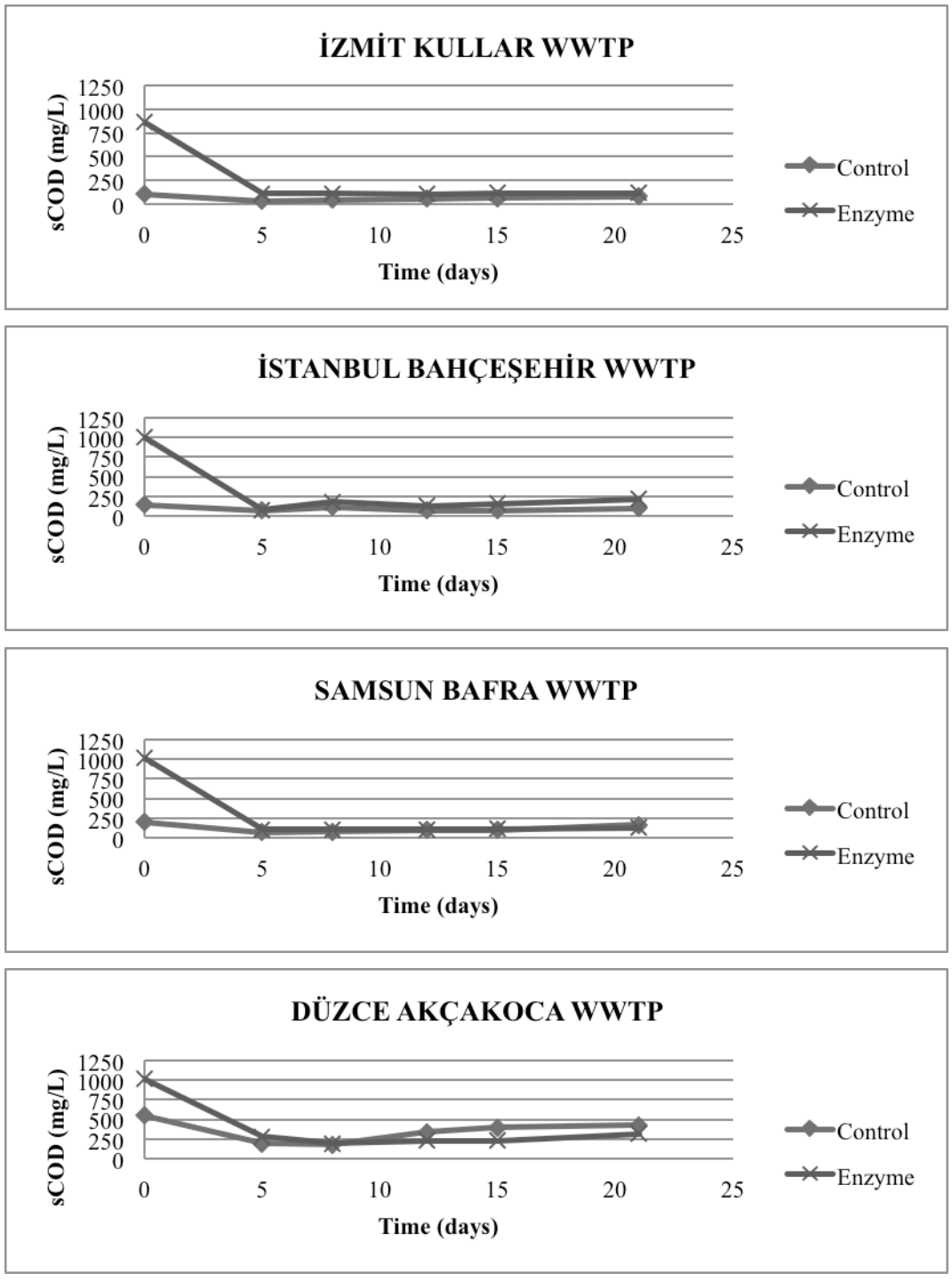


Figure 4.17. sCOD changes in the aerobic digestion reactors.

The enzymatic pre-treatment of sludge causes disruption of cell structure, which leads to the release of cell contents into the solution. Hence, an increase in the sCOD concentrations by the enzymatic pre-treatment is expected. Increased sCOD concentrations are important indicators of a successful cell lysis.

By the application of enzymatic disintegration, sCOD concentrations of the enzymatically pre-treated sludge samples increased. This increase in sCOD concentrations confirms that a large amount of particulate organic matter in the sludge flocs was transferred into soluble organic matter form. It might result from the destruction of flocs structure after the enzymatic hydrolysis, promoting the release of colloidal and soluble organics into the solution. The effect of enzymatic pre-treatment can be evaluated based on the sCOD concentrations. The higher sCOD contents represent a better efficiency of the disintegration of the sludge floc and cell wall structure (Gronroos et al., 2005; Chang et al., 2011). The sCOD concentrations were increased 761 mg/L, 855 mg/L, 810 mg/L, and 470 mg/L in the reactors R2-İK, R4-İB, R6-SB, and R8-DA, respectively. Similar results were reported in the literature (Kim et al., 2000; Chang et al., 2011). Increases in sCOD concentrations suggest that the particulate organic matters in sludge were solubilized and converted into low molecular compounds by the enzymatic disintegration.

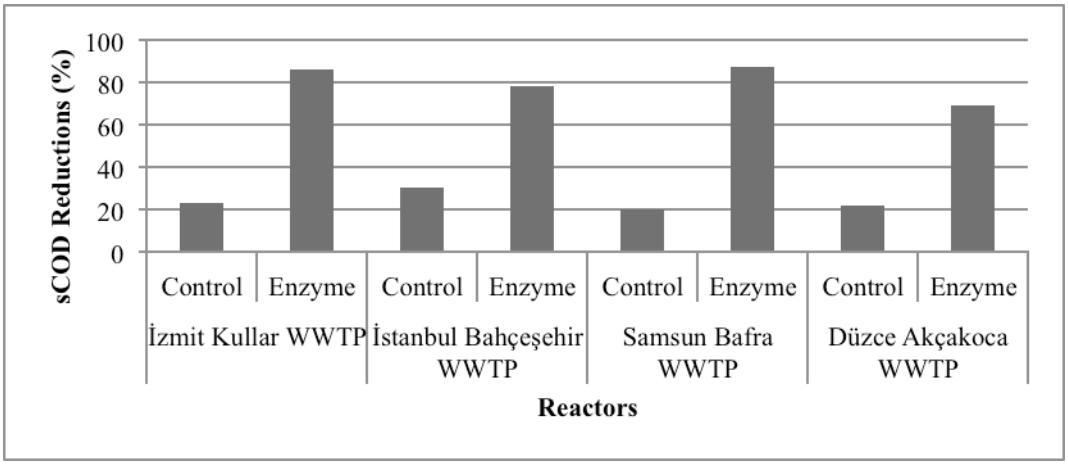


Figure 4.18. sCOD reductions in the aerobic digestion reactors.

Reductions in the sCOD concentrations of the sludge samples were higher in the enzymatically pre-treated reactors compared to control reactors. The sCOD removal was 23%, 30%, 20%, and 22% in control reactors whereas it was 86%, 78%, 87%, and 69% in reactors containing enzyme added sludges of İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. Enzyme addition did not only improve the release of intracellular materials, but also enhanced the degradation of the soluble organic matters.

Table 4.4. sCOD/tCOD and DD in the aerobic reactors.

Treatment Plant	Reactor	Initial sCOD/tCOD (%)	DD (%)
İzmit Kullar WWTP	Control	1,75	
	Enzyme	7,99	80
İstanbul Bahçeşehir WWTP	Control	1,10	
	Enzyme	9,06	73
Samsun Bafra WWTP	Control	1,78	
	Enzyme	6,26	95
Düzce Akçakoca WWTP	Control	4,91	
	Enzyme	6,21	76

Since enzymatic disintegration doesn't have an effect on total COD, sCOD/COD ratio is important in determining the degree of the disintegration. sCOD/COD ratios in control reactors were 1,75%, 1,10%, 1,78%, and 4,91% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively and 7,99%, 9,06%, 6,26%, and 6,21% % for R2-İK, R4-İB, R6-SB, and R8-DA, respectively (Table 4.4). These results show that sCOD/COD ratios were greatly improved by the enzymatic disintegration and also suggest that enzymes play an important role on the degradation and solubilization of the particulate organic matter. As the sCOD concentrations of the pre-treated reactors increased by the enzyme addition, disintegration degree (DD) of the sludge samples was increased, too. An important increase in the DD was observed in the enzymatically pre-treated sludge samples. That increase can be attributed to the release of intracellular materials by the destruction of cell walls.

Linking the high increases in sCOD/COD ratios to the high MLVSS reduction, the addition of enzymes could significantly enhance excess sludge hydrolysis.

4.1.10. Total Organic Carbon and Dissolved Organic Carbon

Total organic carbon (TOC) is the amount of carbons bounded in an organic compound. TOC measurements include all organic carbons in a sludge sample, while dissolved organic carbon (DOC) measurements only include soluble carbons. TOC should be always lower than COD because it only estimates the carbon content, while COD estimates all organic compounds. TOC and DOC analyses were conducted once a week during the digestion process and the results are given in Figure 4.19 and Figure4.20, respectively. In an aerobic digestion process, TOC contents of the sludge samples are expected to decrease since COD concentrations in the reactors decreases, too.

TOC concentrations of the raw sludges taken from treatment plants ranged between 418-732 mg/L. In the Figure 4.19, it is seen that along with the enzymatic disintegration before the aerobic digestion, TOC concentrations in the reactors increased by 20%, 7%, 23%, and 8% in İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca, respectively. This increase in the organic carbon content shows that, enzymes destroyed the cell walls and hydrolysis took place, and then organic contents of the cells became available as substrates.

During the aerobic digestion process, organic carbon contents of the reactors decreased. TOC removal efficiencies in enzymatically pre-treated reactors were higher than the control reactors. Efficiencies in the control reactors were 66%, 46%, 61%, and 43% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively, whereas reductions in the pre-treated reactors were 66%, 77%, 67%, and 57% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. It can be concluded that enzymatic pre-treatment increases the TOC removal efficiencies.

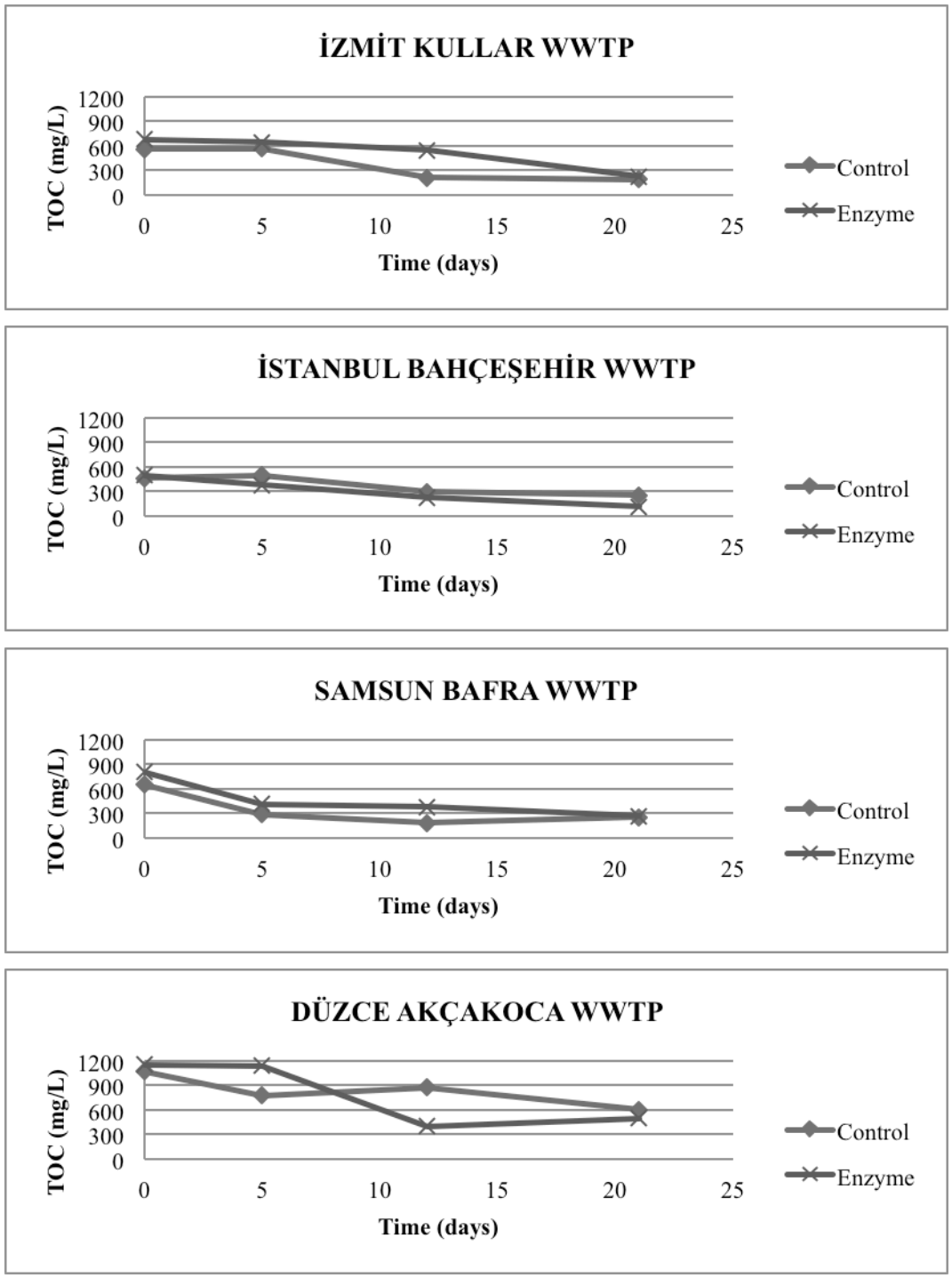


Figure 4.19. TOC changes in the aerobic digestion reactors.

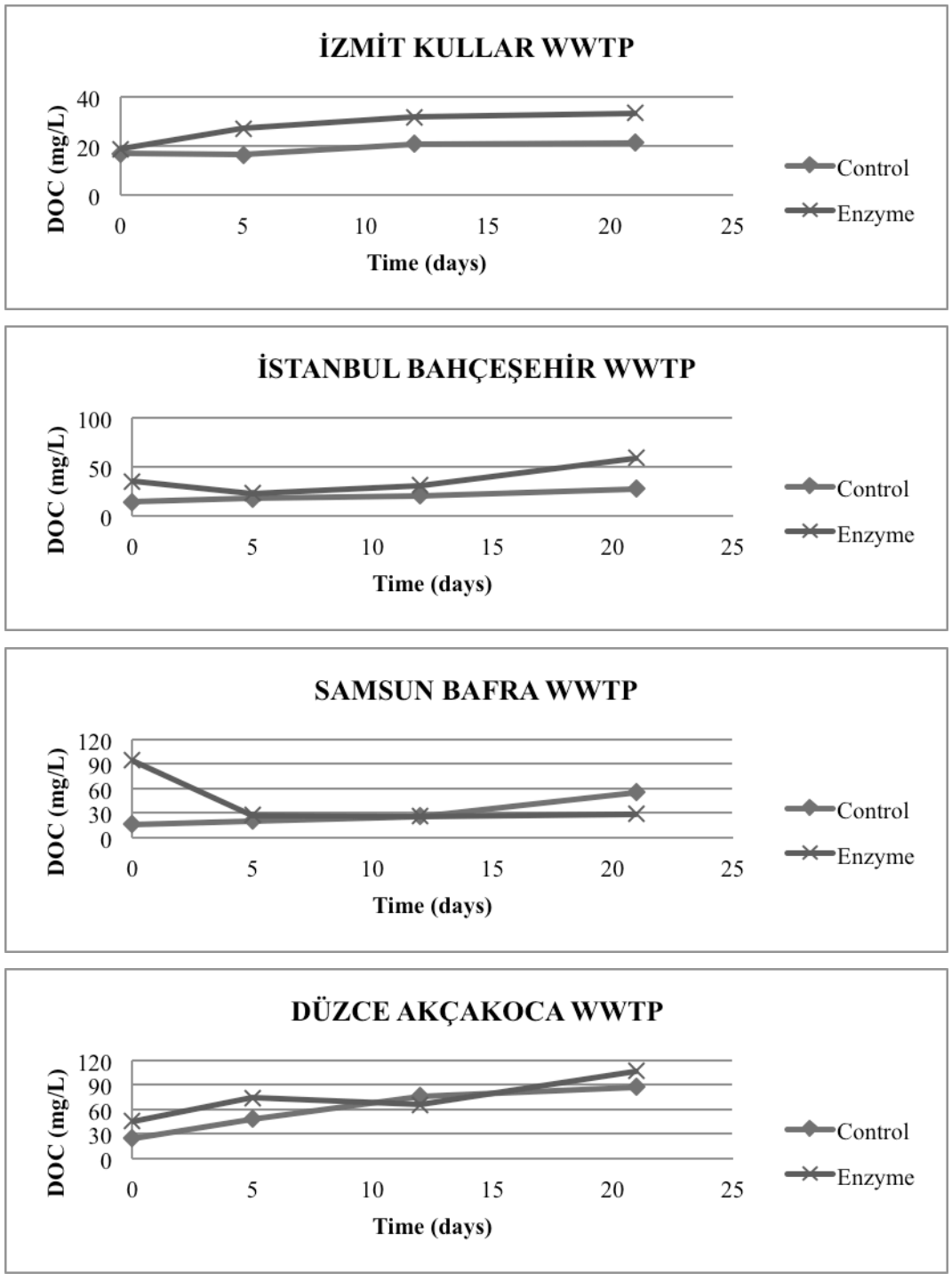


Figure 4.20. DOC changes in the aerobic digestion reactors.

DOC concentrations of the raw sludges taken from treatment plants ranged between 18-25 mg/L. Increases in DOC concentrations were observed along with the enzymatic disintegration before stabilization. A similar increase was also observed in sCOD concentrations, but DOC increment is lower than sCOD increment. In addition, DOC concentrations are lower than sCOD concentrations since DOC only involves carbon fraction of the organic content. During the aerobic digestion process, even though TOC content was removed in given percentages, DOC content of the reactors increased and the concentrations after the stabilization process ranged between 21-108 mg/L. An increase in TOC contents was also observed in Tas's study. It was stated that microorganisms consumed most of the removed TOC and some of the non-biodegradable products were resulted in an increase in the DOC level (Tas 2010).

Same as in the other parameters, a relationship between TOC or DOC removal and the source of the sludge was not found. The highest TOC reductions were achieved in the reactors R2-İK and R6-SB.

4.1.11. Alkalinity

Total alkalinity analyses were conducted two times in a week in order to measure the stability of the reactors. Initial alkalinity concentrations of the raw sludges were 735 mg $CaCO_3$/L, 900 mg $CaCO_3$/L, 1600 mg $CaCO_3$/L, and 500 mg $CaCO_3$/L for İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. Changes in the alkalinity concentrations of reactors are given in Figure 4.21.

Alkalinity of a reactor is linked to its pH level. 0,3 pH units may result in a change of the alkalinity level by 200 mg/L or more. Even though measurement of pH is easier than alkalinity analysis, alkalinity is more reliably monitored than pH. Alkalinity concentrations of İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP and Düzce Akçakoca ranged between 200-1200 mg $CaCO_3$/L whereas Düzce Akçakoca WWTP's concentrations ranged between 500-1500 mg $CaCO_3$/L.

