IMPROVEMENT of ANAEROBIC DEGRADATION of SLUDGE USING ENZYMATIC TREATMENT

by

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İZMİR

IMPROVEMENT of ANAEROBIC DEGRADATION of SLUDGE USING ENZYMATIC TREATMENT

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Diclehan SIR

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Ms.Sc. THESIS EXAMINATION RESULT FORM

We certify that we have read the thesis, entitled **"IMPROVEMENT of ANAEROBIC DEGRADATION of SLUDGE USING ENZYMATIC TREATMENT"** completed by **DICLEHAN SIR** 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.

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IMPROVEMENT of ANAEROBIC DEGRADATION of SLUDGE USING ENZYMATIC TREATMENT

ABSTRACT

A significant amount of sludge has been produced worldwide annually either from municipal and industrial wastewaters or drinking water by physical/chemical, biological treatment processes. Sludges should be treated in accordance with the environmental and health criteria before final disposal. Depending on the sources, sludge characteristics can widely vary. Although processing of the sludges depends on the characteristics and quantities, the core methods used for processing are thickening, stabilization, conditioning, dewatering, and final disposal- incineration, land application (Spinosa et al., 2001).

Sludge stabilization is an important issue in sludge management field for effective reduction of organic matter, removal of pathogen and odor potential. For this purpose, alkaline stabilization, aerobic and anaerobic stabilization, aerobic thermophilic digestion, and composting are introduced. Among the methods, anaerobic digestion has been widely used with its many advantages including efficient degradation of biodegradable particulate organic matters in sludge, and efficient biogas production. Clear of the positive effects, bioprocessing of activated sludge either aerobically or anaerobically leads to some negative effects to the sludge like deprived dewaterability characteristics (Ayol et al., 2007a). The anaerobic sludge stabilization process is very sensitive to 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. 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. 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., 2007a).

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 are released (Dey et al., 2006; Watson et al., 2004).

Based on the recent findings, this research study has examined the effects of hydrolytic enzymes on dewaterability of sludges. Experimental studies have used three anaerobic reactors operated with activated sludge as feed material. Reactor performances with and without enzyme additions were regularly monitored in terms of organic matter reduction, methane and biogas productions, and dewaterability properties at two different operation time as 15 and 25 days. For enzyme additions, two hydrolytic enzymes were used. Initial part of the pilot–scale experiments, the reactors were batchly operated at 25 days at 37 ± 3 °C. Depending on the first part's results, operation time was changed as 15 days.

Experimental results showed that the enzyme additions improved floc disintegration. Enzyme added reactors indicated better performance in terms of organic matter stabilization and gas production when comparing with the control reactor. The protein concentrations in EPS solutions were significantly higher than the polysaccharide concentrations under anaerobic conditions.

This thesis as part of a broader project examining the overall fate and effects of hydrolytic enzymes in biological sludge stabilization presents recent research results to determine the floc disintegration mechanisms by enzymes during anaerobic digestion of sludge.

Keywords: Activated sludge, anaerobic digestion, sludge disintegration, biological hydrolysis, enzymatic treatment, extracellular polymeric substances, dewaterability.

ENZİMATİK ARITMA KULLANILARAK ARITMA ÇAMURLARININ ANAEROBİK BOZUNMASININ GELİŞTİRİLMESİ

ÖZ

Fiziksel/kimyasal, biyolojik prosesler sonucunda, evsel ve endüstriyel atıksulardan veya içme sularından önemli miktarda çamur üretilmektedir. Çamurlar nihai bertaraflarından önce çevre ve sağlık kriterlerine uygun olarak arıtılmalıdır. Kaynaklarına göre, çamurun özelliği önemli ölçüde değişebilir. Çamurların işlenmesi çamurun kaynağına ve özelliklerine bağlı olmasına rağmen, yoğunlaştırma, stabilizasyon, şartlandırma, susuzlaştırma, nihai bertaraf, yakma, toprak uygulaması yaygın kullanılan temel yöntemlerdir.

Çamur stabilizasyonu, organik maddelerin indirgenmesi, patojenlerin uzaklaştırılması ve olası koku problemlerinin önlenmesinden dolayı çamur yönetiminde önemli bir konudur. Bu amaç için, alkali stabilizasyon, aerobik ve anaerobik stabilizasyon, termofilik aerobik çürüme ve kompostlama gibi yöntemler ortaya konmuştur. Bu yöntemler içerisinde, anaerobik çürüme, çamur içindeki organik maddelerin biyolojik olarak ayrışması, biogaz üretimi, vb. gibi birçok avantajlara sahip olması nedeniyle oldukça yaygın kullanılmaktadır. Pozitif etkilerinin yanında, aerobik veya anaerobik biyoproseslerin her ikisi de çamur susuzlaştırma özelliği bakımından bazı olumsuz etkilere neden olmaktadır. (Ayol ve diğer., 2007a). Anaerobik çamur stabilizasyonu atık özelliklerine duyarlı olduğu kadar çevresel koşullara da duyarlıdır. Çamurun biyolojik işlenmesi sırasında flok yapısının bozulması üzerine yapılan laboratuar ölçekli çalışmalarda, yapısal olarak floklardan oluşan hücredışı polimerik maddelerin bakterilere bağlanarak çamurların su verme özelliğini engellemekte olduğu sonucu elde edilmiştir (ör. Novak ve diğer., 2003; Ayol, 2005). Biyolojik olarak parçalanabilen özellikteki kompleks yapılı organik maddelerin hidrolizi ağırlıklı olarak glukozitler, lipazlar ve proteazlar gibi hidrolitik enzimlere bağlıdır (Ayol ve diğer., 2007a). Bazı seçilmiş hidrolitik enzimler çamura ait substrat – biopolimer bağı üzerinde harekete geçer, o bağı parçalar ve düşük moleküler ağırlıklı ürünleri açığa çıkarır. En sonunda, flok

yapısının bozulmasına bağlı olarak, önemli miktarda protein, peptit ve karbonhidrat açığa çıkarılır (Dey ve diğer., 2006; Watson ve diğer., 2004).

Yeni bulgulara dayanarak, bu çalışmada hidrolitik enzimlerin çamurların susuzlaştırılabilme özelliklerine etkisi incelenmiştir. Deneysel çalışmalarda aktif çamur ile beslenen üç adet anaerobik reaktör kullanılmıştır. Enzim ilaveli ve enzim ilavesiz reaktörlerin performansları 15 ve 25 gün gibi iki farklı hidrolik alıkonma süresince düzenli olarak izlenmiş; organik madde giderimi, metan ve biogaz üretimleri ve susuzlaştırma özellikleri gibi parametrelere bakılmıştır. Enzim ilavesi için iki farklı hidrolitik enzim kullanılmıştır. Pilot ölçekli deneylerin ilk kısmında, reaktörler 25 gün boyunca 37 ± 3 °C işletilmiş, ilk bölümün sonuçlarına göre, işletme süresi 15 gün olarak değiştirilmiştir.

Deneysel sonuçlar enzim ilavesinin flok yapısının bozulmasını geliştirdiğini göstermiştir. Enzim ilave edilen reaktörler kontrol reaktörüyle karşılaştırıldığında organik madde stabilizasyonu ve gaz üretimi açısından daha verimli olduğu belirlenmiştir. Anaerobik koşullar altında hücre dışı polimerik maddedeki protein konsantrasyonunun polisakkarit konsantrasyonundan önemli ölçüde daha yüksek olduğu gözlenmiştir.

Bu çalışma, mevcut biyolojik çamur stabilizasyonunda hidrolitik enzimlerin bütün etkilerini inceleyerek çamurun anaerobik çürümesi sırasında flok yapısının enzimlerle parçalanmasını belirlemek için yapılan geniş kapsamlı projenin bir parçasını oluşturmaktadır.

Anahtar kelimeler: Aktif çamur, anaerobik çürüme, çamur dezentegrasyonu, biyolojik hidroliz, enzimatik arıtma, hücre dışı polimerik madde, susuzlaştırma.

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Sludges have been produced from water and wastewater treatment processes. The sludges produced at different treatment stages show tremendous differences in their characteristics as a consequence of their origins and the differences in the design and operation of wastewater treatment plants. They are generally in the form of a liquid or semisolid, which typically contains from 0.25 to 12 percent solids by weight (Metcalf & Eddy, 2003). Depending on the economical and social points, the sludge quantities vary from country to country (Balcerzak W., 2007). Although the amount of sludge is only a few percent by volume of processed wastewater, its handling can account for up 50% of total operating costs (Spinosa, 2007).

