

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES

ENZYMATIC TREATMENT
EFFECTS ON AEROBIC SLUDGE
STABILIZATION

by
Ersan KUZUYAKA

August, 2008
İZMİR

**ENZYMATIC TREATMENT
EFFECTS ON AEROBIC SLUDGE
STABILIZATION**

**A Thesis Submitted to the
Graduate of Natural and Applied Sciences of
Dokuz Eylül University
In Partial Fulfillment of the Requirement for
The Degree of Master of Science in Environmental Engineering,
Environmental Technology Program**

**by
Ersan KUZUYAKA**

**August, 2008
İZMİR**

Ms.Sc. THESIS EXAMINATION RESULT FORM

We certify that we have read the thesis, entitled “**ENZYMATIC TREATMENT EFFECTS on AEROBIC SLUDGE STABILIZATION**” completed by **ERSAN KUZYAKA** under supervision of **PROF. DR. AYŞE FİLİBELİ** and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

Prof. Dr. Ayşe FİLİBELİ

Supervisor

(Jury Member)

(Jury Member)

Dire Prof.Dr.Cahit HELVACI

Director

Graduate School of Natural and Applied Sciences

ACKNOWLEDGMENTS

Foremost, I would like to express my gratefulness to my supervisor Prof. Dr. Ayşe FILIBELI for her guidance, advices and encouragement.

I would like to thank Assoc. Prof. Dr. Azize AYOL for her efforts, altruistic helps, patience, tolerance and moral motivation during my thesis.

I special thank to MSc. Diclehan SIR, my lab-mate, for her continuous efforts, and friendship.

I am also thankful to Dr. Zihni YILMAZ, Dr. Remzi SEYFİOĞLU, Dr. Nazlı BALDAN PAKDİL, MSc. Serkan EKER, MSc. Gülbin ERDEN KAYNAK, MSc. Ebru ÇOKAY ÇATALKAYA, MSc. Oğuzhan Gök, MSc. Gülden GÖK, MSc. Hakan ÇELEBİ, Tech. Yılmaz SAĞER and Tech. Orhan ÇOLAK for their great helps.

I am grateful the personnel of İzmir Güney Batı Municipal WWTP for their assistance in taking samples.

Finally, I am particularly grateful to my family for their love, moral supports, their patience, and their great self-sacrifice during my education. Therefore, I would like to dedicate the thesis to my family.

The author gives his appreciation to the Technical and Scientific Research Council of Turkey (TUBITAK) for their supports during the study under Award #104Y375: A Novel Approach for Enhanced Sludge Dewaterability: Enzymatic Treatment directed by Dr. Azize Ayol.

Ersan KUZUYAKA

İZMİR, 2008

ENZYMATIC TREATMENT EFFECTS ON AEROBIC SLUDGE STABILIZATION

ABSTRACT

Water/wastewater treatment processes have produced sludge in different characteristics and quantities. The sludges should be processed and disposed of in accordance with the environmental health criteria for environmental reasons. For many authorities and engineers, the effective sludge management is still a big challenge since the investment and operational costs of sludge processing have an important part of overall plant's costs. Sludge dewatering process has a central role in sludge management for many operations like storage, transport. But, the dewaterability characteristics of sludges can vary depending on their sources and the applied processes. The methods for effective processing cover the methods - thickening, stabilization, conditioning, dewatering-, and the final disposal alternatives- incineration, land application.

Sludge stabilization has crucial feature since it largely affects sludge dewatering characteristics. For this reason, the both processes should be considered together. As well-known, sludge stabilization is an important process for many reasons including pathogen reduction, removal of organic matters and odor potential. Among the different stabilization processes, aerobic digestion process has been widely used in many wastewater treatment plants. Although the many advantages of the process, it has obviously some negative effects on the sludge dewaterability characteristics as in other biological stabilization processes (Ayol et al. 2007). Previous findings from literature indicated that sludge flocs have different constituents like water, microbial consortia, and extracellular polymeric substances (EPS) (Novak et al. 2003, Ayol 2005). Among the constituents, extracellular polymeric substances (EPS) bridging with bacteria and other constituents in a flocculated matrix is responsible for poor quality of sludge dewatering (Novak et al. 2003). Some researchers have reported that the enzymatic treatment of sludges enhanced the dewaterability properties by

degrading EPS constituents (Novak et al. 2003, Watson et al., 2004, Ayol 2005, Dey et al., 2006).

The main purpose of this thesis was to investigate the effects of hydrolytic enzyme additions on dewaterability performance of activated sludge during aerobic digestion process. Therefore, three 13.5 L aerobic reactors were operated at different operation conditions. Activated sludge was fed to the reactors as a feeding material. Activated sludge samples were taken from Guney Bati Municipal Wastewater Treatment Plant located in Narlidere, Izmir. The parameters – pH, electrical conductivity (EC), salinity, oxidation reduction potential (ORP), capillary suction time (CST), zeta potential, suspended solids content, dry solid content, organic matter- were analyzed to determine the sludge characteristics. Reactor performances with and without enzyme additions were monitored. Experimental results showed that the enzyme additions provided enhanced sludge disintegration. Enzyme added reactors showed considerably superior better performance in terms of organic matter reduction and EPS degradation when comparing the control reactor.

This thesis as part of a project examining the effects of hydrolytic enzymes in biological sludge stabilization gives important research results to determine the floc disintegration mechanisms by enzymes during aerobic sludge digestion.

Keywords: Activated sludge, aerobic digestion, biological hydrolysis, dewaterability, enzymatic treatment, extracellular polymeric substance (EPS), floc disintegration.

ENZİMATİK ARITMANIN AEROBİK ÇAMUR STABİLİZASYONU ÜZERİNE ETKİLERİ

ÖZ

Su ve atıksu arıtma proseslerinde farklı özelliklere sahip ve farklı miktarlarda arıtma çamuru oluşmaktadır. Arıtma çamurları, çevre sağlığı kriterlerine uygun olarak işlenmeli ve bertaraf edilmelidir. Atıksu arıtma tesislerinden sorumlu olan kuruluşlar ve mühendisler için, etkin arıtma çamuru yönetiminin sağlanması, çamur arıtım proseslerinin yüksek ilk yatırım ve işletim maliyeti göz önünde bulundurulduğunda, hala büyük problemdir. Çamur susuzlaştırma prosesi, arıtma çamuru yönetiminde depolama ve taşıma gibi pek çok süreçte büyük bir öneme sahiptir. Bununla birlikte, çamur su verme özellikleri, çamurların geldiği kaynağa ve uygulanan işlemlere bağlı olarak değişkenlik göstermektedir. Arıtma çamurlarının işlenmesi ve bertaraf edilmesinde, yoğunlaştırma, stabilizasyon, şartlandırma ve susuzlaştırma önemli proses adımlarını teşkil ederken, yakma, tarımsal amaçlı kullanım, depolama gibi işlemler de nihai bertaraf alternatiflerini oluşturmaktadır.

Çamur stabilizasyonu, çamurun su verme özelliklerini geniş ölçüde etkilediğinden, çamurların işlenmesinde önemli bir role sahiptir. Bu nedenle, çamur stabilizasyonu ve susuzlaştırma prosesleri bir arada düşünülerek optimize edilmelidir. İyi bilindiği gibi çamur stabilizasyonu, patojen mikroorganizma giderimi, organik madde indirgenmesi ve koku oluşum potansiyelinin azaltılması gibi önemli avantajları sağlamaktadır. Farklı stabilizasyon prosesleri arasında, aerobik çürütme prosesi çoğu atıksu arıtma tesisinde yaygın olarak kullanılmaktadır. Bu proses birçok avantajının olmasına rağmen, çamur su verme özelliklerine olan olumsuz etkisi nedeniyle de diğer biyolojik stabilizasyon uygulamalarında olduğu gibi önemli bir dezavantaja da sahiptir (Ayol ve diğ. 2007). Literatür araştırmalarından elde edilen sonuçlar, çamur floklarının su, mikroorganizmalar ve hücre dışı polimerik maddeler gibi farklı yapılardan oluştuğunu göstermektedir (Novak ve diğ. 2003, Ayol ve diğ. 2005). Hücre dışı polimerik maddeler, bakteri ve diğer bileşenlerle bir flok yapısı oluşturmakta ve çamurların su verme özelliklerini

zayıflatmaktadır (Novak ve diğ. 2003). Bazı araştırmacılar enzimatik arıtma ile çamurdaki hücre dışı polimerik bileşenlerin parçalanması ile su verme özelliklerinin geliştiğini göstermiştir (Novak ve diğ 2003, Watson ve diğ. 2004, Ayol ve diğ 2005, Dey ve diğ. 2006).

Bu tezin başlıca amacı; aerobik çürüme esnasında hidrolitik enzim ilave edilmiş aktif çamurun su verme performansının araştırılmasıdır. Bu nedenle 3 adet 13.5 L hacmindeki aerobik reaktörler, farklı işletim koşullarında çalıştırılmıştır. Bu reaktörler atık aktif çamur ile beslenmiştir. Aktif çamur numuneleri İzmir- Narlıdere’ de bulunan Güney Batı Evsel Atıksu Arıtma Tesisi’nden alınmıştır. pH, elektriksel iletkenlik, tuzluluk, indirgenme-yükseltgenme potansiyeli, kapiler emme süresi, zeta potansiyeli, katı madde ve organik madde parametreleri analizlenerek çamur karakterizasyonu yapılmıştır. Reaktörler, enzim ilaveli ve kontrol amaçlı enzim ilavesiz olarak işletilmiştir. Deneysel sonuçlar enzim ilavesi yapılan reaktörlerden alınan çamurlarda daha iyi çamur parçalanmasının sağlandığını göstermiştir. Enzim ilaveli reaktörler kontrol reaktörü ile karşılaştırıldığında enzim ilaveli reaktörlerin organik madde azaltımı ve hücre dışı polimerik madde bozunmasında daha başarılı sonuçlar verdiği bulunmuştur.

Bu tez, biyolojik çamur stabilizasyonunda hidrolitik enzimlerin etkisini araştırarak kapsamlı bir araştırma projesinden üretilmiştir. Aerobik çamur çürüme esnasında hidrolitik enzimlerin flok parçalama mekanizması üzerindeki etkilerinin belirmesine yönelik araştırma sonuçları tez kapsamında sunulmaktadır.

Anahtar kelimeler: Aktif çamur, aerobik çürüme, biyolojik hidroliz, susuzlaştırma, enzimatik arıtma, hücre dışı polimerik maddeler, flok parçalanması.

CONTENT

	Page
THESIS EXAMINATION RESULT FORM.....	ii
ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
ÖZ.....	vi
CHAPTER ONE – INTRODUCTION.....	1
1.1 Introduction.....	1
1.2 Scope and Research Objectives of the Thesis.....	6
CHAPTER TWO – LITERATURE SURVEY.....	7
2.1. Introduction.....	7
2.2. Sludge Treatment Methods.....	7
2.2.1. Thickening.....	8
2.2.2. Sludge Stabilization.....	8
2.2.2.1 Anaerobic Digestion.....	9
2.2.2.2 Aerobic Digestion.....	9
2.2.2.3 Composting.....	12
2.2.2.4 Lime Stabilization.....	12
2.2.2.5 Pasteurization.....	12
2.2.2.6 Thermal Drying.....	13
2.2.3. Conditioning.....	13
2.2.4. Dewatering.....	13
2.3. Sludge Disposal.....	14
2.3.1. Land Filling.....	14
2.3.2. Incineration.....	14

2.3.3. Agricultural and Other Land Uses	15
CHAPTER THREE – ENZYMES.....	16
3.1. Introduction.....	16
3.2. Enzyme – Substrate Interaction.....	18
3.3 Energy of Reaction.....	19
3.4 Cofactors/Coenzymes.....	20
3.5 Factors Affecting Enzyme Activity.....	22
3.5.1 Enzyme Concentration.....	22
3.5.2 Substrate Concentration.....	23
3.5.3 Effect of pH.....	23
3.5.4 Temperature.....	25
3.6 Source of Enzyme.....	25
3.7 Sludge Enzymatic Treatment Regarding the Extracellular Polymeric Substances in Sludge.....	28
CHAPTER FOUR – MATERIALS AND METHODS.....	30
4.1 Introduction.....	30
4.2 Materials.....	30
4.2.1 Raw Sludges.....	30
4.2.2 Aerobic Reactors.....	30
4.2.3 Chemicals.....	31

4.3 Methods Used in Experimental Studies.....	32
4.3.1 Analytical Methods.....	32
4.3.1.1 Redox Potential, pH Measurements and Oxygen Measurements...	33
4.3.1.2 Particle Size Distribution Analysis.....	33
4.3.1.3 Ca, K, Mg, Na Analysis.....	34
4.3.1.4 Total Nitrogen (TN) and Total Phosphorus (PO ₄ – P) Analysis....	34
4.3.1.5 Summary of Acid Digestion Method.....	35
4.3.1.6 Total Organic Carbon Analysis.....	35
4.3.1.7 EPS Extraction from the Samples.....	36
4.3.1.8 Capillary Suction Time Test.....	37
4.3.1.9 Protein and Polysaccharide Analysis.....	37
4.3.1.10 Zeta Potential Measurement.....	38
4.3.1.11 Viscosity Measurements.....	39
4.3.1.12 Sludge Conditioning.....	39
CHAPTER FIVE – RESULTS AND DISCUSSION.....	41
5.1 Introduction.....	41
5.2 Start-up of the Reactors.....	41
5.3 Sludge Characteristics.....	41
5.4 Enzyme Additions.....	43
5.5 Performance Evaluations for 25 Days of Operation Time.....	43
5.5.1 pH Results.....	43
5.5.2 Temperature Monitoring.....	44
5.5.3 Dissolved Oxygen.....	44
5.5.4 Oxidation Reduction Potential- ORP Results.....	46
5.5.5 Dried Solids.....	47
5.5.6 Volatile Solids.....	47

5.5.7 Alkalinity.....	48
5.5.8 Capillary Suction Time.....	48
5.5.9 Particle Size Distribution Results.....	50
5.5.10 EPS – Protein and Polysaccharide Results.....	50
5.5.11 Sludge Conditioning.....	51
 5.6 Performance Evaluations for 15 Days of Operation Time.....	 53
5.6.1 pH Results.....	53
5.6.2 Temperature Results.....	53
5.6.3 Dissolved Oxygen.....	54
5.6.4 Oxidation Reduction Potential.....	56
5.6.5 Dried Solids.....	57
5.6.6 Volatile Solids.....	57
5.6.7 Alkalinity.....	57
5.6.8 Capillary Suction Time.....	59
5.6.9 EPS – Protein and Polysaccharide Results.....	59
5.6.10 SEM- EDS Results.....	61
5.6.11 Sludge Conditioning.....	63
 CHAPTER SIX – CONCLUSIONS and RECOMMENDATIONS.....	 66
 6.1. Conclusions.....	 66
 6.2 Recommendations.....	 67
 REFERENCES.....	 68

CHAPTER ONE

INTRODUCTION

1.1 Introduction

The activated sludge process has been commonly used in wastewater treatment field. However, this process produces a large mass of waste sludge that needs costly treatment units and large disposal area. The sludge treatment and handling operations are commonly expensive systems, which usually accounts for 30 – 60 % of the total operational cost in a conventional activated sludge treatment plant (Saby, S., Djafer, M., & Chen, G.H., 2003).

Regarding the solid/liquid separation, this process can be operated in a short sludge retention time (SRT) to keep low mixed liquid suspended solid (MLSS) concentration for better gravity separation of sludge. These problems brought an important movement to develop a new technology for minimizing sludge production.

Sludge minimization has received great attention recently to solve the sludge problems, to reduce the investment and operational costs, and to enhance the performance of the subsequent treatment and ultimate disposal processes (Ayol et al. 2007). Many minimization methods have been reported in previous research studies including thermal treatment, acidic or alkali chemical treatment, freeze-thawing, mechanical disintegration using ultrasonic devices, advanced oxidation processes like ozonation, fenton, and biological hydrolysis with enzymatic treatment (Ayol et al., 2007, Kaynak et al., 2007, Ayol 2005, Wei et al. 2003, Liu & Tay 2001, Chu et al. 2001). Ayol (2005) reported 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.

The different ways introduced for reduction of the sludge production from activated sludge process such as ozonization, chlorination, increasing of oxygen concentration in activated sludge systems, and enzymatic treatment have clear

positive effects. In addition to the advantages, these methods significantly increase the operational costs in wastewater treatment plants (Bhatta, C.P., et al. 2004).

To address the solutions for the excess sludge issue, there are three major alternatives:

(1) Pre-treatment of the excess sludge through sludge disintegration and/or mineralization using either chemical or physical treatment, such as ozone, thermal or mechanical treatments;

(2) Limitation of the sludge growth within the process using metabolic uncouples;

(3) To reduce excess sludge production through the oxic-settling-anoxic activated sludge process (OSA system) where activated sludge is exposed to an anoxic zone under no food and low oxidation reduction potential (ORP) conditions periodically (Saby, S., Djafer, M., & Chen, G.H., 2003).

Disposal of sludges from wastewater treatment processes has some difficulties. The sludge treatment processes used in sludge management include:

(1) reduction in sludge volume, primarily by removal of water, which constitutes 97–98% of the sludge;

(2) reduction of the volatile organic content of the sludge, which eliminates nuisance conditions by reducing putrescibility and reduces threats to human health by reducing levels of microorganisms; and

(3) ultimate disposal of the residues (Aerobic sludge digestion).

Among the processes to correspond the environmental reasons given above, sludge stabilization is a very crucial process. This process is very effective for the reduction organic matter, removal of pathogenic and odor problem. For this purpose, different stabilization methods like alkaline stabilization, biological stabilization - aerobic thermophilic stabilization (ATAD), aerobic digestion, anaerobic digestion and composting-, and thermal treatment have been used (Metcalf & Eddy, 2003).

Aerobic sludge digestion is widely used for sludge stabilization and it usually takes more than twenty days to meet the regulation standards (Yu et al, 2007). In this process, microbial hydrolysis of large organic molecules present in the sludge was reported to be the rate-limiting step to achieve rapid degradation (Vavilin et al., 1996).

Beyond the advantages of the processes, biological stabilization processes - bioprocessing of activated sludge either aerobically or anaerobically-cause some negative effects to the sludge like deprived dewaterability characteristics (Ayol et al., 2007). These processes are very sensitive to the environmental conditions as well as waste characteristics since the processes undergo rich microbial consortia. Previous studies (e.g. Novak et al., 2003; Ayol, 2005) have examined the mechanisms of floc destruction during the bioprocessing of the sludge in laboratory scale studies. These studies reported that a flocculated matrix of extracellular polymeric substances (EPS) bridging with bacteria holds back the dewaterability properties of the bioprocessed sludges.

The hydrolysis of complex organic structures in the degradation of biodegradable particulate organic matter like EPS heavily depends on the hydrolytic enzymes like glucosidases, lipases, and proteases (Ayol et al., 2007). Some selected hydrolytic enzymes act on a particular substrate – biopolymer present in the sludge, break down it and release products with lower molecular weight into solution. Ultimately, depending on the floc structure break-up, important amounts of proteins, peptides and carbohydrates can be released (Dey et al., 2006).

Enzymes catalyze the degradation of organic substances in sludge as a function of the substrate. This relation can be explained by enzyme-substrate models like the key and lock model or space filling model.

The upshot of the enzyme additions during biological sludge stabilization process is the enhanced degradation of EPS –proteins, polysaccharides, and humics- and the other biological slimes and gels and improved the releasing capacity of water (Barjenbruch and Kopplow, 2003, Ayol 2005, Dey et al. 2006, Roman et al. 2006).

In sludge disintegration by enzymes or other techniques, it is aimed to improve the biological break down of the sludge by “breaking up the sludge”. Primarily this can be defined as the decomposition of sludge flocs and with more intensive treatment the bacterial cells present are disrupted. When larger energy input is used the structures of the sludge are further decomposed (Luning et al .2007).

Luning et al. (2007) have graphically presented the impact of the energy supply on the sludge disintegration in Figure 1.1.