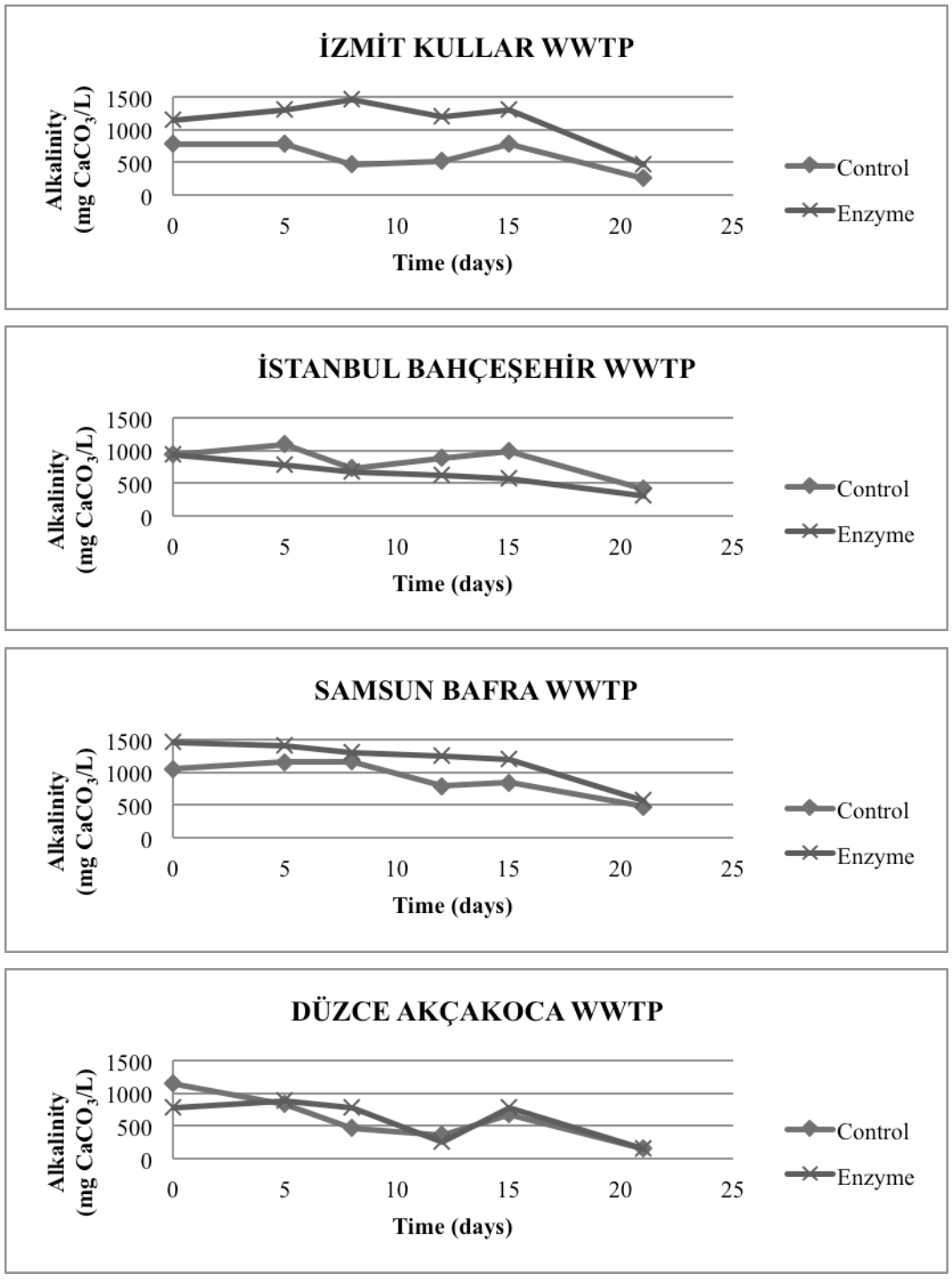


Figure 4.21. Alkalinity changes in the aerobic digestion reactors.

A decrease in the alkalinity concentrations was expected due to the consumption of alkalinity during the aerobic digestion process. In all reactors, reductions in the alkalinity levels were observed. After the aerobic digestion process, alkalinity levels of the reactors were around 200 mg $CaCO_3$/L, except Samsun Bafra WWTP's, which was 500 mg $CaCO_3$/L. The drop in alkalinity can be linked to lowered buffering capacity of the sludge due to the air stripping. In addition to the decrease in the concentrations, alkalinity levels were in the ideal ranges for an aerobic digestion process when the results were examined with pH changes.

4.1.12. Nitrite, Nitrate, Ammonium and Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen (TKN) analyses were conducted once a week and the results are given in Figure 4.22.Nitrite, nitrate and ammonium analyses were performed before and after the digestion and the results are given in Table 4.5.

As can bee seen from Figure 4.22, TKN concentrations in the reactors decreased during the digestion process. TKN is converted to ammonia. TKN removal efficiencies for control and enzyme added reactors are 31% and 31% for R1-İKC and R2-İK, 54% and 50% forR3-İBC and R4-İB, 39% and 33% forR5-SBC and R6-SB, and 61% and 74% forR7-DAC and R8-DA, respectively. The results are compatible with the literature. However, enzymatic disintegration didn't show any enhancement on the TKN removal compared to the control reactors. Though, enzyme addition showed enhancement on TKN removal in the sludge samples of Düzce Akçakoca WWTP, 74% TKN removal was achieved in the enzymatically pre-treated sludge compared to %61 TKN removal in the control reactor.

Bacteria remove nitrogen from sludge by two-step biological processes: nitrification followed by denitrification. In nitrification, bacteria known as *Nitrosomonas* convert ammonia and ammonium to nitrite. Then, *Nitrobacter* complete the conversion by converting nitrite to nitrate. Nitrification can occur under aerobic conditions at DO levels more than 1 mg/L simultaneously. Nitrification process lowers the pH, and consumes alkalinity.

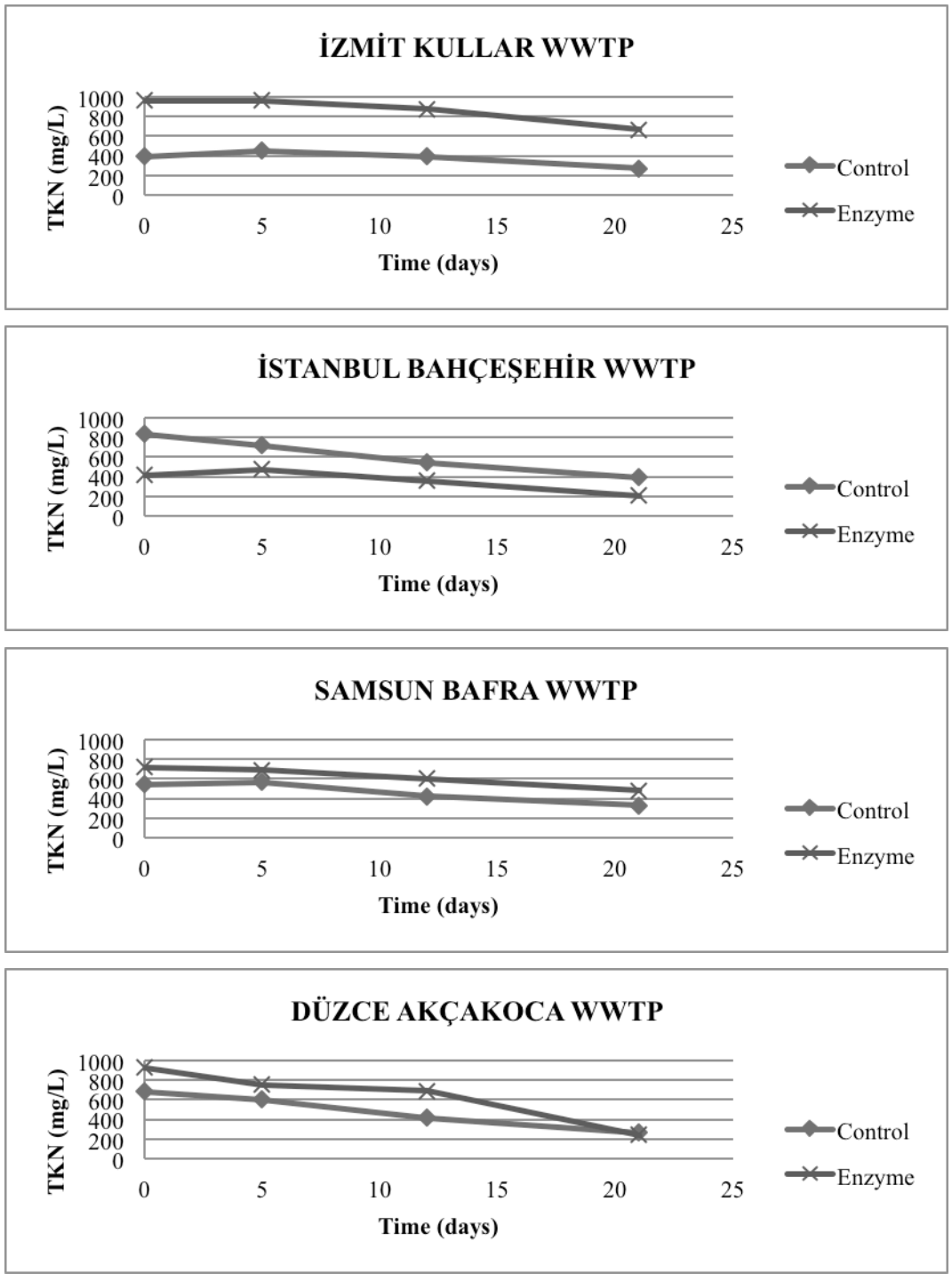


Figure 4.22. TKN changes in the aerobic digestion reactors.

Before the aerobic digestion, nitrite concentrations of the reactors ranged between 4-13 mg/L and this value decreased above 8 mg/L after the digestion. Nitrite concentrations of the reactors decreased along with the enzyme addition before the digestion. Nitrate concentrations in all reactors increased with the aerobic digestion, except the control reactor of Samsun Bafra WWTP. In addition, ammonium nitrogen concentrations decreased in all reactors. Low levels of nitrite and nitrate concentrations were also reported by Novak et al. (2011).

Table 4.5. Nitrite, nitrate, and ammonium changes in the aerobic digestion reactors.

Days	**İzmit Kullar WWTP**		**İstanbul Bahçeşehir WWTP**		**Samsun Bafra WWTP**		**Düzce Akçakoca WWTP**	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
NO_2^- (mg/L)								
0	10	7	5	4	11	5	13	9
21	4	2	2	2	2	3	8	1
NO_3^- (mg/L)								
0	14,3	0	2,9	3	33	18	0,1	15,1
21	17,9	3	4,2	13,4	9,3	13,4	81,5	67,7
NH_4^+-N (mg/L)								
0	7	11,25	19,76	1,52	11,25	7,3	61,71	43,77
21	2,7	7,8	13,25	0,25	4,1	6,4	36,75	43,75

During an aerobic digestion process, nitrate production takes place through the ammonia oxidation by nitrifying microorganisms. It is expected to observe an increase in nitrate concentrations resulting from the mineralized nitrogen from decayed activated sludge that is nitrified through nitrification process (Tas, 2010). The results of this study show that nitrite removal and nitrate generation in the reactors show that complete nitrification took place during the aerobic digestion process.

4.1.13. Total Phosphorous

Total phosphorous (TP) analyzes were conducted once in a week and the results are given in Figure 4.23. Initial raw sludge TP concentrations of treatment plants varied between 200-350 mg/L.

In the first 5 days of the stabilization, an increase in the TP concentrations was observed for İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP. However, an increase in İzmit Kullar WWTP took place between 5-10 days. This increase in the phosphorous concentrations can be related with the release of phosphorous content of cells as a result of hydrolysis in the beginning of the digestion process. In addition, it can be interpreted that, hydrolysis in İzmit Kullar WWTP started later than the other reactors.

As the stabilization process continued, reductions in the phosphorous contents were observed. Reductions in the control and enzyme added reactors were 50% and 63% at İzmit Kullar WWTP, 54% and 53% at İstanbul Bahçeşehir WWTP, 50% and 51% at Samsun Bafra WWTP, and 44% and 60% at Düzce Akçakoca WWTP, respectively. Enzyme addition showed better phosphorous reduction efficiencies for İzmit Kullar WWTP and Samsun Bafra WWTP. Reductions in other two treatment plants were close to each other.

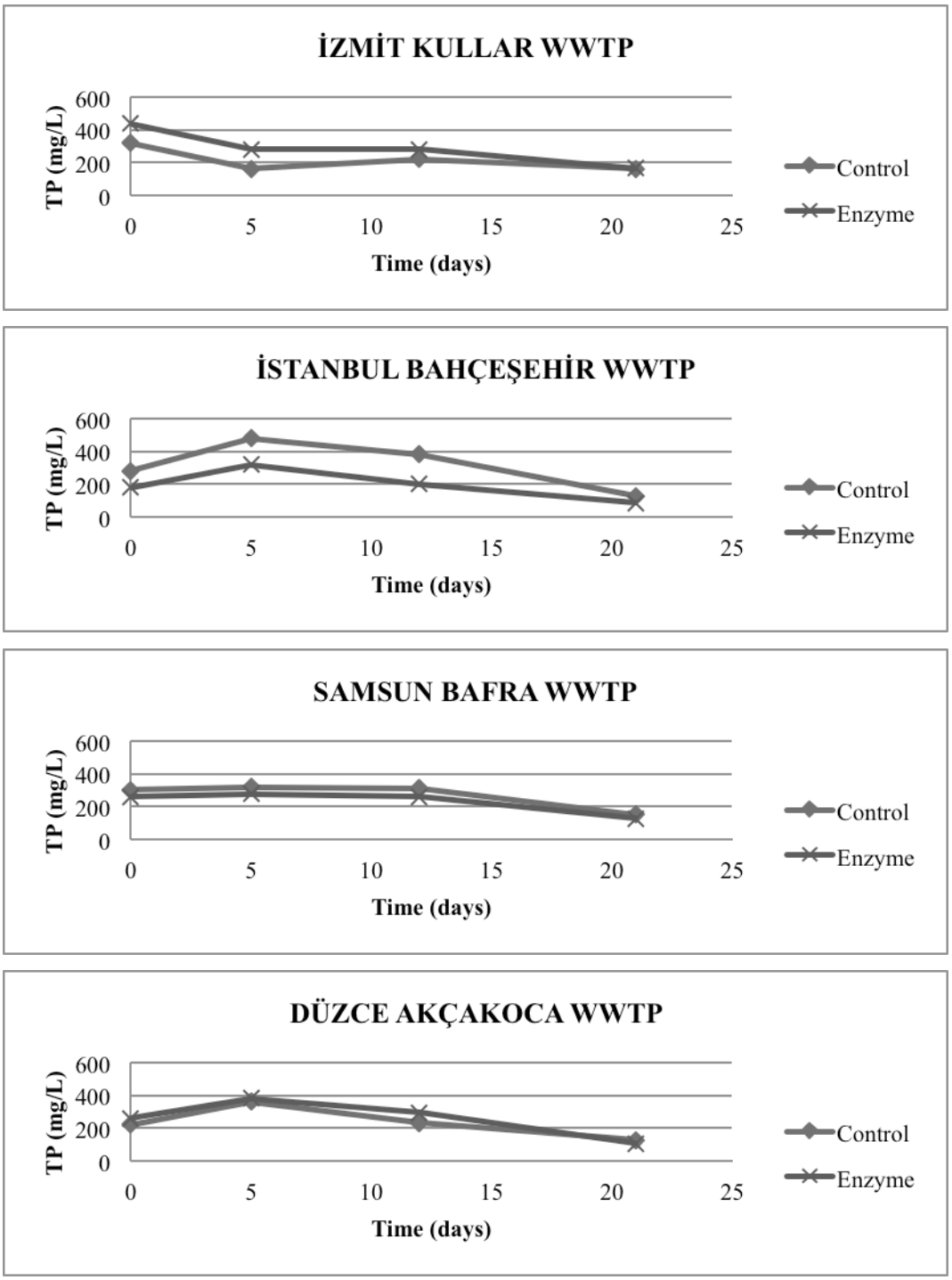


Figure 4.23.TP changes in the aerobic digestion reactors.

4.1.14. Sulfate and Chloride

During the operation of reactors, sulfate and chloride analyzes were conducted before and after the digestion and the results are given in Table 4.6.

From the Table 4.5, it can be seen that along with the enzymatic pre-treatment, chloride concentrations of the reactors increased were increased by 166%, 166%, 100%, and 132% for İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. Chloride results were in accordance with salinity and electrical conductivity measurements. Salinity and EC also increased as a result of enzyme addition. Chloride levels were in the optimum ranges so that an inhibitory affect was not observed.

On the other hand, sulfate concentrations of the reactors decreased along with enzyme addition 33%, 3%, 46%, and 2% for İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. At the end of the digestion process, chloride concentrations of the reactors increased. On the other hand, changes in sulfate concentrations were different for each reactor. Sulfate concentrations increased except İzmit Kullar WWTP control, İstanbul Bahçeşehir WWTP control and enzyme added reactors.

Table 4.6. Sulfate and chloride changes in the aerobic digestion reactors.

Days	**İzmit Kullar WWTP**		**İstanbul Bahçeşehir WWTP**		**Samsun Bafra WWTP**		**Düzce Akçakoca WWTP**	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
Chloride (mg/L)								
0	104	277	104	277	173	346	69	242
21	138	380	169	380	207	484	173	415
Sulfate (mg/L)								
0	225	150	145	140	235	125	190	185
21	205	210	105	120	255	240	270	255

4.1.15. Capillary Suction Time

The sludge dewaterability is measured by capillary suction time (CST). CST analyses were conducted once in a week in order to determine the effect of enzymatic pre-treatment on the sludge dewaterability and the changes within time are given in Figure 4.24. The control reactors' initial CST values were higher from the characteristics that were given in Table 3.1 since sludge samples were thickened in order to achieve 1% TS content. Dewaterability of the sludge depends on the sludge characteristics and the source of the wastewater treatment plant. For instance, CST values of İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, and Samsun Bafra WWTP's initial CST varied between 23-65 seconds whereas Düzce Akçakoca WWTP's CST was 320 seconds. Compared to the others, Düzce Akçakoca WWTP has the worst dewaterability.

From Figure 4.24, it can be seen that, by the application of pre-treatment before the digestion, CST values of pre-treated sludge samples increased. This implies that application of the enzymatic pre-treatment tends to worsen the dewaterability characteristics of the sludge. Enzymatic pre-treatment causes a reduction in the floc size by destroying cell walls, which results in the increased surface areas. Consequently, more surface water was bound that led to an increase in the CST of pre-treated sludge samples.

Moreover, EPS present in the sludge strongly affects the dewaterability of the sludge. Sludge dewaterability depends on the protein and carbohydrate content of the sludge EPS (Figure 4.25 and Figure 4.26). After the release of EPS components to the medium, CST of the pre-treated sludges increased showing worsen dewaterability. Çetin and Erdinçler (2004) explained that the contrary relationship between the protein part of the EPS and the sludge dewaterability depends on the water-holding capacity of protein part of EPS. Since protein was the dominant component of the sludge's EPS, filterability of the sludge was deteriorated by the release of proteins. Then, enzymatic biodegradation of EPS contents took place during the aerobic digestion of sludge.