In sludge management field, it is aimed to apply suitable treatment operations for utilization or disposal at minimum cost. Low initial solids concentration (2 – 3%) and the presence of pathogens, heavy metals, organic pollutants and potentially putrescible organic matter in produced sludges are the main problems to be faced and solved. There is a link between the problems and the increased difficulties in complying with environmental quality requirements imposed by legislation, and with properly locating disposal works (Spinosa, 2007). The difficulties have driven the force seeking effective sludge processing from the environmental and economical point views.

Sludge minimization strategies are paid more attention recently to solve the sludge related problems, reduce the investment and operational costs, and enhance the performance of the subsequent treatment and final disposal processes. Many minimization methods have been reported in previous research studies (e.g. Wei et al. 2003, Liu & Tay 2001, Chu et al. 2001). Mostly applied methods for sludge reduction to date include thermal treatment, acidic or alkali chemical treatment, freeze-thawing, mechanical disintegration using ultrasonic devices, advanced

oxidation processes like ozonation, and biological hydrolysis with enzymatic treatment (Ayol et al., 2007a). Biological hydrolysis of sludges with enzyme additions seems to be an effective and cost reducing process for the treatment plants in many respects.

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 objective of the thesis was to investigate the improvement of anaerobic degradation of sludge with adding hydrolytic enzymes. The objectives were therefore:

- To investigate the effects of enzyme additions on accelerated hydrolysis of the organic matter content of sludge using enzymes,
- 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 anaerobic conditions,
- To compare different hydrolytic enzymes effects on the hydrolysis and degradation of biodegradable particulate organic matter in sludge,
- To improve dewaterability capacity of sludge,
- To determine whether enzyme pre-treatment changes enhance the dewaterability capacity of sludge,
- To determine whether enzyme pre-treatment can be optimized with polymer dosage to provide a feasible means of improving biosolids dewatering.

CHAPTER TWO
LITERATURE SURVEY

2.1 Introduction

Chapter 2 represents some definitions on sludge management and enzymes in sludge treatment.

2.2 Definitions of Sludge and Disposal Methods

As a consequence of wastewater treatment, sludge produces at different treatment units depending on the operations and process used. Sludges are generally in the form of a liquid or semisolid, which typically contains from 0.25 to 12 percent solids by weight (Spinosa et al., 2001). Sludges are very complex materials to be characterized and they should be handled using appropriate environmental technologies.

The problem of dealing with sludge is complex because;

1. they largely contain the substances of untreated wastewater,
2. the portion of sludge produced from biological wastewater treatment contains the organic matter contained in the wastewater and will decompose and become more offensive,
3. it contains only a small part of solid matter (Metcalf & Eddy, 2003).

In principal, sludge management targets to reduce the water and organic content of sludge and to render the processed solids suitable for reuse or final disposal. The principle methods used for sludge processing and their functions are summarized in Table 2.1.

Table 2.1 Solids processing methods (Metcalf&Eddy, 2003)

Unit operation, unit process, or treatment method	Function
Pumping	Transport of sludge and liquid biosolids
Preliminary operations:	
Grinding	Particle size reduction
Screening	Removal of fibrous materials
Degritting	Grit removal
Blending	Homogenization of solids streams
Storage	Flow equalization
Thickening:	
Gravity thickening	Volume reduction
Flotation thickening	Volume reduction
Centrifugation	Volume reduction
Gravity – belt thickening	Volume reduction
Rotary – drum thickening	Volume reduction
Stabilization:	
Alkaline stabilization	Stabilization
Anaerobic digestion	Stabilization, mass reduction
Aerobic digestion	Stabilization, mass reduction
Autothermal aerobic digestion (ATAD)	Stabilization, mass reduction
Composting	Stabilization, product recovery
Conditioning:	
Chemical conditioning	Improve dewaterability
Other conditioning methods	Improve dewaterability
Dewatering:	
Centrifuge	Volume reduction
Belt – filter press	Volume reduction
Filter press	Volume reduction
Sludge drying beds	Volume reduction
Reed beds, Lagoons	Storage, volume reduction
Heat drying:	
Direct dryers	Weight and volume reduction
Indirect dryers	Weight and volume reduction
Incineration:	
Multiple – hearth incineration	Volume reduction, resource recovery
Fluidized – bed incineration	Volume reduction
Co-incineration with solid waste	Volume reduction
Application of biosolids to land:	
Land application	Benefical use, disposal
Dedicate land disposal	Disposal, land reclamation
Landfilling	Disposal
Conveyance and storage	Solids transport and storage

Thickening, conditioning, dewatering, and drying are the methods used primarily for removing moisture from sludge while digestion, composting, and incineration are the methods used primarily for stabilization of the organic matter content of sludge (Metcalf & Eddy, 2003).

2.2.1 Preliminary Operations

Grinding, de-gritting, blending, and storage of sludge are important operations to provide a relatively constant, homogenous feed to subsequent processing facilities. The main targets of the operations are briefly explained in this section.

2.2.1.1 Grinding

The large and stringy material contained in sludge is generally cut or sheared into small particles to prevent clogging or wrapping around rotating equipment.

2.2.1.2 Screening

Sludge screening is an alternative to grinding. Screening is advantageous in that nuisance material is removed from the solid stream.

2.2.1.3 Degritting

The process might be necessary to remove the grit before further processing of the sludge especially in the plants where separate grit – removal facilities are not used ahead of the primary sedimentation tanks.

2.2.1.4 Blending

Sludge is blended to produce a uniform mixture to downstream operations and processes. Uniform mixtures are most important in short – detention – time systems, such as sludge dewatering, heat treatment, and incineration. Sludges can be blended

in primary settling tanks, pipes, processing facilities with long detention times, and separate blending tanks (Metcalf & Eddy, 2003).

2.2.1.5 Storage

It can be used for preventing the fluctuations in the rate of sludge production and to allow solids to accumulate during periods when subsequent processing facilities are not in operation. It is particularly important in providing a uniform feed rate ahead of the processes: Mechanical dewatering, lime stabilization, heat drying, and thermal reduction. While short–term solids storage can be accomplished in settling tanks or in thickening tanks, long–term solids storage can be done in stabilization processes- aerobic and anaerobic digestion, or in specially designed separate tanks.

2.2.2 Thickening

The solids content of sludge can vary depending on their origin and the sludges can be named as primary, activated, trickling filter, or mixed sludge. The thickening process is generally accomplished by physical means, including co – settling, gravity settling, flotation, centrifugation, gravity belt, and rotary drum. In the design of the unit, project engineers should consider the enough capacity to meet peak demands and prevent the septic conditions with its attendant odor problems, during the thickening process (Metcalf & Eddy, 2003).

2.2.3 Stabilization

This process is used to reduce sludge pathogenic content and potential odors from the sludge and to inhibit, reduce, or eliminate the potential for putrefaction. The main methods used for sludge stabilization are;

1. alkaline stabilization- usually with lime,
2. composting,

3. sludge digestion-aerobic digestion, including autothermal thermophilic aerobic digestion, and anaerobic digestion,

Alkaline stabilization is used to eliminate the nuisance conditions in sludge through the use of alkaline material. In the lime stabilization process, lime is added to untreated sludge in sufficient quantity to raise the pH to 12 or higher to create the environment that halts or substantially retards the microbial reactions that can otherwise lead to odor production and vector attraction (Metcalf & Eddy, 2003).

Composting is another method used for stabilization purpose and introduced as cost–effective and environmentally sound alternative. In this process, organic material in the sludge undergoes biological degradation to a stable end product-humus like material. Approximately 20 to 30 percent of the volatile solids are converted to carbon dioxide and water. While the organic material in the sludge decomposes, the compost heats to temperatures in the pasteurization range of 50 to 70 ^{0}C, and enteric pathogenic organisms are destroyed. Properly composted biosolids may be used as soil conditioners in agricultural or horticultural applications if they provide the limitations based on the constituents in the composed biosolids. Although this process may be accomplished under anaerobic or aerobic conditions, generally all municipal wastewater biosolids are composted under aerobic conditions (Metcalf & Eddy, 2003).

Apart from the composting, aerobic and anaerobic digestion is the biological stabilization methods applied to the sludges. In biological stabilization methods, the term "***Digestion***" refers to the breakdown of complex organic substances through the action of bacteria and other microorganisms (http://www.biosolids.state.va.us/research.htm#S). It is related to the volatile or organic fraction of the solids and biosolids. Survival of pathogens, release of odors, and putrefaction occur when microorganisms are allowed to flourish in the organic fraction of the sludge. Therefore the elimination of the nuisance conditions is mainly related to the reduction of the volatile content. Digestion results in the destruction of

pathogens and reduction in volatile solids and odors either anaerobically or aerobically (Novak et al., 2003).