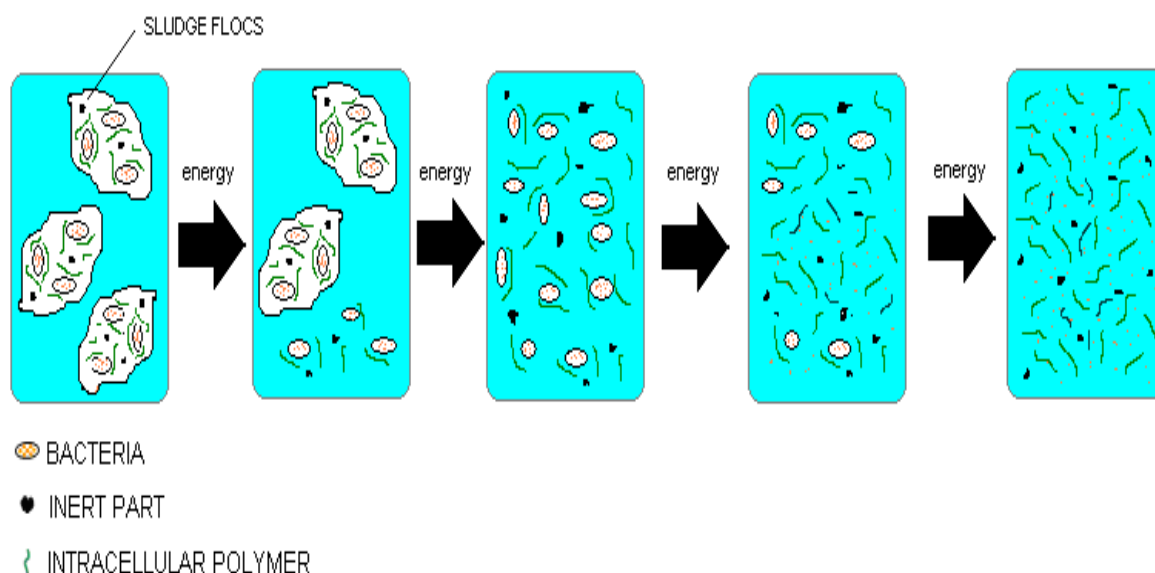


Figure 1.1 Effect of increased energy input on disintegration (Luning, L., et al .2007)

Neyens et al. (2004) have reported that the presence of the main components of EPS, i.e., proteins and polysaccharide, in untreated sludges. These constituents - proteins and polysaccharides- are the most water retaining components, hence influencing the dewaterability characteristics of the sludge. Since the mass of total EPS (EPS and water held in its structure) represents up to 80% of the mass of the activated sludge (Neyens et al., 2004).

The effects of EPS constituents on sludge dewaterability have been investigated in various studies. Kang et al. (1989) have been determined that extracellular polymer substance extracted from one activated sludge then added to another activated sludge has detrimental effect on the dewatering process of the latter sludge. Houghton et al. (2001) have been reported that there appears to be levels of EPS at which the sludge should be easiest to dewater. Sanin and Vesilind (1994) have pointed out that the increasing relatively low levels of EPS can aid sludge dewaterability by improving the level of sludge flocculation.

Extracellular polymeric substances (EPS) and cells form bioaggregates such as biofilms and sludge flocs (Nielsen and Jahn, 1999). EPS in sludge flocs were composed of soluble EPS (i.e., slime) and bound EPS. Research studies showed that the bound EPS exhibited a dynamic double-layer-like structure, which was composed of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Poxon and Darby, 1997; Li and Yang, 2007). Almost all the extracellular enzymes are immobilized in flocs (Frolund et al., 1995). On the other hand, the hydrolysis of EPS and/or cells together within the sludge flocs limits the rate and extent of degradation has been hypothesised (Higgins and Novak, 1997). Park and Novak (2007) have pointed out that since EPS rather than cells represent the major organic fraction determining flocs structure, integrity and the strength, the disruption of EPS matrix could enhance the rate and extent of sludge biodegradation during aerobic digestion (Park and Novak, 2007)

Extracellular enzymes play an important role in sludge processing (Ayol, 2005). Researchers suggested that the measurement of extracellular enzymes as an

alternative method to assess microbial biomass and activity (Nybroe et al., 1992). Protease, α -amylase and α -glycosidase were reported to play essential roles in the hydrolysis of two major fractions of EPS: proteins and polysaccharides (Goel et al., 1998). Therefore, these extracellular enzymes were used in the experimental studies reported in this thesis.

The thesis as part of a broader project examining the overall fate and effects of hydrolytic enzymes in biological sludge stabilization gives the recent research results on enzymatic treatment of sludges during anaerobic digestion. This research was financially supported by the Scientific and Technical Council of Turkey- TUBITAK under award #104Y375 “A Novel Approach for Enhanced Sludge Dewaterability: Enzymatic Treatment”. Experimental study was done at the Department of Environmental Engineering, Dokuz Eylul University, over a period of one year.

1.2 Scope and Research Objectives of the Thesis

The scope of the thesis was to investigate the improvement of aerobic degradation of sludge with adding hydrolytic enzymes. The objectives of the study were given below:

- To investigate the effects of enzyme additions on accelerated hydrolysis of the organic matter content of sludge using enzymes during aerobic sludge digestion,
- To determine whether enzyme additions increase the degradation of extracellular polymeric substances – EPS in sludge and the disrupt the flocculated matrix to obtain increased floc disintegration under aerobic conditions,
- To determine whether enzyme additions enhance the dewaterability capacity of sludge,
- To determine whether enzymatic treatment can be optimized with polymer dosage to provide a feasible means of improving sludge dewatering.

CHAPTER TWO

LITERATURE SURVEY

2.1. Introduction

This chapter gives the commonly used processes in sludge treatment and focuses on the stabilization processes especially aerobic stabilization process regarding the research study.

2.2. Sludge Treatment Methods

Sludge must undergo various types of processing for economic and environmental reasons. The purpose of all types of sludge processing is mainly to reduce the volume of sludge, to stabilize for reducing the pathogen microorganisms, organic matter, and odor potential. To address this, a sequence of treatments such as conditioning, thickening, dewatering and stabilization have been used (European Commission, 2001, p 37).

Sludge suspensions include different types of water that can be categorized according to their physical bonding to the sludge particles. These are:

- *free water, which is not bound to the particles;*
- *interstitial water, which is bound by capillary forces between the sludge flocs;*
- *surface water, which is bound by adhesive forces;*
- *intracellular water* (Spinosa; Vesilind, 2001).

The free water content represents the largest part (70-75%) of sewage sludge that can move freely between the individual sludge particles is not adsorbed by them and is not influenced by capillary forces. It can be separated by gravity and mechanically, for example by centrifugal forces or filtration. Spinosa and Vesilind (2001) defined the interstitial water is kept in the interstice of the sludge particles and

microorganisms in the sludge floc while the surface water covers the entire surface of the sludge particles in several layers of water molecules and is bound by adsorptive and adhesive forces. The important point is that the surface water is physically bound to the particles and cannot move freely. Similarly, the intracellular water contains the water in cells and can only be determined together with the surface water. This type of water fraction namely bound water can be removed by thermal processes (Spinosa; Vesilind, 2001, p. 24-25).

In principal, sludge management aims to reduce the water and organic content of sludge and to provide the processed solids suitable for reuse or final disposal. The principle methods used for sludge processing and their functions are explained in the following subsections.

2.2.1 Thickening

The sludge produced at the wastewater treatment plant contains a lot of water and it has to be thickened. The thickening methods- gravity thickening, flotation thickening, and centrifugation- vary with the source of the sludge and the purpose is to remove some of its free water. Flotation may be a suitable method for chemical and biological sludge while primary sludge is best thickened by various sedimentation processes. Centrifugation can be used either for thickening or dewatering purposes (Metcalf & Eddy, 2003).

2.2.2 Sludge Stabilization

Raw sludge is biologically active and includes many biodegradable compounds. Stabilization processes aim to reduce the organic matter, to remove the pathogenic microorganisms, and to lessen the odor potential of sludge. This process includes three methods for sludge stabilization:

1. biological sludge digestion-aerobic digestion, anaerobic digestion, composting,
2. alkaline stabilization- usually with lime,
3. thermal stabilization- pasteurization, thermal drying.

Stabilization has an importance in regard to hygienization since the odors are reduced and therefore do not attract vectors via smell. This can prohibit re-infection and re-growth of pathogens. After stabilization, the organic volume is decreased and hygienization has occurred. Therefore the sludge is no longer regarded as active (Metcalf&Eddy, 2003; European Commission, 2001).

2.2.2.1 Anaerobic Digestion

Anaerobic digestion is a stabilization method that will reduce the volume and stabilize the sludge in the absence of oxygen. It will also give a partial hygienization. The total quantity of the sludge is reduced by about 35% (European Commission, 2001). The decomposition process in an anaerobic digester has several stages. Carbohydrates, fats and proteins are broken down in different steps and finally converted into methane gas and carbon dioxide and in which a molecule other than oxygen is the final electron acceptor (Metcalf & Eddy, 2003). For instance sulphate-reducing bacteria transfer electrons to sulphate (SO_4^{2-}) reducing it to H_2S . while nitrate reducers transfer the electrons to nitrate (NO_3^-) reducing it to nitrite (NO_2^-) nitrous oxide (NO) or nitrogen gas (N_2) (Metcalf & Eddy, 2003). The temperature is normally kept around 35°C for mesophilic conditions and the retention time should be more than 20 days in order to receive a good stabilization and hygienization but other retention times and temperatures also exists (European Commission, 2001). Figure 2.1 shows the stages of anaerobic digestion process.

2.2.2.2 Aerobic Digestion

Wastewater treatment plants produce organic sludge as wastewater is treated; this sludge must be further treated before ultimate disposal. Aerobic digestion is a process where the sludge is aerated in processing tanks. A basic goal of aerobic digestion is that the degradation of floc-forming microbes, pollutants or any organic material as reduction the mass of the solids for disposal. While a fraction of the organic material is used for the synthesis of new microorganisms, resulting in an increase in biomass, the remaining material is channeled in to metabolic energy and

oxidized to carbon dioxide, water, nitrates sulphates and phosphates to provide energy for both synthesis and cellular functions. This situation is schematized in Figure 2.2 (Metcalf & Eddy, 2003; Whiteley, C.G., Lee, D.-J., 2006).

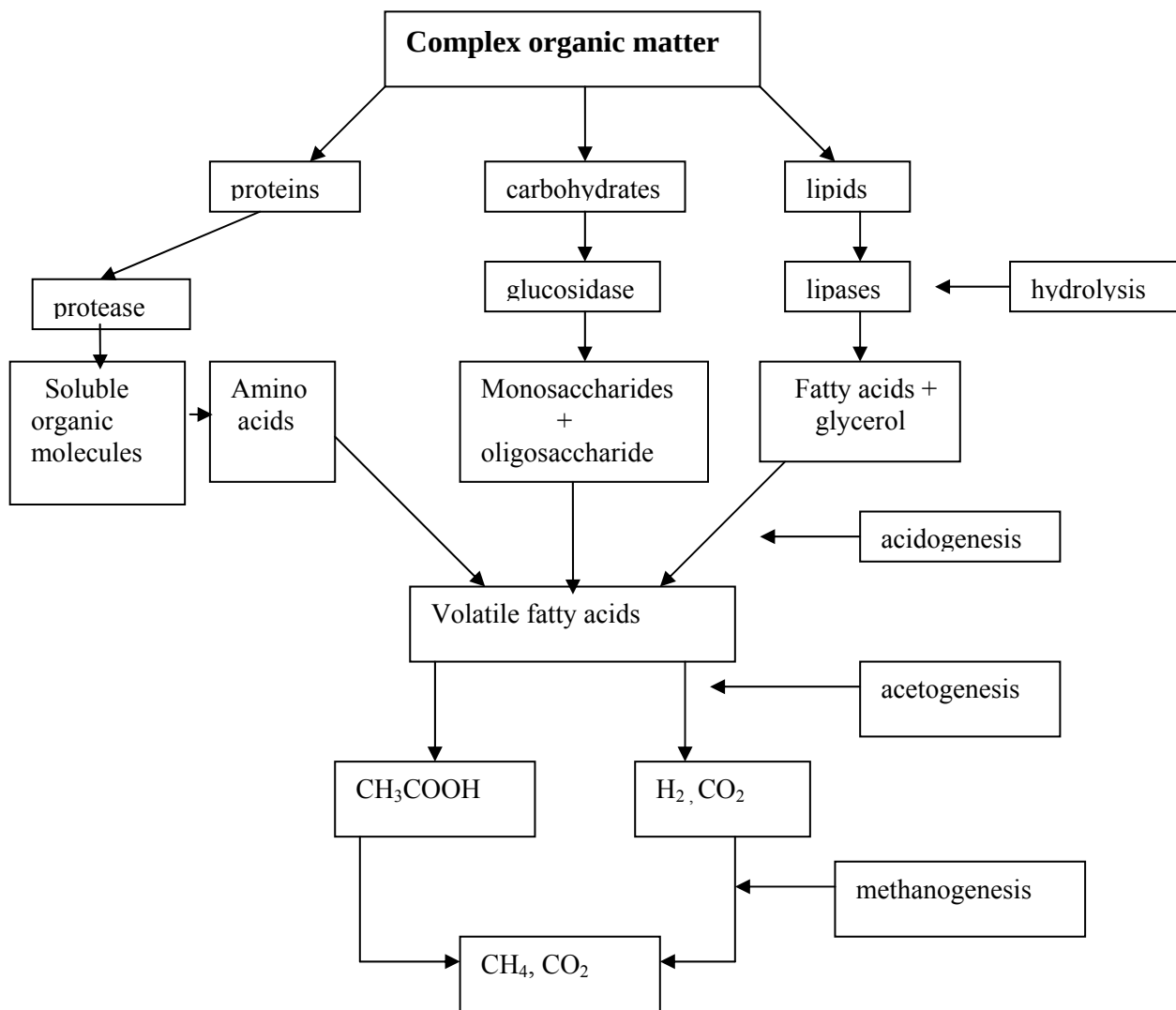


Figure 2.1. Anaerobic digestion of waste containing microorganisms (Whiteley, C.G., Lee, D.-J., 2006)

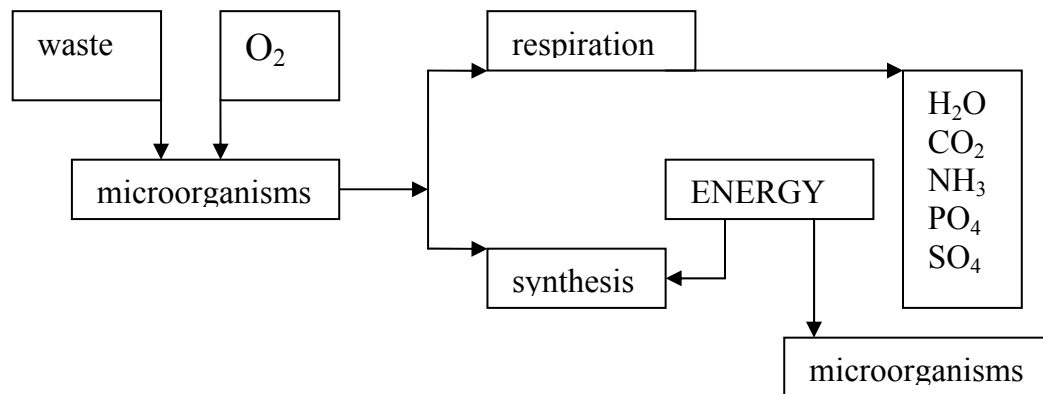


Figure 2.2 Aerobic digestion of waste containing microorganisms (Whiteley, C.G., Lee, D.-J., 2006)

The decomposition is performed by aerobic microorganisms and this generates heat. If the process is working adequately, more than $70^{\circ}C$ can be reached. Usually the sludge is subjected to 50 to $65^{\circ}C$ for 5 to 6 days and most of the harmful organisms are destroyed. One drawback is that the energy costs are 5 to 10 times higher than for anaerobic digestion (European Commission, 2001).

The settled biosolids are subsequently recycled or aeration tanks in order to maintain the required biomass concentration. Once the organic waste material becomes exhausted then the organisms will begin endogenous respiration to oxidize cellular material. An important disadvantage of aerobic treatment is the production on large amounts of sludge which contains volatile organic solids, nutrients, pathogens, heavy metals, inorganic ions, toxic organic chemicals and the original problem of dissolved organic waste is now transformed into a problem of particulate (Whiteley, C.G., Lee, D.-J., 2006).

Aerobic respiration is defined as the aerobic catabolism of nutrients to carbon dioxide and water involving glycolysis, the tricarboxylic cycle, an electron transport system and molecular oxygen as final electron acceptor: this type of aerobic digestion is notable in organisms that require molecular oxygen, and facultative anaerobes that are capable of aerobic respiration but can switch to fermentation if oxygen is (Whiteley, C.G., Lee, D.-J., 2006).

2.2.2.3 Composting

Composting is another method for stabilization of sludge. In this process, the organic components of the sludge are biologically decomposed under controlled conditions to a state where the resulting material can be handled, stored or applied to land without adversely affecting the environment. This process can undergo either in anaerobic or aerobic conditions. In aerobic process, the microorganisms are mainly mesophilic bacteria (growing at 25-45°C) and thermophilic bacteria (growing at temperatures above 45°C) (Spinosa; Vesilind, 2001). The sludge should be mixed with a biodegradable material such as sawdust or animal manure. The moisture content in the compost should be around 50 to 60% in order to keep the microbial activity at its optimum. The appropriate ratio between carbon and nitrogen is also important factor. The optimum ratio is about 25-30:1 (C:N) (European Commission, 2001).

2.2.2.4 Lime Stabilization

Lime stabilization takes advantage of the fact that all biological activity is effectively terminated when the pH rises above 12. Enough lime, about 30% of the dry solid content, has to be added in order to ensure that no fermentation takes place in the long run. Pathogenic microorganisms are killed effectively during liming. The only disadvantage of this process is increases in dry solids amounts (European Commission, 2001).

2.2.2.5 Pasteurization

Pasteurization is another stabilization process in sludge managements. Sludge is heated to a temperature of 70°C to 80°C for a short period of time (about 30 minutes). The amount of pathogens is reduced during pasteurization process (European Commission, 2001, p. 44) but afterwards the sludge must undergo a secondary process in order to stabilize the sludge. The sludge is sensitive to re-

infection directly after pasteurization. Therefore, pasteurization is often followed by anaerobic digestion (Spinosa; Vesilind, 2001).

2.2.2.6 Thermal Drying

Thermal drying of the sludge will result in the elimination of interstitial water and reduction of the volume. The process also allows stabilization and hygienization when the dry solids content exceeds 90%. Heat is transferred directly or indirectly to the sludge. *At direct transfer, hot gas is used and two important methods are the rotating drum dryer and the fluidized bed dryer. At indirect transfer, a heat transfer surface is used and the heat is transferred via heat conduction. The drying takes place at different temperatures and if higher temperatures are used (above 300°C) it is important to control that no dioxins or furan compounds have been formed.* A dry matter content of 35 to 90% is reached and re-growth of pathogens is inhibited mainly due to the reduced water content (European Commission, 2001).

2.2.3 Conditioning

Conditioning of the sludge causes some important modifications in the sludge structure so that more water can be easily separated. This process improve the performance of further treatment processes such as thickening or dewatering and it may also restrict the fine particle content of the reject water. There are different conditioning processes like chemical conditioning and thermal processes. In chemical conditioning, mineral agents such as lime or salts and organic agents like polymer have been used. In thermal conditioning, sludge is heated to 150-200°C in 30 to 60 minutes. Heating to 40°C or 50°C is also possible and will give a partial thermal conditioning (Metcalf & Eddy, 2003)

2.2.4 Dewatering

Dewatering can be defined as the volume reduction of the sludge by separating water. Raw sludge contains high amounts of water, usually more than 95% by

weight (Metcalf& Eddy, 2003, European Commission, 2001). It is only possible to remove a certain proportion of free, adhered and capillary water with the technology. Different dewatering processes are available such as natural systems like drying beds and mechanical units like centrifuging, belt filter press, and filter press. All processes except for drying beds require the chemical conditioning. The water content of the sludge after dewatering depends on the treatment and can reach about 30% (European Commission, 2001).

2.3 Sludge Disposal

There are several disposal methods for sewage sludge. In many countries for regarding to sustainable activities, agricultural use of sludge is becoming more attractive instead of land filling. Incineration is another option for sludge disposal whereas the end product is not suitable for beneficial use. In the following subsections, the disposal alternatives will be discussed.

2.3.1 Land Filling

In this operation, the treated sludge is transported to a landfill area. The waste is then covered with some landmasses. Some countries have restrictions in the design, for example stating that the leakage from the landfill should be minimized. The leakage from landfills is hazardous and can often contain heavy metals and other pollution. There are landfill gases emitted to the air mainly consisting of methane and carbon dioxide. There is also a risk for spreading of pathogens. When depositing sludge, the sludge is disposed without making use of nutrients, energy or material (European Commission, 2001).

2.3.2 Incineration

The sludge must be dried before incineration of the sludge. When sludge is incinerated, it is possible to make use of the energy in the sludge. This energy is in the form of heated water or air.

There are several pollutants that may be emitted to the air when incinerating sludge. The treatment of flue gases is also important. The pollutants from the process are particulates and fly ash, nitrogen oxides, carbon dioxide, organic compounds and heavy metals bound to particles, heavy metals in gaseous form, dioxins, acid gases and volatile organic compounds (U.S. EPA, 1999). Pathogens and unwanted organic substances are destroyed in the process.