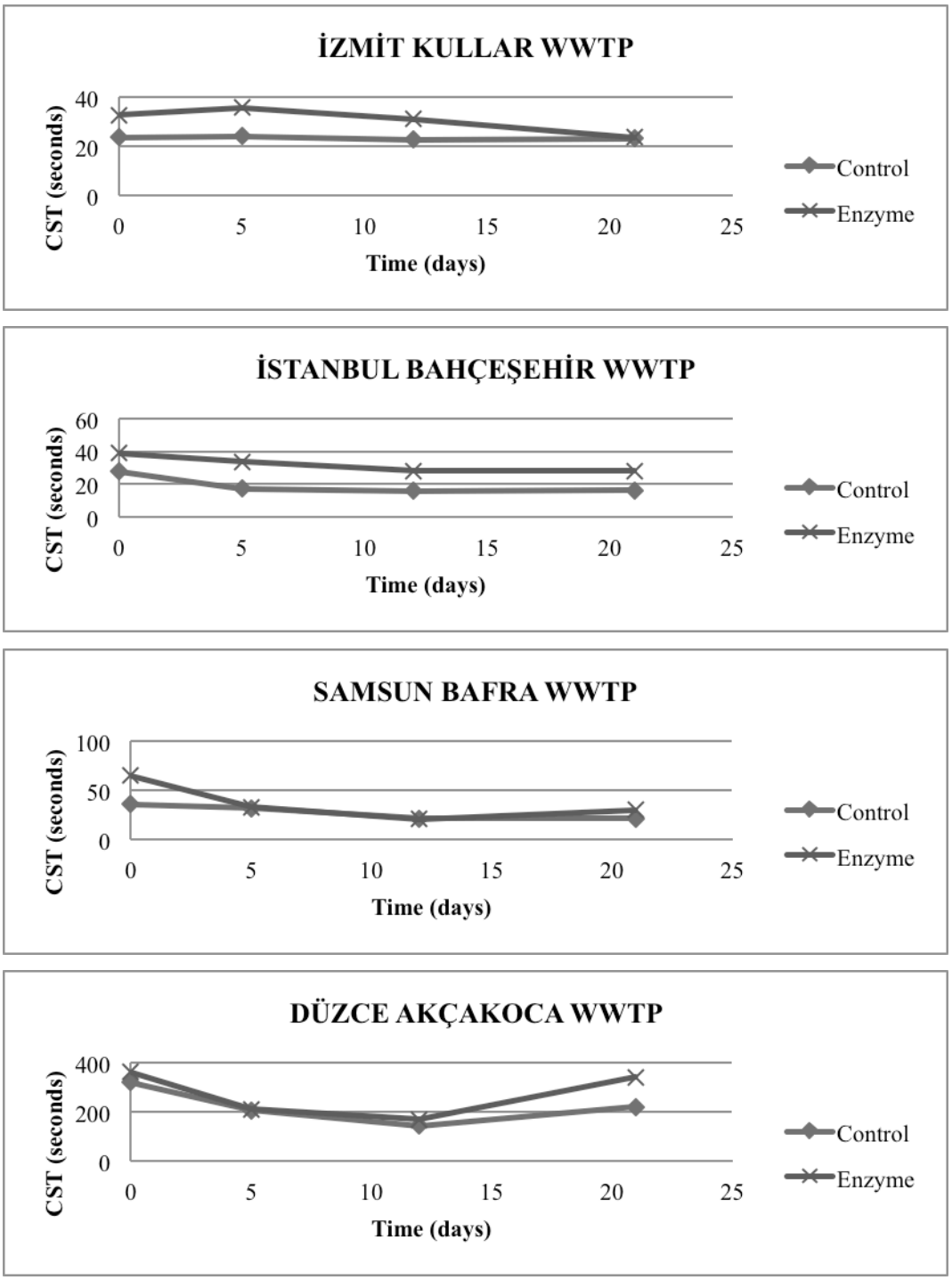


Figure4.24. CST changes in the aerobic digestion reactors.

Neyens et al. (2004) indicated that the degradation of EPS reduces the water retention properties of sludge as a result of releasing the bound water of EPS and increasing the dewaterability efficiency of sludge. Improved degradation of EPS content in the pre-treated sludge samples resulted in the better dewaterability.

At the first 12 days of the digestion, dewaterability improved with the enzymatic pre-treatment. But after the 12^{th} day, the dewaterability of the samples started to decrease. At the end of the aerobic digestion, the dewaterability of enzymatically pre-treated samples was slightly deteriorated or almost same compared to control samples. The CST values were very close to each other to make a good correlation between the control and enzymatically pre-treated sludge samples. The final CST values of the enzymatically pre-treated sludge samples were higher than the sludge samples in the control reactors. The difference in the CST values control and enzymatically pre-treated for four different sludge samples ranged between 2-10 seconds. It seemed that enzymatic pre-treatment did not affect the dewaterability of sludge remarkably. The release of soluble sludge colloidal material from the gel matrix of the sludge might have contributed to lower dewaterability.

Other authors reported results showing that enzymatic pre-treatment improves the sludge dewaterability. Dursun et al. (2006) investigated the effect of enzymatic pre-treatment on the dewaterability of the sludge and stated that enzymes weaken the gel structure of the sludge floc by the hydrolysis of EPS, which results in improved dewaterability. Ayol et al. (2008) also reported that regarding the improved degradation of EPS, enzymatically pre-treated reactors showed better filterability.

4.1.16. Particle Size

The changes in the particle size distributions of the reactors were analyzed weekly. Samples taken from İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP reactors and their results are given in Table 4.7, Table 4.8, Table 4.9, and Table 4.10, respectively.

Table 4.7. Particle size distributions of R1-İKC and R2-İK.

Days	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
İzmit Kullar WWTP Control					
0	16,009	52,23	142,582	31,169	83,197
5	12,888	35,598	98,905	22,902	58,669
12	14,289	40,994	111,234	26,467	66,812
21	13,755	37,404	101,076	25,173	51,545
İzmit Kullar WWTPEnzyme					
0	15,823	49,424	164,57	30,512	99,385
5	14,589	44,91	124,018	27,737	64,225
12	14,697	45,126	115,312	28,037	59,893
21	14,45	42,629	137,021	27,406	76,342

Table 4.8. Particle size distributions of R3-İBC and R4-İB.

Days	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
İstanbul Bahçeşehir WWTP Control					
0	20,965	58,634	186,703	41,061	95,101
5	21,921	58,805	179,522	40,101	88,289
12	22,83	61,026	168,616	41,352	88,253
21	21,843	57,52	184,979	39,597	104,587
İstanbul Bahçeşehir WWTP Enzyme					
0	21,673	61,153	204,616	40,359	100,758
5	24,312	68,474	186,008	44,51	91,815
12	24,378	65,914	181,736	43,803	95,015
21	21,491	58,254	147,8	38,536	76,728

Table 4.9. Particle size distributions of R5-SBC and R6-SB.

Days	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
Samsun Bafra WWTP Control					
0	13,125	42,654	102,067	26,175	51,636
5	13,426	41,646	98,219	25,807	52,708
12	13,551	98,073	86,1	24,312	48,58
21	12,349	33,36	79,848	21,712	45,004
Samsun Bafra WWTP Enzyme					
0	12,603	38,085	87,169	23,108	46,556
5	15,998	50,647	124,768	31,368	67,371
12	15,929	48,723	112,263	30,611	63,033
21	15,249	44,115	100,713	28,542	58,002

Table 4.10. Particle size distributions of R7-DAC and R8-DA.

Days	Surface Weighted Mean D[3.2]	Volume Weighted Mean D[4.3]	d (0.9) µm	d (0.5) µm	d (0.1) µm
Düzce Akçakoca WWTP Control					
0	7,634	23,911	82,561	15,215	48,594
5	7,444	23,583	113,942	13,795	52,941
12	6,535	24,971	72,663	13,29	39,734
21	5,231	21,305	98,583	11,355	45,444
Düzce Akçakoca WWTP Enzyme					
e	7,746	24,119	90,033	14,03	45,944
5	8,834	28,032	85,78	16,58	49,624
12	8,91	29,792	121,033	16,936	61,569
21	6,436	24,567	78,982	12,333	37,374

Particle size distribution and composition determine the mechanism and rate of sludge hydrolysis and degradation in wastewater treatment. Most of the biodegradable organic matter ranges from 10^3 to 100 μm. Microorganisms can directly take up particles that are smaller than 10^3 μm (Ferenci, 1980; White, 2000).

The application of enzymatic disintegration results in the destroyed sludge flocs. Sludge flocs are broken down by cell lysis and the particle size of the cells is reduced. The changes in the particle size distributions show that, enzymatic disintegration was effective on the destruction of the sludge flocs.

4.1.17. Microbiology

As previously stated before, one of the targets of the stabilization process is to achieve pathogen reduction in the sludge samples. Total Coliform (TC), Fecal Coliform (FC) and Fecal Streptococci (FS) measurements were completed in order to represent the stabilization efficiency.

Coliforms and fecal streptococci are commonly used as bacterial indicators of food and water sanitary quality. They are commonly present in human and animal feces, thus they are indicators of possible sewage contamination. Even though they don't cause illnesses, they are indicator of other pathogenic organisms that might be present. Therefore, it can be concluded that their presence suggests that pathogenic microorganisms might also be present. Since testing for pathogenic organisms is difficult, time-consuming, and expensive, sludge is usually tested for coliforms and fecal streptococci instead.

According to US EPA, total coliforms can occur in human and animal feces, fecal coliforms are a subset of total coliform bacteria, and fecal streptococci occur in the digestive systems of human and other warm-blooded animals and those bacteria should be monitored for the water quality.

Microbial counts of the reactors before and after the stabilization are given in Table 4.11. It is observed that, along with the application of enzymatic pre-treatment, an increase in the microbial population was observed in all enzyme added reactors.

Table 4.11. Microbial analyses of the reactors before and after the aerobic digestion.

Days	İzmit Kullar WWTP		İstanbul Bahçeşehir WWTP		Samsun Bafra WWTP		Düzce Akçakoca WWTP	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
Total Coliform (CFU/100 mL)								
0	$4,1x10^7$	$9x10^7$	$1x10^8$	$2,3x10^8$	$1,5x10^8$	$1,5x10^8$	$1,7x10^8$	$2,4x10^8$
21	$1,1x10^5$	$2,7x10^5$	$2,3x10^5$	$4,5x10^5$	$8x10^4$	$2,7x10^5$	$3,1x10^5$	$7,5x10^5$
Fecal Coliform (CFU/100 mL)								
0	$3,7x10^7$	$6,7x10^7$	$5,9x10^7$	$1x10^8$	$5,5x10^7$	$1x10^8$	$1x10^8$	$1x10^8$
21	$7x10^4$	$9x10^4$	$1,4x10^5$	$2,1x10^5$	$4x10^4$	$1,9x10^5$	$6x10^4$	$3,8x10^5$
Fecal Streptococci (CFU/100 mL)								
0	$3x10^6$	$4,7x10^7$	$3x10^7$	$4x10^7$	$1,8x10^7$	$3,3x10^7$	$3,8x10^7$	$4,8x10^7$
21	$3x10^4$	$7x10^4$	$1,5x10^4$	$2x10^3$	$1,2x10^3$	$6x10^4$	$3,8x10^3$	$8x10^3$

At the end of the digestion process, TC, FC, and FS reductions in enzymatically pre-treated reactors were more than the control reactors in each treatment plant's sludge samples. However, final bacteria concentrations of control and enzyme added reactors were very close to each other since enzyme added reactors' initial concentration was increased due to enzyme addition. The mean value of TC, FC, and FS reductions in all reactors are 99,78%, 99,84%, and 99,82%, respectively. As a result, stabilization was achieved in both reactors with overall 99,8% bacteria removal since it was an indicator of a successful stabilization. In addition, enzymes accelerate the removal of pathogens from sludge samples.

4.1.18. Extracellular Polymeric Substances

Enzymes have been used and proven to be effective for the degradation of the multi structured EPS of sludge (Johansen et al., 1997; Melo et al., 1997; Lequette et al., 2010). Enzymatic hydrolysis of sludge can disrupt EPS matrix, which results in enhanced sludge solubilization of the sludge flocs. As the flocs are disintegrated, macromolecular substances that were previously protected from enzyme attack are exposed and may be degraded by hydrolytic enzymes.

Proteins and carbohydrates are the most dominant components of EPS and they make up a large portion of sCOD and COD in sludge (Yu et al., 2007). Therefore, the measurement of proteins and carbohydrates can provide a more thorough understanding of the influences of enzymatic pre-treatment on the digestion process (Liu and Fang, 2003).

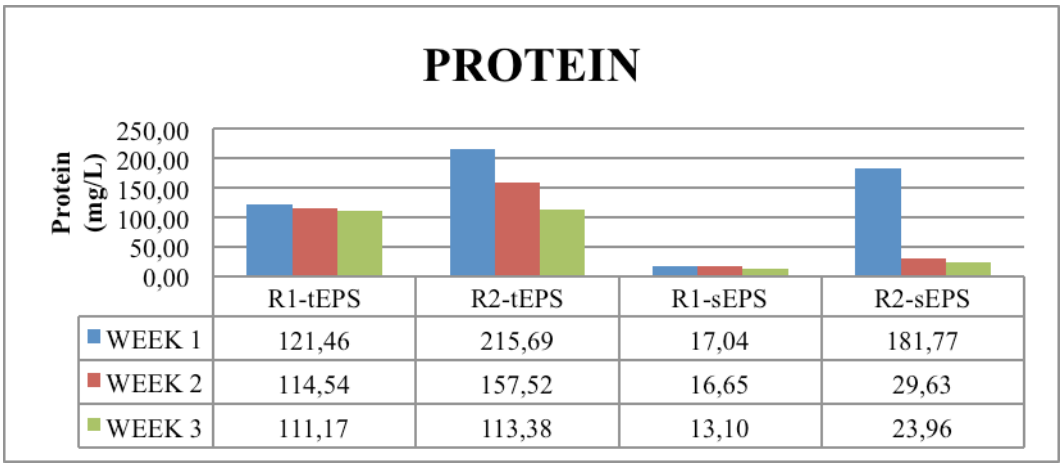
EPS extraction analyses were conducted in sludge samples taken from İstanbul Bahçeşehir WWTP in order to find out the effect of enzyme addition on the sludge's EPS characteristics. EPS extraction was performed by using the cation exchange resin method. The composition of EPS was determined by performing carbohydrate and protein analyses in the extracted EPS samples. R1 and R2 symbolize control and enzyme added reactors for İstanbul Bahçeşehir WWTP, respectively.

EPS in a sludge floc is composed of soluble EPS and bound (total) EPS. The latter exhibits a dynamic double-fractioned structure and is made up of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Liming et al., 2008). Based on the extraction method, LB-EPS and TB-EPS can be classified separately (Poxon and Darby, 1997; Ramesh et al., 2006; Li and Yang, 2007; Yu et al., 2007; Yu et al., 2009). Hence, from the outer surfaces to the cores of the granules, the sludge flocs possess a multi-fractioned structure consisting of slime, LB-EPS, TB-EPS, and pellet (Liming et al., 2008). 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 total EPS were conducted by using completely extracted samples.

As previously stated before, proteins are the most dominant compounds in EPS, followed by carbohydrates. Over 85% of the organic carbon in the untreated sludge can be attributed to proteins and carbohydrates (Neyens et al., 2004). However, in some studies, it was indicated that carbohydrates are the main constituents of the EPS. In this study, protein concentrations in both control and enzyme added reactors were higher than carbohydrate concentrations in both reactors at the initial stage of the aerobic digestion. This confirms that protein is the most dominant component of the sludge's EPS structure that was used during this study. However, the protein concentration in the EPS of enzyme added reactor (215 mg/L) was higher than the protein concentration in the control EPS (121 mg/L) (Figure 4.25). Similarly, carbohydrate concentrations were higher in the enzyme added reactor (62 mg/L) than control EPS (29 mg/L) (Figure 4.26).

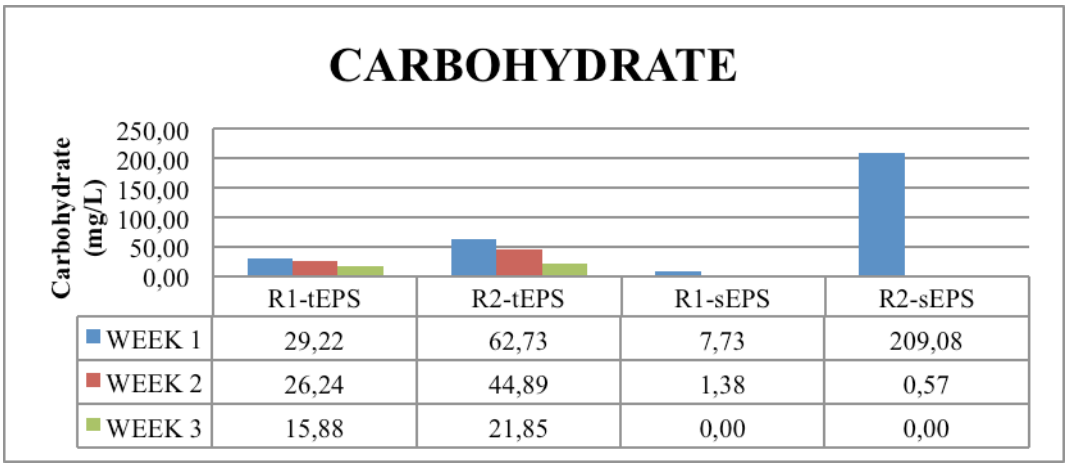
Since carbohydrate and protein components of EPS are released during the extraction process, it is possible to find out the protein and carbohydrate concentrations of the sludge samples throughout the aerobic digestion process and the EPS extraction analyses were carried out once in a week. From Figure 4.25 and Figure 4.26, it is clear to see that at the initial stage of the aerobic digestion process, protein and carbohydrate concentrations in the pre-treated reactors were higher than the control reactors. Protein concentrations in tEPS and sEPS were increased by 77% and 966%, respectively, compared to R1. Furthermore, carbohydrate concentrations in tEPS and sEPS at R2 were increased by 114% and 2604%, respectively, compared to R1.

The increases in protein and carbohydrate concentrations clearly show that enzymes are effectively able to destroy sludge flocs by breaking microorganism cell walls, release the intracellular contents of the to the medium. After enzyme addition to the sludge sample, EPS content present within the sludge flocs were released into the solution. The release of proteins and carbohydrates was higher in soluble form since loosely bound EPS was released to the medium. Even though it is hard to destroy an EPS structure, the results show that enzymes successfully destroy EPS. So, applying enzymatic pre-treatment to the sludge sample caused an improvement on the concentrations of proteins and carbohydrates.



	R1-tEPS	R2-tEPS	R1-sEPS	R2-sEPS
WEEK 1	121,46	215,69	17,04	181,77
WEEK 2	114,54	157,52	16,65	29,63
WEEK 3	111,17	113,38	13,10	23,96

Figure 4.25. Protein concentrations in extracted EPS of reactors.



	R1-tEPS	R2-tEPS	R1-sEPS	R2-sEPS
WEEK 1	29,22	62,73	7,73	209,08
WEEK 2	26,24	44,89	1,38	0,57
WEEK 3	15,88	21,85	0,00	0,00

Figure 4.26. Carbohydrate concentrations in extracted EPS of reactors.

Meanwhile, the EPS content of sludge flocs during aerobic digestion process changed with time. The sludge flocs are broken first and then EPS of the sludge flocs is released into the bulk solution in the hydrolysis process. During the aerobic digestion, concentrations of EPS components in all forms decreased. At the end of the aerobic digestion process, enzymatically pre-treated reactor was more effective in the degradation of proteins and carbohydrates. Protein contents of the reactors were degraded by 8% and 47% in tEPS form, and by 23% and 86% in sEPS form at R1 and R2, respectively. In addition, carbohydrate

contents of the reactors were degraded by 45% and 65% in tEPS form, and by 100% and 100% in sEPS form at R1 and R2, respectively.

Degradation of the EPS reduces their water retention properties thereby releasing the EPS-bound water and increasing the dewatering efficiency of sludge. The advanced methods such as enzymatic disintegration degrade proteins and polysaccharides of sludge EPS, which constitute almost 60% of EPS (Neyens et al., 2004). In this study, degradation of EPS resulted in bettered dewaterability characteristics.

4.2. Anaerobic Digestion

4.2.1. pH

In anaerobic digesters, pH is an important parameter that should be monitored throughout the digestion process. Each microorganism group in the sludge has a different optimum pH range. For instance, methonogens are extremely sensitive to the pH and they work best when it is between 6,5 and 7,5.On the other hand, fermentative microorganisms are less sensitive and can function in pH levels ranging between 4,0 and 8,5 (Hwang et al., 2004). The changes in the pH levels have a great influence on the methane production.

During this study, changes in the pH levels of sludges were observed and the data are given in Figure 4.27. Initial pH of sludges in the control reactors ranged between 7,27 and 7,44. This level decreased to 6,6 by the enzyme addition in the pre-treated reactors. The drop of pH shows that the hydrolysis stage of the anaerobic digestion started to take place as soon as enzymes were added to the sludge samples. Enzymes accelerated the destruction of large organic compounds into small organic compounds. Amino acids, fatty acids, and sugars were formed which lead to the drop of pH levels in the pre-treated reactors.