Aerobic digestion can be applied to waste – activated sludge only,mixtures of waste – activated sludge or trickling – filter sludge, and primary sludge waste sludge from extended aeration plants. It is similar to the activated – sludge process. When the available substrate (food) is consumed, the microorganisms begin to consume their own protoplasm to obtain energy for cell maintenance reactions. In the endogenous phase, microorganisms obtain the energy needed from cell tissue that is oxidized aerobically to carbon dioxide, water, and ammonia. Approximately, 75 to 80 percent of the cell tissue can be oxidized while the rest of them stay as inert and non-biodegradable organic compounds (Metcalf & Eddy, 2003). The advantageous and disadvantageous of the process is given in Table 2.2.

Table 2.2 The major advantages and disadvantages of aerobic digestion

Advantages	Disadvantages
• volatile solids reduction in a well – operated aerobic digester is approximately equal to that obtained anaerobically • lower BOD concentrations in supernatant liquor • production of an odorless, humuslike, biologically stable end product • recovery of more of the basic fertilizer values in the sludge • Operation is relatively easy lower capital cost suitability for digesting nutrient – rich biosolids.	• high power cost is associated with supplying the required oxygen • digested biosolids produced have poorer mechanical dewatering characteristics • the process is affected significantly by temperature, location, tank geometry, concentration of feed solids, type of mixing/aeration device , and type of tank material.

Anaerobic digestion which is the one of the oldest processes used for the stabilization is an accepted and effective alternative. It involves the decomposition of

organic matter and inorganic matter (principally sulfate) in the absence of molecular oxygen (Metcalf&Eddy, 2003; Speece, 1996). Since the thesis deals with the anaerobic digestion of sludge and effects of enzyme additions to the process, anaerobic digestion will be given in details in the following sections of the thesis.

2.2.4 Conditioning

Sludges are conditioned to enhance their dewaterability characteristics before sludge dewatering. The applied methods for this purpose are given as follows:

- Chemical conditioning,
- Heat treatment,
- Sludge pre-heating, and
- Freeze-thawing process.

Among the methods, ***chemical conditioning*** is the widely applied method worldwide. The use of chemicals to condition sludge is economical in many respects. This method can help to reduce the 90 to 99% of water content of sludge to 65 to 85%, depending on the nature of the solids to be treated and the dewatering unit used subsequently such as centrifugation, belt – filter press, and pressure – filter presses. The conditioning method generates coagulation of the solids and release of the absorbed water from sludge flocs. Ferric cloride, lime, alum, and organic polymers are the chemicals used for the method (Metcalf & Eddy, 2003).

Heat treatment is another process for conditioning and stabilization of sludge. It involves heating the sludge for short periods of time under high pressure. It coagulates the solids, breaks down the gel structure of the sludge, and reduces the water affinity of sludge solids. As a result, the sludge is sterilized and is dewatered readily (Metcalf & Eddy, 2003).

Sludge can be ***pre-heated*** with heat dryers before centrifuges. High cake solids concentrations can be achieved by conditioning the sludge with heat to 60^0C (Metcalf & Eddy, 2003).

Freeze – thaw conditioning is used to convert the gel-like consistency of the residuals to granular–type material that drains readily especially in cold climates. Sludge includes several types of water as free water, interstitial water, surface water, and bound water. Free water is defined as the water that surrounds the sludge floc but does not move with solids. Interstitial water defines the trapped water within the floc structure and travels with floc or is held *by capillary forces between particles.* Surface water is held on the surface of the floc and cannot be removed by mechanical means. Finally, bound water is that is "*bound to the particles and can only be released by thermochemical destruction of the particles*" (Vesilind & Martel, 1990). In freeze-thawing process, the free water begins to freeze first with binding by crystallization. It trends to get more free water to bind and grow with while pushing the floc particles to the ice front. Therefore, after free water is frozen, the interstitial water is extracted by diffusion and added to the growing crystalline structure (Vesilind & Martel, 1990).

2.2.5 Dewatering

Dewatering is a physical unit operation used to reduce the water content of sludge and biosolids for the reasons including the cost reduction for transporting and trucking of sludge to the disposal sites, easily handling of dewatered sludge, increasing the calorific value of sludge if incineration will be applied, and reducing the bulking agent requirements prior to the composting, and reducing the leachate production in landfills.

While some dewatering techniques rely on natural evaporation and percolation-drying beds, and lagoons -, mechanically assisted techniques- centrifuges, belt–filter presses, recessed–plate filter presses- are used to dewater the sludge more quickly. The physical means of dewatering include filtration, squeezing, capillary action, and

centrifugal separation and compaction (Spinosa et al., 2001). The factors affecting dewaterability of sludges are given as follows:

1. morphological aspects (size distribution, filament index, fractal dimension),
2. physical properties (flocculating ability, hydrophobicity, surface charge and viscosity),
3. chemical constituents of polymeric substances (protein, humic substances and carbohydrate), and
4. metallic ions (Ca^{++}, Mg^{++}, Fe^{+3} and Al^{+3}), (Jin, B., Wilén B.R., & Lant, P., 2004).

2.2.6 Heat Drying

Heat drying involves the application of heat to evaporate water and to reduce the moisture content of biosolids that is achievable by conventional dewatering methods. The advantages of heat drying include reduced product transportation costs, further pathogen reduction, improved storage capability, and marketability (Metcalf & Eddy, 2003).

2.2.7 Incineration

Incineration of sludge involves the total conversion of organic solids to oxidized end product, primarily carbon dioxide, water and ash. The major advantages of incineration are the maximum volume reduction, destruction of pathogens and the toxic compounds, and energy recovery potential (U.S. EPA, 1985a).

2.3 Anaerobic Digestion

Anaerobic digestion is one of the more commonly used stabilization processes in sludge management, providing effective pathogen destruction, reduction of volatile solids and odour potential and an energy source in the form of biogas (Novak et al., 2003). Many progresses have been made in the fundamental understanding and

control of the anaerobic digestion process, design and the application of the equipment. This process is one of the dominant processes for stabilizing sludge because of the energy conservation and recovery. Anaerobic digestion of municipal wastewater sludge can, in many cases, produce sufficient digester gas to meet most of the energy needs for plant operation (Metcalf & Eddy, 2003).

Although, it has these advantages, the stabilization process may lead to poorer dewatering characteristics, which means higher chemical requirements in biosolids conditioning, lower quality of final processed biosolids, and higher operation and disposal costs (Ayol et al. 2007b).

Microorganisms and microbial–environment conditions are crucial in this process as in other biological treatment processes. During the anaerobic digestion of biosolids, the first stage in the degradation of particulate organic matter is the solubilization and enhanced hydrolysis of complex polymeric organic carbon structures. Bacteria are usually contained within a flocculated matrix of exopolymeric substances (Ayol 2005a, Ayol and Dentel 2005b). Novak et al. (2003) have stated that biocolloids releasing from floc structure hinder effective dewatering of the biosolids and are responsible for a significant portion of the polymer demand in the chemical conditioning (Novak et al. 2003).

2.3.1 Process Definition

Anaerobic digestion breaks down the organic material of sludge into biogas (CH_4, CO_2) in the absence of oxygen. The final electron acceptor is different from oxygen, for example, sulphate-reducing bacteria transfer electrons to sulphate (SO_4^{2-}) reducing it to H_2S, while others (nitrate reducers) transfer the electrons to nitrate (NO_3^-) reducing it to nitrite (NO_2^-), nitrous oxide (NO) or nitrogen gas (N_2) (Whitely & Lee, 2006).

During anaerobic treatment, a complex microbial community consisting of many interacting microbial species degrades natural polymers such as polysaccharides,

proteins, nucleic acids, and lipids, in the absence of oxygen, into methane and carbon dioxide (McInerney, 1999; Grotenhuis, 1992).

This process has four distinct steps given below and schematized in Figure 2.1:

(a) complex organic matter is decomposed into simple soluble organic molecules using water (hydrolysis) and hydrolase enzymes (glucosidases, lipases, proteases, sulphatases, phosphatases);
(b) chemical decomposition of these single monomeric unit molecules (monosaccharides, amino acids, fatty acids, glycerol) into volatile fatty acids by a process termed acidogenesis;
(c) acetogenic fermentation into acetate, H_2 and CO_2;
(d) methanogenic conversion into CH_4 and CO_2 (Whitely & Lee, 2006).