2.3.3 Agricultural and Other Land Uses

Regarding the sustainability concept, beneficial uses of sludge is taking significant attention. Therefore, the researches and applications on agricultural uses of sludge focused on the insertion of this to the life cycle by utilization of sludge beneficially.

The term ‘biosolid’ is recommended as the preferred product term for when the properly treated sludge is used beneficially as a soil conditioner and fertilizer in accordance with the regulations (Spinosa; Vesilind, 2001). Biosolid is land applied to improve the structure of the soil and as a fertilizer to supply nutrients to crops and other vegetation grown in the soil. It is commonly applied to agricultural land, forests, reclamation sites, public contact sites (e.g., parks, turf farms, highway median strips, golf courses), lawns, and home gardens (U.S. EPA, 1995,). Physical properties of the soil can also be improved through the application of sludge.

While there are many advantages given above, it has also some drawbacks. The presence of heavy metals, organic molecules and pathogenic microorganisms raises questions about the quality of biosolid being spread on land (Capizzi-Banaz, et al, 2004). Limiting the amounts of biosolid applied to the soil and reducing the pathogens by sludge stabilization can control the risks (Sanchez-Monedero, et al. 2003).

CHAPTER THREE

ENZYMES

3.1 Introduction

Enzymes can be defined as very effective organic catalysts produced by living cells (Sawyer, McCarty & Parkin, 2003). Enzymes are highly specific for their substrates and function of enzymes is very sensitive under different conditions such as temperature and pH. Sawhney & Singh (2000) have stated that “enzymes accelerate the rate of chemical reaction without altering the equilibrium of the chemical reaction”. Enzymes are not consumed during enzyme-substrate reactions shown in Figure 3.1 (Sawhney & Singh, 2000).

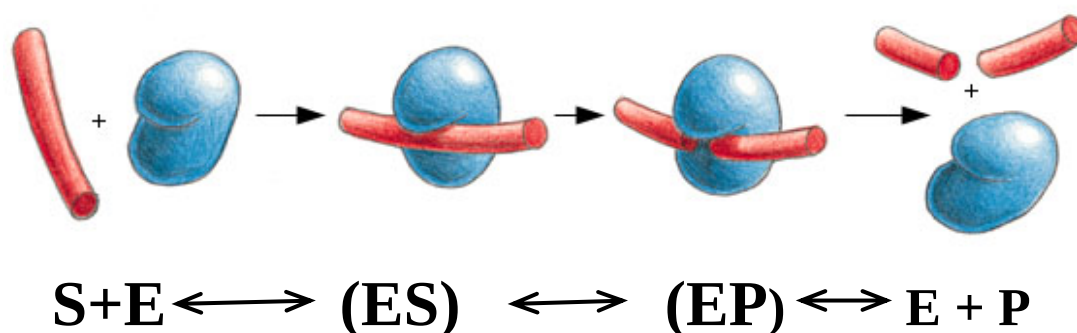


Figure 3.1 Enzyme-substrate complex (Choromosone Terminology)

More than 2000 enzymes are known and named by adding the suffix-ase to the end of the substrate, such as protease, or the reaction catalyzed, such as alcohol dehydrogenase. Classification of enzymes is given in Table 3.1.

Table 3.1 Classification of enzyme (Sawyer, McCarty & Parkin, 2003)

Enzymes	Substrate	Product
Hydrolases 1. Carbohydrases: a. Glycosidases (sugar splitters) Sucrase Maltase Lactase b. Amylases Diatase Ptyalin c. Cellulase 2. Esterases a. Lipases: Lipase b. Phosphatases 3. Proteases: a. Proteinases Pepsin Trypsin b. Peptidases 4. Amidases a. Urease 5. Deaminases:	Sucrose Maltase Lactase Starch Starch Cellulose Glycerides Phosphoric esters Proteins Proteins Polypeptides Urea Aminoacids	Glucose + Fructose Glucose Glucose + Glactose Maltose Maltose Cellobiose Glycerol + Fatty acids H ₃ PO ₄ + alcohol Polypeptides Polypeptides Aminoacids NH ₃ + CO ₂ NH ₃ + CO ₂
Oxido-reductases (oxidation-reduction reactions) 1. Dehydrogenases 2. Hydroxylase 3. Reductive dehalogenase 4. Oxidases 5. Oxygenases 6. Methane monooxygenase 7. Toluene monooxygenase 8. Toluene dioxygenase 9. Ammonia monooxygenase	PCE Methane Phenol Benzene Toluene Ammonia	TCE Methanol Cotechol Dihydroxy benzene Dihydroxy toluene Hydroxylamine
Transferases (transfer of functional groups) 1. Transaminases 2. ATP		

Enzyme technologies has been using many industries like food industry, pharmaceutical industry and also waste treatment so they have been receiving great attention with the improvements in biological techniques (Whiteley & Lee, 2006).

Enzymatic treatment can be an important technique with biological discovery, protein engineering and environmental biotechnology for wastewater treatment. In the biological treatment, biological agents (e.g., living microorganisms or enzymes) for treatment process have been used and involve biological remediation techniques that generally fall under aerobic or anaerobic digestion (Whitley, C.G., & Lee, D.-J, 2006). Hydrolytic enzymes may be extracellular, or exoenzyme that act outside the cell wall in order to break large molecules into smaller ones that can pass through the cell.

3.2 Enzyme – Substrate Interaction

Enzymes interact with their specific substrates to form an enzyme–substrate complex [ES] that can be revealed by either a ‘Lock-and-Key’ or ‘Induced Fit’ model shown in Fig. 3.2.



Whiteley and Lee (2005) have compared, the lock and key model with induced fit model as “‘Lock-and-Key’ model, the active site of the enzyme is complementary in shape to that of the substrate. With the, more favored, ‘Induced Fit’ model, however, an initial weak interaction between enzyme and substrate rapidly induces conformational changes in the enzyme thereby strengthening the binding and bringing catalytic sites and scissile substrate bonds close together”.

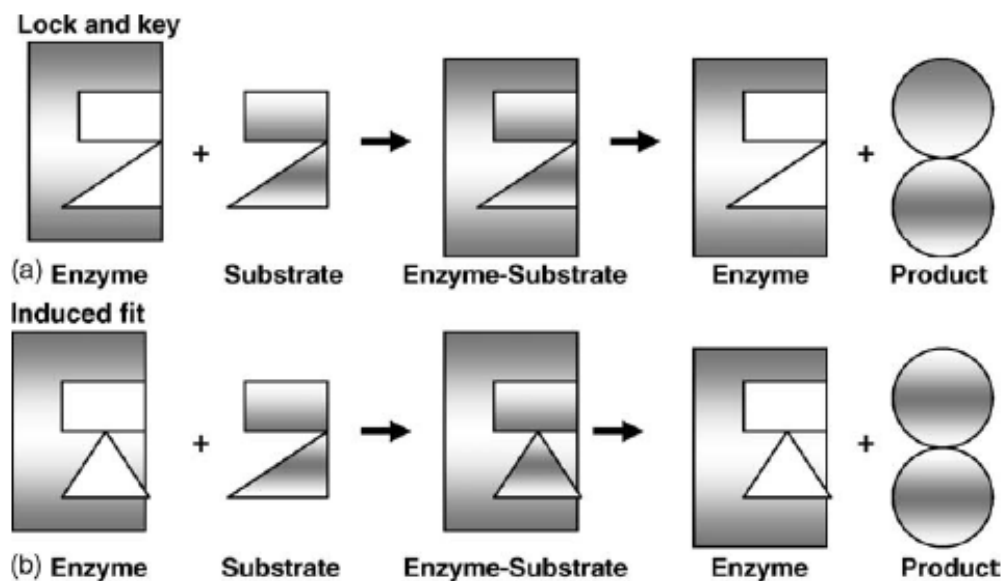


Figure 3.2 Enzyme–substrate complex with (a) Lock-and-Key and (b) Induced Fit model (Whiteley, C.G., Lee, D.-J., 2005)

3.3 Energy of Reaction

Enzymes are responsible for supporting almost every type of chemical reaction can speed up ,by at least 1000-fold, the rates of reactions by decreasing the amount of energy required to form a complex of reactants, known as the transition state complex that is competent to produce reaction products.

The free energy required to form an activated complex is much lower in the catalyzed reaction and consequently at any instant a greater proportion of the molecules in the population can achieve this transition state shown in Fig. 3.3. (Choromosone Terminology).

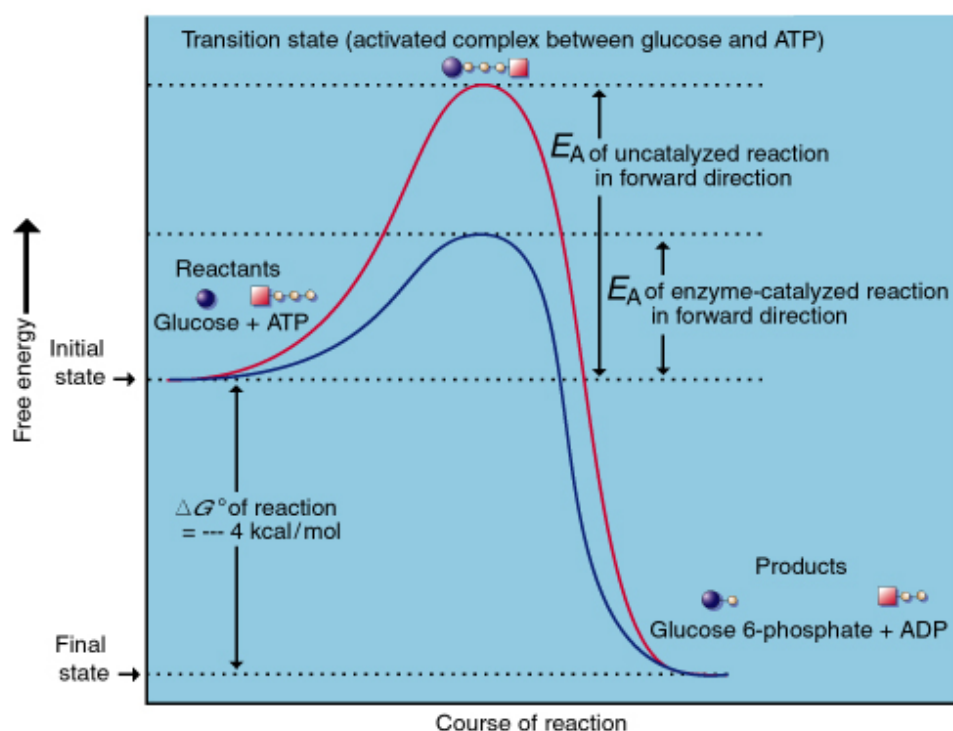


Figure 3.3 Energy of reactants and products and activation energy with, and without an enzyme (Choromosone Terminology)

3.4 Cofactors/Coenzymes

Shuler and Kargi (2002) have explained cofactor as “some protein enzymes require a nonprotein group called cofactor for their activity”. Some metal ions, Mg, Zn, Mn, Fe, or a coenzyme, such as a complex organic molecule, NAD, FAD, CoA, or some vitamins can be cofactor. An enzyme containing a nonprotein group is called a holoenzyme. The protein part of this enzyme is the apoenzyme (holoenzyme = apoenzyme + cofactor)” (Shuler, M., L., Kargi, F., 2002). Even if the substrate is present at the active region of the enzyme catalysis does not occur until the second component is present (biochemistry of enzyme). The binding of substrate and cofactor to an enzyme is shown in Fig. 3.4. A summary of some of the principal coenzymes and the particular enzymatic reactions are given in Tables 3.2 and 3.3.

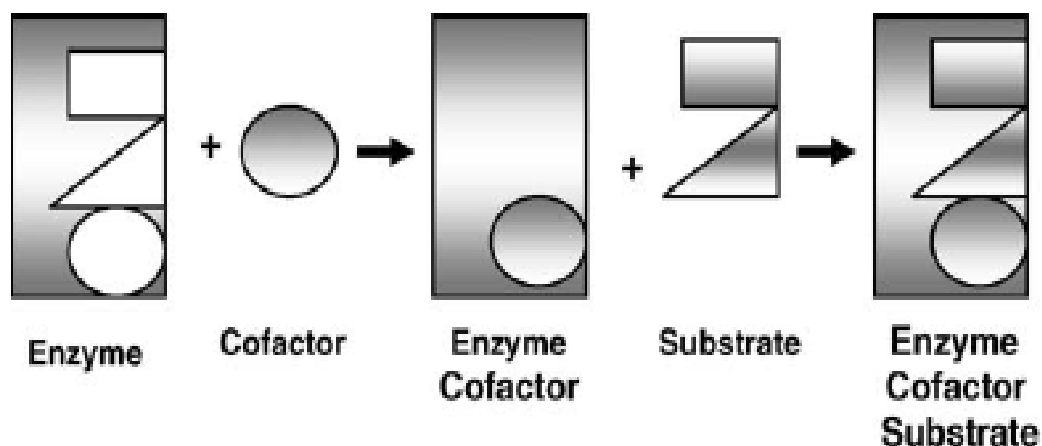


Figure 3.4. Binding of substrate and cofactor to an enzyme (Whiteley, C.G., Lee, D.-J., 2005).

Table 3.2 Metal cofactors, the enzymes they activate, and the enzyme function (Shuler, M., L., Kargi, F., 2002)

Metal Cofactor	Enzyme or Function
Co	Transcarboxylase, Vitamin B ₁₂
Cu	Cytochrome c oxidase, proteins involved in respiration, some superoxide dismutases
Fe	Activates many enzymes, catalases, oxygenases, cytochromes, nitrogenases, peroxidases
Mn	Activates many enzymes, oxygenic photosynthesis, some superoxide dismutases
Mo	Nitrate reductase, formate dehydrogenases, oxotransferases, molybdenum, nitrogenase
Ni	Carbon monoxide dehydrogenases, coenzyme F ₄₃₀ of methanogens, urease
Se	Some hydrogenases, formate dehydrogenase
V	Vanadium nitrogenase, some peroxidases
W	Oxotransferases of hyperthermophiles, some formate dehydrogenases
Zn	RNA and DNA polymerase, carbonic anhydrase, alcohol dehydrogenase

Table 3.3 Coenzyme involved in group-transferring reactions (Shuler, M., L., Kargi, F., 2002)

Group Transferred	Coenzyme	Acronym
Hydrogen atoms (electrons)	Nicotinamide adenine dinucleotide	NAD
	Nicotinamide adenine dinucleotide phosphate	NADP
	Flavin adenine dinucleotide phosphate	FAD
	Flavin mononucleotide	FMN
	Coenzyme Q	CoQ
	Coenzyme F ₄₂₀	F ₄₂₀
Acyl groups	Lipomide	
	Coenzyme A	HSCoA
One-carbon units	Tetrahydrofolate	
	Methanofuran	
	Tetrahydromethanopterin	
	Coenzyme M	CoM
Carbon dioxide	Biotin	
Methyl	S-adenosylmethionine	
Glucose	Uridinediphosphate glucose	
Nucleotides	Nucleotide triphosphates	
Aldehyde	Thiamine pyrophosphate	

3.5 Factors affecting enzyme activity

The main factors affecting enzymatic reactions are enzyme concentration, substrate concentration, pH, temperature, and the presence of activators or inhibitors. Some explanation about the factors is given in the following subsections.

3.5.1 Enzyme Concentration

The enzyme reaction velocity is directly related to the enzyme concentration. However, deviation may sometimes occur from the linear relationship. The effect of enzyme concentration to the reaction velocity is shown in Figure 3.5. (Sawhney, S., K., Singh, R., 2000).

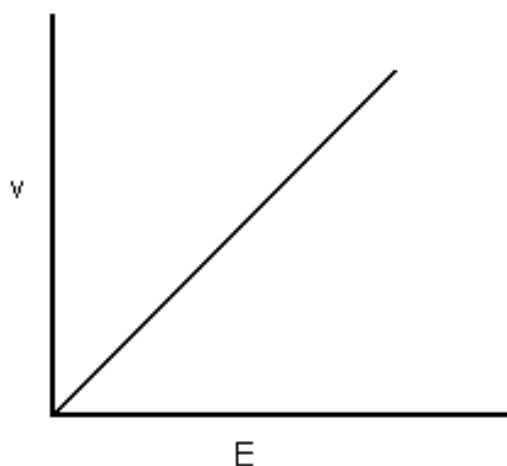


Figure 3.5. Typical effect of enzyme concentration reaction velocity reaction (Sawhney, S., K., Singh, R., 2000)

3.5.2 Substrate Concentration

The amount of substrate concentration is also another important factor affecting the rate of enzyme reactions. The effect of varying substrate on initial velocity of an enzyme catalyzed reaction is shown in Fig. 3.6 (Sawhney, S., K., Singh, R., 2000). *At relatively low substrate concentration, initial velocity increases almost linearly with an increase in the substrate (in this region of the reaction follows first order kinetics). At higher substrate concentration, initial velocity increases by smaller and smaller extent in response to increases in substrate (in this region of the curve, the reaction is of mixed order type). Finally a point is reached beyond which there is small and/or no increase in the velocity with increase in the concentration of the substrate (the reaction follows zero order kinetics in this region). This plateau is called the V_{max} . At this stage, the enzyme is fully saturated with the substrate.*

3.5.3 Effect of pH

pH value is important parameter since they are active only over a limited range of pH. The effect of pH is depicted in Figure 3.7 (Sawhney, S., K., Singh, R., 2000). The irreversible destruction of the enzyme could be possible as shown in Curve B exposing the enzyme to a range of pH values and then testing the activity after

readjusting the pH optimum. In Figure 3.7, Curve A shows a standard pH curve for an enzyme. The fall on the alkaline side is due to destruction of enzyme (Sawhney, S., K., Singh, R., 2000).

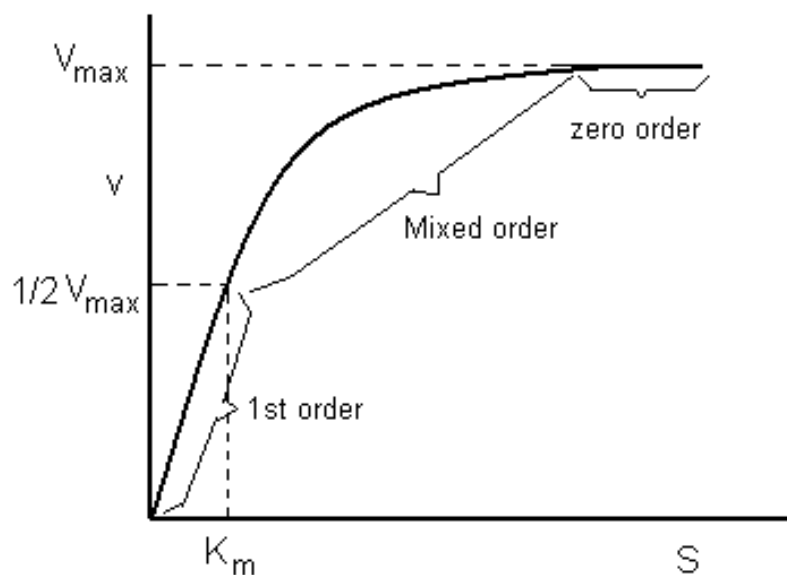


Fig 3.6. Effect of substrate concentration on the initial velocity of an enzyme catalyzed reaction (Sawhney, S., K. Singh, R., 2000)

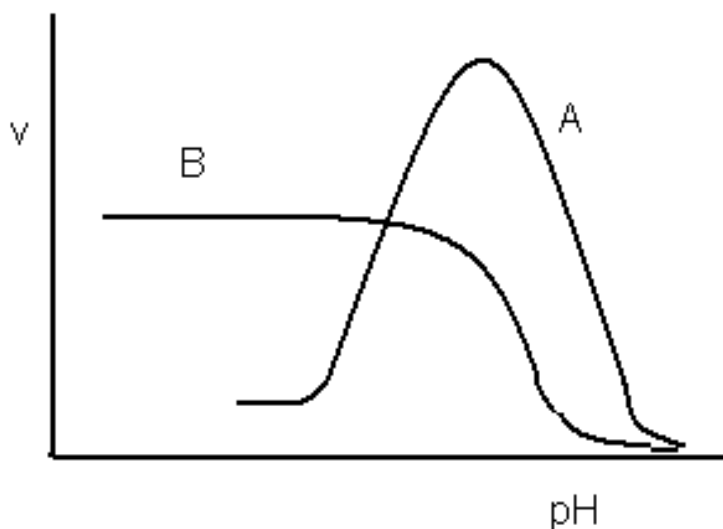


Figure 3.7 Effect of pH on the initial velocity of an enzyme catalyze reaction A – pH profile curve and B – the stability curve (Sawhney, S., K., Singh, R. 2000)

3.5.4 Temperature

The rate of enzyme catalyzed reaction increases with the increasing temperature until an optimum point. The velocity decreases on further increasing the temperature as shown in Figure 3.8. Sawhney and Singh (2000) have reported that the temperature effect may be due to several reasons: the stability of the enzyme, the actual velocity of the breakdown of the complex determined by the reaction heat activation, the enzyme substrate affinity; pH functions of any or all of the components due to an alteration of their pK_a s influenced by the heats of ionization, the activators or inhibitors, if any. If a system has two or more enzyme with different temperature coefficients, temperature may affect transfer of rate limiting functions from one enzyme to another.