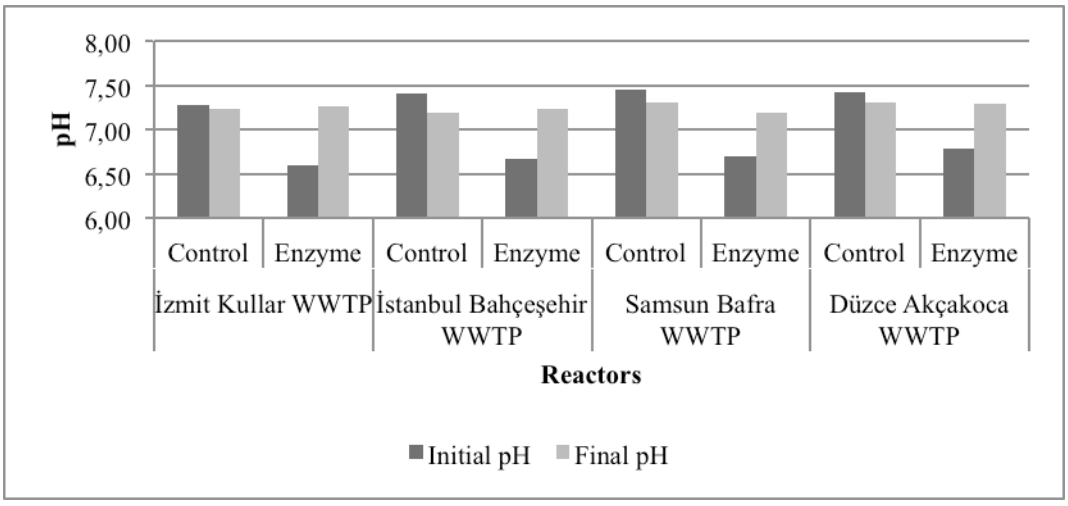


Figure 4.27. pH changes in the anaerobic digestion reactors.

At the end of the anaerobic digestion process, pH levels of the sludges in the control reactors slightly decreased while pH levels of the pre-treated sludges increased. The final pH of the pre-treated sludges rose from 6,66 to 7,25. That increase in the pH level can be linked to the reduction of volatile fatty acids that were accumulated in the sludge at the beginning of the digestion. Throughout the anaerobic digestion process, pH values of all reactors ranged between 6,6 to 7,44 that indicates optimum pH conditions were preserved and no inhibition was observed in the reactors.

4.2.2. Oxidation Reduction Potential

ORP is an important indicator of an anaerobic environment. In an anaerobic digester, ORP should be in the negative range. Even small amounts of DO in an anaerobic digestion system can increase the ORP of the sludge and reduce the anaerobic activity. Anaerobic microorganisms survive and degrade substrates most efficiently when ORP in their environment is between -400 and -200 mV (Gerardi, 2003).

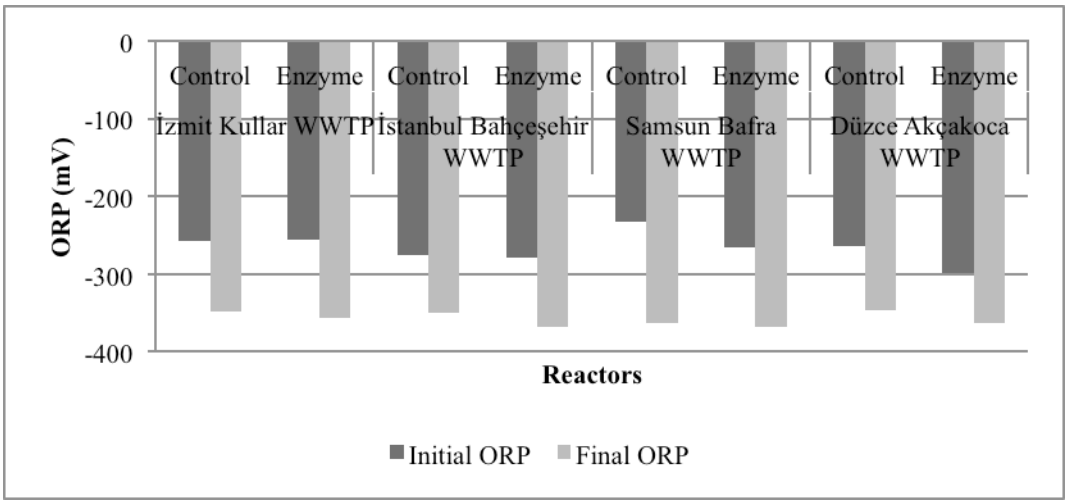


Figure 4.28. ORP changes in anaerobic digestion reactors.

The ORP measurements of this study were recorded before and after the anaerobic digestion and they are given in Figure 4.28. ORP values of the sludge samples ranged between -232 to -299 mV at the beginning of the anaerobic digestion process and these values decreased to approximately -360 mV at the end. This decrease in the ORP values indicates that the anaerobic conditions were preserved throughout the process and the environment had become more anaerobic as the stabilization process continued. So, the necessary conditions for anaerobic microorganisms to degrade substrates were conserved in the process.

4.2.3. Electrical Conductivity

In this study, EC of the sludges were measured before and after the anaerobic digestion, and the results are given in Figure 4.29.At the beginning of the digestion, EC levels of the pre-treated reactors were higher than the EC levels of the control reactors because of enzyme addition. At the end of the digestion, conductivity of the reactors increased from 7,43 mS/cm to 14 mS/cm in the control reactors, and from 8,9 mS/cm to 16,7 mS/cm in the pre-treated reactors. These increases indicate that enzyme addition to the sludge and the anaerobic digestion process increased the ability of sludge to conduct electrical current through it.

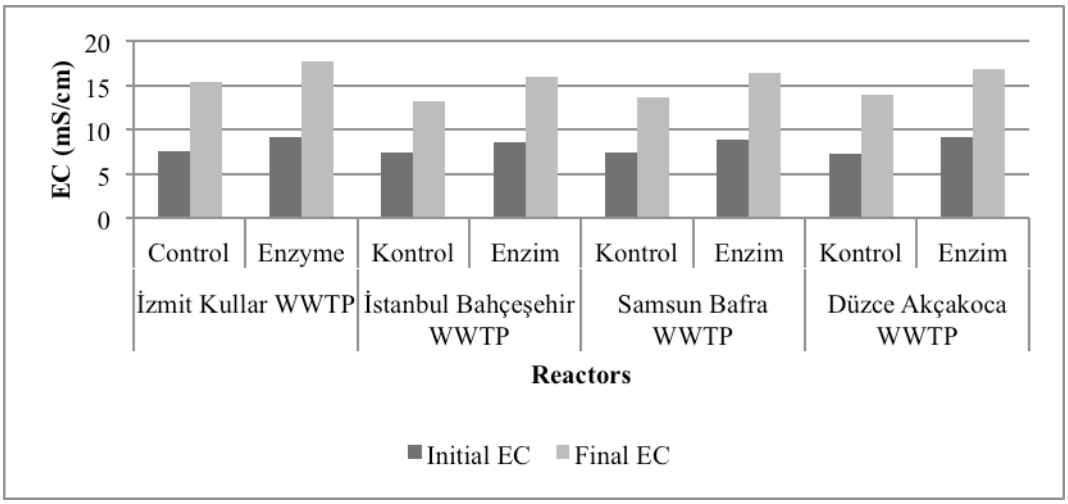


Figure 4.29. EC changes in the anaerobic digestion reactors.

4.2.4. Salinity

Salinity measurements were conducted before and after the anaerobic digestion process and the results are given in Figure 4.30.The values ranged between 5,1 – 9,6 ‰ for İzmit Kullar WWTP sludge samples, 5 – 8,5 ‰ for İstanbul Bahçeşehir WWTP sludge samples, 5,1 – 8,9 ‰ for Samsun Bafra WWTP sludge samples and 4,9 – 9 ‰ for Düzce Akçakoca WWTP sludge samples. Salinity values of sludges were in accordance with the EC data. At the initial stage, increases in the salinity values were observed due to the enzyme addition in the pre-treated reactors. Also, salinity of all sludge samples increased at the end of the digestion.

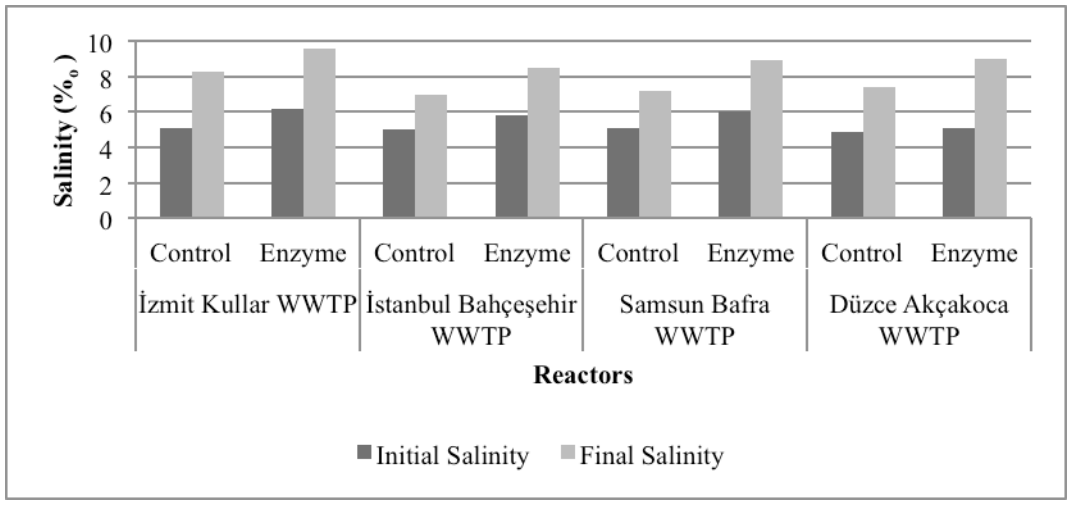


Figure 4.30. Salinity changes in the anaerobic digestion reactors.

4.2.5. Total Solids and Volatile Solids

During this study, solids contents of the sludge samples were monitored by conducting TS and VS analyses and the results are given in Figure 4.31 and Figure 4.33, respectively. Even though initial TS and VS contents of the reactors were close to each other, solids contents of the pre-treated reactors were slightly higher than the control reactors. The same increase in the TS and VS levels was also observed during the aerobic digestion process and it was linked to the increased microbial growth as a result of enzyme addition. Since enzyme addition to the sludge samples led to an increase in the microbial population at the beginning of the anaerobic digestion too, the increase in the solids contents can be explained by the increased microbial growth as a result of enzyme addition (Table 4.15).

At the end of the anaerobic digestion process, solids contents of the reactors decreased. Each reactor had different TS removal efficiency and the TS removal efficiencies are shown in Figure 4.32. TS reductions of control reactors were 22%, 13%, 11%, and 12% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively. TS reductions of pre-treated reactors were 24%, 17%, 20% and 25% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively.

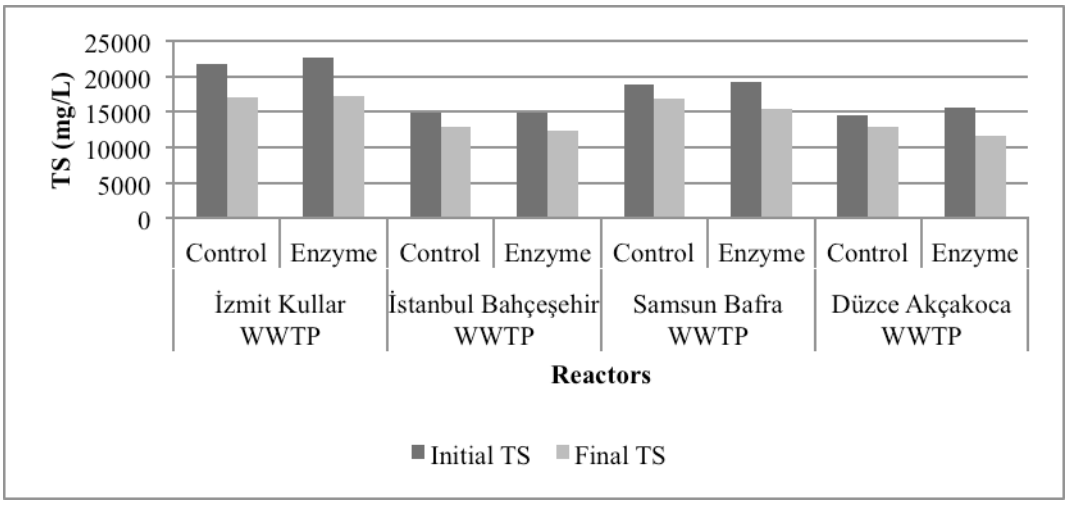


Figure 4.31. TS changes in the anaerobic digestion reactors.

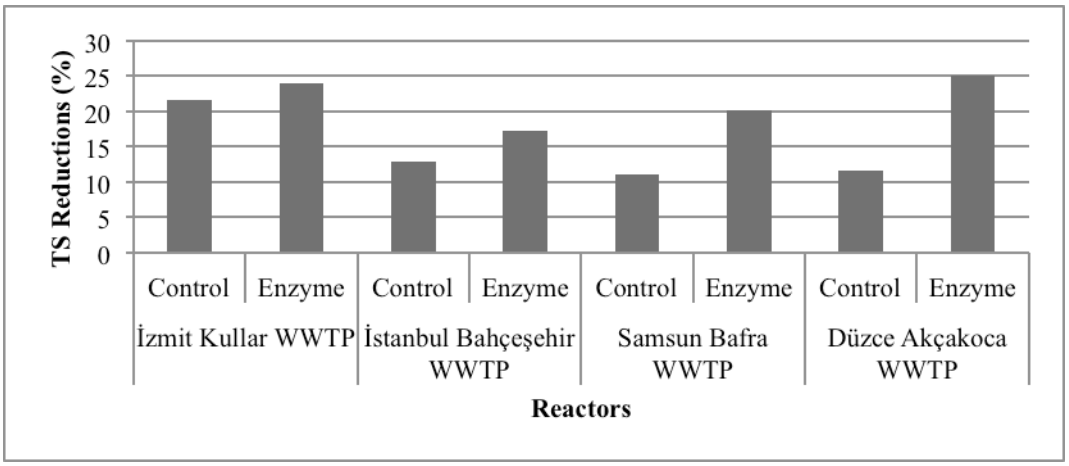


Figure 4.32. TS reductions in the anaerobic digestion reactors.

From the Figure 4.32, it can be seen that the pre-treated reactors showed better performance on the removal of total solids compared to the control reactors. Enzyme addition led to an increase in the TS removal. One of the aims of the disintegration was to achieve sludge minimization and application of enzymatic pre-treatment prior to anaerobic digestion in this study led to less sludge production.

In anaerobic digestion systems, VS removal is used to evaluate the degradation of organic matter contents of the sludges. The volatile solids that are degraded during anaerobic digestion are converted into biogas (Appels et al., 2008). During this study, volatile solids of the reactors were degraded and the removal efficiencies are given in Figure 4.34.VS reductions in the control reactors were 26%, 20%, 19%, and 21% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively. In the enzyme added reactors, VS reductions were 29%, 25%, 37%, and 36% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. In all sludge samples that were taken from different WWTPs, VS removal efficiency was improved by the addition of enzymes to the sludge samples. Since enzymes accelerate the destruction of complex organic matters into micro organic matters, degradation of organic materials got easier in the presence of enzymes.

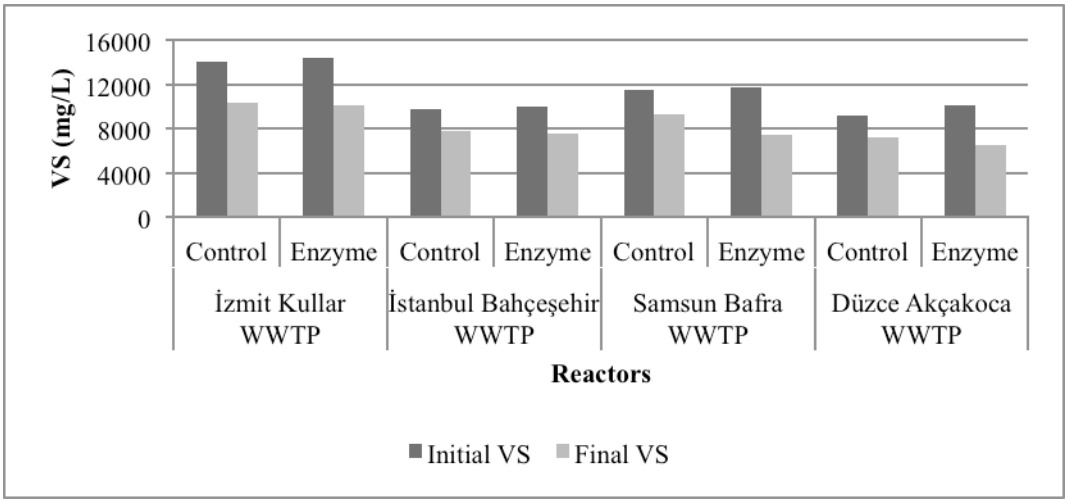


Figure 4.33. VS changes in the anaerobic digestion reactors.

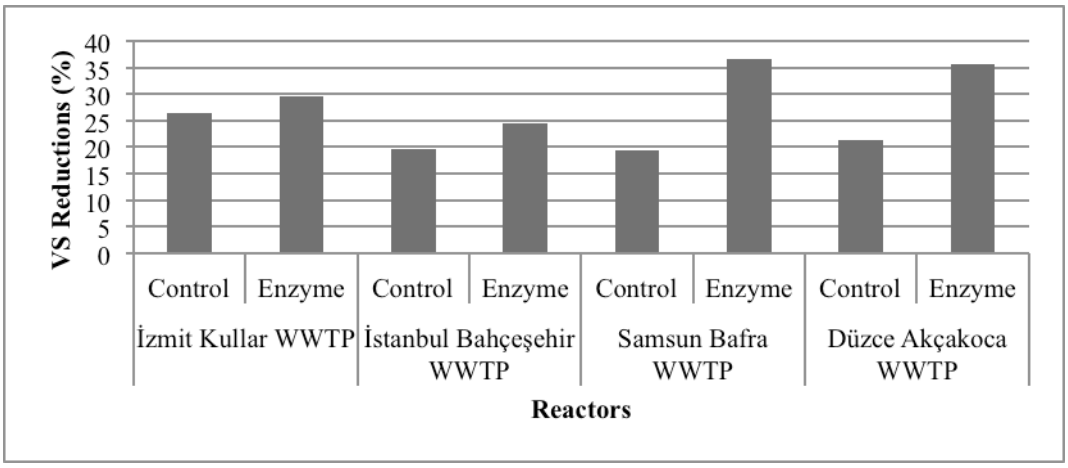


Figure 4.34. VS reductions in the anaerobic digestion reactors.

As stated before, one of the main aims of the stabilization process was to improve the volatile solids reductions in the reactors and obtain higher removal percentages. In an anaerobic digestion system, degraded volatile solids are converted to CH_4 and CO_2. The results of TS and VS analyses show that application of enzymatic disintegration increased the biodegradability of the sludge as well as increasing the efficiency of the digestion. Also, it reduced the mass of sludge produced at the end of the digestion. Application of enzymatic disintegration enhanced the VS reduction performances of the reactors, which is expected to lead increased biogas production. The results are compatible with the literature. Parmar et al. (2001) achieved 29% solids reduction when the sludge was pre-treated with an enzyme mixture compared to a reduction of 6,1% in the control reactor. Rashed et al. (2010) also showed that enzyme addition prior to anaerobic digestion results in increased VS removal efficiencies.