The efficiency of an anaerobic digester depends on the dynamics and kinetics of the microbial populations within the reactor and on the narrow limits that thermodynamics places on the ensuing reactions (McInerney, 1999). Acetate is a major product of the fermentation of intermediate organic molecules as shown in Figure 2.1 (Dolfing, 1988). About 70% of methanogenesis is through the acetate route (Mountford and Asher, 1978; Boone, 1982; Vogels et al., 1988; Grotenhuis, 1992; Winter, 1999; Poulsen, 2003). The important environmental factors for this process are;

1. solids retention time
2. hydroulic retention time
3. temperature
4. alkalinity
5. pH
6. the presence of inhibitory substances, i.e., toxic materials
7. the bioavailability of nutrients and trace metals

The first three factors are important in process selection while alkalinity is a function of feed solids and is important in controlling the digestion process (Metcalf & Eddy, 2003).

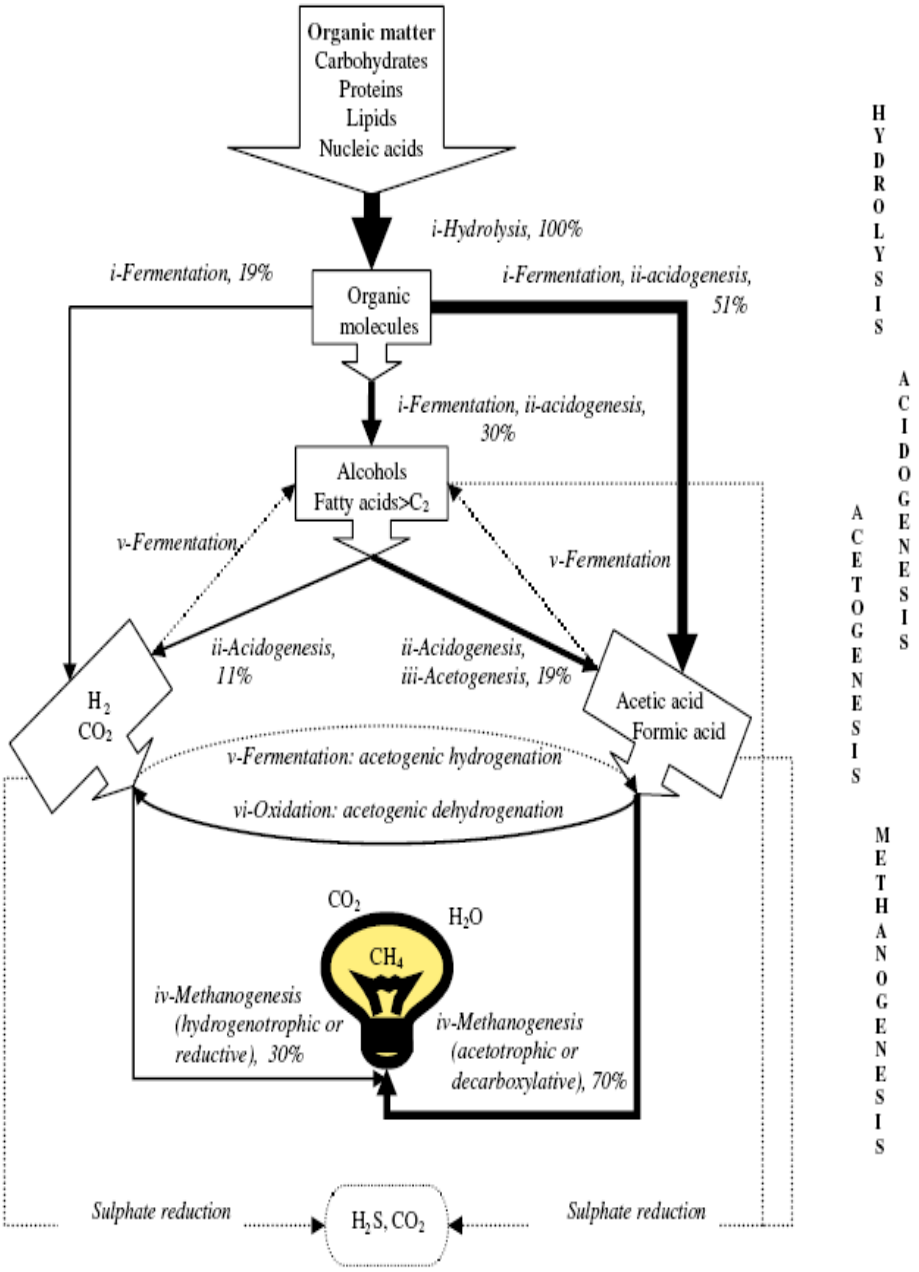


Figure 2.1 Schematic of the different metabolic steps and microbe groups involved in the complete degradation of organic matter to methane and carbon dioxide (Aiyuk, S., Forrez, I., Lieven, D.K.,Haandel, A.V., & Verstraete, W., 2006).

2.3.1.1 Solids and Hydraulic Retention Times

Anaerobic digester design is heavily depent on providing sufficient residence time in well–mixed reactors to allow significant destruction of volatile suspended solids (VSS) to occur. Sizing criteria used are given as follows:

1. solids retention time (SRT), the average time the solids are held in the digestion process
2. the hydraulic retention time θ, the average time the liquid is held in the digestion process.

For digestion systems without recycle, SRT = θ.

The three reactions (hydrolysis, fermentation, and methanogenesis) are directly related to SRT. An increase or decrease in SRT results in an increase or decrease in the extent of each reaction. There is a minimum SRT for each reaction. If the SRT is less than the minimum SRT needed, bacteria cannot grow rapidly enough and the digestion process will fail eventually (Metcalf & Eddy, 2003).

2.3.1.2 Temperature

Temperature is an important factor for the process that can progress under psychrophilic (<25^{0}C), mesophilic (25 - 40^{0}C) and thermophilic (>45 ^{0}C) conditions (Gomec, Letsiou, Ozturk & Wilderer, 2007). Methane forming bacteria are very sensitive to temperature changes.

Temperature not only influences the metabolic activities of the microbial population but also has a profound effect on such factors as gas transfer rates and the settling characteristics of biological solids. In anaerobic digestion, temperature is also important in determining the rate of digestion, particularly the rates of hydrolysis and methane formation. Most anaerobic digestion systems are designed to operate in the mesophilic conditions between 30 and 38^{0}C. Other systems are

designed for operation in the thermophilic temperature range of 50 to 57^0C. All biological treatment processes' performance is affected from temperature (Banik & Dague, 1997) and low temperatures in anaerobic treatment leads to low reactor performance (Kato et al., 1997).

2.3.1.3 Alkalinity

Calcium, magnesium, and ammonium bicarbonates are examples of buffering substances found in a digester. The digestion process produces ammonium bicarbonate from breakdown of protein in the raw sludge feed: the others are found in the feed sludge. The concentration of alkalinity in a digester is, to a great extent, proportional to the solids feed concentration. A well–established digester has a total alkalinity of 2000 to 5000 mg/L (Metcalf & Eddy, 2003). The principal consumer of alkalinity in a digester is carbon dioxide, and not volatile fatty acids as is commonly believed (Speece, 1996). Carbon dioxide is produced in the fermentation and methanogenesis phases of the digestion process as shown in Figure 2.1. Due to the partial pressure of gas in a digester, the carbon dioxide solubilizes and forms carbonic acid. The carbon dioxide concentration in the digester gas is therefore reflective of the alkalinity requirements. Supplemental alkalinity can be supplied by the addition of sodium bicarbonate, lime, or sodium carbonate (Speece, 1996).

In practice, two types of anaerobic digester are widely used (Metcalf & Eddy, 2003);

1. Single – Stage High – Rate Digestion
2. Two – Stage Digestion

The design of anaerobic sludge digestion processes are correlable with the understanding of the fundamental principles of biochemistry and microbiology. The design methods are based on:

1. solids retention time,
2. the use of volumetric loading factors,
3. volatile solids destruction,
4. observed volume reduction, and
5. loading factors based on population (Metcalf & Eddy, 2003).

2.4 Sludge Minimization

Sludge minimization strategies are paid more attention recently to solve the sludge related problems, reduce the investment and operational costs, and enhance the performance of the subsequent treatment and final disposal processes. Numerous strategies on sludge minimization have been reported in previous research studies (e.g. Wei et al. 2003; Liu and Tay 2001; Chu et al. 2001). Mostly applied methods for sludge reduction to date include:

- thermal treatment,
- acidic or alkali chemical treatment,
- freeze-thawing,
- mechanical disintegration using ultrasonic devices,
- advanced oxidation processes like ozonation, and
- biological hydrolysis with enzymatic treatment.