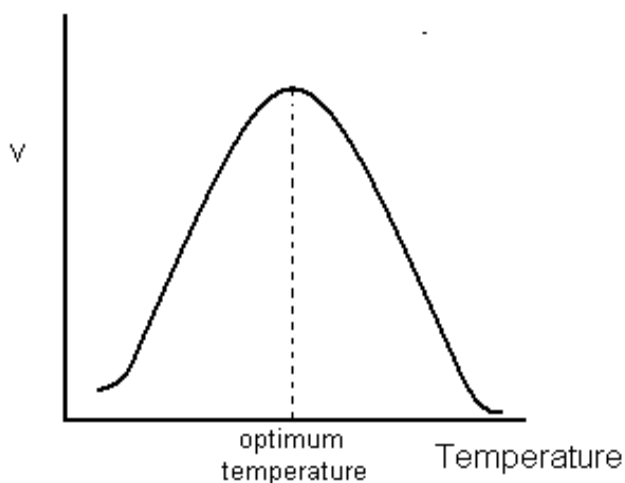


Figure 3.8. Effect of temperature on the initial velocity of an enzyme catalyzed reaction (Sawhney, S., K., Singh, R. 2000)

3.6 Source of Enzyme

Biologically active enzymes may be extracted from any living organism. A very wide range of sources are used for commercial enzyme production from *Actinoplanes* to *Zymomonas*, from spinach to snake venom. Many enzymes have been used industrially, over a half are from fungi and yeast and over a third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources as given in Table 3.4. . Non-microbial sources provide a larger proportion of these at

the present time. Microbes are preferred to plants and animals as sources of enzymes because:

1. *they are generally cheaper to produce.*
2. *their enzyme contents are more predictable and controllable,*
3. *reliable supplies of raw material of constant composition are more easily arranged, and*
4. *plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds (from plants), endogenous enzyme inhibitors and proteases (Enzyme Library)*

Table 3.4 Industrial enzymes and their sources (<http://www.lcbu.ac.uk/biology/enztech/source.html>).

Enzyme	Source	Intra/Extra-Cellular	Scale of production	Industrial use
Animal enzymes				
Catalase	Liver	I	-	Food
Chymotrypsin	Pancreas	E	-	Leather
Lipase ^e	Pancreas	E	-	Food
Rennet ^f	Abomasum	E	+	Cheese
Trypsin	Pancreas	E	-	Leather
Plant enzymes				
Actinidin	Kiwi fruit	E	-	Food
α -Amylase	Malted barley	E	+++	Brewing
α -Amylase	Malted barley	E	+++	Brewing
Bromelain	Pineapple latex	E	-	Brewing
β -Glucanase ^g	Malted barley	E	++	Brewing
Ficin	Fig latex	E	-	Food
Lipoxygenase	Soybeans	I	-	Food
Papain	Pawpaw latex	E	++	Meat
Bacterial enzymes				
α -Amylase	<i>Bacillus</i>	E	+++	Starch
α -Amylase	<i>Bacillus</i>	E	+	Starch
Asparaginase	<i>Escherichia coli</i>	I	-	Health
Glucose isomerase ^h	<i>Bacillus</i>	I	++	Fructose syrup
Penicillin amidase	<i>Bacillus</i>	I	-	Pharmaceutical
Protease ⁱ	<i>Bacillus</i>	E	+++	Detergent
Pullulanase ^j	<i>Klebsiella</i>	E	-	Starch

Table 3.4 Industrial enzymes and their sources (Cont'd)

Enzyme	Source	Intra/Extra-Cellular	Scale of production	Industrial use
Fungal enzymes				
α -Amylase	<i>Aspergillus</i>	E	++	Baking
Aminoacylase	<i>Aspergillus</i>	I	-	Pharmaceutical
Glucoamylase ^k	<i>Aspergillus</i>	E	+++	Starch
Catalase	<i>Aspergillus</i>	I	-	Food
Cellulase	<i>Trichoderma</i>	E	-	Waste
Dextranase	<i>Penicillium</i>	E	-	Food
Glucose oxidase	<i>Aspergillus</i>	I	-	Food
Lactase ^l	<i>Aspergillus</i>	E	-	Dairy
Lipase ^e	<i>Rhizopus</i>	E	-	Food
Rennet ^m	<i>Mucor miehei</i>	E	++	Cheese
Pectinase ⁿ	<i>Aspergillus</i>	E	++	Drinks
Pectin lyase	<i>Aspergillus</i>	E	-	Drinks
Protease ^m	<i>Aspergillus</i>	E	+	Baking
Raffinase ^o	<i>Mortierella</i>	I	-	Food
Yeast enzymes				
Invertase ^p	<i>Saccharomyces</i>	I/E	-	Confectionery
Lactase ^l	<i>Kluyveromyces</i>	I/E	-	Dairy
Lipase ^e	<i>Candida</i>	E	-	Food
Raffinase ^o	<i>Saccharomyces</i>	I	-	Food

^a The names in common usage are given. As most industrial enzymes consist of mixtures of enzymes, these names may vary from the recommended names of their principal component. Where appropriate, the recommended names of this principal component are given below.

^c I - intracellular enzyme; E - extracellular enzyme

^d +++ > 100 ton year⁻¹; ++ > 10 ton year⁻¹; + > 1 ton year⁻¹; - < 1 ton year⁻¹.

^e triacylglycerol lipase;

^f chymosin;

^g Endo-1,3(4)- β -glucanase;

^h xylose isomerase;

ⁱ subtilisin;

^j α -dextrin endo-1,6- α -glucosidase;

^k glucan 1,4- α -glucosidase;

^l β -galactosidase;

^m microbial aspartic proteinase;

ⁿ polygalacturonase;

^o α -galactosidase;

^p β -fructofuranosidase

3.7 Sludge Enzymatic Treatment Regarding the Extracellular Polymeric Substances in Sludge

Polymeric network of activated sludge flocs is composed of extracellular polymeric substances (EPS) (Liu and Fang, 2003). The EPS originate from active bacteria (Prescott et al., 2002) and organic or inorganic materials present in sewage sludge itself (Tchobanoglous et al., 2003). EPS are composed of a variety of organic substances: carbohydrates, proteins, humic substances, uronic acids, lipid compounds and deoxyribonucleic acids. The EPS composition depends on wastewater type and the operation conditions of the treatment plant (Sponza, D.T., 2003). EPS act together with multivalent ions to aid the formation and settling of sludge flocs in both aerobic and anaerobic treatment systems. On the other hand, an excess of EPS may hinder dewatering of sludge, bioflocculation and settling of sludge (Wawrzynczyk et al. 2007; Liu and Fang, 2003; Pere et al., 1993; Urbain et al., 1993)

In recent years, new technologies have been introduced to advance the biological decomposition of activated 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 appears to be an efficient alternative in this field. Enzymes catalyze the degradation of organic substances in sludge as a function of the substrate. The result of the enzyme additions during biological sludge stabilization process is the enhanced degradation of EPS – proteins and polysaccharides – and the other biological slimes and gels and improved the releasing capacity of water (e.g. Barjenbruch & Kopplow, 2003; Ayol 2005; Dey et al., 2006; Roman et al., 2006).

Previous studies (e.g. Novak et al., 2003; Ayol, 2005) have examined the mechanisms of floc destruction during the bioprocessing of the sludge in laboratory scale studies. Concluding remark from the studies is that a flocculated matrix of extracellular polymeric substances (EPS) bridging with bacteria holds back the dewaterability properties of the bioprocessed sludges (Ayol et al. 2007). Novak et al. (2003) reported the loss of enzyme activity explicated polysaccharide accumulation

while protein is degraded under aerobic conditions. They indicated the decreasing enzyme activity for both protein and polysaccharide degradations under anaerobic conditions, however, this case is better than those under aerobic conditions (Ayol et al., 2007).

The hydrolysis of complex organic structures in the degradation of biodegradable particulate organic matter heavily depends on the hydrolytic enzymes like glucosidases, lipases, and proteases. Ayol et al. (2007) have stated that the enrichment of active enzymatic systems during aerobic or anaerobic sludge digestion might provide floc disintegration that logically means enhanced degradation of EPS in the sludge. Disruption of the flocculated matrix enhances the weakness of EPS which is within the floc and protected from enzymatic degradation. This destruction leads to improved solubilization of sludge by attacking of the hydrolytic enzymes to polymeric substances forming enzyme-substrate complexes (Ayol et al. 2007).

Aerobic or anaerobic biological processes have a rate – limiting step during biological hydrolysis and this step causes a process slowdown with consequent requirement of large digester volumes (Eastman and Ferguson, 1981; Shimizu et al., 1993). The effects of enzymatic treatment on anaerobic and aerobic digestion processes were investigated in a research project (TUBITAK-104Y375). As a part of this project, the effects of the enzymes on aerobic digestion process to improve the dewaterability characteristics of digested sludge were reported in this thesis.

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Introduction

This chapter gives the information on the materials and methods used in this thesis.

4.2 Materials

4.2.1 Raw Sludges

Waste activated sludge was periodically taken from Guney Bati Wastewater Treatment Plant located in Narlıdere, Izmir City, Turkey. The sludge was fed to the aerobic reactors.

4.2.2 Aerobic Reactors

Aerobic digestion studies were done using three pilot scale aerobic reactors with 13.5-L volume each. The reactors were operated at ambient conditions around 30 ± 3 °C. The temperature was kept constantly by heat transfer oil jacket for each reactor.

The aerobic reactors were constructed from stainless-steel and operated with PLC. The reactors were aerated by blowers with a capacity of 10 L air/min. The air was distributed to the reactors by diffuser systems. Figure 4.1 illustrates the reactors used in this work. First reactor was used as control reactor without enzyme addition while the others were operated with different enzymes additions.



Figure 4.1. Aerobic tanks used in this study and some pictures from the reactor operations

4.2.3 Chemicals

Different hydrolytic enzymes were added to two reactors while first reactor was used as control. The enzymes added to the reactors were denoted as Enzyme1 and

Enzyme2. They were purchased from Novoenzymes Inc as pure enzymes and belong to Alpha-amylase and Beta-glucanase endo-1, 3(4)-) enzyme class, respectively.

In sludge conditioning studies, a high molecular weight cationic organic polymer (Zetag 7557, Ciba Specialty Chemicals) was used. This is an acrylamide based copolymer (DMAEAQ: MeCl). A 0.5 % stock polymer solution was prepared according to Dentel et al. (1993) for additions. The monomer structure of the polymer is given in Figure 4.2.

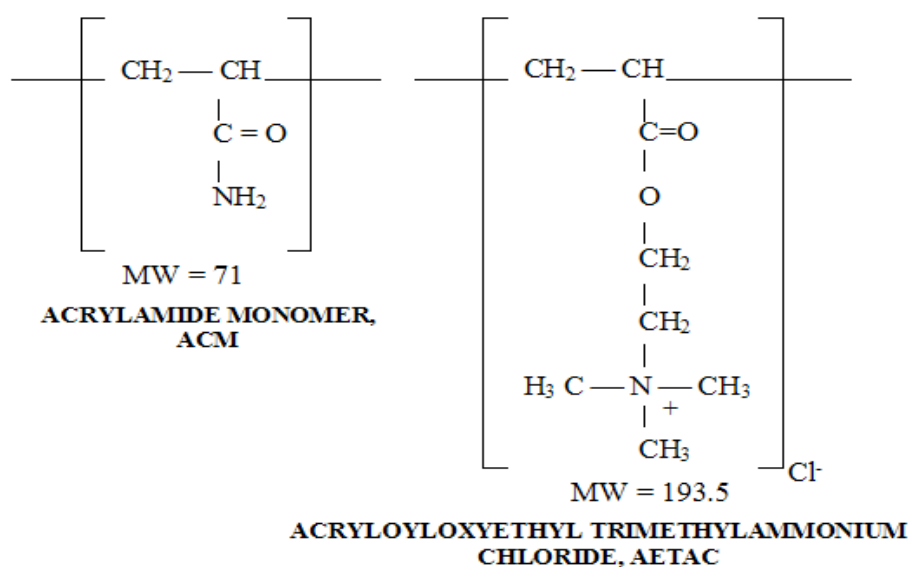


Figure 4.2 The structure of polymer used Zetag 7557, 80% cationic copolymer ACM/AETAC), molecular mass $\sim 1.8 \times 10^6$ (Ayol et al. 2006)

4.3 Methods Used in Experimental Studies

4.3.1 Analytical Methods

To monitor the reactor performances, dry solids content (DS), water content (WC), volatile organic matter content (VS), suspended solids(SS), temperature, alkalinity, dissolved oxygen, oxygen uptake rate (OUR), redox potential, pH, total organic carbon (TOC), capillary suction time (CST), zeta potential (ZP), particle size distributions, EPS – protein and polysaccharide parameters were analyzed in laboratory studies.

All analyses were regularly done according to Standard Methods (American Public Health Association [APHA], 2005). Lacking any standard methodology, analyses were performed according to the most accepted methods in research studies. Most of the measurements in this study were done in triplicate.

4.3.1.1 Redox Potential, pH Measurements and Oxygen Measurements

Redox potential, pH and oxygen value were measured by WTW model 340i multi analyzer.



Figure 4.3 WTW model 340i

4.3.1.2 Particle Size Distribution Analysis

Particle size distributions were determined using a Malvern Mastersizer 2000QM analyzer shown in Figure 4.4.



Figure 4.4 Malvern Mastersizer 2000QM particle size analyzer.

4.3.1.3 *Ca, K, Mg, Na Analysis*

Ca, K, Mg, Na concentrations for some sludge samples were analyzed using an ICP-QMS (Perkin Elmer- Optima 2100DV) shown in Figure 4.5.

4.3.1.4 *Total Nitrogen (TN) and Total Phosphorus ($PO_4 - P$) Analysis*

Total Nitrogen (TN) (Merc cell kit # 14537) and Total Phosphorus ($PO_4 - P$) (Merc cell kit # 14543) were analyzed by using spectroquant cell test purchased from Merck. Photometric measurements were done using Merc Photometer SQ 300. Acid digestion method was used as extraction method for total phosphorus, total nitrogen, Ca, K, Mg, Na (EPA, Method 3050B).



Figure 4.5 ICP-QMS Perkin Elmer-Optima 2100DV

4.3.1.5 Summary of Acid Digestion Method

A representative 1 gram (dry weight) sample is digested with addition of hydrochloric acid (HCl) (EPA, Method 3050B). The resultant digestate is reduced in volume while heating and then diluted to a final volume of 100 mL. This digestate is filtered by Whatman blue band filter paper. Then, it was kept for further analysis.

4.3.1.6 Total Organic Carbon Analysis

TOC analysis was measured by DOHRMANN DC-190 high temperature analyzer. For TOC measurements, sludge samples taken from reactors were centrifuged at 3000 rpm for 15 minute and the centrat samples were diluted to 1/25 with pure water before analysis.



Figure 4.6 DOHRMANN DC-190

4.3.1.7 EPS Extraction from the Samples

Extracellular polymeric substances (EPS) were extracted from the samples using the heat extraction technique originated by Goodwin and Forster (1985) and Frolund et al. (1996) as detailed in elsewhere (Ayol 2005).

4.3.1.8 Capillary Suction Time Test

Capillary Suction Time (CST) values of the sludge samples were measured using Triton A 304 M CST-meter. A standard CST sample cylinder of 1.8 cm diameter was used during experiments with Whatman # 17 filter paper. All CST measurements were conducted in triplicates. Figure 4.7 shows the capillary suction time analyzer used in this study.



Figure 4.7. Capillary Suction Time Analyzer used in this work

4.3.1.9 Protein and Polysaccharide Analysis

Extracellular polymeric substances (EPS) were extracted from the samples using the heat extraction technique originated by Goodwin and Forster (1985). Sludge samples were first centrifuged at 3000 rpm for 15 minutes; the supernatant was then decanted and the samples resuspended to their original volume in a buffer solution as

described by Frolund et al. (1996). This step is intended to remove the various dissolved solids and extracellular "slime" in the biosolids samples (Ayol, 2005). Protein contents of EPS samples were analyzed using protein assay kits (Procedure No. TP0300 Micro Lowry, Sigma).

Polysaccharides in the EPS samples were determined using a phenol-sulphuric acid method developed by Dubois et al. (1956) as given in elsewhere (Ayol, 2005).

4.3.1.10 Zeta Potential Measurement

The zeta potential measurements were done using Zeta-meter System 3.0+ in order to determine optimum polymer dose range using electrokinetic evaluations. The results were also compared with CST results. Figure 4.8 shows the zeta meter used in this study.



Figure 4.8. Zeta Meter system 3.0+ used in experimental evaluations

4.3.1.11 Viscosity Measurements

Rheological analyses for viscosity measurements used the method explained in Ayol et al. (2006) and Abu-Orf and Dentel (1999). Rheological analyses were done using a Brookfield RVDV III type rheometer with coaxial sensors. Figure 4.9 shows the rheometer used in this study.



Figure 4.9. Brookfield RVDV III type rheometer with coaxial sensors

4.3.1.12 Sludge Conditioning

Polymer stock solutions were prepared at a 0.5% w/v concentration. Standard 1-liter beakers were used with 500 ml sample. A household-blending mixer (Moulinex Handblender) was used for 20 seconds to mix the sludge and different amounts of polymer solution.

In order to investigate the enzymatic treatment effects on polymer conditioning and dewatering of the aerobically digested samples, a bench scale dewatering unit simulating full scale belt filter press was used. This device called Crown press was

purchased from Phipps and Bird, USA. Dry solid contents of dewatered samples were analyzed. Prior to this press, water drainage rate of the samples were determined using a gravity drainage plow simulator kit. The detailed information about the press and the gravity drainage plow simulator kit can be found in Severin et al. (1999); Ayol & Dentel (2005); and Ayol et al. (2005). Figure 4.10 shows the drainage kit and the Crown press used in this work.



Figure 4.10. Gravity drainage plow simulator kit and Crown press used in this study (Ayol et al. 2007)

CHAPTER FIVE

RESULTS AND DISCUSSION

5.1 Introduction

This chapter gives results and evaluations of the experimental studies to determine the effects of hydrolytic enzymes on aerobic digestion. Although, 25 day of hydraulic retention time was initially used in the batch experiments, it was decided to reduce this operation time to 15 days depending on the first experimental evaluations. The reactors were operated at ambient conditions and the temperature was recorded as 28 ± 3 °C. This chapter presents the experimental results for 25 days and 15 days of operation time.

5.2 Start-up of the Reactors

Waste activated sludge was taken from Guney Bati municipal Wastewater Treatment Plant, Narlıdere, Izmir. Inoculum sludge was taken from a full scale aerobic digester of Manisa Municipal Wastewater Treatment Plant. Three aerobic reactors with a 13.5 L of working volume each were operated. Before feeding the sludges, the reactors were operated with tap water to control the tanks, aeration system, oil jacket and PLC system for 15 days. After doing this, approximately 13 L of inoculum sludge was first fed to the reactors and the sludge was withdrawn each day till 2/3 volume of the reactor. The same amount of raw waste activated sludge was step by step fed to the reactors.

5.3 Sludge Characteristics

The characteristics of waste activated sludge taken from Guney Bati MWWTP for 25 days and 15 days of operation time, and the inoculum sludge taken from Manisa MWWTP were reported in Table 5.1.