Furthermore, sludge samples that were taken from domestic WWTPs showed better performance on the sludge minimization if they were enzymatically pre-treated. R2-İK and R8-DA had the highest TS removals by 24% and 25%, respectively. However, no relationship was found between VS removal efficiencies and the type of WWTP.

4.2.6. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

Suspended solids contents of the reactors were evaluated by conducting MLSS and MLVSS analyses and the results are given in Figure 4.35 and Figure 4.37, respectively.

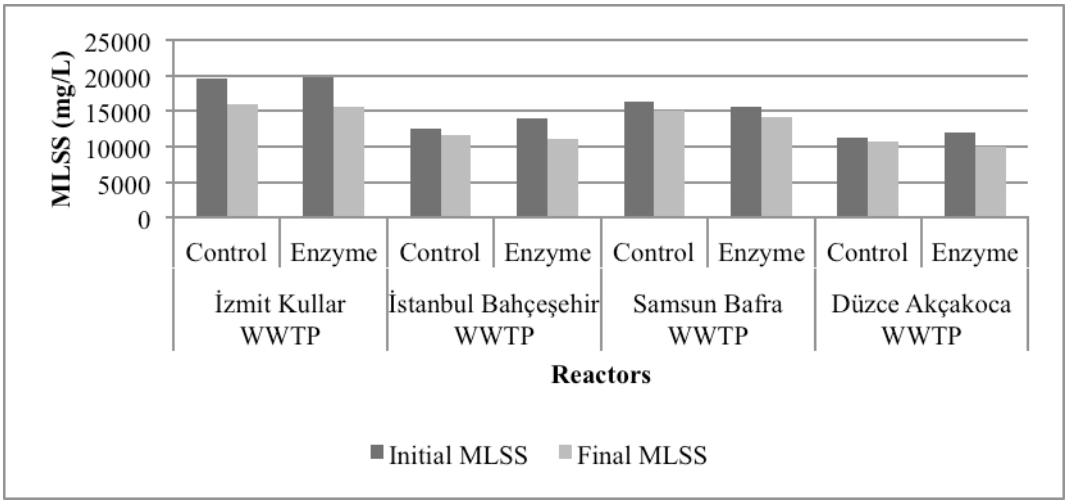


Figure 4.35. MLSS changes in the anaerobic digestion reactors.

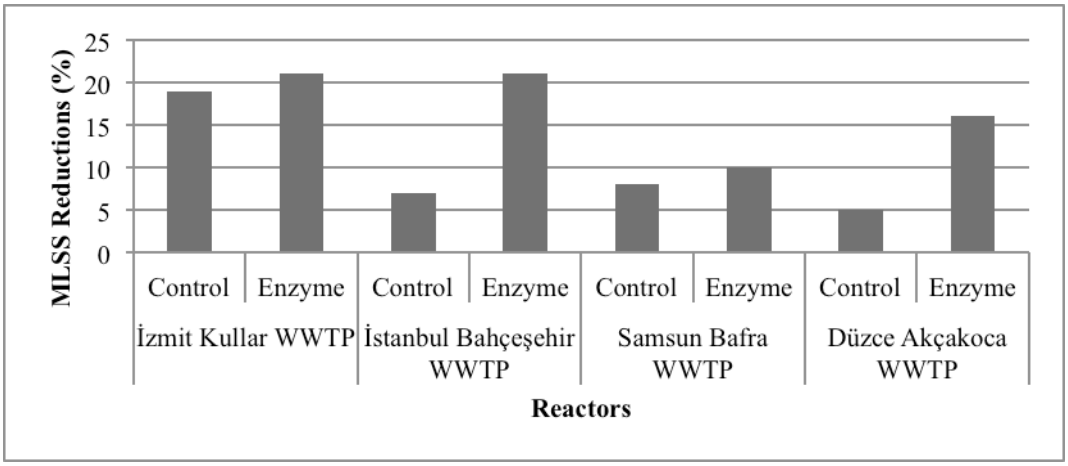


Figure 4.36.MLSS reductions in the anaerobic digestion reactors.

At the end of the anaerobic digestion, MLSS contents of the sludge samples decreased. Each reactor showed different removal efficiency. MLSS concentrations in the control reactors

decreased by 19%, 7%, 8%, and 5%, at R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively (Figure 4.36). These reductions are lower than the results given in the literature. Fortunately, MLSS reductions in enzyme added reactors were higher than the control reactors, which is an indicator of the positive effect of enzymatic disintegration. MLSS reductions in the enzyme added reactors were 21%, 21%, 10%, and 16%, for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. The highest improvements on MLSS removal efficiency were obtained in reactors containing disintegrated sludge samples of İstanbul Bahçeşehir WWTP and Düzce Akçakoca WWTP.

Improved MLSS reductions were also observed by Roman et al. (2006). It was found that sludge samples treated with pronase E and cellulase reduced MLSS by 36% and 29%, respectively compared to 20% in control reactor. However, an 80% reduction was achieved when sludge was treated with pronase E and cellulase mixture, which is too much higher than the results of this study. On the other hand, Parmar et al. (2001) achieved 29% MLSS reduction in anaerobically stabilized, enzyme mixture added sludge samples, which is more similar to the current study's foundings.

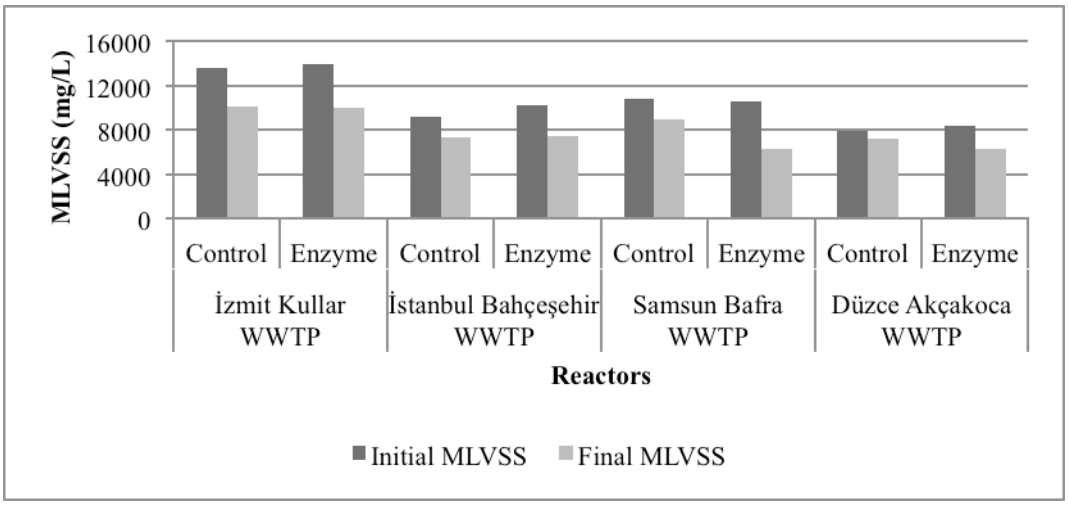


Figure 4.37. MLVSS changes in the anaerobic digestion reactors.

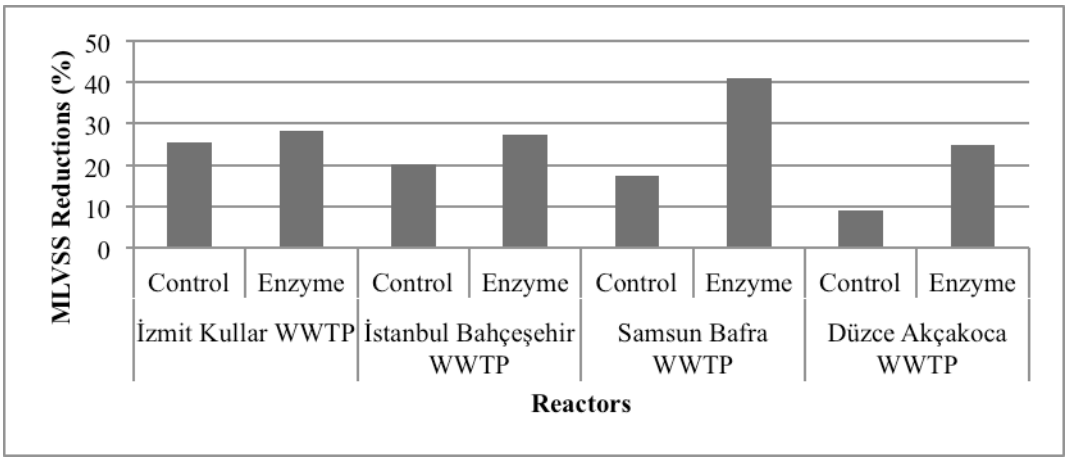


Figure 4.38.MLVSS reductions in the anaerobic digestion reactors.

MLVSS is responsible for sludge hydrolysis during an anaerobic digestion and it is an important parameter. At the end of the anaerobic digestion process, reductions in MLVSS concentrations were observed and the reduction efficiencies are given in Figure 4.38. MLVSS reductions in the control and enzyme added reactors were 26% and 28% for R1-İKC and R2-İK, 20% and 27% for R3-İBC and R4-İB, 25% and 41% for R5-SBC and R6-SB, and 9% and 58% for R7-DAC and R8-DA, respectively. Same as in MLSS reduction efficiencies, MLVSS reduction efficiencies are lower than the removal efficiencies given in the literature. However, the effect of enzymatic disintegration can be seen in the improved MLVSS reductions in the pre-treated reactors.

Yang et al. (2010) indicated that MLVSS reduction is a critical parameter responsible for sludge hydrolysis and achieved 50% in average MLVSS removal when sludge was treated with mixed enzymes, compared to 10% in control reactors (Yang et al., 2010). On the other hand, Luo et al. (2012) reported 14,5% MLVSS reduction when the sludge was pre-treated with amylase enzyme at 40^{o}C, which is closer to this study's findings.

4.2.7. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand

COD analyses were conducted before and after the anaerobic digestion process and the results are given in Figure 4.39.COD concentrations of raw sludges that were used during anaerobic digestion process were 38470 mg/L, 14327 mg/L, 8337 mg/L, 7486 mg/L, and 5299 mg/L in Fritolay seed sludge, İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively. Since sludge samples that were taken from WWTPs were mixed with Fritolay seed sludge, an increase in the COD concentrations of the reactors was observed.

Destruction and removal of organic matters by acidogens and methanogens in an anaerobic digestion system leads to a decrease in the COD concentrations. Organic matters are consumed by bacteria in order to produce CH_4 and CO_2. From the Figure 4.40, it can be seen that enzymatic pre-treatment played an important role on increasing the removal efficiency of the organic material. The COD removal was 33%, 32%, 31%, and 35% in control reactors whereas it was 41%, 44%, 40%, and 41% in pre-treated sludges of İzmit Kullar WWTP, İstanbul Bahçeşehir WWTP, Samsun Bafra WWTP, and Düzce Akçakoca WWTP, respectively.

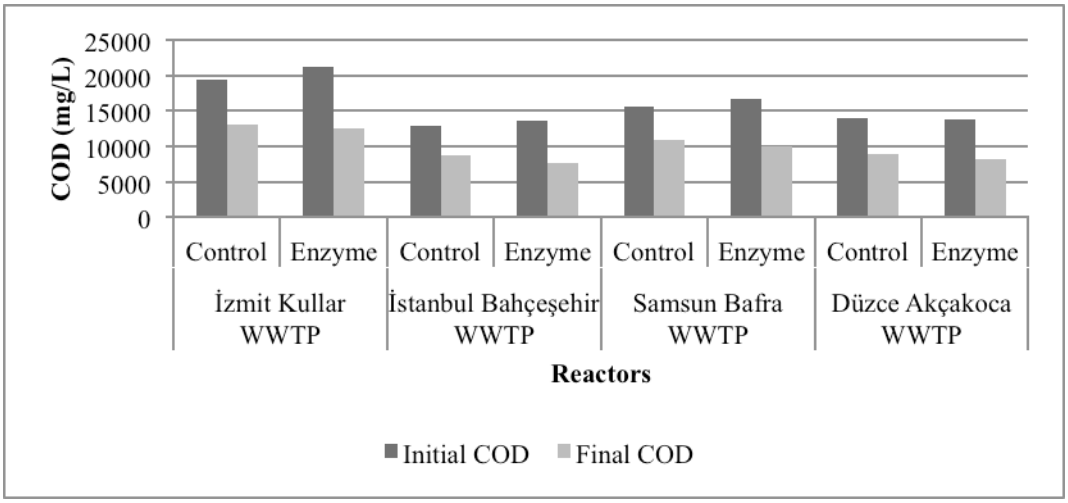


Figure 4.39. COD changes in the anaerobic digestion reactors.

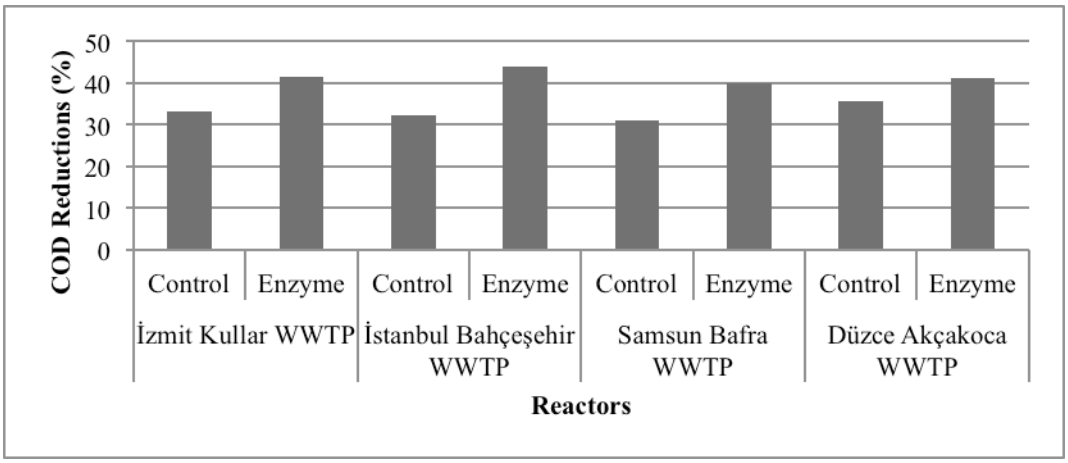


Figure 4.40. COD reductions in the anaerobic digestion reactors.

Improved COD removal efficiencies were also observed by other researchers. Roman et al. (2006) reported that COD concentrations of sludge samples were decreased at the end of the anaerobic digestion process and enzyme mixture added sludge samples showed better COD reduction performance compared to the reactors containing raw sludge, cellulase, or pronase E enzyme added sludges.

Indicator of solubilization, sCOD analyses were also conducted before and after the anaerobic digestion process and the results and the sCOD removal efficiencies are given in Figure 4.41 and Figure 4.42, respectively.

At the beginning of the anaerobic digestion process, sCOD concentrations in all pre-treated reactors were greatly improved. Up to 3 to 4 times of the initial sCOD levels, concentrations of enzyme added reactors were increased. Same increases were also reported by other researchers. Roman et al. (2006) related the increase in sCOD concentrations with the solubilization of the organic particles. Scheidat et al. (1997) observed a sCOD increase from 15% to 40% by the addition of enzymes. Romano et al. (2009) also showed that the addition of enzymes increased the release of sCOD of sludge by 31%.

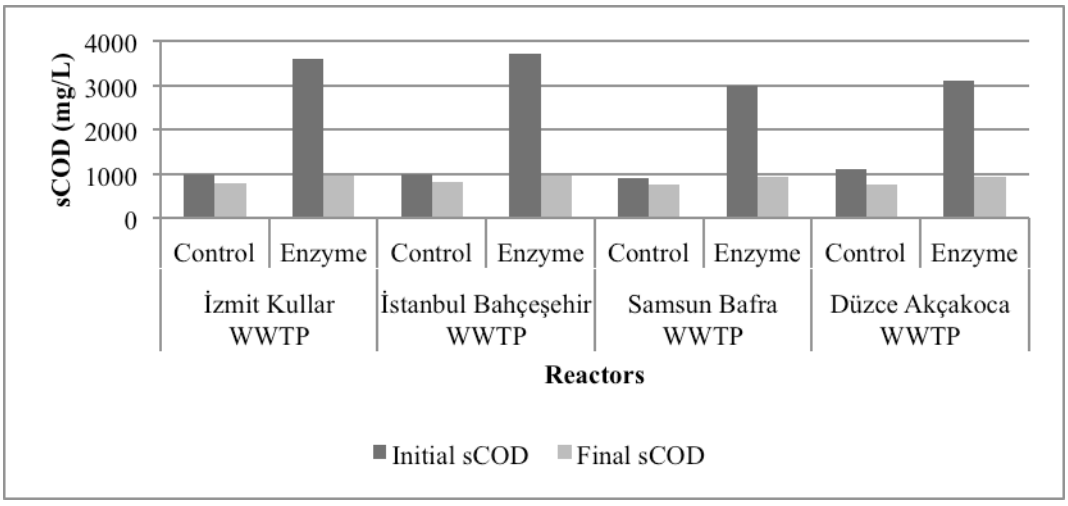


Figure 4.41. sCOD changes in the anaerobic digestion reactors.

In the control reactors, the level of sCOD decreased from 993 to 781 mg/L (21%) in R1-İKC, from 1005 to 832 mg/L (17%) in R3-İBC, from 894 to 764 mg/L in R5-SBC, and from 1105 to 772 (30%) in R7-DAC. On the other hand, sCOD reductions were greatly improved by the enzyme addition. sCOD concentrations in the pre-treated reactors decreased from 3602 to 961 mg/L in R2-İK (73%), from 3702 to 955 in R4-İB (74%), from 2981 to 927 mg/L (69%) in R6-SB, and from 3093 to 923 (70%) in R8-DA.

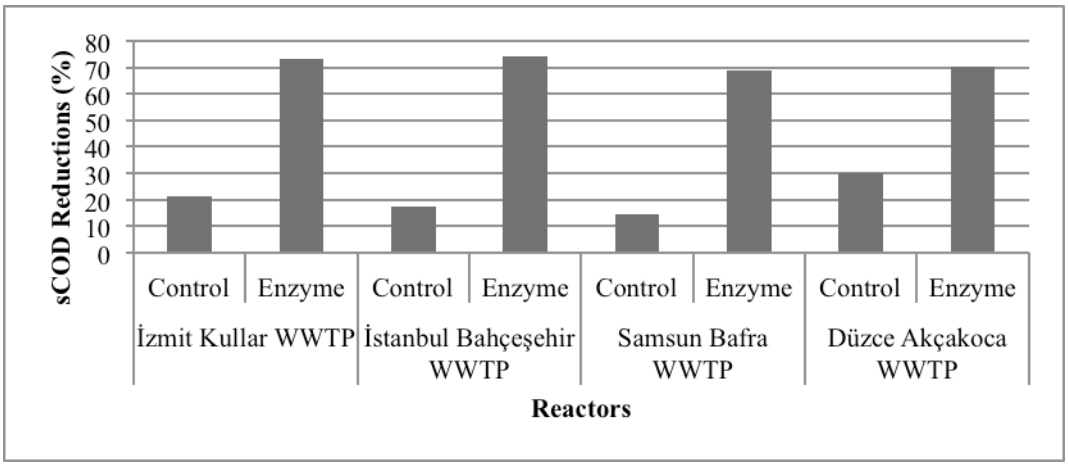


Figure 4.42. sCOD reductions in the anaerobic digestion reactors.

Initial increases in the sCOD concentrations indicated that the organic particles were being solubilized (Roman et al., 2006). Subsequent decreases in COD and sCOD demonstrated that the digestion process and the degradation of the organic material present were improved by the addition of enzymes (Roman et al., 2006). The pattern of increased followed by decreased sCOD and decreased final COD suggested that the complex substances in the sludge were first solubilized into readily biodegradable dissolved substances, which were then converted into methane (Roman et al., 2006). Thus, the solubilization of sludge by hydrolytic enzymes plays an important role on the improvement of anaerobic digestion and this theory should be supported by the observation of increases in the amounts of VFA produced.