Sludge disintegration in biological systems incorporates the mechanisms:

- cell lysis transforming cell content into the medium,
- breakdown of EPS fractions in the flocculated matrix, and
- biodegradation of the end products by microbial metabolism (Mueller, 2004).

Among the methods, biological hydrolysis seems to be an effective and cost reducing process for the treatment plants using aerobic or anaerobic digesters for sludge stabilization purpose since it is at present on the chain of the sludge treatment.

Since this thesis deals with the enzymatic treatment of sludges during anaerobic digestion, enzymes and the enzyme models will be given in details in the following section of this Chapter.

2.5 Enzymes

2.5.1 Introduction

Enzymes are organic catalysts produced by microorganisms. They speed up the rate of the thousands of individual energy – yielding and cell – building reactions occuring within the cell. Generally, they are known with their two important characteristics: enzyme specificity and the rate of the reaction that it catalyzes. Specificity can be defined as an enzyme channels the transformation of a chemical along a desired pathway while the rate refers to the speed at which the desired reaction occurs when the enzyme is available. However, by having the right enzymes in the correct amounts, microorganisms can gain control and direct the pathways through which chemical reactions occur (Rittmann, B.E., & Mc Carty, P.L., 2001).

Enzyme technology has been receiving great attention with the improvements in biological techniques (Whiteley & Lee, 2006). Enzymes are used in many industries like food industry, pharmeticual industry, medical care, and also waste treatment. In envrionmental technology, previous researchers have used enzymes in activated sludge systems by the means of indicator of specific microbial populations (Hankin, L., & Sands, D.C., 1974), measure of active biomass (Teuber, M., 1977; Richards, S.R. et al., 1984), and as indicators of processes such as chemical oxygen demand and phosphorous removal (Lotter & Merwe, 1987). According to Aitken (1993), enzymes were first proposed for the treatment of waste in the 1930s, but it was not until the 1970s that enzymes were used to target specific pollutants in waste. The rapid developments and associated increase in complexity of understanding activated sludge processes and wastewater remediation warrants exploitation of different strategies for their degradation (Whiteley & Lee, 2006).

Enzymology or enzymatic treatment can be regarded as being at the interface between, on the one hand, biological discovery and protein engineering and environmental biotechnology on the other. Bioprocesses that convert one material into other using biological agents (e.g., living microorganisms or enzymes) involve biological remediation techniques that generally fall under aerobic or anaerobic digestion (Whitley, C.G., & Lee, D.-J, 2006). Lee and Whitley (2006) categorize the activated wastewater sludge processes into several divisions as shown in Figure 2.2

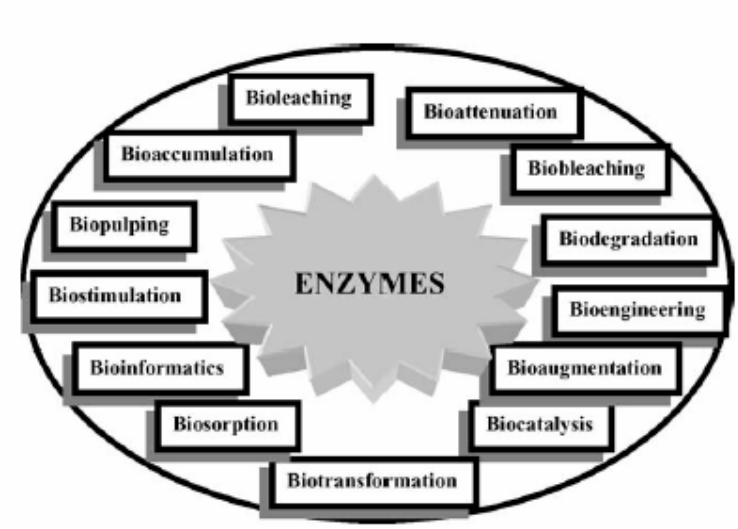


Figure 2.2 Overview of the enzymology of biological remediation (Whitley & Lee, 2006).

The overall performance of any wastewater treatment, whether in activated aerobic sludge processes or anaerobic digesters, is particularly controlled by the internal structure, composition and microbial ecology of the floc. The flocs are commonly characterized by being fragile, easily compressible, porous and permeable to fluids, irregular in shape with a networked multi-level structure (Li et al., 1990; Li et al., 1989).

2.5.2 Enzyme – Substrate Interactions

Enzymes interact with their specific substrate to form an enzyme–substrate complex [ES] that can be explained by either a 'Lock – and – Key' given in Figure 2.3 (http://www.elmhurst.edu/~chm/vchembook/571lockkey.html) or 'Induced Fit'

model shown in Figure 2.4 which then passes to a transition state [ES*] and eventually to an EP complex which dissociates into product and free enzyme (Whitley & Lee, 2006).

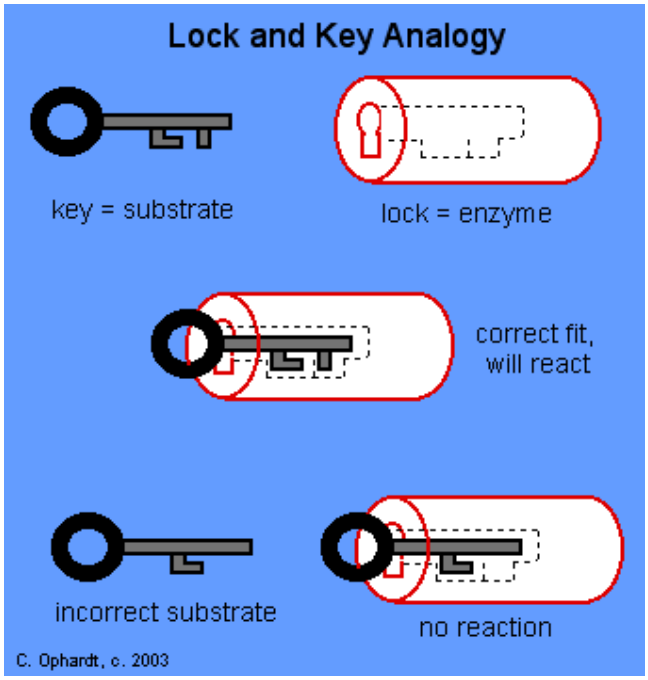


Figure 2.3 Lock-and-Key

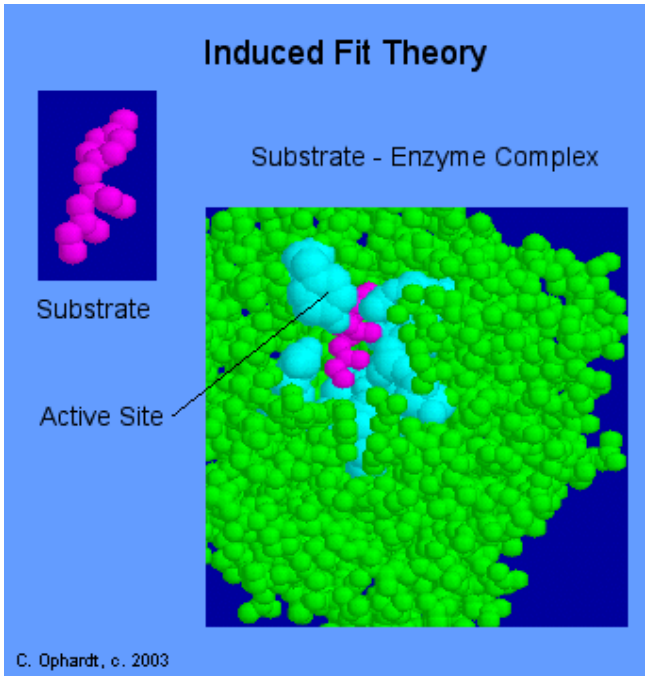


Figure 2.4 Induced Fit model

$$E + S \leftrightarrow ES \leftrightarrow ES^* \leftrightarrow EP \leftrightarrow E + P$$

In the 'Lock-and-Key' model, the active site of the enzyme is complementary in shape to that of the substrate. With the, more preferential, '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. Such catalysis takes place at the active site, within the enzyme, which provides specific, high affinity binding of substrates and provides a favorable environment for catalysis (Whitley & Lee, 2006).