Table 5.1 Waste activated sludge and aerobic inoculum characteristics

Parameters	25 days of operation time	15 days of operation time	Aerobic inoculum sludge
Dry solids (DS, %)	1.69	1.21	2.05 ± 0.02
Volatile solids (VS, %)	54.26±0.47	66.41±0.52	58.11 ± 0.04
Suspended solids (SS, mg/L)	10300.25	7100.00	18590.91
pH	8.02	6.88	8.36
Oxidation reduction potential (ORP, mV)	+37.0	+98.6	+51.00
Dissolved oxygen (DO, mg/L)	2.10	2.50	2.14
Capillary suction time (CST, sec.)	35±14.27	16±0.36	345.53 ± 33.49
Zeta potential (mV)	-22.00±1.25	16±0.36	-21.07 ± 1.12
Particle size (µm)			
Surface weighted mean D[3,2]	24.389	29.365	15.731
Volume weighted mean D[4,3]	70.134	94.136	135.775
d (0.5)	51.165	65.144	46.082
d (0.9)	135.859	157.845	438.694
d (0.1)	14.255	25.336	7.271
Viscosity (mPa.s,@30 1/s shear rate)	7.36	9.54	5.55

5.4 Enzyme Additions

Different hydrolytic enzymes belonging to Alpha-amylase and Beta-glucanase endo-1, 3(4)-) enzyme class were added to second and third reactors, respectively. The first aerobic reactor was operated as control reactor with no enzyme addition. The effects of enzyme additions at different amounts (0.1% and 0.5%) on reactor performances were examined during the operation time. The reactors of aerobic unit were denoted as R1 (control reactor), R2 and R3, respectively.

5.5 Performance Evaluations for 25 Days of Operation Time

5.5.1 pH Results

pH is an important parameter for biological system operations in wastewater treatment technology. The pH values can change between 6.0 and 8.5 in aerobic digestion process. During the operation period, pH values of the sludge samples taken from the reactors were measured between 7.92 and 8.53. The pH measurements are depicted in Figure 5.1.

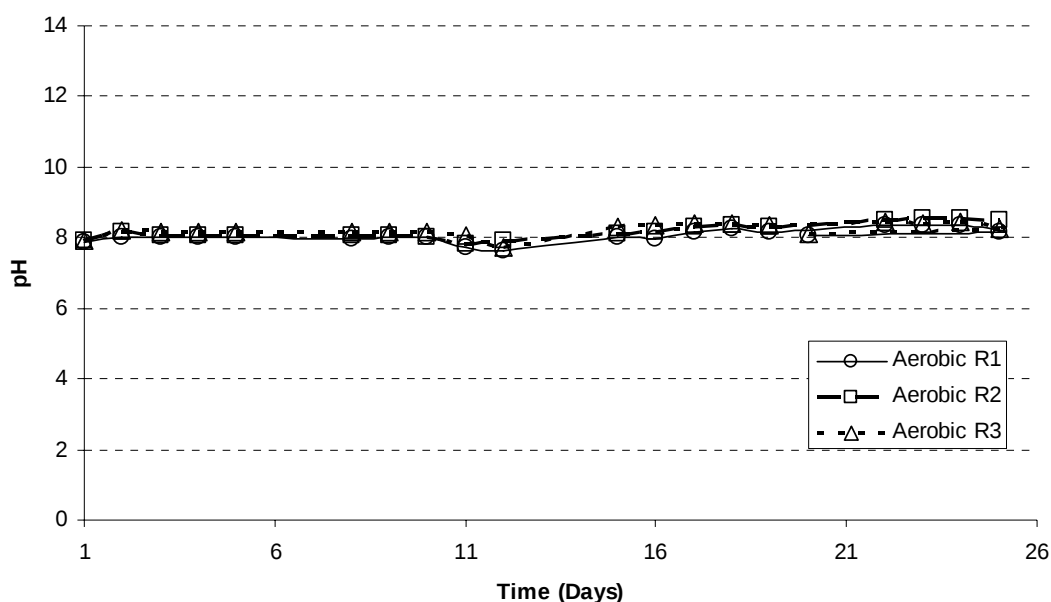


Figure 5.1 pH values during the operation time – 25 days

5.5.2 Temperature Monitoring

The operation temperature was recorded as 28 ± 3 °C for the aerobic unit. The operation temperature is important for the aerobic microbial activity. It was recommended that the temperature of aerobic systems should not become less than 15 °C for suitable activity (Metcalf & Eddy, 2003). The measured temperature values are given Figure 5.2.

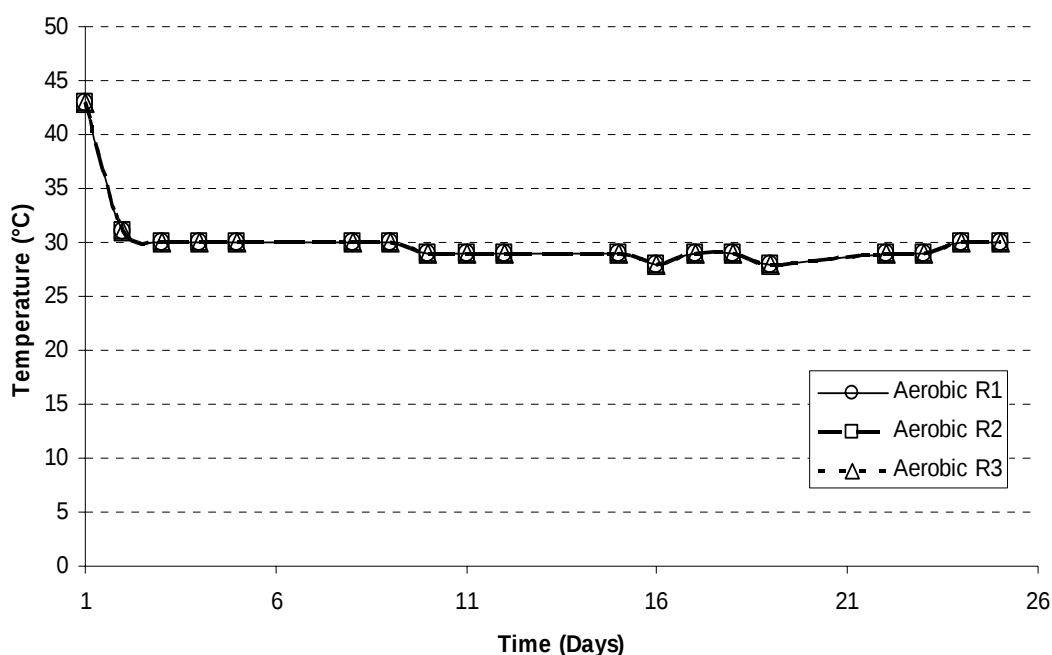


Figure 5.2 Temperature values during the operation time – 25 days

5.5.3 Dissolved Oxygen

Dissolved oxygen values (DO) were kept levels higher than 3 mg/L in the aerobic reactors. It is recommended that DO concentrations should not be less than 1 mg/L for aerobic digesters (Metcalf & Eddy, 2003). Some fluctuations were observed in DO levels during the operation. The DO levels are shown in Figure 5.3 Oxygen uptake rate (OUR) profile has been used to assess the effects of enzyme additions to the aerobic reactors. OUR results are given in Figure 5.4.

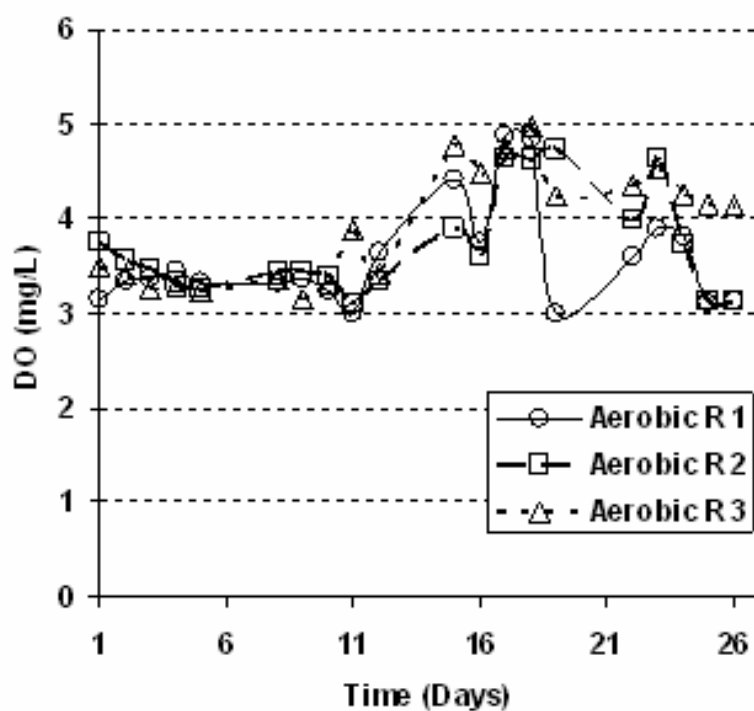


Figure 5.3 DO levels in the aerobic reactors and OUR for enzyme added reactors and control reactor.

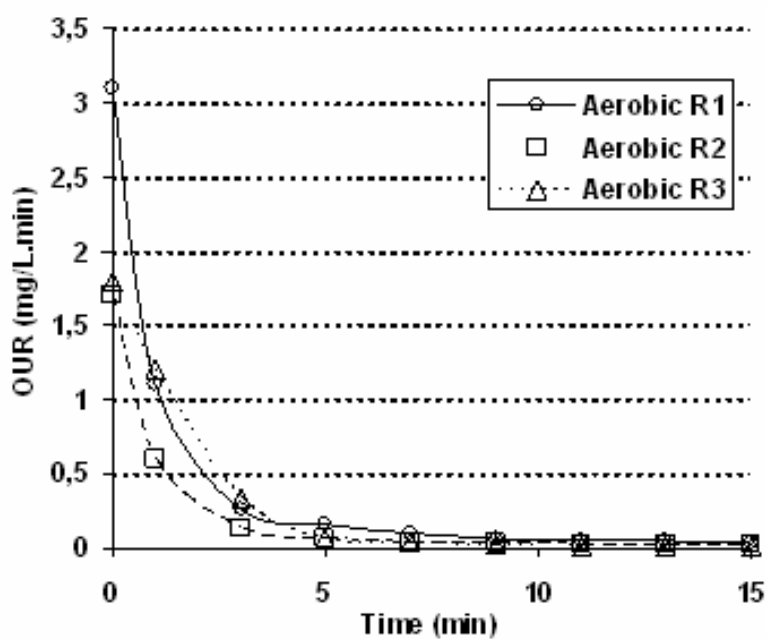


Figure 5.4 DO levels in the aerobic reactors and OUR for enzyme added reactors and control reactor.

At the beginning of the test, great differences were noticed between enzyme added reactors and control reactor. Higher OUR values could be attributed to higher microbial activity. But, it is not quite conclusive to say that enzyme added reactors did not have high activities in terms of substrate utilization. OUR should be monitored at longer periods rather than 15 min. for this work. Cokgor et al. (2007) noted that the amount of dissolved oxygen utilized after an appropriate reaction time is a much better index for the assessment of the inhibitory and other effects.

5.5.4 Oxidation Reduction Potential- ORP Results

For performance evaluations of the reactors, Redox potential (ORP) was daily measured. ORP values higher than 30 mV were obtained for aerobic reactors. These measurements are given in Figure 5.5. The values were about 60 mV and became less than 30 mV with the increasing of the operation time. It came close to anoxic zone for some reason. Meantime, mixing conditions were checked in aerobic reactors, some revisions were done in the reactors such as changing blower capacities and renewing of the diffusers.

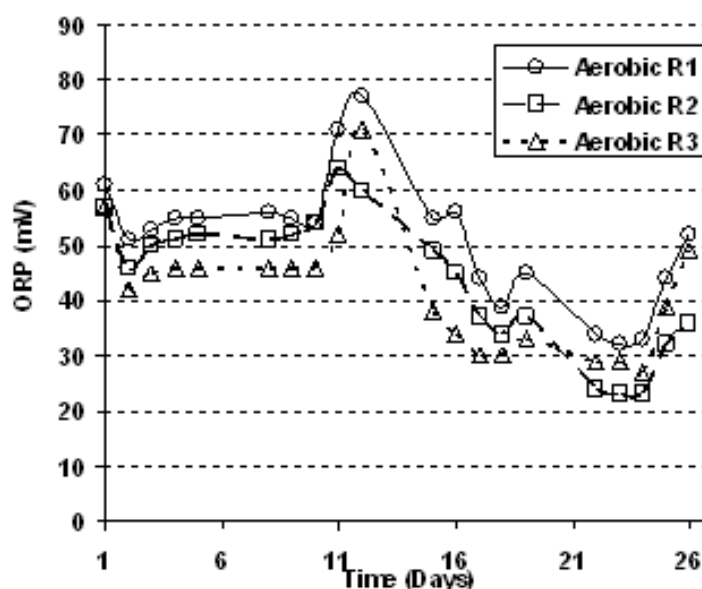


Figure 5.5 ORP results as a function of operation time.

5.5.5 Dried Solids

Dried solid (DS) concentrations changed between 1% and 4%. The results are given in Figure 5.6. DS values decreased and gave a maximum point at first week of operation. After the maximal point, reductions in DS values were obtained and a plateau was observed.

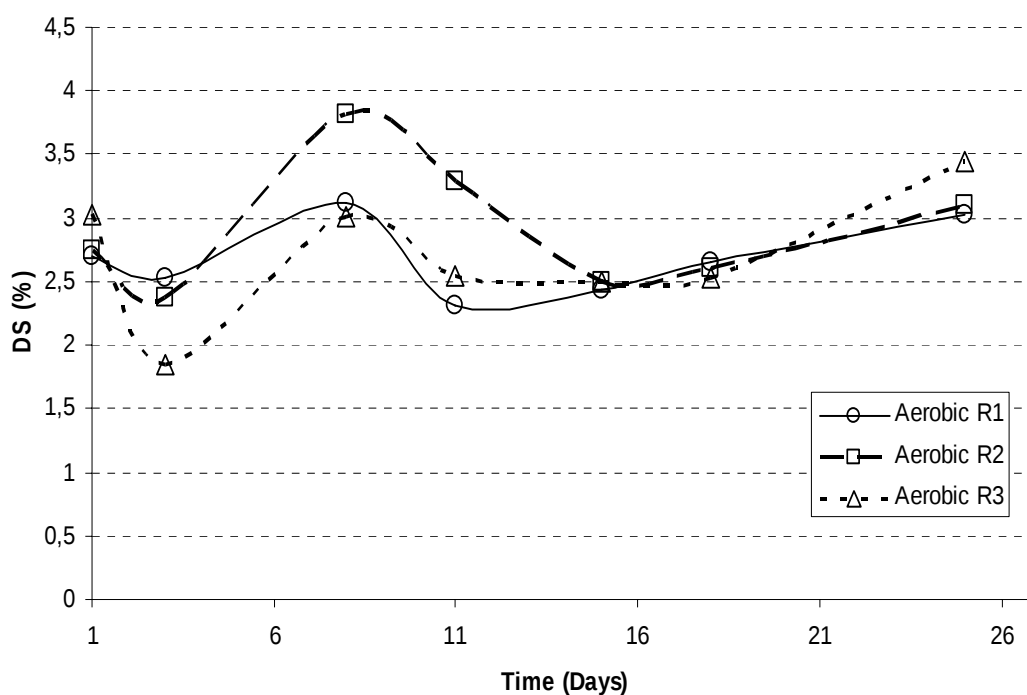


Figure 5.6 DS values during the operation time – 25 days

5.5.6 Volatile Solids

Very close VS reductions were observed for aerobic unit among the control and enzyme treated reactors. Volatile solids results are given in Figure 5.7.

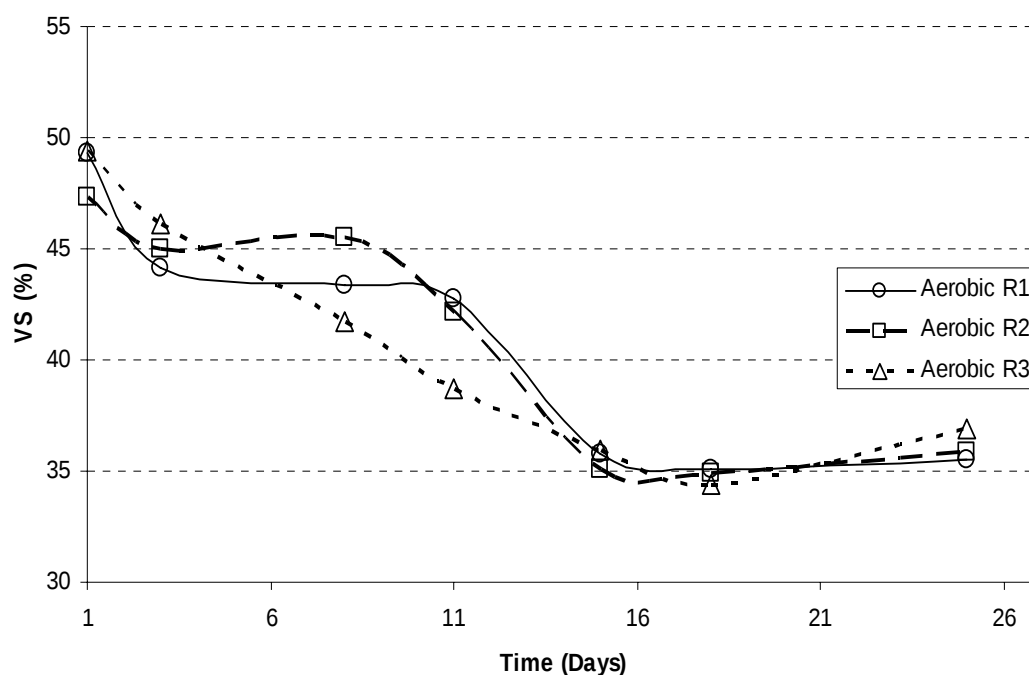


Figure 5.7 Volatile solids changes as a function of operation time – 25 days

5.5.7 Alkalinity

Total alkalinity values were measured three times a week and an alkalinity range of 592-to 1294 mg CaCO_3/L . The measured alkalinity results are shown in Figure 5.8.

5.5.8 Capillary Suction Time

Capillary suction time (CST) is important parameter for the evaluation of sludge dewaterability performance. CST measurements were daily done for dewaterability and filterability performances of the operated reactors. The results are also consisted with the EPS analyses given in the following sections of this chapter. The CST measurements are given in Figure 5.9. It can be said that there was no positive effect of these enzyme additions regarding the CST data. But, it is not strong conclusive since superior EPS degradation occurred in the enzyme added reactors. The dose of enzyme additions and other environmental factors should be examined.

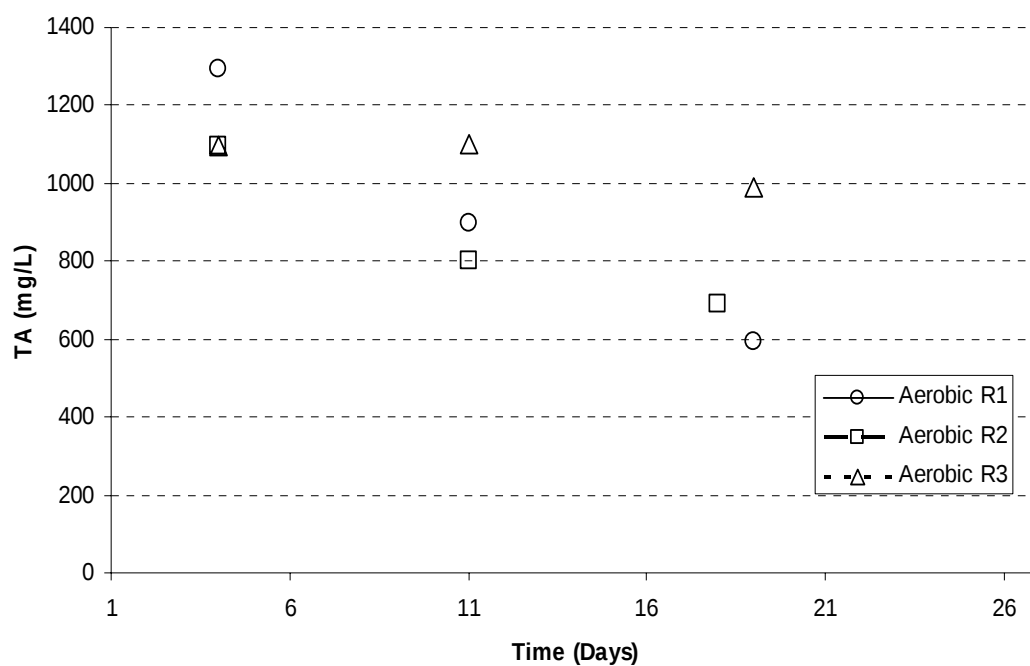


Figure 5.8 Total alkalinity results of reactors on operation time – 25 days

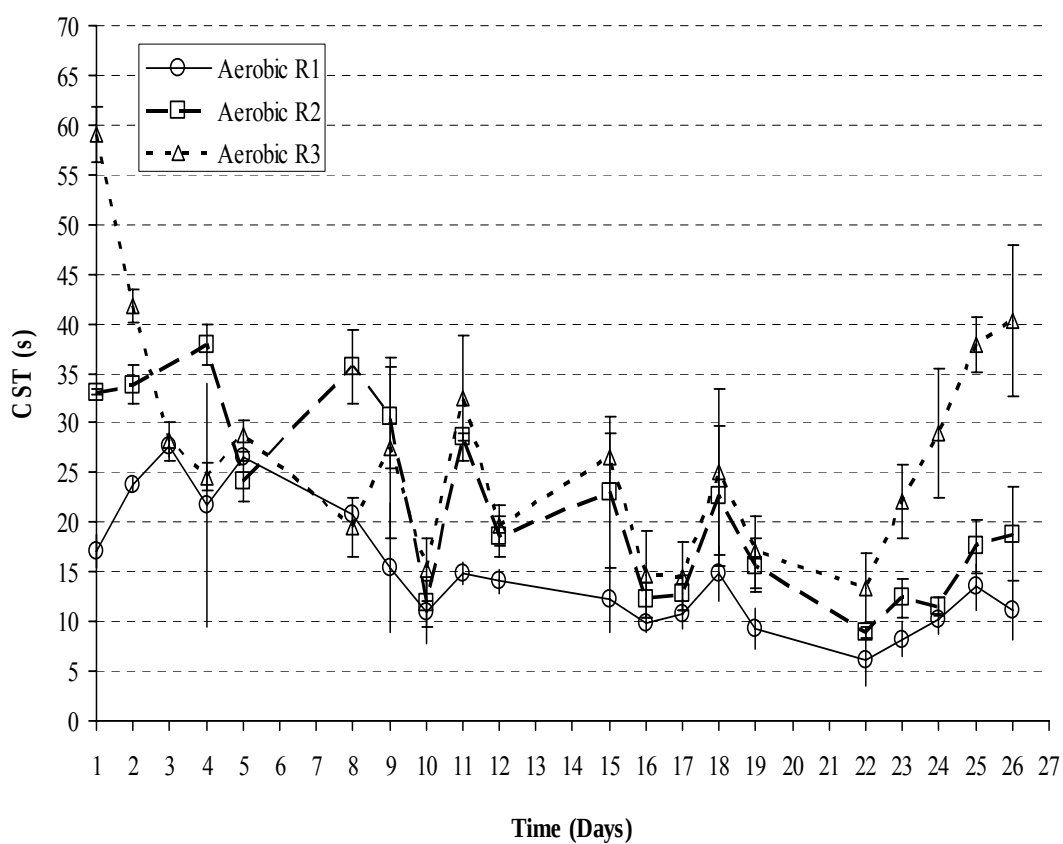


Figure 5.9 CST changes during the operation time – 25 days

5.5.9 Particle Size Distribution Results

Particle size distributions indicated floc disintegration stemming with EPS data reported in the subsequent section. For selected operation days, the changes in the particle size distributions are shown in Table 5.1. Reductions in particle size can be clearly seen from these results. Watson et al. (2004) reported dramatic decreases in floc sizes occurred for enzyme added sludges.