Since enzyme addition has no effect on the total COD of the sludge, therefore the ratio of sCOD/COD represents the release of the organic matter from the solid state to the liquid form after the disintegration. Table 4.11 shows that the maximum sCOD/COD ratio for treated sludge increased from 5,1% to 16,9% in İzmit Kullar WWTP, from 7,8% to 27,3% in İstanbul Bahçeşehir WWTP, from 5,7% to 17,9% Samsun Bafra WWTP, and from 7,9% to 22,4% in Düzce Akçakoca WWTP at control and enzyme added reactors, respectively. The disintegration degree of the sludge was also increased by the pre-treatment. Application of enzymatic pre-treatment to the sludge samples increased the DD of the sludge by increasing the release and destruction of intracellular materials. The increase in the DD levels proves that the disintegration of sludge cells was achieved successfully when enzymatic pre-treatment was applied to the sludge.

Similar increase in sCOD/COD ratios was obtained by Yang et al. (2010). sCOD/COD ratio of raw sludge was 1,2% while 22,1% and 26,2% ratios were obtained when sludge was treated with enzymes, which suggests that large amount of particulate organics in sludge flocs was transferred into soluble organic as a result of enzymatic hydrolysis (Yang et al., 2010). Luo et al. (2012) also reported increased sCOD/COD ratios by the enzyme addition to the sludge samples.

The increase in sCOD/COD ratio is due to the release of EPS (proteins, carbohydrates etc.), which are embedded in the floc matrix that is disintegrated due to the enzyme addition. This shows that enzyme addition influences solubilization of particulate COD as the ratio increases with enzyme addition.

Table 4.12. sCOD/tCOD changes and DD in the anaerobic reactors.

Treatment Plant	Reactor	Initial sCOD/tCOD (%)	Final sCOD/tCOD (%)	DD (%)
İzmit Kullar WWTP	Control	5,1	4,5	
	Enzyme	16,9	5,8	67
İstanbul Bahçeşehir WWTP	Control	7,8	7,2	
	Enzyme	27,3	9,4	70
Samsun Bafra WWTP	Control	5,7	5,3	
	Enzyme	17,9	6,9	70
Düzce Akçakoca WWTP	Control	7,9	6,4	
	Enzyme	22,4	8,5	61

Linking the high ratios of sCOD/COD to the improved MLVSS reduction, the addition of enzymes could significantly enhance excess sludge hydrolysis.

4.2.8. Dissolved Organic Carbon

DOC analyses of the sludge samples were conducted before and after the anaerobic digestion and the results are given in Figure 4.43. Initial DOC concentrations of sludges in the control reactors ranged between 80-120 mg/L. Enzyme addition caused a huge increase in the DOC concentrations at the initial stage of the anaerobic digestion. DOC concentrations were increased by 273%, 598%, 195%, and 419% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively and the concentrations ranged between 267-572 mg/L. The increases in the DOC concentrations show that particulate organic carbons were broken down into soluble organic carbons by the enzyme addition. A similar increase was also observed in sCOD

concentrations, but DOC increment is lower than sCOD increment since it is only comprised of carbonaceous material of the organic content.

At the end of the anaerobic digestion, DOC concentrations were decreased by 15%, 9%, 6%, and 20% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively and 81%, 83%, 79%, and 83% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. It can be concluded that enzymatic pre-treatment improved the solubilization of the dissolved organic matter and increased the degradation of it through anaerobic digestion.

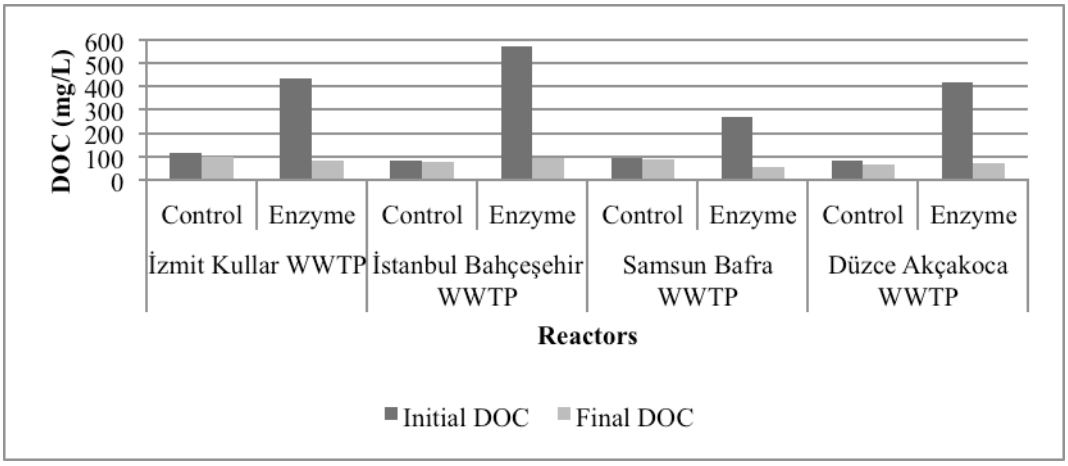


Figure 4.43. DOC changes in the anaerobic digestion reactors.

4.2.9. Alkalinity

Alkalinity of an anaerobic digestion system is important because it represents the buffering capacity of the system (the capacity to resist variations in pH and volatile fatty acids accumulation). It is important to maintain enough alkalinity in the digester and to keep alkalinity below 1000 mg $CaCO_3$/L for a successful digestion process. A typical anaerobic reactor should have alkalinity concentrations in a range between 2000 to 3000 mg $CaCO_3$/L (Calli, 2011) and alkalinity of a balanced digestion system should not be less than 1500 mg $CaCO_3$/L (Gunaseelan, 1997). In this study, initial alkalinity concentrations of the reactors

ranged between 2000-2900 mg $CaCO_3$/L. At the end of the anaerobic digestion, alkalinity concentrations increased by 30%.

During the acidification, alkalinity is expected to decrease because VFAs and carbon dioxide are generated. During the methanogenesis, alkalinity is expected to increase because H^+ is consumed by methanogens. This increase in alkalinity in the digesters possibly can be related by the transformation of organic nitrogen to ammonia nitrogen and hydrogen carbonate. A decrease in the alkalinity concentration is a failure parameter of the anaerobic digestion process and such a condition was not observed.

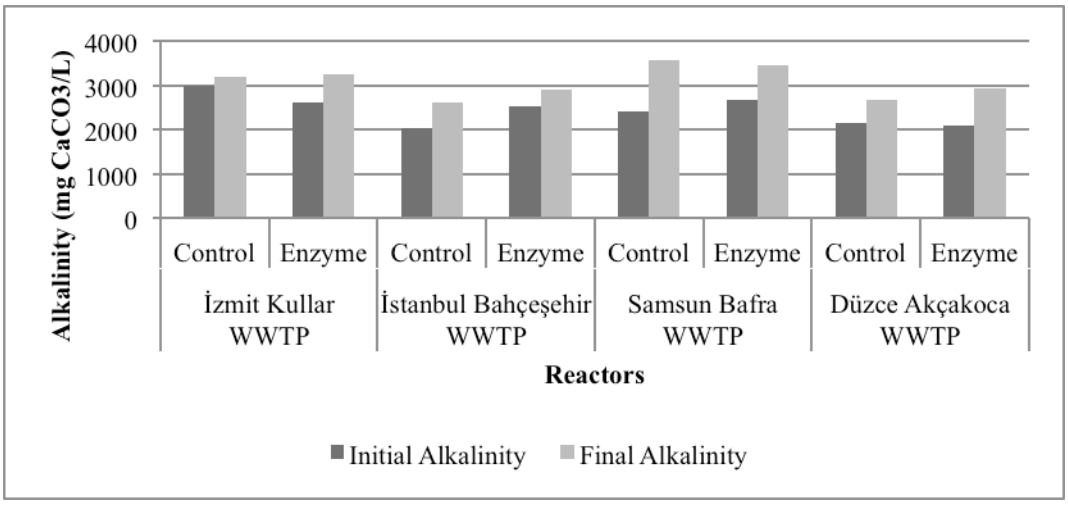


Figure 4.44. Alkalinity changes in the anaerobic digestion reactors.

4.2.10. Ammonium and Total Kjeldahl Nitrogen

TKN and ammonium analyses were conducted before and after the anaerobic digestion process and the results are given in Table 4.13. Along with the digestion process, TKN concentrations decreased and ammonium levels increased due to the hydrolysis of large molecular compounds such as proteins.

Initial TKN concentration of İzmit Kullar WWTP was higher compared to the other reactors, and this can be attributed to the different sludge characteristics. In an anaerobic digestion system, TKN is expected to remain constant as organic nitrogen is converted to ammonia. Even though ammonia concentrations in the reactors increased during the digestion, TKN concentrations of the reactors decreased. In terms of TKN reductions, all reactors showed relatively close performances. Table 4.13 shows that enzymatic disintegration enhances the TKN degradation. TKN removals were up to 20% in enzyme added reactors, while control reactors only removed 10% of TKN content. However, the initial and final TKN concentrations of the sludge samples are close to each other. The difference in the concentrations can originate from the sensitiveness of the experiments.

Table 4.13. TKN and ammonium changes in the anaerobic digestion reactors.

Days	İzmit Kullar WWTP		İstanbul Bahçeşehir WWTP		Samsun Bafra WWTP		Düzce Akçakoca WWTP	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
TKN (mg/L)								
0	1140	1290	840	900	1050	990	900	960
22	950	1080	840	750	810	720	820	750
NH_4^+-N (mg/L)								
0	294	496	330	362	230	300	202	340
22	680	704	464	476	446	496	518	496

During the anaerobic digestion of sludge, ammonium is produced by the biological degradation of nitrogenous substrates. As a result, in anaerobic digesters, high amounts of ammonium-nitrogen are produced. In this study, NH_4^+-N production increased during the anaerobic digestion. Initial NH_4^+-N concentrations of control reactors ranged between 200 to 330 mg/L. However, an increase in NH_4^+-N was observed in enzyme added reactors at the initial stage of the anaerobic digestion. Compared to the control reactors, the concentration of NH_4^+-N for R2-İK, R4-İB, R6-SB, and R8-DA were increased by 68%, 10%, 30%, and 68%, respectively. Reactors containing urban WWTP sludge samples showed better efficiency by releasing 68% of NH_4^+-N. On the other hand, NH_4^+-N concentrations increased at the end of

the anaerobic digestion process by 131%, 40%, 93%, and 156% for R1-İKC, R3-İBC, R5-SBC, and R7-DAC, respectively, while in enzyme added reactors, they were only increased by 41%, 31%, 65%, and 45% for R2-İK, R4-İB, R6-SB, and R8-DA, respectively. The difference in the increases is related with the fact that NH_4^+-N was already increased in the enzyme added reactors before the anaerobic digestion process. Still, final NH_4^+-N concentrations of control and enzyme added reactors were only slightly different from each other. Since increased ammonium concentrations in anaerobic digesters can cause toxicity, it is crucial to control ammonium levels in the anaerobic digesters.

Even though, it is expected to increase during the anaerobic digestion processes, high amounts of NH_4^+-N accumulations results in toxicity to the digestion system. If ammonium inhibits methanogenesis, acetic acid is accumulated, which causes an inhibition to acetogenesis, and a consequent accumulation of propionic and butyric acids, leading to inhibition of acidification (Lyberatos and Skiadas, 1999). Liu and Sung (2002) stated that ammonium concentrations below 200 mg/L are beneficial to the anaerobic digestion process since nitrogen is an essential nutrient for anaerobic microorganisms (Liu and Sung, 2002). On the other hand, every anaerobic digestion system has its own characteristics depending on the sludge characteristics that are used. For instance, ammonium levels of the sludge samples that were used during this study were more than 200 mg/L but this difference didn't cause any toxic effect to the system. This theory is supported by the decreased volatile fatty acids concentrations.

4.2.11. Total Phosphorous

Changes in the TP concentrations of sludge samples are given in Figure 4.45. In an anaerobic digestion system, microorganisms do not consume phosphorous so that the degradation of TP content is not expected. Usually, total mass of phosphorous remains constant. In this study, increments in TP concentrations were observed in reactors R1-İKC, R2-İK, R3-İBC, R4-İB, and R5-SBC. Increments were also observed in reactors R6-SBC, R7-DAC, and R8 DA but the differences between initial and final TP concentrations were so close to each other. The increased TP concentrations at the end of the anaerobic digestion process

can be related with the solubilization of phosphorous as a result of the hydrolysis. As can be seen from Figure 4.45, the increase in the TP concentrations in the enzymatically pre-treated sludge samples is higher than the untreated sludge samples. The difference might stem from the improved release of intracellular materials by the enzymatic pre-treatment.

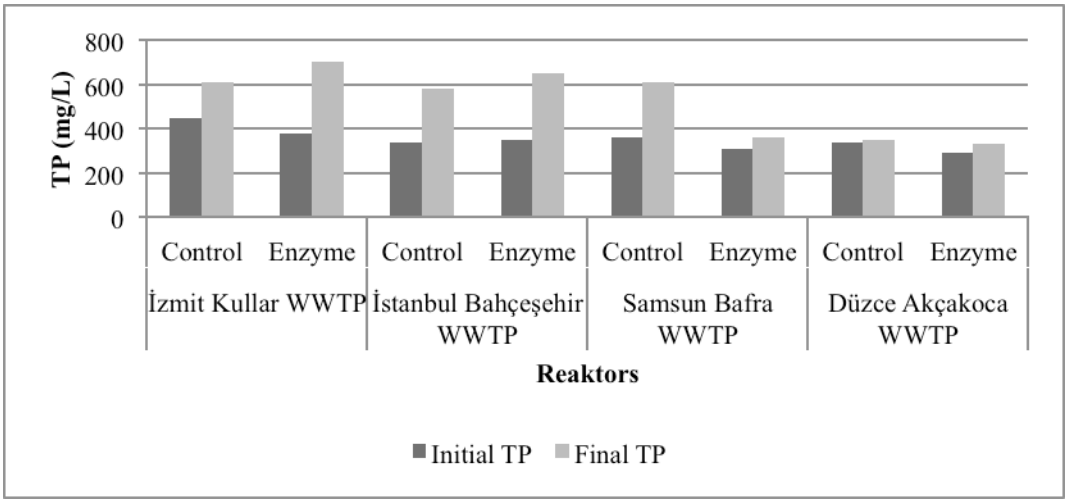


Figure 4.45. TP changes in the anaerobic digestion reactors.

4.2.12. Sulfate and Chloride

The changes in sulfate and chloride concentrations along with the anaerobic digestion process are given in Table 4.14.

In anaerobic reactors, sulfate is reduced to sulfide by the sulfate reducing bacteria. Sulfate reducing bacteria utilizes hydrogen or organic matters, and sulfate as electron donors and acceptors, respectively. It is known that methanogenic bacteria can be influenced by the sulfate reduction as sulfide is produced as an end product. The sludge samples that were used during this study had low sulfate concentrations, less than 100 mg/L. So, any toxicity related with the sulfate reduction was not expected. Sulfate concentrations of İzmit Kullar WWTP were lower than the other reactors but it can be related with the different sludge characteristics. As expected, sulfate concentrations were reduced at the end of the anaerobic

digestion process. However, enzymatic pre-treatment didn't show enhancement on the degradation of sulfate concentrations. Sulfate reductions in the reactors ranged between 50-90% and the final sulfate concentrations were between 5-20 mg/L.

Table 4.14. Sulfate and chloride changes in the anaerobic digestion reactors.

Days	İzmit Kullar WWTP		İstanbul Bahçeşehir WWTP		Samsun Bafra WWTP		Düzce Akçakoca WWTP	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
Sulfate (mg/L)								
0	20	20	100	80	50	70	30	70
22	10	5	10	10	15	20	10	10
Chloride (mg/L)								
0	213	354	213	319	283	425	206	354
22	319	461	354	461	283	461	248	461

It is well known that the presence of the chloride radical is likely become inhibitory on digestion as the concentration rises. Before the anaerobic digestion process, chloride contents of the reactors were affected from the enzyme addition and increments in the concentrations were observed in the enzyme added reactors. Chloride contents of enzyme added reactors were increased by 66%, 49%, 50%, and 71% for R2-İK, R4-İB, R6-SB, and R8-DA. The increments were higher in sludge samples that were obtained from urban WWTPs. At the end of the anaerobic digestion process, chloride concentrations were increased and the concentrations were in the optimum ranges. So, there was no inhibition in the reactors as a result of increased chloride concentrations.

4.2.13. 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 conducted in order to represent the anaerobic digestion efficiency. The results of the analyses are given in Table 4.15.

Table 4.15. Microbial analyses of the reactors before and after the anaerobic digestion.

Days	İzmit Kullar WWTP		İstanbul Bahçeşehir WWTP		Samsun Bafra WWTP		Düzce Akçakoca WWTP	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
Total Coliform (CFU/100 mL)								
0	$1,1x10^7$	$1,8x10^7$	$4,8x10^6$	$5,8x10^7$	$5,3x10^7$	$1,3x10^8$	$6,3x10^7$	$1,5x10^8$
22	$5,4x10^4$	$1x10^4$	$3x10^4$	$8x10^4$	$5,5x10^3$	$3x10^4$	$1x10^3$	$1,7x10^5$
Fecal Coliform (CFU/100 mL)								
0	$4,9x10^6$	$7x10^6$	$4,7x10^5$	$4,6x10^6$	$4,2x10^6$	$5,5x10^7$	$3,8x10^6$	$7,3x10^7$
22	$2x10^3$	$1x10^2$	$1,1x10^3$	$3x10^3$	$1x10^2$	$1x10^2$	$1,4x10^2$	$5x10^3$
Fecal Streptococci (CFU/100 mL)								
0	$1,3x10^5$	$2,7x10^5$	$1,7x10^5$	$9x10^5$	$3,2x10^5$	$8x10^6$	$7,1x10^6$	$1,1x10^7$
22	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10
Salmonella (CFU/100 mL)								
0	$1,8x10^4$	$1x10^5$	$1,9x10^4$	$1x10^5$	$3x10^4$	$9x10^5$	$4,4x10^5$	$8x10^6$
22	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10

After the anaerobic digestion process, TC, FC, FS and Salmonella reductions in sludge samples of enzyme added reactors were more than the control reactors for each WWTP sample. However, final bacteria concentrations of control and enzyme added reactors were very close to each other since enzyme added reactors' initial concentration was increased due to enzyme addition. The mean value of TC, FC, FS and Salmonella reductions in all reactors were 99,82%, 99,96%, 99,99%, and 99,99%, respectively. As a result, stabilization was achieved in both reactors with overall 99,94% bacteria reduction since it was an indicator of a successful stabilization.

4.2.14. Volatile Fatty Acids

Volatile fatty acids (VFA) are acids with a carbon chain of six carbons or fewer. They originate from the anaerobic biodegradation of organic matters. Acetic, propionic, isobutyric, butyric, isovaleric, valeric and isocaproic acids are VFAs that are produced by bacteria. The results of VFA analyses are given in Table 4.16.

Prior to the anaerobic digestion process, only acetic acid and propionic acid were formed in the control reactors, with 25 mg/L concentrations in average. On the other hand, pre-treatment of sludge by enzyme addition resulted in a significant increase in VFA production. In enzyme added reactors not only acetic acid and propionic acid were present, but also, isobutyric acid, butyric acid, isovaleric acid, valeric acid, and isocaproic acid were formed. Acetic acid and propionic acid were the most abundant acids that were produced followed by isovaleric acid, isobutyric acid, valeric acid, and isocaproic acid. The degradation of particulate organic matter could be assumed to result in increased volatile fatty acids concentrations. Therefore, when the enzyme sludge was used as a substrate, the VFA production was higher than the control reactors. The increase in the VFA concentrations of enzyme added reactors were more than 500% of control reactors. The reason for this is that the acidogenic bacteria can easily use the soluble substrates released during enzymatic disintegration. Significant enhancement of VFA production within a short period of fermentation is important in the anaerobic digestion process, especially from an economic point of view because large digesters are not needed (Tan et al., 2012).