2.5.3 Enzyme Classification

Enzymes are grouped into six functional classes by the Enzyme Commission of the International Union of Biochemists (Report of the Comission on Enzyme of the International Union of Biochemistry, 1961; Nomenclature Commite of the International Union Biochemistry and Molecular Biology, 1992) assigning each enzyme a unique four-digit number (Whitley & Lee, 2006).

- The first number gives the enzyme class,
- the second one symbolizes the class bond of the substrate,
- the third one to a sub-class or functional group of the substrate and
- the last one to the actual molecule

The nomenclature committee of the International Union of Biochemistry and Molecular Biology (IUBMB) defines subclasses and sub-subclasses of enzymes. Each enzyme is assigned an EC (Enzyme Commission) number. For example, the EC number of catalase is EC1.11.1.6. The first digit indicates that the enzyme belongs to oxidoreductase (class 1). Subsequent digits represent subclasses and sub-subclasses (http://www.web-books.com/MoBio/Free/Ch2E1.htm). Regarding the IUBMB committee, the classification is recommended as given in below;

- ***Oxidoreductases*** catalyze a variety of oxidation-reduction reactions. This group includes dehydrogenase, oxidase, reductase and catalase.
- ***Transferases*** catalyze transfers of groups (acetyl, methyl, phosphate, etc.). It includes acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.
- ***Hydrolases*** catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water.
- ***Lyases*** catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation. It includes decarboxylase and aldolase.
- ***Isomerases*** catalyze atomic rearrangements within a molecule. Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase.
- ***Ligases*** catalyze the reaction which joins two molecules. Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.

Among the groups given above, hydrolytic enzymes like glucosidases, lipases, and proteases have an imperative function at the hydrolysis stage of complex organic structures in the degradation of biodegradable particulate organic matter. As a key factor, extracellular polymeric substances – EPS control the extracellular hydrolytic enzymes in this degradation mechanism. A flocculated matrix of EPS bridging with bacteria holds back the dewaterability properties of the bioprocessed sludges. Disruption of the flocculated matrix leads to improved solubilization of sludge solids by attacking of the hydrolytic enzymes to polymeric substances forming enzyme-substrate complexes (Ayol et al., 2007).

2.5.4 Extracellular Polymeric Subtances In Sludge

Lee and Whiteley have stated that the extracellular polymeric substances (EPS), produced from bacterial cellular metabolism, cell autolysis and the wastewater itself, cement sludge components together and consequently any hydrolysis of the EPS would lead to significant changes in sludge structure (Wingender et al., 1999).

The production of EPS is accepted as a general property of microorganisms in natural environments (Wingender et al.,1999). EPS form a highly hydrated biofilm matrix, in which the micro-organisms are embedded. Hence, they have importance in the removal of pollutants from wastewater, in bioflocculation, in settling and in dewatering of activated sludge (Neyens et al., 2004).

Effective dewatering requires the attack of the microorganisms. The vast majority of these micro-organisms live in aggregates such as films, flocs and sludges. Films, flocs and sludges are accumulations of microorganisms, EPS, multivalent cations, biogenic and inorganic particles as well as colloidal and dissolved compounds (Neyens et al., 2004). EPS as the key to the physicochemical and biological properties are mainly responsible for the structural and functional integrity of the aggregates in sludge (Flemming & Wingender, 2001). EPS form a three-dimensional, gel-like, highly hydrated and often charged biofilm matrix, in which the micro-organisms are embedded and more or less immobilized (Wingender et al., 1999).

EPS fractions like polysaccharides, proteins, DNA, and humics- have crucial role in the removal of pollutants from wastewater, in bioflocculation and settling (Eriksson & Alm, 1991; Bruus et al., 1992; Urbain et al., 1993), and in the dewatering of activated sludge (Urbain et al.,1993; Pere et al., 1993; Forster & Lewin, 1972; Li & Ganczarczyk, 1990; Sanin & Vesilind, 1994).

In activated sludge, between 70 and 80% of the extracellular organic carbon could be attributed to proteins and polysaccharides (Dignac et al., 1998). Li and Ganczarczyk (1990) found that EPS form the third biggest component in an activated sludge floc after the cells and water (Neyens et al., 2004). Some researchers have suggested that polysaccharides play the major role in flocculation: Bruus et al. (1992) indicated that divalent cations bridge negatively charged groups on alginate-like polysaccharides within bioflocs (Neyens et al., 2004).

The water retained in the EPS-structure is bound mainly by the polysaccharides and proteins of the EPS in the activated sludge. The molecular mechanisms of water

binding are of crucial importance for a rational basis of the improvement of dewatering techniques (Neyens et al., 2004).

In recent years, new technologies have been introduced to advance the biological decomposition of activated sludge by anaerobic digestion. 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. The major EPS fractions are proteins, polysaccharides, and humics. 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, it is privileged than aerobic conditions (Ayol et al., 2007a).

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. Previous work regarding hydrolysis kinetics on anaerobic degradation of particulate organic matter has pointed out that the EPS as a network detains the extracellular hydrolytic enzymes (Vavilin et al., 1996). 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., 2007a).

As given above, anaeobic digestion is one of the most extensively applied process for sludge treatment, but the process has a rate – limiting step in biological hydrolysis that causes generally a process slowdown with consequent requirement of large digester volumes (Eastman & Ferguson, 1981; Shimizu et al., 1993). In order to improve hydrolysis of anaerobic digestion performance and to improve the dewaterability characteristics of digested sludge, enzymatic treatment of sludge was investigated in this research.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

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

3.2 Materials

3.2.1 Raw Sludges

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

At the start-up of the reactors as inoculum, granular anaerobic sludge was taken from a full scale Upflow Anaerobic Sludge Blanket (UASB) reactor treating beer industry wastewaters of Efes Pilsen Inc., Izmir.

3.2.2 Anaerobic Reactors

Anaerobic digestion studies used three pilot scale anaerobic reactors with 13.5-L volume each. The reactors were operated at 37 ± 3 °C under mesophilic conditions. The reactors were heated by thermostats and the temperature was kept constantly by heat transfer oil jacket.

The anaerobic reactors were constructed from stainless-steel and operated with PLC. The reactors were mixed by mechanical mixers. Figure 3.1 illustrates the reactors used in this work.



Figure 3.1 Anaerobic reactors used in this study

First reactor was used as control reactor without enzyme addition while the others were operated with different enzymes additions.

3.2.3 Chemicals

Different hydrolytic enzymes were added to the reactors. The enzymes used in experimental studies 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 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 3.2.

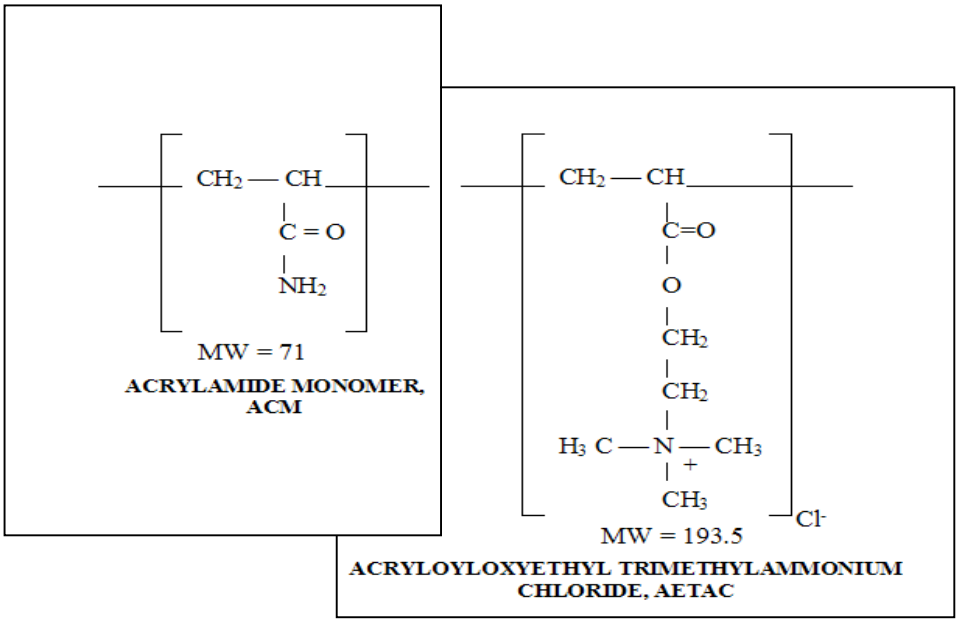


Figure 3.2 The structure of polymer used Percol 757, 80% cationic copolymer (ACM/AETAC), molecular mass ~ 1.8x 106 (Ayol et al. 2006)

3.3 Methods Used in Experimental Studies

3.3.1 Analytical Methods

For performance evaluations, dry solids content (DS), water content (WC), volatile organic matter content (VS), suspended solids(SS), temperature, alkalinity, total gas and methane productions, gas composition in the biogas (CH_4, CO, CO_2, H_2S), redox potential, pH, total organic carbon (TOC), volatile fatty acid (VFA), capillary suction time (CST), zeta potential (ZP), 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. Confidence intervals are at the 95% significance level.