Table 5.1 Particle size changes in 1st and 22nd operation days for enzyme treated sludges

Sludge ID	Particle Size(μm)				
	Surface weighted mean D[3,2]	Volume weighted mean D[4,3]	d (0.1)	D (0.5)	d (0.9)
Aerobic R1- 1day	33.301	91.463	18.467	58.961	190.165
Aerobic R2- 1day	28.356	88.821	17.014	58.177	162.020
Aerobic R3- 1day	27.627	82.337	16.888	56.616	153.643
Aerobic R1- 22day	38.432	98.848	22.512	71.601	163.703
Aerobic R2- 22day	24.796	74.523	15.881	50.339	144.390
Aerobic R3- 22day	20.721	71.125	12.701	42.954	130.961

5.5.10 EPS – Protein and Polysaccharide Results

Enzyme1 (alpha-amylase) and Enzyme2 (beta-glucanase endo-1, 3(4)) improved the degradation of extracellular polymeric substances. Protein and polysaccharide concentrations of the samples decreased with the operation time in all reactors. In the reactors, protein concentrations were obtained about half the level of polysaccharides. The polysaccharide and protein results are given in Figure 5.10 and 5.11, respectively. Novak et al. (2003) have pointed out that the glucosidase activity, indicating polysaccharide degradation potential, dropped to zero by day 10 of digestion time in aerobic sludge digestion process. The loss of the activity explained why polysaccharide accumulates in aerobic digestion process while most of the protein is degraded.

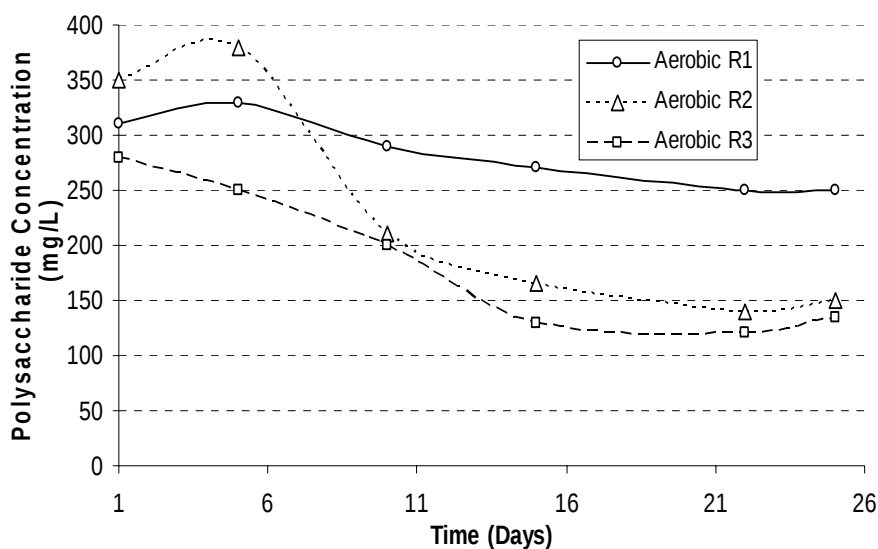


Figure 5.10 Polysaccharide concentration results of operation time – 25 days

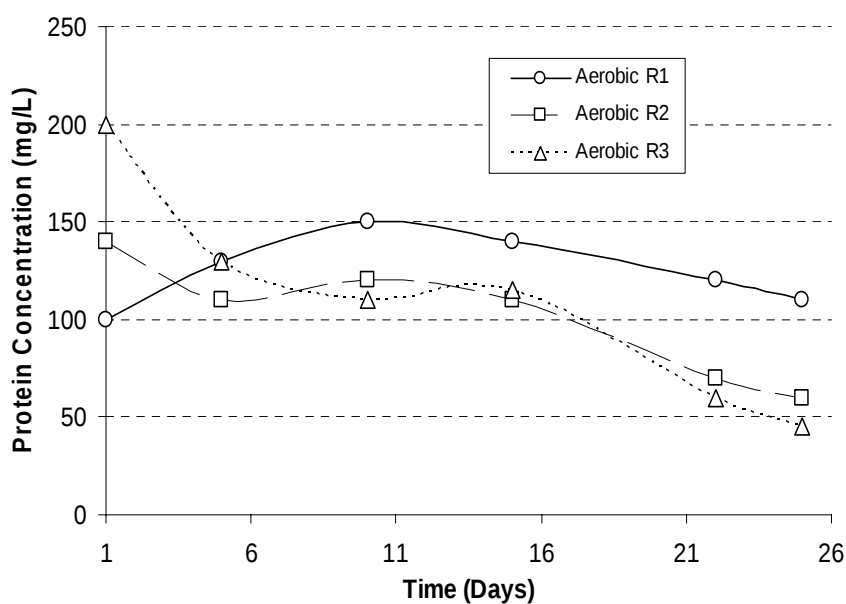


Figure 5.11 Protein concentrations as a function of operation time – 25 days

5.5.11 Sludge Conditioning

Cationic polymer addition was done for all sludge samples taken from the reactors in the end of operation time for sludge conditioning purpose. Thus optimum cationic polymer dosages were determined for aerobic sludge. Regarding the optimal doses, lower polymer doses were required for enzyme added sludges. These values are given in Tables 5.2-5.4, respectively.

Table 5.2 CST results of aerobic reactor 1 at the end of operation time – 25 days

Polymer Addition (mg/L)	Polymer Addition (kg/ton)	Average of CST (second)
0	0	9.57
25	0.083	6.37
50	0.166	5.97
100	0.331	5.63
200	0.662	6.50
250	0.828	6.33

Table 5.3 CST results of aerobic reactor 2 at the end of operation time – 25 days

Polymer Addition (mg/L)	Polymer Addition (kg/ton)	Average of CST (second)
0	0	9.97
25	0.081	7.03
50	0.161	6.23
100	0.323	6.43
200	0.645	7.77
250	0.806	9.53

Table 5.4 CST results of aerobic reactor 3 at the end of operation time – 25 days

Polymer Addition (mg/L)	Polymer Addition (kg/ton)	Average of CST (second)
0	0	12.33
25	0.073	8.30
50	0.145	7.20
100	0.291	6.33
200	0.581	7.17
250	0.727	8.50

5.6 Performance Evaluations for 15 Days of Operation Time

At the end of the operation period of 25 days, the experimental results showed that the digestion of the sludge was completed within the first 15 days. So it was decided that the second batch experiments used 15 days of operation time. The results for 15 days operation for the same amount of enzyme addition (0.5%) are shown in the following sections.

5.6.1 pH Results

The pH values for the operation period were similar results with the first operation period – 25 days. The pH measurements are given in Figure 5.12.

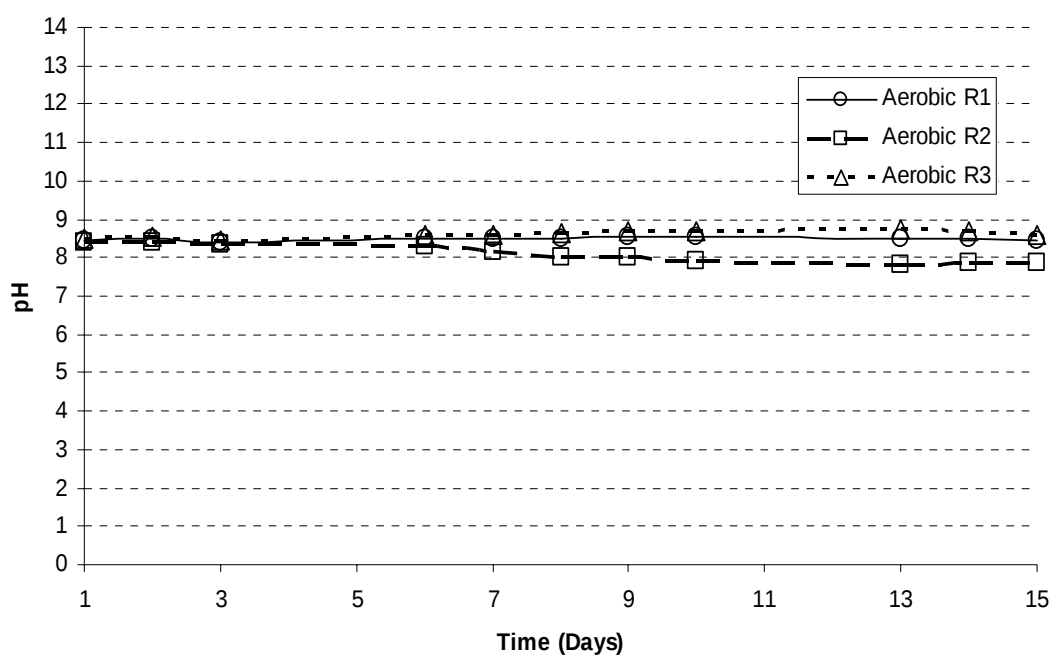


Figure 5.12 pH measurement during the operation time – 15 days

5.6.2 Temperature Results

Temperature values were measured around 29 ± 3 °C for 15 days operation time. The temperature measurement results are given in Figure 5.13.

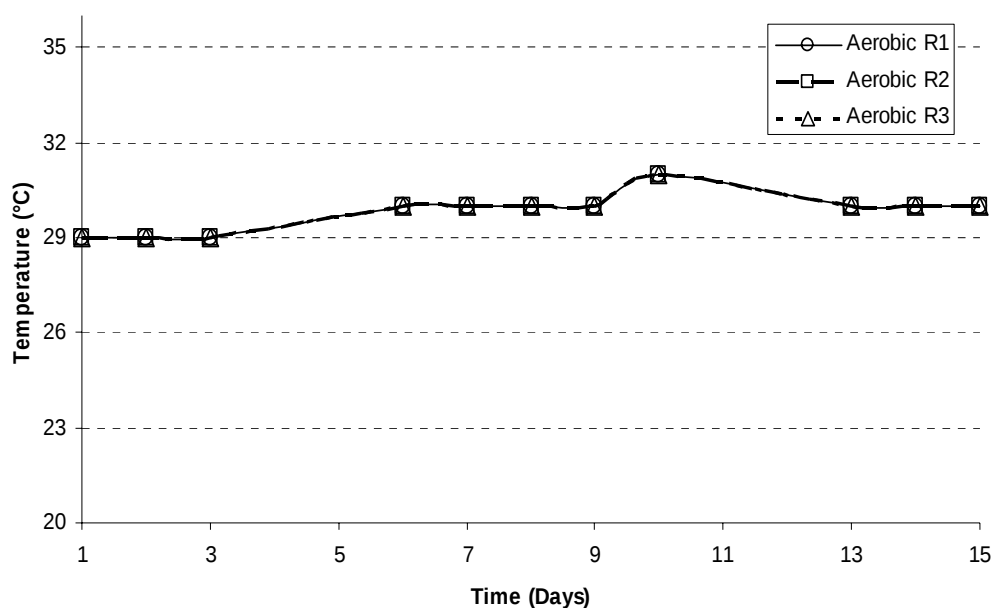


Figure 5.13 Temperature measurements during the operation time – 15 days

5.6.3 Dissolved Oxygen

Dissolved oxygen (DO) values were observed too much. In order to prevent settling sludge flocs in the tank, extensive mixing was applied to the reactors. This situation led to the higher DO levels. For subsequent series, DO levels were reduced by adjusting the aeration. On the other hand, high DO levels provided enhanced disintegration of sludges. DO measurements are given in Figure 5.14.

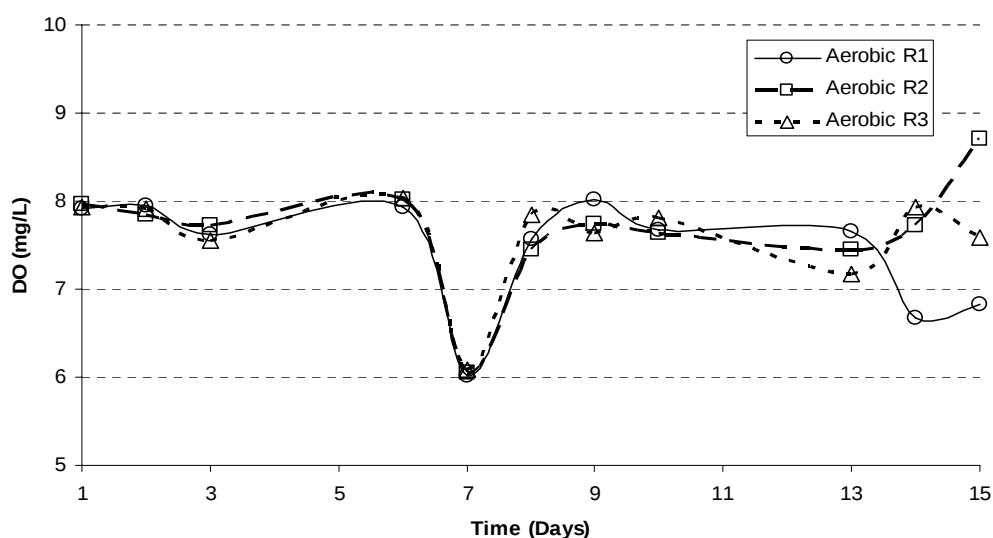


Figure 5.14 DO levels during the operation time – 15 days

As mentioned previously, higher OUR values could be attributed to higher microbial activity. OUR measurements were monitored for 15 min. in this work. The OUR results for the samples taken at 1, 7, and 15 days of operation time were given in Figures 5.15- 5.17. It can be seen from these figures, the results of 7th day of operation are higher, which can be attributed to the high microbial activity.

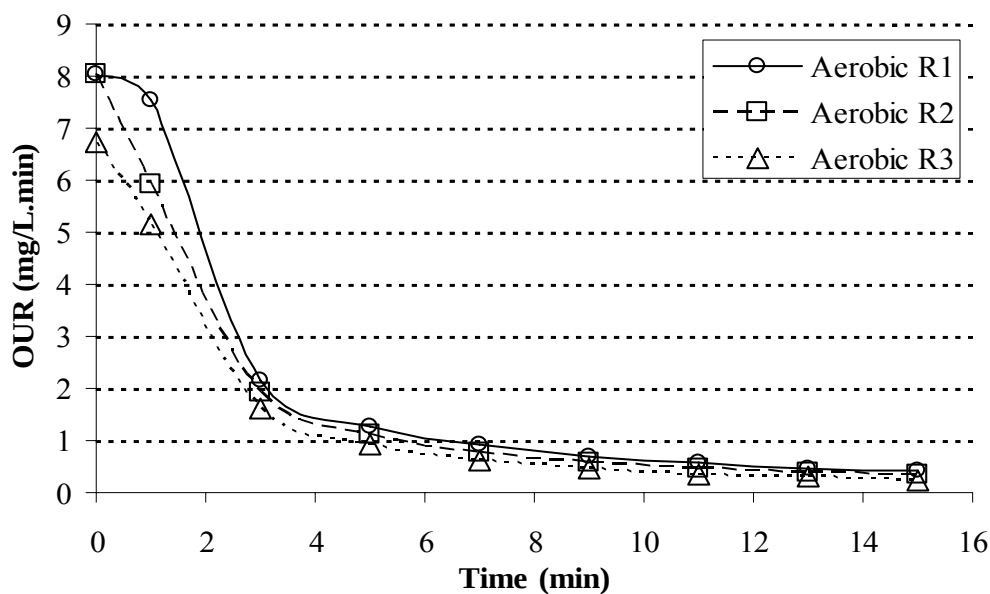


Figure 5.15 OUR changes- demonstration for 1st day sludges

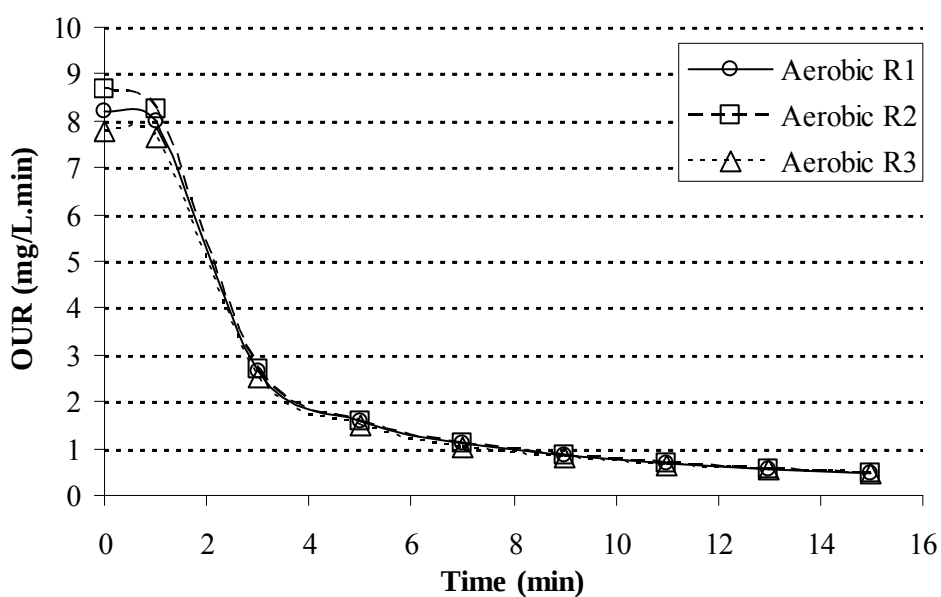


Figure 5.16 OUR changes- demonstration for 7th day sludges

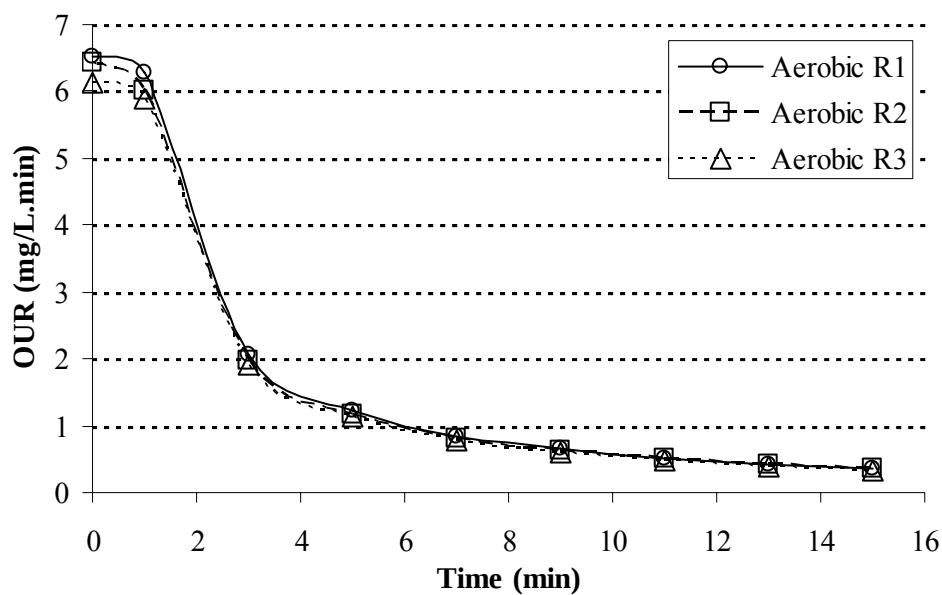


Figure 5.17 OUR changes- demonstration for 15th day sludges

5.6.4 Oxidation Reduction Potential

Redox potential (ORP) was daily measured and found higher than 30 mV. These measurements are given in Figure 5.18. The values showed high fluctuations because of the mixing conditions and rich microbial activities.