At the end of the anaerobic digestion process, VFAs that were produced were degraded in all reactors. The degradation efficiency was higher in enzyme added reactors compared to the control reactors. Almost all of VFAs were consumed by bacteria. The remained VFAs were negligible which means methanogenic microorganisms successfully used all of them and they were consumed in order to produce CH_4 and CO_2.

Table 4.16. VFA analyses of the reactors before and after the anaerobic digestion.

Days	İzmit Kullar WWTP		İstanbul Bahçeşehir WWTP		Samsun Bafra WWTP		Düzce Akçakoca WWTP	
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Control	Enzyme
Acetic Acid (mg/L)								
0	24	136	21	339	26	146	27	211
22	15	13	12	14	18	25	12	17
Propionic Acid (mg/L)								
0	9	510	3	347	-	202	-	278
22	-	-	-	-	1	-	1	-
Isobutyric Acid (mg/L)								
0	-	13	-	18	-	11	-	14
22	-	-	-	-	-	-	-	-
Butyric Acid (mg/L)								
0	-	7	-	17	-	4	-	12
22	-	-	-	-	-	-	-	-
Isovaleric Acid (mg/L)								
0	-	26	-	34	-	21	-	27
22	-	-	-	-	-	-	-	-
Valeric Acid (mg/L)								
0	-	5	-	7	-	4	-	5
22	-	-	-	-	-	-	-	-
Isocaproic Acid (mg/L)								
0	-	1	-	-	-	-	-	2
22	-	-	-	-	-	-	-	-

In some cases, the accumulation of VFA (e.g., acetate and propionate), can lead to digester failure. These compounds lower the pH (below 6,8) and alkalinity, and inhibit the activity of the methanogenic bacteria, which results in decreased methane gas production (Recktenwald et al., 2008; Bjornsson et al., 2000; Hori et al., 2006). In this study, such an effect did not occur, which can be assumed as an indicator of a successful stabilization process.

It was found that enhanced anaerobic digestion should be result in higher VFA production by Park et al. (2005). However, the VFAs produced in digestion are generally utilized by methanogenic bacteria, so it is possible that a portion of the VFAs produced were utilized and did not remain available for measurement. Roman et al. (2006) and Song et al. (2004) also reported decreased VFA concentrations during anaerobic digestion and linked their VFA concentrations to an increase in methane production (Song et al., 2004; Roman et al., 2006). Roman et al. (2006) stated that reduction in VFA concentrations is an operational advantage since long term VFA accumulation could lead pH to decrease below 6,5 and inhibit complete stabilization of the solids. Recktenwald et al. (2008) also observed low VFA concentrations throughout the process but the level of methane production was not increased and it was explained with the unchanged concentrations of the substrate feed.

4.2.15. Biogas Analyses

One of the main aims of enzymatic pre-treatment application to the sludge is to improve the methane content of the biogas produced during the anaerobic digestion process. Also, to improve the quality of the biogas produced is aimed.

4.2.15.1. Cumulative Biogas Production. Biogas production in an anaerobic system is related to the amount of stabilized biodegradable organic matter and the methanogens in the reactor (Grady et al., 1999). Since the hydrolysis of sludge is the rate-limiting step for the anaerobic digestion, the improvement of this step led to an increased production of biogas. The results of the cumulative biogas production in the reactors are given in Figure 4.46.

As can be seen from Figure 4.46, at the initial stage of the anaerobic digestion, biogas production increased rapidly in the reactors. Then, the biogas production was slowed, but continued. After the completion of 20 days, the production almost stopped.

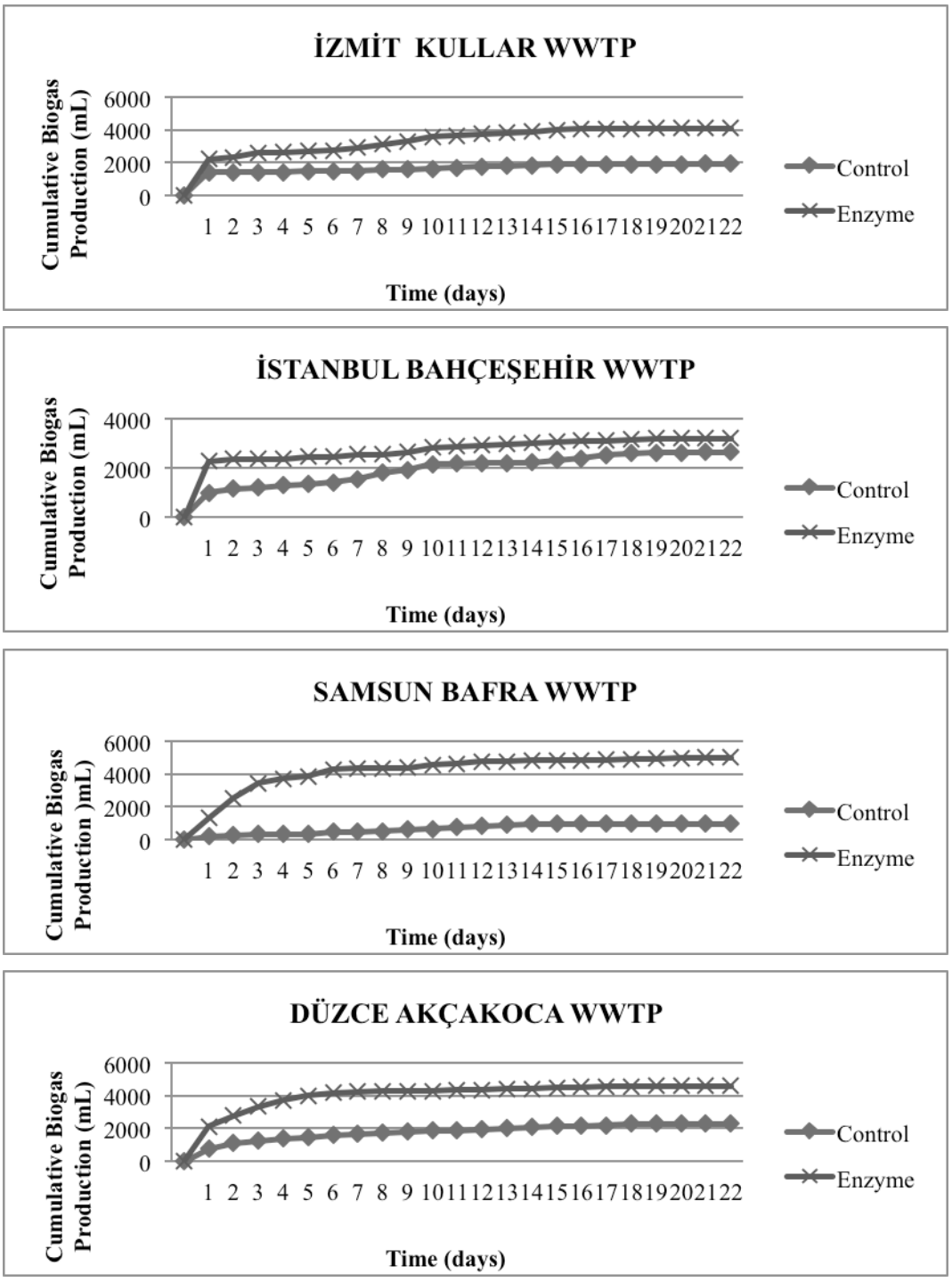


Figure 4.46. Cumulative biogas productions in the anaerobic reactors.

The enzyme added sludge samples showed a sudden increase in the biogas production in comparison to the control reactor. The hydrolytic enzymes improved to hydrolysis step prior to acidogenesis. It is previously stated that the hydrolysis of substrate is the rate-limiting step for the anaerobic digestion; therefore the improvement of this step led to an increased production of biogas.

The results are compatible with those in the literature. Recktenwald et al. (2008) found that enzyme treated sludge showed an increase in the biogas production by 10% to 20% compared to the control reactors and linked the increase to the improved hydrolysis step prior to acidogenesis (Recktenwald et al., 2008).

4.2.15.2. CH_4 %. The main by-product of an anaerobic digestion process is biogas, which is mainly comprised of methane and carbon dioxide as major components. Other gases such as nitrogen constitute smaller fractions. Methane content in the biogas indicates the stability and performance of an anaerobic digester and it depends on the fraction of organic matter destroyed. The CH_4 percentages in the anaerobic digesters are shown in Figure 4.47.

In the anaerobic digestion process, CH_4 measurements were conducted twice a week. The methane content of a biogas determines its quality and a CH_4content higher than 50% is accepted as good quality. As can be seen from Figure 4.47, methane contents of the reactors were improved by enzymatic pre-treatment.

In the anaerobic digestion of R1-İKC, methane content of the biogas produced was about 67%, and the methane content of the enzyme added reactor (R2-İK) was about 73%. Methane contents of biogas produced from R3-İBC, R5-SBC, and R7-DAC were about 67%, 64%, and 70% while methane contents were about 73%, 74%, and 70% of reactors R4-İB, R6-SB, and R8-DA.

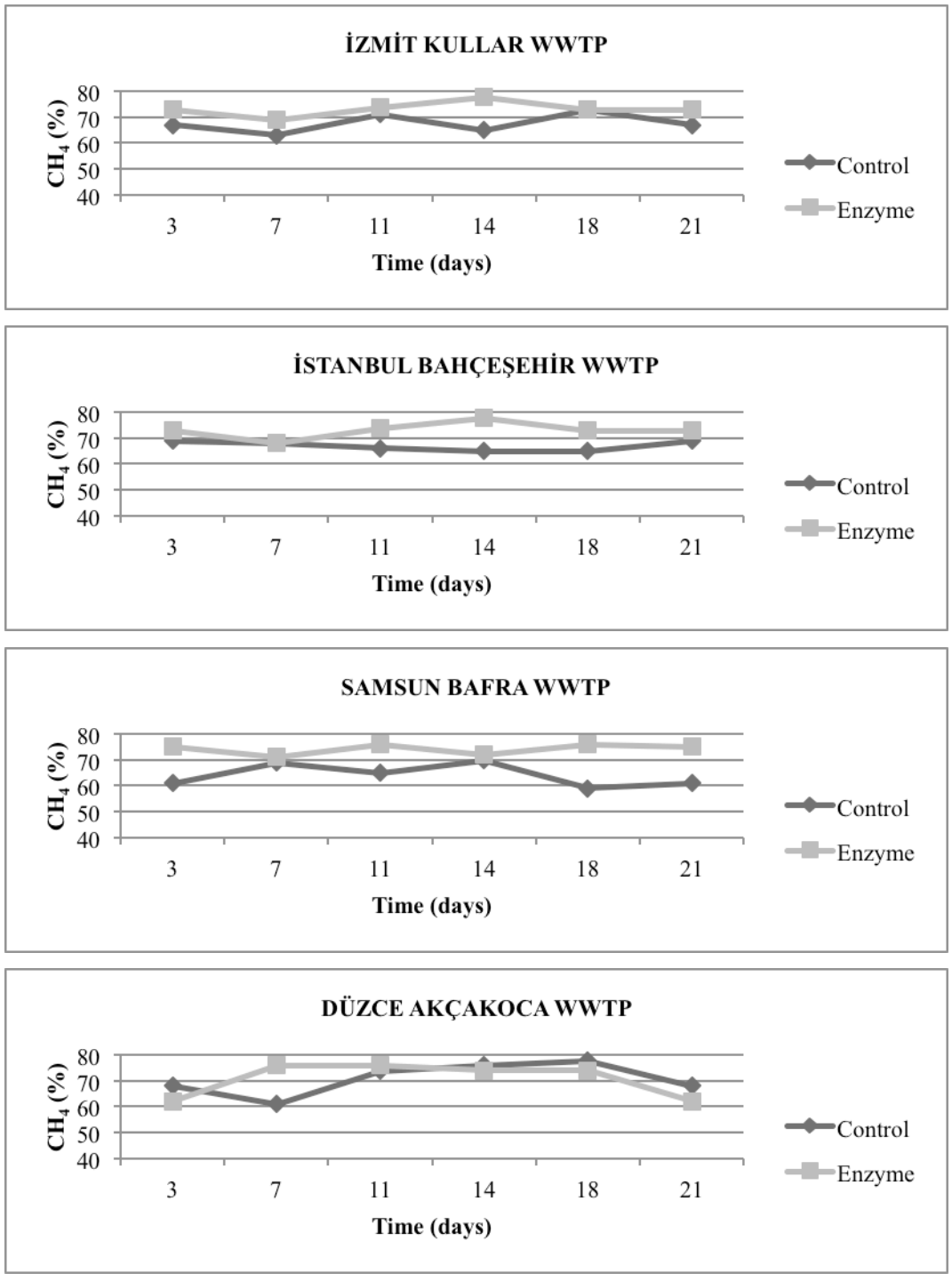


Figure 4.47. CH_4 percentages in the anaerobic digestion reactors.

The enzyme added reactors showed better performance on the methane production, compared to the control reactors. In the anaerobic digestion process, VS, MLVSS, and COD degradation was also improved by the enzyme addition. Higher removal of VS, MLVSS, and COD were resulted in more organic matters destruction. As a result, as more organic matters were destroyed, the quality of the biogas produced was enhanced.

5. CONCLUSIONS

This study investigated the effect of enzymatic pre-treatment on aerobic and anaerobic digestion of wastewater sludges. The following are the conclusions of the study.

- Enzymatic pre-treatment increased the solubility of the organic materials in the sludge, which led to an improvement in the biodegradability of the organic materials. The sCOD concentrations of the sludges were increased by 500% and 200% in average for the enzymatically pre-treated sludge samples during aerobic and anaerobic digestion, respectively.

- The improvement in the biodegradability of the organic matters resulted in the increased organic matter removal efficiencies in the enzymatically pre-treated reactors. The organic matter removal was increased by 70% and 60% in average in aerobic and anaerobic reactors, respectively.

- Enzymatic pre-treatment applied reactors showed better performance on the minimization of the excess sludge production. Less sludge was produced in the disintegrated reactors. Total solid removal efficiencies increased from 24% to 42% and from 15% to 22% in aerobic and anaerobic reactors, respectively.

- Digestion of the organic matter was improved in the enzymatically pre-treated reactors as a result of the increased biodegradability of sludge. Volatile solids removal in the enzymatically pre-treated sludges increased from 31% to 50% during the aerobic digestion, and from 21% to 32% during the anaerobic digestion.

- Destruction of large, organic, particulate matters into small organic compounds increases the surface area available for the degradation of them in order to improve the

efficiency of the digestion process. Particle sizes of the sludge flocs were decreased as a result of the enzymatic pre-treatment.

- Enzymatic pre-treatment decreased the dewaterability of the sludge samples. CST was worsened by the enzymatic pre-treatment in the initial stage. Disintegration caused to an increase in the capillary suction time, CST, of the sludge samples by decreasing the floc size and viscosity. Enzymatic pre-treatment firstly deteriorated the sludge dewaterability by increasing CST, whereas subsequent aerobic digestion balanced this effect and CST decreased by the degraded EPS. However, the sludge dewaterability was slightly deteriorated by the enzymatic pre-treatment at the end of the aerobic digestion process but the difference with the control samples was so small. It seemed that enzymatic pre-treatment did not effect the dewaterability of sludge remarkably.

- Enzymatic pre-treatment enhanced the pathogen removal in the sludge. At the end of the aerobic digestion, the reductions in TC, FC, and FC were about 99,78%, 99,84%, and 99,82%, respectively. At the end of the anaerobic digestion, the reductions in TC, FC, FS, and salmonella were found to be about 99,82%, 99,96%, <99,99%, and 99,98%, respectively.

- The EPS of the control and pre-treated sludge samples taken from reactors R3-İBC and R4-İB were mainly composed of proteins. The concentration of proteins in the EPS structure of sludge was 4 times greater than the concentration of carbohydrates.

- Enzymatic pre-treatment increased the solubilization of proteins and carbohydrates by destroying the structure of EPS. As a result of the release of protein and carbohydrate parts of the EPS, protein and carbohydrate concentrations in the soluble EPS increased by 966% and 2604%, respectively.

- Significant reductions in EPS were obtained for enzymatically pre-treated reactors. Protein degradation was improved by 455% in total EPS and by 274% in soluble EPS while carbohydrate degradation was improved by 45% in total EPS.

- Biological excess sludge hydrolysis can be enhanced by the enzymatic pre-treatment. Increased solubility and decreased particle sizes proved that the hydrolysis of complex organic matters was more rapid and effective in the enzymatically pre-treated reactors.

- Solubilization of complex organic matters into readily biodegradable substances and accessibility to soluble organic matters resulted in the significantly increased VFA productions in the enzymatically pre-treated sludge samples during anaerobic digestion. VFAs were mainly formed by acetic acid and propionic acid.

- Higher biogas productions were obtained in the enzymatically pre-treated reactors. The increased destruction of organic components resulted in the increased biogas production.

- The enzymatically pre-treated reactors showed better performance on the methane production. Methane contents of the produced biogas were increased from 67% to 73% in the disintegrated reactors.

Enzymatic pre-treatment is a promising technology in this field with its many advantages. It can be successfully used as a pre-treatment technique when treating domestic or urban wastewater sludges. As a promising technology, it should be studied deeper to get decisive results for the large-scale applications in the future. A cost analysis for the comparison of the effect of enzymatic pre-treatment on aerobic and anaerobic digestion systems is suggested.

REFERENCES

Aehle, W., 2007.Enzymes in Industry, WILEY-VCH, Weinheim.

Ahuja, S.K., Ferreira, G.M., Moreira A.R., 2004. Utilization of enzymes for environmental applications. Critical Reviews in Biotechnology, 24, 125-154.

Amano Enzyme. Home Page. Retrieved December 2012, from http://www.amano-enzyme.co.jp/eng/index.html.

APHA/AWWA/WPCF, 1988.Standard Methods for the examination of Water and Wastewater. 20th ed. Washington, DC: American Public Health Association/American Water Works Association/Water Pollution Control Federation, USA.

Appels, L., Baeyens, J., Degreve, J., Dewil, R., 2008.Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 34, 755-781.

Ayol, A., Filibeli, A., Sır, D., Kuzyaka, E., 2008. Aerobic and anaerobic bioprocessing of activated sludge: floc disintegration by enzymes. Journal of Environmental Science and Health, 43, 1528-1535.

Banik, S., Bandyopadhyay S., Ganguly, S., 2003. Bioeffects of microwave- a brief review. Bioresource Technology, 87, 155-159.

Barjenbruch, M., Kopplow, O., 2003. Enzymatic, mechanical and thermal pre-treatment of surplus sludge. Advances in Environmental Research, 7, 715-720.

Barragan Sanchez, J., Quiroga Alonso, J.M., Coello Oviedo, M.D., 2006. Use of microbial activity parameters for determination of a biosolid stability index. Bioresource Technology, 97, 562-568.

Bernard, S., Gray, N.F., 2000. Aerobic digestion of pharmaceutical and domestic wastewater sludges at ambient temperature. Water Research, 34, 725–734.

Bitton, G., 2005.Wastewater Microbiology, 3rd edition. John Wiley & Sons, Inc, Hoboken, New Jersey.

Bjornsson, L.,Murto, M., Mattiasson, B., 2000.Evaluation of parameters for monitoring anaerobic co-digestion process. Applied Microbiology and Biotechnology, 54, 844-849.

Bougrier, C., Carrere, H., Delgenes, J.P., 2005. Solubilisation of waste-activated sludge by ultrasonic treatment. Chemical Engineering Journal, 106, 163-169.

Burgess, J.E., Pletschke, B.I., 2008. Hydrolytic enzymes in sewage sludge treatment: A mini-review. South African Water Research Commision, 34, 343-349.

Carrere, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenes, J.P., Steyer, J.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. Journal of Hazardous Materials, 183, 1-15.

Chang, T., You, S., Damodar, R.A., Chen, Y., 2011. Ultrasound pre-treatment step for performance enhancement in an aerobic sludge digestion process. Journal of Taiwan Institute of Chemical Engineers, 42, 801-808.