3.3.1.1 Total Gas and Methane Analysis

Total gas and methane productions of the anaerobic reactors were determined by liquid displacement method which gas passes through distilled water including 2%(v/v) H_2SO_4 and 10% (w/v) NaCl for total gas and 3%(w/v) NaOH for methane. Figure 3.3 shows the gas measuring system used in the study.



Figure 3.3 Total and methane gas measuring system used in this work.

To measure the amount of gas produced, reactors' gas valves were first closed about 1 hour and then they were opened. The liquid displacements were measured at 1 minute intervals about 20 minutes. The total liquid displacements were converted to the daily productions.

Gas components in the total gas produced (CO, CO_2, CH_4, and H_2S) were daily analyzed using a Dräger model X-am 7000 multigas analyzer. Figure 3.4 shows the multi gas analyzer used in the experimental studies.



Figure 3.4 Dräger model X-am 7000 multigas analyzer

3.3.1.2 Volatile Fatty Acid (VFA) Analysis

High Pressure Liquid Chromatography was used in VFA analyses. A HPLC, Agilent 1100 model equipped with C18 column was used in the experimental studies.

3.3.1.3 Redox Potential and pH Measurements

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

3.3.1.4 Particle Size Analysis

Particle size distributions were monitored using a Malvern Mastersizer 2000QM analyzer shown in Figure 3.5.



Figure 3.5 Malvern Mastersizer 2000QM particle size analyzer.

3.3.1.5 Ca, K, Mg, Na Analysis

Ca, K, Mg, Na concentrations were analyzed using a ICP-QMS (Perkin Elmer-Optima 2100DV).

3.3.1.6 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 obtained from Merc. 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).

3.3.1.7 Summary of Acid Digestion Method

For the digestion of samples, a representative 1 gram (dry weight) sample is digested with addition of hydrocloric 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.

3.3.1.8 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 centrats were diluted to 1/25 with pure water before analysis.

3.3.1.9 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).

3.3.1.10 Protein and Polysaccharide Analysis

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 explained in elsewhere (Ayol, 2005).

3.3.1.11 Capillary Suction Time Test

Capillary Suction Time Test (CST) was also used for the evaluation of the dewatering performances of the sludge samples either taken from reactors or polymer conditioned. CST values were determined 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 3.6 shows the capilary suction time analyzer used in this study.



Figure 3.6 Capillary Suction Time Analyzer used in this work

3.3.1.12 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 compared with CST results. Sample preparation for zeta potential measurement and zeta potential test procedure are done according to procedure given by manufacturer. Figure 3.7 shows the zeta meter used in this study.

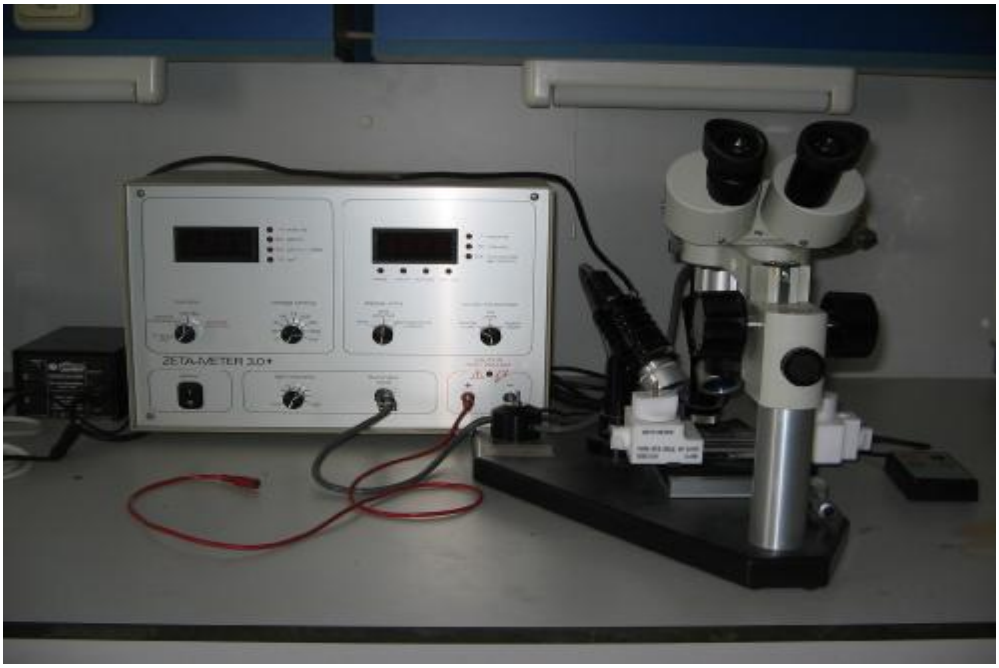


Figure 3.7 Zeta Meter system 3.0+ used in experimental evaluations

3.3.1.13Viscosity 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.

3.3.1.14 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 anaerobically digested samples, more recently developed mechanical device called Crown press (purchased from Phipps and Bird, USA),

simulates full scale belt filter presses, was used. 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 3.8 shows the drainage kit and the Crown press used in this work.



Figure 3.8 Gravity drainage plow simulator kit and Crown press used in this study

To evaluate the filterability of these samples, capilary suction time (CST) analysis were done and the dry solid contents of the dewatered samples were analyzed according to Standard Methods (APHA, 2005).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Introduction

This thesis mainly focused on the effects of hydrolytic enzymes on anaerobic digestion. This chapter gives the results and evaluations of the experimental studies. The experimental approach initially used 25 days of hydraulic retention time in the batch experiments at the mesophilic conditions, 37 ± 3 °C for sludge digestion with and without enzyme additions. Depending on the first batch results, the operation time decreased to 15 days. This chapter gives the experimental results depending on the operation time used in the experimental studies.

4.2 Start-up of the Reactors

Inoculum sludge was fed to the anaerobic reactors which was provided from a full scale UASB reactor treating beer industry wastewaters of Efes Pilsen Inc., Izmir. Three anaerobic reactors with 13.5 L of working volume were operated. At start-up phase, 12 L of inoculum sludge was first fed to the reactors and the sludge was witdrawn 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. The same operation was done again up to 1/3 volume of the reactor content. After the start-up phase, 1/3 volume of the reactor content was kept constantly by using automatic valves while rest of them was withdrawn.

4.3 Sludge Characteristics

The characteristics of waste activated sludge taken from Guney Bati Municipal Wastewater Treatment Plant, Izmir and the granular anaerobic sludge used as inoculum taken from Efes Pilsen WWTP were given in Table 4.1 for 25 days and 15 days of operation time, respectively.

Table 4.1 Activated sludge and anaerobic inoculum sludge characteristics for 25 days and 15 day of operation time

Parameters	**25 days of operation time**		**15 days of operation time**	
	Activated sludge	**Anaerobic inoculum sludge**	**Activated sludge**	**Anaerobic inoculum sludge**
Dried solids (DS, %)	1.69	3.44 ± 0.47	1.21±0.23	7.01 ± 0.85
Volatile solids (VS, %)	54.26 ± 0.47	82.17 ± 0.14	66.41 ± 0.52	84.82 ± 0.36
Suspended solids (SS, mg/L)	10300.25	45566.67	7100.00	45100.42
pH	8.02	8.52	6.88	8.52
Redox potential (ORP, mV)	+37.0	-198.00	+98.6	-198.00
Dissolved oxygen (DO, mg/L)	2.10	0.30	2.50	0.20
Capillary suction time (CST, s)	35±14.27	92.93 ± 8.21	16±0.36	83.26 ± 7.16
Zeta potential (mV)	-22.00 ± 1.25	-52.83 ± 1.19	-24.00 ± 4.37	-50.06 ± 7.00
Particle Size (μm)-				
Surface weighted mean D[3,2]	24.389	93.705	29.365	101.124
Volume weighted mean D[4,3]	70.134	526.432	94.136	458.148
d (0.5)	51.165	433.559	65.144	425.136
d (0.9)	135.859	1202.893	157.845	954.122
d (0.1)	14.255	37.299	25.336	45.387
Viscosity (mPa.s,@30 1/s shear rate)	7.36	124.86	9.54	142.55

4.4 Enzyme Additions

The first reactor of anaerobic unit denoted as R1 was the control reactor with no enzyme addition. Different hydrolytic enzymes (0.1%) were dosed to the second and third reactors. Enzyme 1 and Enzyme 2 were added to the second (R2) and third (R3) reactors, respectively.