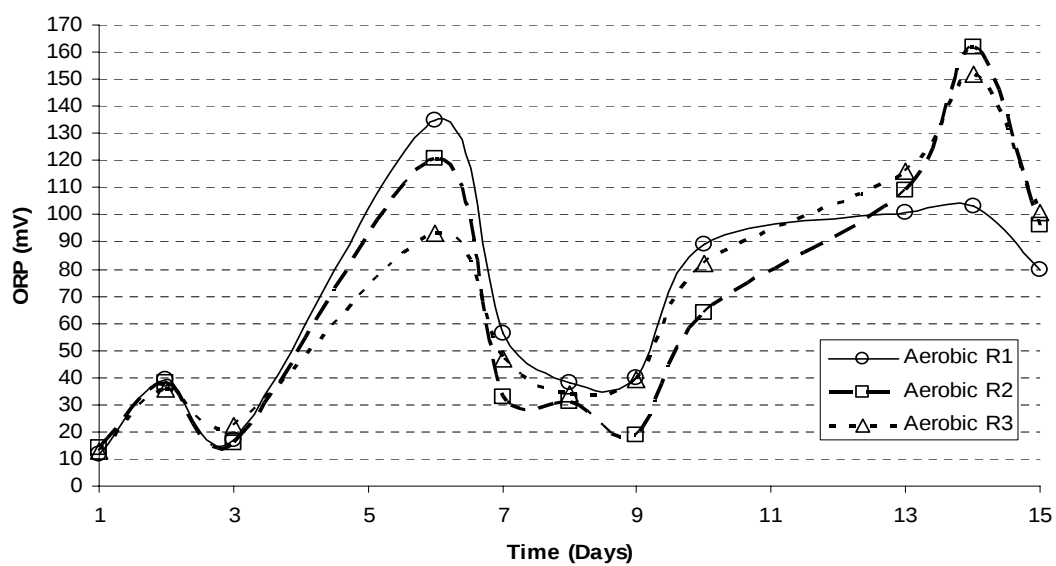


Figure 5.18 ORP measurement during 15 days operation time

5.6.5 Dried Solids

Dried solid (DS) concentrations are between 1.5% and 3%. There were no more changes in DS values after the first week of the operation. Dried solid values are shown Figure 5.19.

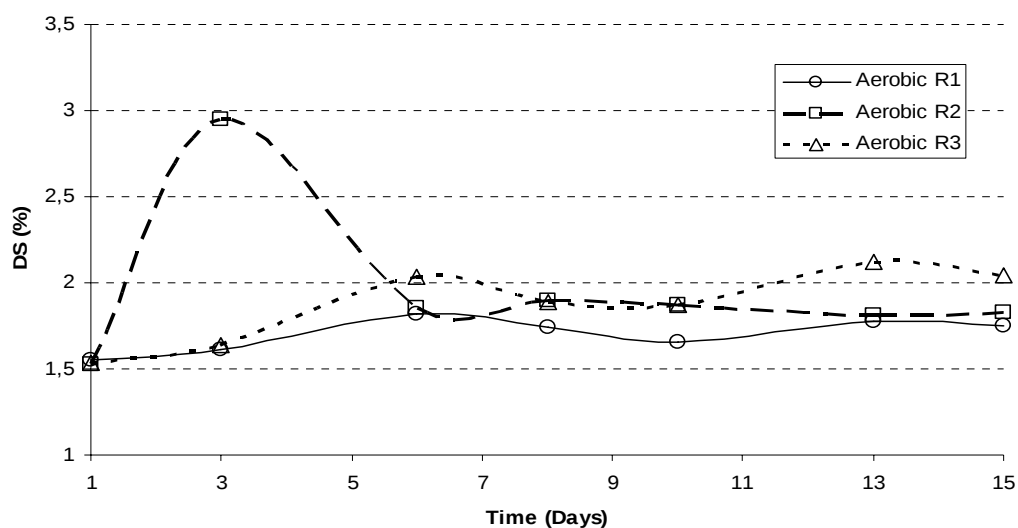


Figure 5.19 DS values during the operation time – 15 days

5.6.6 Volatile Solids

The volatile solids results are given in Figure 5.20. It can be said that the enzyme additions improved the volatile solid reductions when comparing the control reactor.

5.6.7 Alkalinity

Total alkalinity values were measured three times in a week and an alkalinity range of 500 to 800 mg CaCO₃/L was determined. The alkalinity results are shown in Figure 5.21.

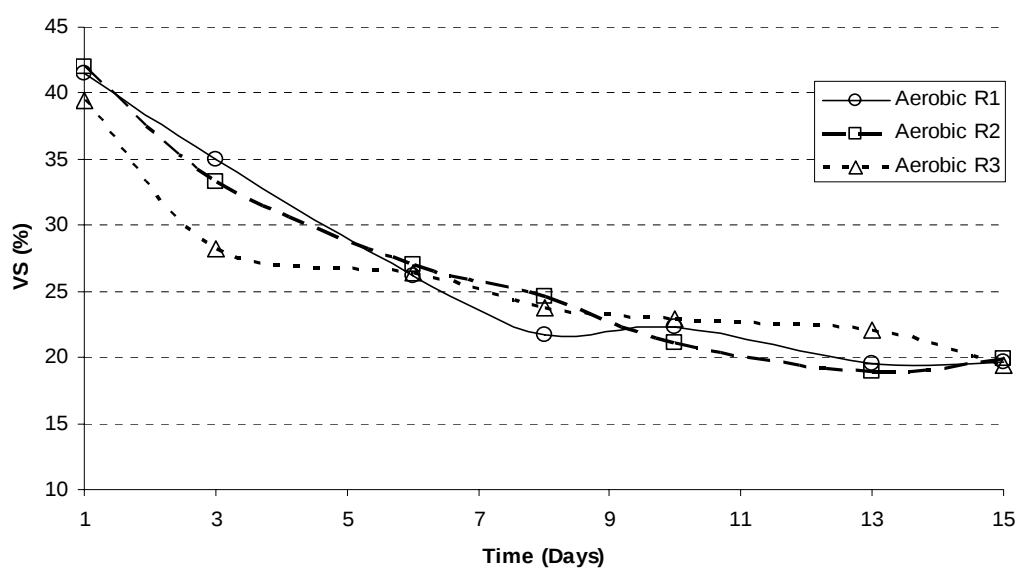


Figure 5.20 Volatile solids changes as a function of operation time – 15 days

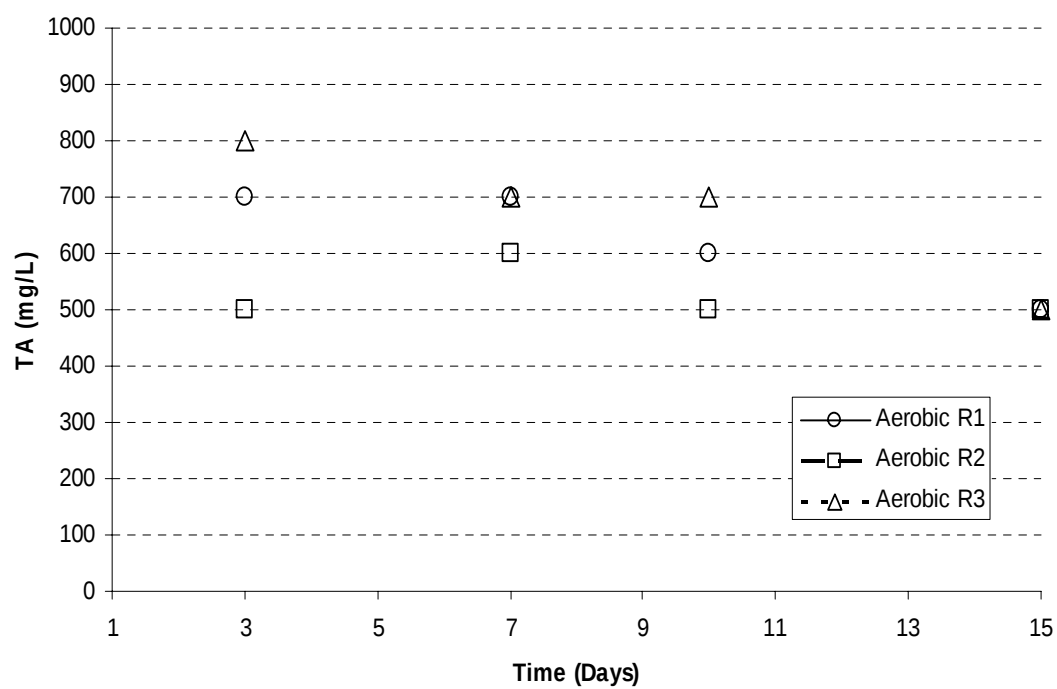


Figure 5.21 Total alkalinity results of reactors on operation time – 15 days

5.6.8 Capillary Suction Time

Figure 5.22 showed the CST results during the aerobic digestion. Enzyme additions improved the dewaterability of sludges even the results are better than the first experimental series which uses lower enzyme additions.

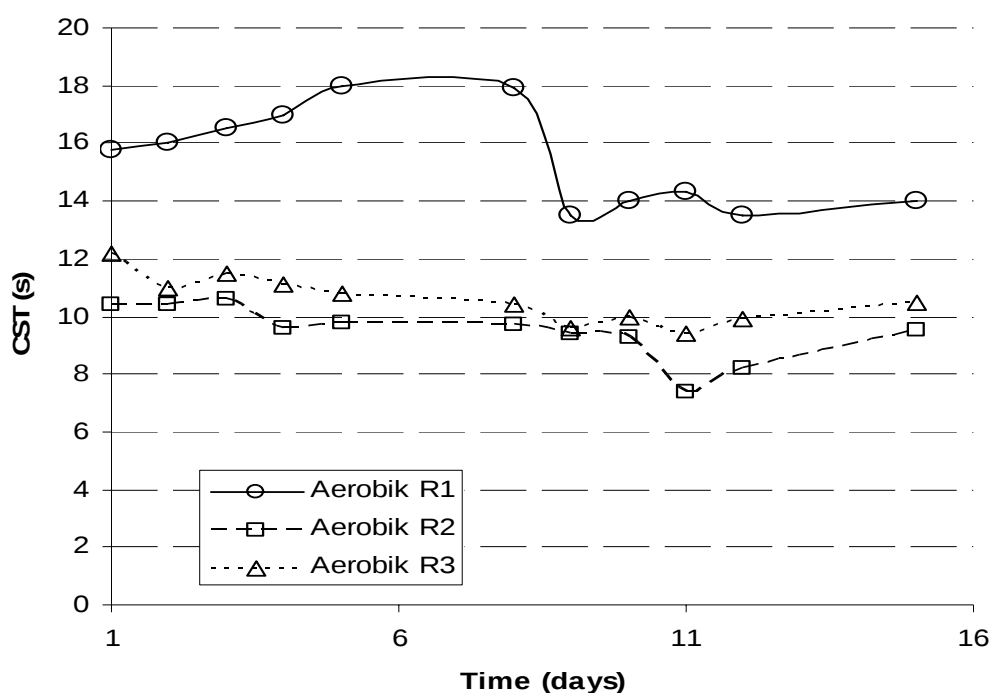


Figure 5.22 CST changes as a function of the operation time – 25 days

5.6.9 EPS – Protein and Polysaccharide Results

In aerobic reactors, protein concentration values were found half the level of polysaccharide concentration values. The results are also consisted to work done by Novak et al. (2003). The improved degradation in EPS of the samples indicated better dewaterability properties for enzyme added reactors depending on the CST results plotted in Figure 5.22. The EPS concentrations are given in Figures 5.23 and 5.24.

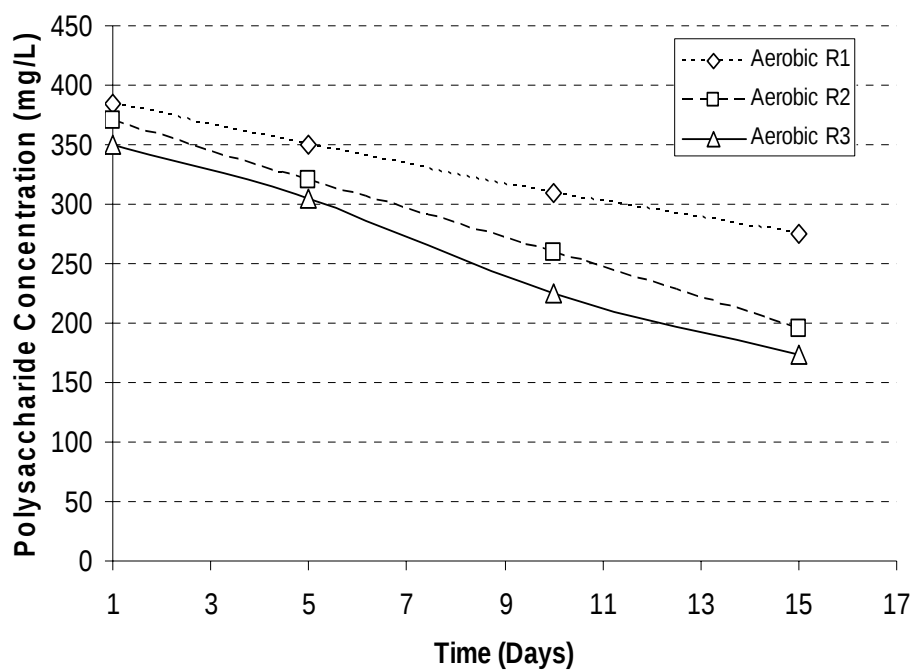


Figure 5.23 Polysaccharide concentration results of operation time – 15 days

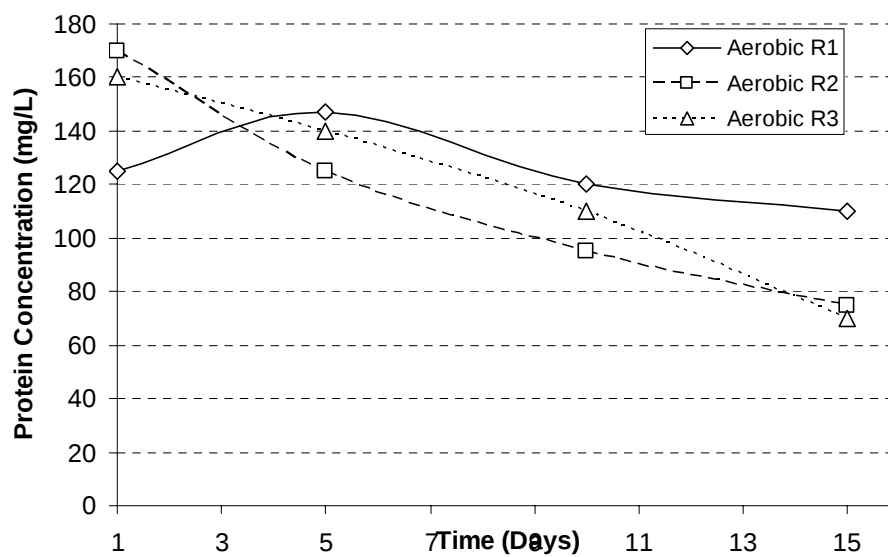
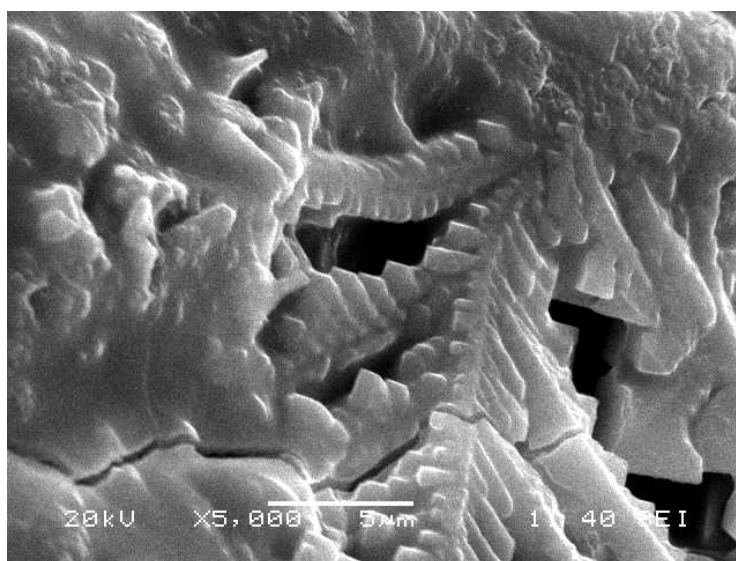


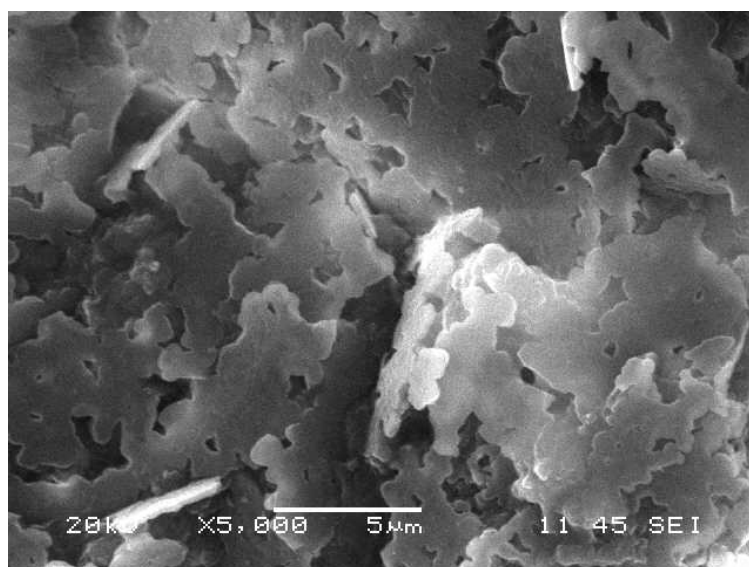
Figure 5.24 Protein concentrations as a function of operation time – 15 days

5.6.10 SEM- EDS Results

Photomicrographs of some sludge samples were taken using a Scanning Electron Microscope (SEM- Jeol 6060) and the components of the samples were determined by the SEM equipped with an energy dispersive spectrometer (EDS). SEM results belonging to 1st and 10th day of operation for the samples taken from R2 are given in Figures 5.25 a and b. Structural changes can be seen from these figures.



a. The sample taken at 1st day of the operation of R2



b. The sample taken at 10th day of the operation of R2

Figure 5.25 SEM results belonging to 1st and 10th day of operation for the samples taken from R2

The components of the samples determined by EDS are given in Figure 5.26. It can be seen from these results that the structural changes occurred during the operation period. The changes in some components showed the enhanced disintegration achievements regarding the Ca results which higher Ca values indicate flocculation. Similar results (not reported here) were also obtained for other reactor samples.