Chu, C.P., Chang, B.V., Liao, G.S., Jean, D.S., Lee, D.J., 2001. Observation on changes in ultrasonically treated waste-activated sludge. Water Research, 35, 1038 – 1046.

Coello Oviedo, M.D., Barragan Sanchez, J., Quiroga Alonso, J.M., 2005. Enzymatic estimation of biosolids stability in aerobic digestion systems. Enzyme and Microbial Technology, 36, 191-197.

Çetin, S., Erdinçler, A., 2004.The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges. Water Science and Technology, 50, 49-56.

Davidsson, A., Jansen, J.L.C., 2006. Pre-treatment of wastewater sludge before anaerobic digestion – Hygienisation, ultrasonic treatment and enzyme dosing. Vatten, 62, 335-340.

Del Borghi, A., Converti, A., Palazzi, E., Del Borghi, M., 1999.Hydrolysis and thermophilic anaerobic digestion of sewage sludge and organic fraction of municipal solid waste. Bioprocess Engineering, 20, 553–560.

Dewil, R., Baeyens, J., Neyens, E., 2005. Fenton peroxidation improves the drying performance of waste activated sludge. Journal of Hazardous Materials, 117, 161–170.

Dey, E.S., Szewczyk, E., Wawrzynczyk, J., Norrlöw, O., 2006.A novel approach for characterization of exopolymeric material in sewage sludge. Journal of Residuals Science and Technology, 3, 97-103.

Dursun, D., Turkmen, M., Abu-Orf, M., Dentel, S.K., 2006. Water Science and Technology, 54, 33-41.

Eastman, J.A., Ferguson, J.F., 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. Journal of the Water Control Federation, 53, 352-366.

Elefsiniotis, P., Wareham, D.G., Oldham, W.K., 1996. Particulate organic carbon solubilization in an acid phase upflow anaerobic sludge blanket system. Environmental Science and Technology, 30, 1508–1514.

Enzyme Nomenclature, 1992.Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Academic Press, New York.

Erdinçler, A.U., Vesilind, P.A., 2000. Effect of sludge cell disruption on compactibility of waste activated sludge, Water Science and Technology, 42, 119-126.

Ferenci, T., 1980.The recognition of maltodextrions by E.coli. European Journal of Biochemistry, 108, 631–636.

Foladori, P., Andreotola, G., Ziglio, G., 2010. Sludge reduction technologies in wastewater treatment plants, 1st Edition. IWA Publishing, London.

Forster, C.F., Lewin, D.C., 1972. Polymer interactions at activated sludge surfaces. Effluent Water Treatment Journal, 12, 520-525.

Friends of the Earth Web Page, 2007. Briefing: Anaerobic Digestion. Retrieved November 2012, from http://www.foe.co.uk/resource/briefings/anaerobic_digestion.pdf.

Frolund, B., Griebe, T., Nielsen, P.H., 1995.Enzymatic activity in the activated-sludge floc matrix. Applied Microbiology Biotechnology, 43, 755–761.

Gerardi, M., 2003.The microbiology of anaerobic digestion. A John Wiley & Sons, Inc., Publication, New Jersey, USA.

Gianfreda, L., Rao, M.A., 2004. Potential of extracellular enzymes in remediation of polluted soils: a review. Enzyme and Microbial Technology, 35, 339-354.

Goel, R., Mino, T., Satoh, H., Matsuo, T., 1998. Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. Water Research, 32, 2081–2088.

Graczyk, M., 1984.The stabilization criteria of concentrated wastewater and sludge. Ochrona Srodowiska, 2, 25–33.

Grady, L., Diagger, G., 1999. Biological Wastewater Treatment, 2nd Edition. Marcel Dekker, Inc., New York, NY.

Gronroos, A., Kyllonen, H., Korpijarvi, K., Pirkonen, P., Paavola, T., Jokela, J., Rintala, J., 2005. Ultrasound assisted method to increase soluble chemical oxygen demand (SCOD) of sewage sludge for digestion. Ultrasonics Sonochemistry, 12, 115-120.

Gujer, W., Zehnder, A.J.B., 1983. Conversion processes in anaerobic digestion. Water Science and Technology, 15, 127-167.

Haner, A., Mason, C.A., Hamer, G., 1994. Death and lysis during aerobic thermophilic sludge treatment: characterization of recalcitrant products. Water Research, 28, 863-869.

Hori, T., Haruta, S., Ueno, Y., Ishii, M., Igarashi, Y., 2006.Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester. Applied Environmental Microbiology, 72, 1623-1630.

Hwang, M.H., Jang, N.J., Hyum, S.H., Kim, I.S., 2004. Anaerobic bio-hydrogen production from ethanol fementation: the role of pH. Journal of Biotechnology, 111, 297–309.

Johansen, C., Falholt, P., Gram, L., 1997. Enzymatic removal and disinfection of bacterial biofilms. Applied Environmental Microbiology, 63, 3724- 3728.

Karam, J., Nicell, J.A., 1997. Potential applications of enzymes in waste treatment. Journal of Chemical Technology and Biotechnology, 69, 141.

Kim, D.H., Jeong, E., Oh, S.E., Shin, H.S., 2010. Combined (alkaline+ultrasonic) pretreatment effect on sewage sludge disintegration. Water Research, 44, 3093-3100.

Kim, Y.K., Kwak, M.S., Lee, W.H., Choi, J.W., 2000. Ultrasonic pre-treatment for thermophilic aerobic digestion in industrial waste activated sludge treatment. Biotechnology and Bioprocess Engineering, 5, 469-474.

Lai, T.E., Nopharatana, A., Pullammanappallil, P.C., Clarke, W.P., 2001. Cellulolytic activity in leachate during leach-bed anaerobic digestion of municipal solid waste. Bioresources Technology, 80, 205–210.

Lay, J.J., Li, Y.Y., Noike, T., Endo, J., Ishimoto, S., 1997. Analysis of environmental factors affecting methane production from high-solids organic waste.Water Science and Technology, 36, 493–500.

Lequette, Y., Boels, G., Clarisse, M., Faille, C., 2010. Using enzymes to remove biofilms of bacterial isolates samples in the food industry. Biofouling, 4, 421-431.

Li, X.Y., Yang, S.F., 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. Water Research, 41, 1022–1030.

Liu, S., Zhu, N, Ning, P., Li, L.Y., Gong, X., 2012. The one-stage autothermal thermophilis aerobic digestion for sewage sludge treatment: Effects of temperature on stabilization process and sludge properties. Chemical Engineering Journal, 197, 223-230.

Liu, Y., Fang, H.H.P., 2003. Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge. Critical Reviews in Environmental Science and Technology, 28, 193-201.

Liu, Y., 2003. Chemically reduced excess sludge production in the activated sludge process. Chemosphere, 50, 1-7.

Liu, T., Sung, S., 2002. Ammonia inhibition on thermophilic aceticlastic methanogens. Water Science and Technology, 45, 113–120.

Liu, Y., Tay, J.H., 2001. Strategy for minimization of excess sludge production from the activated sludge process. Biotechnology Advances, 19, 97 – 107.

Lossedrecht, M.C.M., Henze, M., 1999.Maintenance, endogenous respiration, lysis, decay and predation. Water Science and Technology, 39, 11-20.

Luo, K., Yang, Q., Li, X., Yang, G., Liu, Y., Wang, D., Zheng, W., Zeng, G., 2012. Hydrolysis kinetics in anaerobic digestion of waste activated sludge enhanced by alpha-amylase. Biochemical Engineering Journal, 62, 17-21.

Lyberatos, G., Skiadas, I.V., 1999. Modelling of anaerobic digestion – a review. Global Nest: The International Journal, 1, 63-76.

Mason, C.A., Hamer, G., 1987. Cryptic growth in *Klebsiella pneumoniae*. Applied Microbiology, 25, 577-584.

Melo, L.F., Bott, T.R., 1997. Biofouling in water systems. Experimental Thermal and Fluid Science, 14, 375-381.

Midlandstech Lecture Notes, 2012. Microbial Metabolism. Retrieved December 2012, from http://classes.midlandstech.edu/carterp/courses/bio225/chap05/lecture2.htm.

Montusiewicz, A., Lebiocka, M., Rożej, A., Zacharska, E., Pawłowski, L., 2010.Freezing/thawing effects on anaerobic digestion of mixed sewage sludge. Bioresource Technology, 101, 3466-3473.

Müller, J.A, Winter, A., Strünkmann, G., 2004. Investigation and assessment of sludge pretreatment processes. Water Science and Technology, 49, 97-104.

Müller, J.A., 2001. Prospects and problems of sludge pre-treatment processes. Water Science and Technology, 44, 121-128.

Müller, J.A., Pelletier, L., 1998. Désintégration mécanique des boues activées.L'eau, l'industrie, les nuisances, 217, 19-26.

Neu, T.R., Lawrence, J.R., 2010. Extracellular polymeric substances in microbialbiofilms.Holst, O., Brennan, P.J., Itzstein, M. (Eds.), Microbial Glycobiology Academic Press, San Diego.

New World Encyclopedia, 2006.Enzyme. Retrieved December, 2012, from http://www.newworldencyclopedia.org/entry/Enzyme.

Neyens, E., Baeyens, J., Dewil, R., De heyder, B., 2004. Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. Journal of Hazardous Materials, 106, 83-92.

Nickel, K., Tiehm, A., Neis, U., 1999.Ultrasound in environmental engineering, Hamburg University of Technlogy Reports on Sanitary Engineering, 25, 205-216.

Novak, J.T., Banjade, S., Murthy, S.N., 2011. Combined anaerobic and anaerobic digestion for increased solids reduction and nitrogen removal. Water Research, 45, 618-622.

Odegaard, H., 2004. Sludge minimization technologies – an overview. Water Science and Technology, 49, 31-40.

Park, C., Lee, C., Kim, S., Chen, Y., Chase, H.A., 2005. Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. Journal of Bioscience and Bioengineering, 100, 164-167.

Park, K.Y., Ahn, K.H., Maeng, S. K., 2002. Feasibility of sludge ozonation for stabilization and conditioning. Ozone Science Engineering, 25, 73–80.

Parkin, G.F., Owen, W.F., 1986.Fundamentals of anaerobic digestion of wastewater sludges.Journal of Environmental Engineering, 112, 867-920.

Parmar, N., Singh, A., Ward, O.P., 2001. Enzyme treatment to reduce solids and improve settling of sewage sludge. Journal of Industrial Microbiology and Biotechnology, 26, 383-386.

Parmar, N., Singh, A., Ward, O. P., 2001. Characterization of the combined effects of enzyme, pH and temperature treatments for removal of pathogens from sewage sludge. World Journal of Microbiology and Biotechnology, 17, 169-172.

Pena, M., Perez-Elvira, S., Fdz-Polanco, F., 2008.Enzymatic autohydrolysis of secondary sludge. Unpublished raw data.

Pere, J., Alen, R., Viikari, L., Eriksson, L., 1993. Characterization and dewatering of activated sludge from the pulp and paper industry. Water Science and Technology, 28, 193-201.

Pérez-Elvira, S.I., Diez, P.N., Fdz-Polanco, F., 2006. Sludge minimization technologies. Reviews in Environmental Science and Biotechnology, 5, 375–398.

Poxon, T.L., Darby, J.L., 1997. Extracellular polyanions in digested sludge: measurement and relationship to sludge dewaterability. Water Research, 31, 749– 758.

Ramesh, A., Lee, D.J., Hong, S.G., 2006. Soluble microbial products (SMP) and extracellular polymeric substances (EPS) from wastewater sludge. Applied Microbiology and Biotechnology, 73, 219–225.

Rashed, I.G.A., Akunna, J., El-Halwany, M.M., Atiaa, A.F.F.A., 2010. Improvement in the efficiency of hydrolysis of anaerobic digestion in sewage sludge by the use of enzymes.Desalination and Water Treatment, 21, 280-285.

Flaig, W., 1962.Report of the Commission on Enzymes of the International Union of Biochemistry.The Journal of Biological Chemistry, 237, 979-980.

Roman, H.J., Burgess, J.E., Pletschke, B.I., 2006. Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. African Journal of Biotechnology, 5, 963– 967.

Romano, R.T., Zhang, R.H., Teter, S., McGarvey, J.A., 2009.The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. Bioresource Technology, 100, 4564–4571.

Ronja, B., 2008.Enzymatic treatment of wastewater sludge in presence of a cation binding agent-improved solubilisation and increased methane production. MSc Thesis. Linkopings University, Sweden.

Roxburgh, R., Sieger, R, Johnson, B., Rabinowit, B., Goodwin, S., Crawford, G., Daigger, G., 2006. Sludge minimization technologies – doing more to get less. Proceedings of the Water Environment Federation, WEFTEC 2006: Session 1- 10, 506-525.

Rudolfs, W., 1932. Enzymes and sludge digestion. Sewage Works Journal, 4, 782-789.

Sadava, D., Hillis, D.M., Heller, H.C., Berenbaum, M., 2009. Life: The Science of Biology. W.H. Freeman, 9th edition, Sunderland, Massachusetts.

Saha, M., Eskicioglu, C., Marin, J., 2011. Microwave, ultrasonic and chemo-mechanical pretreatments for enhancing methane potential of pulp mill wastewater treatment sludge. Bioresource Technology, 102, 7815-7826.

Salsabil, M.R., Prorot, A., Casellas, M., Dagot, C., 2009. Pre-treatment of activated sludge: Effect of sonication on aerobic and anaerobic digestibility. Chemical Engineering Journal, 148, 327-335.

Sangave, P.C., Pandit, A.B., 2006. Enhancement in biodegradability of distillery wastewater using enzymatic pre-treatment. Journal of Environmental Management, 78, 77-85.

Scheidat, D., Kasche, V., Sekoulov, I., 1997. Solubilization of primary sludge by addition of hydrolytic enzymes. Technishe Universitat Hamburg-Harburg. Biotechnologie, Biotransformation und Biosensorik.

Scheminski, A., Krull, R., Hempel, D.C., 1999.Oxidative treatment of digested sewage sludge with ozone. Water Science and Technology, 42, 151–158.

Sheng, G.P., Yu, H.Q., Li, X.Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biosource Advances, 28, 882-894.

Spath, R., Flemming, H.C., Wuerts, S., 1998. Sorption properties of biofilms. Water Science and Technology, 37, 207-210.

Speece, R.E., 1983. Anaerobic biotechnology for industrial wastewaters. Environmental Science and Technology, 17, 416-427.

Tan, R., Miyanaga, K., Toyama, K., Uy, D., Tanji, Y., 2010. Changes in composition and microbial communities in excess sludge after heat-alkaline treatment and acclimation .Biochemical Engineering Journal, 52, 151-159.

Tas, D.O., 2010. Respirometric assessment of aerobic sludge stabilization. Bioresource Technology, 101, 2592-2599.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Meltcalf & Eddy, Inc.'s Wastewater Engineering: Treatment, Disposal, and Reuse, 4th Edition. McGraw-Hill, Inc., New York.

Thomas, L., Jungschaffer, G., Sprossler, B., 1993. Improved sludge dewatering by enzymatic treatment, Water Science and Technology, 28, 189-192.

Tokumura, M., Sekine, M., Yoshinari, M., Znad, H. T., Kawase, Y., 2007.Photo-fenton process for excess sludge disintegration. Process Biochemistry, 42, 627-633.

Urbain, V., Block, J.C., Manem, J., 1993. Bioflocculationin activated sludge: an analytic approach. Water Resource, 27, 829-838.

US EPA., 1992. Environmental regulations and technology: control of pathogens and vector attraction in sewage sludge under 40 CFR Part 503. EPA/625/R-92/013, Washington, DC.

van Haandel, A.C., van der Lubbe, J., 2012. Handbook of biological wastewater treatment. Design and optimization of activated sludge systems. 2nd edition, IWA Publishing.

Vesilind, P.A., 1979. Treatment and Disposal of Wastewater Sludges. Ann Arbor Science Publishers, Inc. Michigan, USA.

Vesilind, P.A., Spinosa, L., 2001. Sludge into Biosolids - Processing, Disposal, Utilization. First Edition, IWA Publishing U.K (ISSN Online: 1476-1777).

Vranitzky, R., Lahnsteiner, J., 2005. Sewage sludge disintegration using ozone – a method of enhancing the anaerobic stabilization of sewage sludge. Unpublished raw data.

Wang, F., Wang, Y., Ji, M., 2005. Mechanisms and kinetics models for ultrasonic waste activated sludge disintegration. Journal of Hazardous Materials, 123, 145–150.

Watson, S.D., Akhurst, T., Whiteley, C.G., Rose, P.D., Pletschke, B.I., 2004. Primary sludge floc degradation is accelerated under biosulphidogenic conditions: enzymological aspects. Enzyme and Microbial Technology, 34, 595-602.

Wawrzynczyk, J., Szewczyk, E., Norrlow, O., Dey, E.S., 2007a.Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of extracellular polymeric substances from sewage sludge Characterization of the extracted polysaccharides/glycoconjugates by a panel of lectins. Journal of Biotechnology, 130, 274-281.

Wawrzynczyk, J., Recktenwald, M., Norrlow, O., Dey, E.S., 2007b. Solubilisation of sludge by combined chemical and enzymatic treatment. African Journal of Biotechnology, 6, 1994-1999.

Wawrzynczyk, J., Recktenwald, M., Norrlow, O., Dey, E.S., 2007c.The function of cation-binding agents in the enzymatic treatment of municipal sludge. Water Research, 42, 1555-1562.

Wawrzynczyk, J., Dey, E. S., Norrlow, O., Jansen, J. I. C., 2003. Alternative method for sludge reduction using commercial enzymes. In: Aqua Environment Technology Transfer:Eighth CLWEM/Aqua Enviro European Biosolids and Organic Residuals Conference: Wakefield West Yorkshire UK, pp. 1–5.

Weemaes, M., Grootaerd, H., Simoens, F., Huysmans, A., Verstraete, W., 2000.Ozonation of sewage sludge prior to anaerobic digestion, Water Science and Technology 42, 175 –178.

Wei, Y., Houten, R.T.V., Borger, A. R., Eikelboom, D. H., Fan, Y., 2003. Minimization of excess sludge production for biological wastewater treatment. Water Research, 37, 4453-4467.

White, D., 2000. The Physiology and Biochemistry of Pprokaryotes, 2nd edition, Oxford University Press Inc., New York.

Whiteley, C.G., Lee, D.J., 2006. Enzyme technology and biological remediation. Enzyme and Microbial Technology, 38, 291-396.

Worthington Biochemical Corporation, Manual of Clinical Enzyme Measure, 1972. Worthington Diagnostics, Freehold, NJ, 2-17.

Yang, Q., Luo, K., Li, X., Wang, D., Zheng, W., 2010. Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. Biosource Technology, 101, 2924-2930.

Yu, Y., Chan, W., Liao, P., Lo, K., 2010. Disinfection and solubilization of sewage sludge using the microwave enhanced advanced oxidation process. Journal of Hazardous Materials, 181, 1143-1147.

Yu, G.H., He, P.J, Shao, L.M., 2009. Characteristics of extracellular polymeric substances fractions from excess sludges and their effects on bioflocculability. Bioresource Technology, 100, 3193-3198.

Yu, G.H., He, P.J., Shao, L.M., Lee, D.J., 2007. Enzyme activities in activated sludge flocs. Applied Microbiology and Biotechnology, 77, 605–612.

Zeikus, J.G., 1980. Microbial populations in digesters, In: *Anaerobic Digestion.* D.A. Stafford, B.I. Wheatley and D.E. Hughes (ed.), Applied Science Publishers, London, U.K.

Zhang, G., Zhang, P., Yang, J., Liu, H., 2008. Energy-efficient sludge sonication: Power and sludge characteristics. Bioresource Technology, 99, 9029–9031.

Zhou, J., Wang, Y.H., Chu, J., Luo, L.Z., Zhuang, Y.P., Zhang, S.L., 2009. Optimization of cellulase mixture for efficient hydrolysis of steam-exploded corn stover by statistically designed experiments. Bioresource Technology, 100, 819–825.