Elt.	Line	Intensity (c/s)	Error 2-sig	Conc		Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	
N	Ka	0.00	0.000	0.000	wt. %	N	Ka	0.25	0.100	1.935	wt. %
O	Ka	5.72	0.478	23.202	wt. %	O	Ka	15.69	0.792	42.855	wt. %
Na	Ka	4.94	0.444	4.753	wt. %	Na	Ka	5.38	0.464	4.800	wt. %
Mg	Ka	5.87	0.485	4.160	wt. %	Mg	Ka	16.46	0.811	10.800	wt. %
P	Ka	32.95	1.148	16.779	wt. %	P	Ka	4.35	0.417	2.155	wt. %
Cl	Ka	18.81	0.867	10.527	wt. %	Cl	Ka	11.96	0.692	5.841	wt. %
K	Ka	3.03	0.348	1.802	wt. %	K	Ka	6.67	0.517	3.504	wt. %
Ca	Ka	40.29	1.269	25.653	wt. %	Ca	Ka	30.49	1.104	17.481	wt. %
Cr	Ka	0.43	0.131	0.441	wt. %	Cr	Ka	0.07	0.055	0.070	wt. %
Mn	Ka	0.17	0.082	0.198	wt. %	Mn	Ka	0.17	0.084	0.189	wt. %
Fe	Ka	3.49	0.374	4.635	wt. %	Fe	Ka	1.24	0.222	1.500	wt. %
Co	Ka	0.27	0.104	0.425	wt. %	Co	Ka	0.18	0.084	0.253	wt. %
Ni	Ka	0.58	0.152	1.039	wt. %	Ni	Ka	0.21	0.092	0.346	wt. %
Cu	Ka	0.33	0.115	0.747	wt. %	Cu	Ka	0.28	0.106	0.586	wt. %
Zn	Ka	0.40	0.127	1.109	wt. %	Zn	Ka	0.28	0.105	0.696	wt. %
Cd	La	0.00	0.000	0.000	wt. %	Cd	La	0.00	0.000	0.000	wt. %
Pb	La	0.19	0.087	4.531	wt. %	Pb	La	0.32	0.112	6.988	wt. %
100.000 wt. % Total						100.000 wt. % Total					
kV 20.0						kV 20.0					
Takeoff Angle 35.0°						Takeoff Angle 35.0°					
Elapsed Livetime 100.0						Elapsed Livetime 100.0					

a. The sample-1st day of the operation of R2

b. The sample- 10th day of the operation of R2

Figure 5.26 EDS results belonging to 1st and 10th day of operation for the samples taken from R2

5.6.11 Sludge Conditioning

Cationic polymer additions led to improved filterability of the samples corresponding to lower CST values. In this experimental series, optimum polymer doses were determined by CST and Zeta potential results for all aerobic sludges.

Decreases in polymer doses were observed for enzyme added sludges when comparing the sludge taken from control reactor. Enzyme1 application gave better results than the Enzyme2 additions in this experimental series. CST results are given in Tables 5.5-5.7, respectively. Figures 5.27 and 5.29 give the ZP results which are consistent with the CST data.

Table 5.5 CST results of aerobic reactor 1 at the end of operation time – 15 days

Polymer Addition (mg/L)	Polymer Addition (kg/ton)	Average of CST (second)
0	0	8.57
10	0.139	7.67
25	0.278	5.30
50	0.556	5.83
100	1.111	8.00
200	1.389	12.73

Table 5.6 CST results of aerobic reactor 2 at the end of operation time – 15 days

Polymer Addition (mg/L)	Polymer Addition (kg/ton)	Average of CST (second)
0	0	8.40
10	0.126	7.33
25	0.253	5.03
50	0.505	5.73
100	1.010	8.23
200	1.263	10.70

Table 5.7 CST results of aerobic reactor 3 at the end of operation time – 15 days

Polymer Addition (mg/L)	Polymer Addition (kg/ton)	Average of CST (second)
0	0	15.13
10	0.152	12.07
25	0.305	9.07
50	0.610	8.17
100	1.220	8.00
200	1.524	10.67

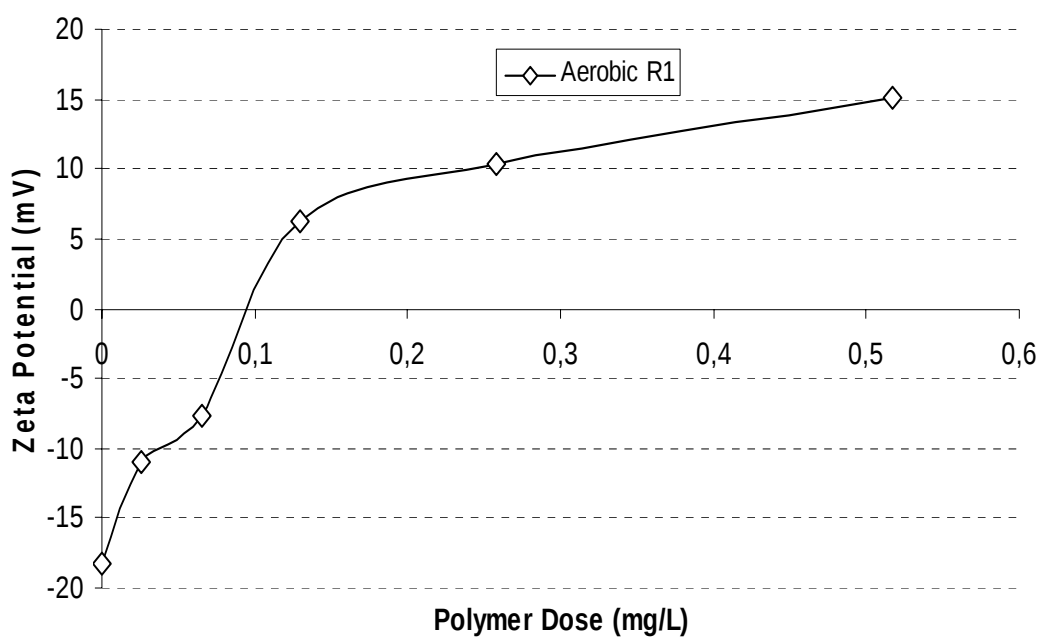


Figure 5.27 Zeta Potential results as a function enzyme addition for R1

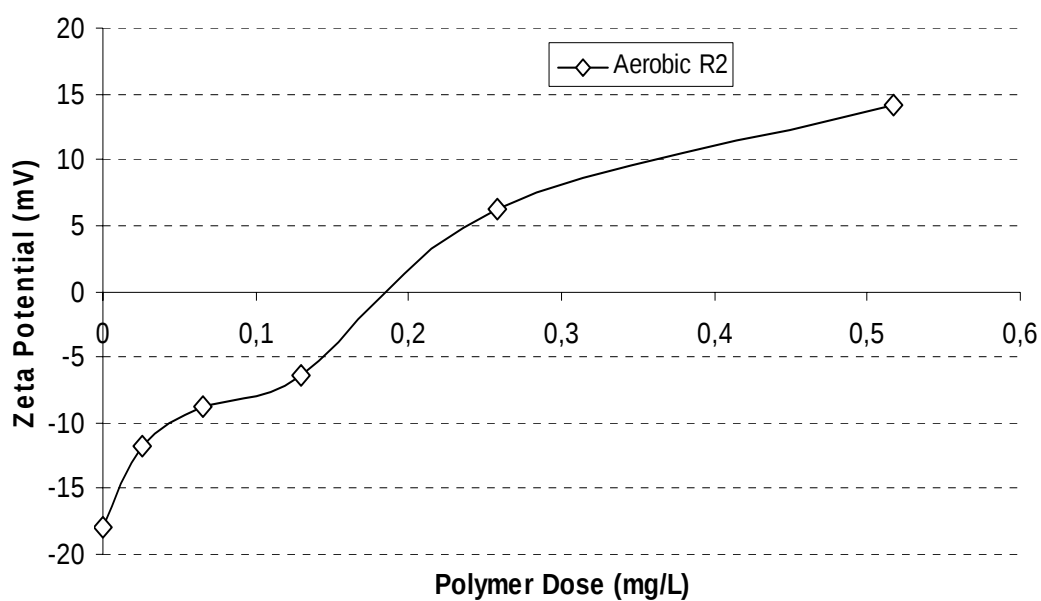


Figure 5.28 Zeta Potential results as a function enzyme addition for R2

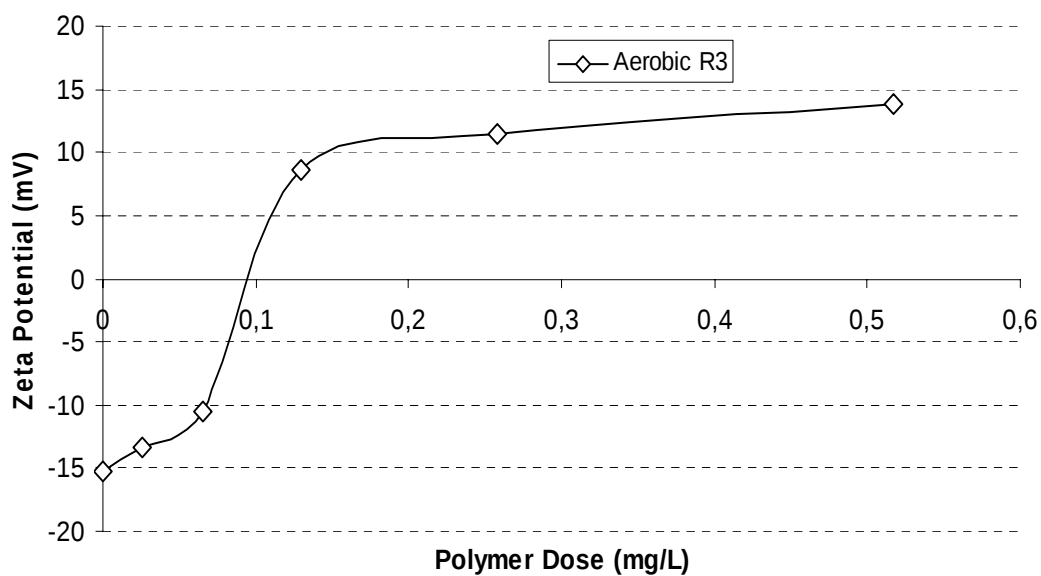


Figure 5.29 Zeta Potential results as a function enzyme addition for R3

CHAPTER SIX

CONCLUSIONS and RECOMMENDATIONS

6.1. Conclusions

As a new approach enzymatic treatment effects on aerobic sludge were examined in this study. Three aerobic reactors were operated at different operational conditions with and without enzyme additions. Experimental results showed that the enzyme additions led to the improved processing of the sludges. Enhanced degradations in protein and polysaccharides concentrations were obtained, which correspond better dewaterability performance of the sludges. The concluding remarks from this thesis can be given as follows:

- Experimental studies showed that enzymatic treatment was positively effected the EPS degradation and improving dewaterability performance. Especially at the first 10 days of the operation time.
- Although both enzyme additions Enzyme1 and Enyme2 improved the sludge processing, higher additions (0.5%) resulted better degradations than the 0.1% enzyme application.
- Increased degradations of the EPS with the enzyme additions improved the dewatering properties of the waste activated sludges during aerobic digestion process. These results were also confirmed by CST and Zeta potential data.
- Experimental studies have showed that the hydrolytic enzyme additions led to the better sludge disintegration as sampled by SEM-EDS data.

6.2 Recommendations

Experimental studies were done in laboratory scale. To make more conclusive results on enzymatic treatment effects on aerobic sludge processing, full-scale trials should be done.

As promising technology, enzymatic treatment of the sludges requires inclusive research studies including costs analysis to show whether the enzymatic treatment is appropriate in practice or not.

The experimental results should be modeled by commonly used biokinetic models to determine the biokinetic constants for full scale application of enzymatic treatment.

REFERENCES

- Abu-Orf, M. M., & Dentel, S. K. (1999). Rheology as a tool for polymer dose assessment and control. *J. Environ. Eng.*, 125, pp. 1133–1141.
- Aerobic Sludge Digestion*, October 11, 2007 from http://findarticles.com/p/articles/mi_km4449/is_200510/ai_n16261144
- APHA, AWWA, WEF. Standard methods, 19th ed. Washington, DC; 2005
- Ayol, A. (2005). Enzymatic treatment effects on dewaterability of anaerobically digested biosolids-I: performance evaluations. *Process Biochemistry*, 40, pp. 2427 – 2434.
- Ayol, A., & Dentel, S.K. (2005). Enzymatic treatment effects on dewaterability of anaerobically digested biosolids-II: laboratory characterizations of drainability and filterability. *Process Biochemistry*, 40, pp. 2435–2442.
- Ayol, A. (2006). Evaluation of conditioning responses of thermophilic mesophilic anaerobically and mesophilic aerobically digested biosolids using rheological properties. *Water Sci. Technol.*, 54, pp 23 – 31.
- Ayol, A., Filibeli, A., Sir, D., & Kuzyaka, E. (2007). Aerobic and anaerobic bioprocessing of activated sludge: floc disintegration by enzymes. *IWA Specialist Conference on Facing Sludge Diversities*, March 28–30, 2007, Antalya, Turkey, pp. 755 – 764.
- Barjenbruch, M., & Kopplow, O. (2003). Enzymatic, mechanical and thermal pre treatment of surplus sludge. *Advances in Environmental Research*, 7, pp. 715 – 720.

Bhatta, C.P., Matsuda, A., Kawasaki, K., & Omori, D. (2004). Minimization of sludge production and stable operational condition of a submerged membrane activated sludge process. *Water Sci. and Technol.*, Vol 50, No 9, pp 121 – 128.

Biochemistry of Enzyme, January, 18, 2008, from <http://tsailab.tamu.edu/biochem4102004/13-EnzymesIntroTransSActE.pdf>.

Capizzi-Banas, S., Deloge, M., Remy, M. & Schwartzbrod, J. (2004). Liming as an advanced treatment for sludge sanitasation: helminth eggs elimination- Ascaris eggs as models. *Water Research*, 38, pp 3251 – 3258.

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(4), pp. 1038 – 46.

Choromosone Terminology, December 23, 2007, from www.uwlax.edu/biology/faculty/Howard/04MCAT%20review%20A.ppt#4

Dey, E., S., Szewczyk, E., Wawrzynczyk, J., & Norrlöw, O. (2006). A novel approach for characterization of exopolymeric material in sewage sludge, *J. Resid. Sci. Technol.*, 3(2), pp. 97 – 103.

Eastman, J.A., Ferguson, J.F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. WPCF* 53 (3) pp 352 – 366.

Enzyme Technology, October, 3, 2007, from <http://www.lcbu.ac.uk/biology/enztech/source.html>

European Commission. (2001). *Disposal and Recycling Routes for Sewage Sludge - Scientific and Technical sub-component report*.

- Frolund, B., Palmigren, R., Keiding, G.K., & Nielsen, P.H. (1996). Extraction of extracellular polymer from activated sludge using a cation exchange resin. *Water Research*, 30(8), pp. 1749 – 58.
- Goel, R., Mino, T., Satah, H., Matsuo, T. (1998). Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Res.* 32, pp 2081 – 2085.
- Goodwin, J.A.S. and Forster, C.F., A further examination into the composition of activated sludge surfaces in relation to their settlement characteristics. *Water Research*, 1985, 19/4 527-533.
- Higgins, M.J., Novak, J.T. (1997). Characterization of exocellular protein and its role in bioflocculation. *J. Environ. Eng.* 123 (5), pp 479 – 485.
- Houghton, J.I., Quarmby, J., Stephanson, T. (2001). Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer. *Water Sci. Technol.* 44 (2-3), pp 373 – 379.
- Kang, S.M., Kishimoto, M., Shioya, S., Yoshida, T., Suga, K.I.H, Taguchi, H. (1989). Dewatering characteristics of activated sludges and effect of extracellular polymer. *J. Ferment Bioeng.* 68 (2), pp 117 – 122.
- Kaynak, G., Filibeli, A., (2007). Sludge disintegration using fenton's peroxidation. *IWA Specialist Conference on Facing Sludge Diversities*, March 28 – 30, 2007, Antalya, Turkey. pp 453 – 460
- Li, X.Y., Yang, J.F. (2007). Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res.* 41, pp 1022 – 1030.

- Liu, Y. & Tay, J.H. (2001). Strategy for minimization of excess sludge production from the activated sludge process. *Biotechnol. Adv.*, 19 (2), pp. 97 – 107.
- Luning, L., Roeleveld, P.J., Claessen, V.W.M. (2007). Sludge minimization by desintegration at different WWTPs. *WASA Specialist Conference on Facing Sludge Diversities*, March 28 – 30, Antalya, Turkey. pp 453 – 460.
- Metcalf & Eddy (2003). *Wastewater Engineering: Treatment, Disposal, Reuse* McGraw Book Company, New York, USA.
- Muslu, Y., 2001, “Su ve Atıksu Mühendisliği”, Cilt No:2, *Su Vakfı Yayınları*, İstanbul, pp 384
- Neyens, E., Baeyens, J., Dewil, R., and De heyder, B. (2004). Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *Journal of Hazardous Materials* 106, pp. 83 – 92.
- Novak, J.T., Sadler, M.E., & Murthy, S.N. (2003). Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Water Research*, 37, pp. 3136 – 3144.
- Nybroe, O., Jorgensen, P.E., Henze, M.(1992). Enzyme activities in waste water and activated sludge. *Water Res.* 26, pp. 579 – 584.
- Pere, J., Alen, R., Viikari, L., Eriksson, L., (1993). Characterization and dewatering of activated sludge from the pulp and paper industry. *Water Science Technology* 28, 193 – 201.
- Poxon, T.L., Darby, J.L. (1997). Extracellular polyanions in digested sludge: measurement and relationship to sludge dewaterability. *Water Res.*, 31, pp 749 – 758.

- Rittmann, B., E., McCarty, P., L., (2001). Environmental Biotechnology Principles and Application, *McGraw – Hill*, New York, USA
- Roman, H. J., Burgess, J. E., & Pletschke, B. I. (2006). Enzyme treatment to decrease solids and improve digester efficiency of primary sewage sludge. *African Journal of Biotechnology*, 5(10), pp. 963 -
- Saby, S., Djafer, M., & Chen, G.H. (2003). Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling-anoxic activated sludge process. *Water Research* 37, pp 11 – 20.
- Sanchez- Monedero, M. A., Mondini, C., Nobili, M., Leita, L. & Roig, A. (2003). Land application of biosolids. Soil response to different stabilization degree of the treated organic matter. *Waste Management*, 24, pp 325 – 332.
- Sanin, F.D., & Vesilind, P.A. (1994). Effect of centrifugation on the removal of extracellular polymers and physical properties of activated sludge, *Water Sci. Technol.*, 30(8), pp. 117–127.
- Sawhney, S., K., Singh, R., (2000). Introductory practical biochemistry, *Narosa Publishing House, London, UK*.
- Sawyer, C. N., McCarty, P. L., Parkin, G. F. (2003). Chemistry for Environmental Engineering and Science. *McGraw - Hill Companies, USA*.
- Severin, B.F., Jeffrey, V.N., & Kim B.J. (1995). Model and analysis of belt drainage thickening. *J. of Environmental Engineering*, 125/9, pp. 807-815.
- Shimizu, T. Kudo, K., Nasu, Y. (1993). Anaerobic waste activated sludge digestion a bioconversion and kinetic model. *Biotechnol. Bioeng.* 41, pp 1082 – 1091.

- Shuler, M.,L., Kargi, F., (2002). *Bioprocess Engineering Basic Concepts*, Second Edition, *Prentice Hall PTR*.
- Spinosa, L. & Vesilind, P.A. (2001). *Sludge into biosolids: processing, disposal and utilization*, *IWA Publishing*, UK.
- Sponza, D.T. (2003). Investigation of extracellular polymer substances (EPS) and physicochemical properties of different activated sludge flocs under steady-state conditions. *Enzyme Microb. Tech.* 32, pp 375 – 385.
- Tchobanoglous, G., Theisen, H. ve Vigil S. A. (1993). *Integrated Solid Waste Management*. *McGraw-Hill Inc.*, New York.
- Urbain, V., Block, J.C., Manem, J. (1993). Bioflocculation in activated sludge: an analytic approach. *Water Res.* 27, 829–838.
- U.S. Environmental Protection Agency. (1999). *Biosolids Generation, Use, and Disposal in The United States*, EPA530-R-99-009, Washington.
- U.S. Environmental Protection Agency. (1995). *Process Design Manual for Land Application of Municipal Sludge*, Washington.
- Vavilin, V.A., Rytov, S.V., & Lokshina, L.Y.(1996). A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Biosour. Technol.*, 56, pp. 229–37.
- 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, pp. 595-602.
- Wawrzynczyk, J., Szewczyk, E., Norrlöw, O., & Dey, S.E. (2007). Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of

extracellular polymeric substance from sewage sludge characterization of the extracted polysaccharide/glycoconjugates by panel of lection. *Journal of Biotechnology*, 130, pp 274 – 281.

Wei, Y., Van Houten, R.T., Borger, A.R., Eikelboom, D.H., & Fan Y. (2003). Minimization of excess sludge production for biological wastewater treatment. *Water Research* 37, pp. 4453–4467.

Whiteley, C.G., & Lee, D.J. (2006). Enzyme technology and biological remediation. *Enzyme and Microbial Technology*, 38, pp. 291–316.