The effects of enzyme additions to the reactors were examined and volatile solids reduction, gas production, dewatering properties for control and enzyme added reactors were evaluated at the operation periods.

4.5 Performance Evaluations for 25 Days of Operation Time

4.5.1 pH Results

pH is an indicator for stability of anaerobic digestion process. All operation period, pH of the control and enzyme added reactors was measured between 8.0 to 8.8. It shows the appropriate conditions for methanogenic activity. The measurements were daily done and no high range differences between pH values was obtained. The results are given Figure 4.1.

4.5.2 Temperature Monitoring

Most of the anaerobic digestion systems are designed in the mesophilic temperature range between 30 and 38^0C. It affects the gas transfer, especially methane formers and their activities. The temperature of anaerobic reactors for this operation period was kept at 37 ± 3 °C. The temperature measurements are shown in Figure 4.2.

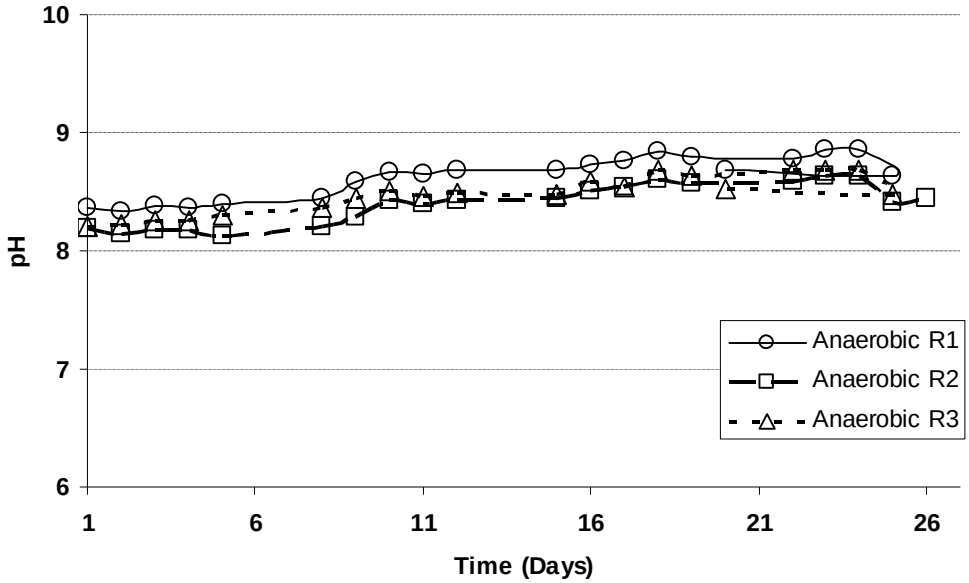


Figure 4.1 pH values during the operation time-25 days.

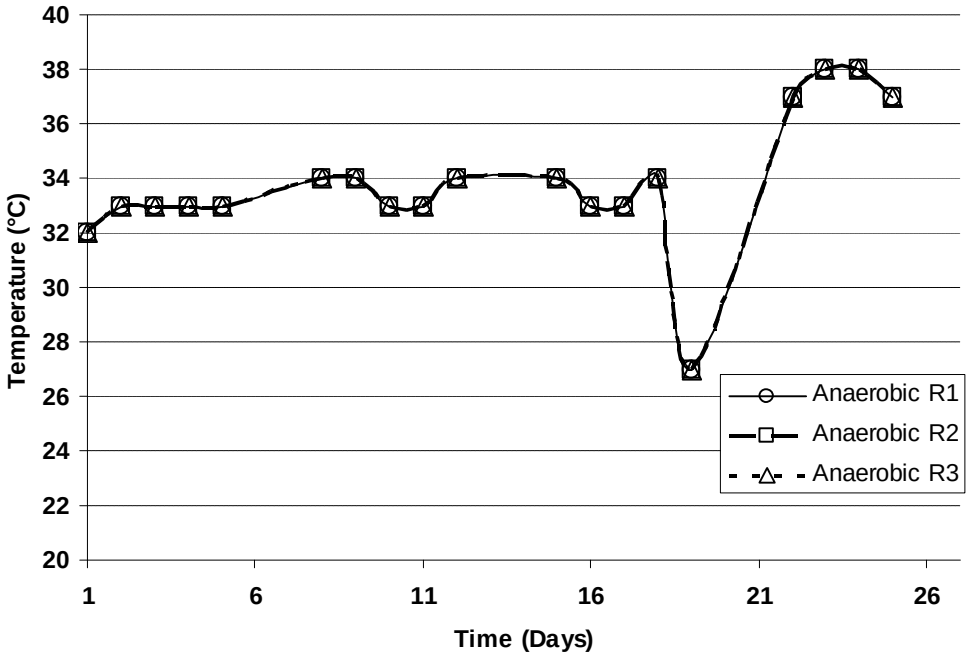


Figure 4.2 Temperature changes during the operation time-25 days.

4.5.3 Oxidation Reduction Potential (ORP)

Oxidation – reduction potential (ORP) "redox" indicates the relative capacity of a solution to oxidize or reduce. For anaerobic digestion, ORP values should be in very negative range. Daily ORP measurements were obtained lower than -200 mV. The measurements are depicted in Figure 4.3.

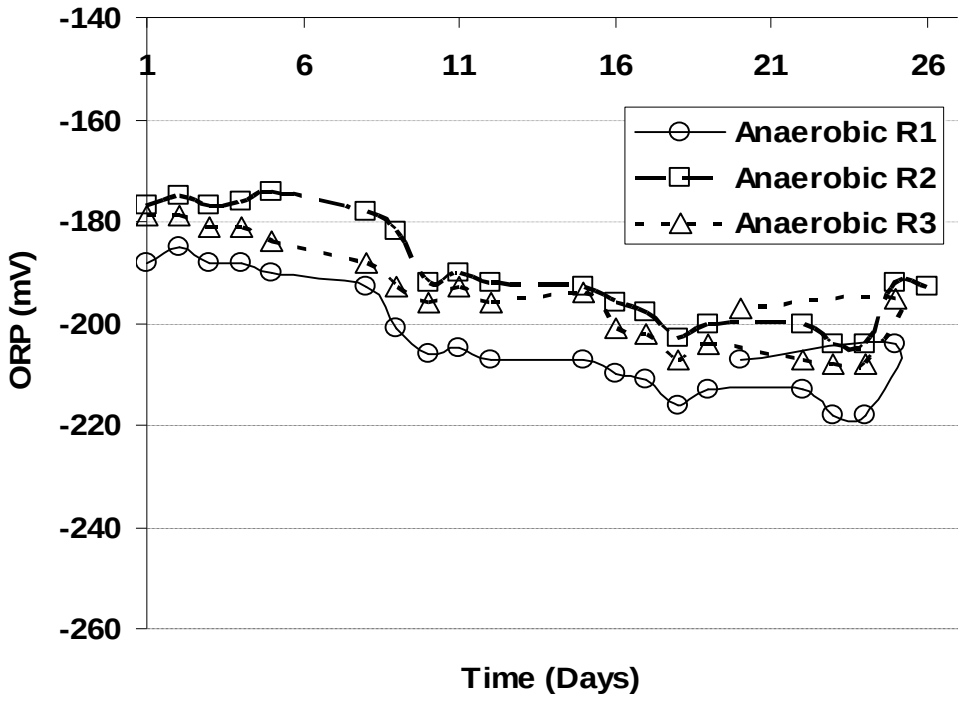


Figure 4.3 Oxidation-Reduction potential results during the operation period-25 days

4.5.4 Alkalinity

In the consequence of breakdown of protein in sludge, ammonium bicarbonate is produced by digestion period. A well–established digester has a total alkalinity of 2000 to 5000 mg/L (Metcalf & Eddy, 2003). Total alkalinity measurements in the reactors were done three times in a week. The range of alkalinity was measured 1688 – 4000 mg $CaCO_3$/L. Figure 4.4 illustrates the alkalinity results.

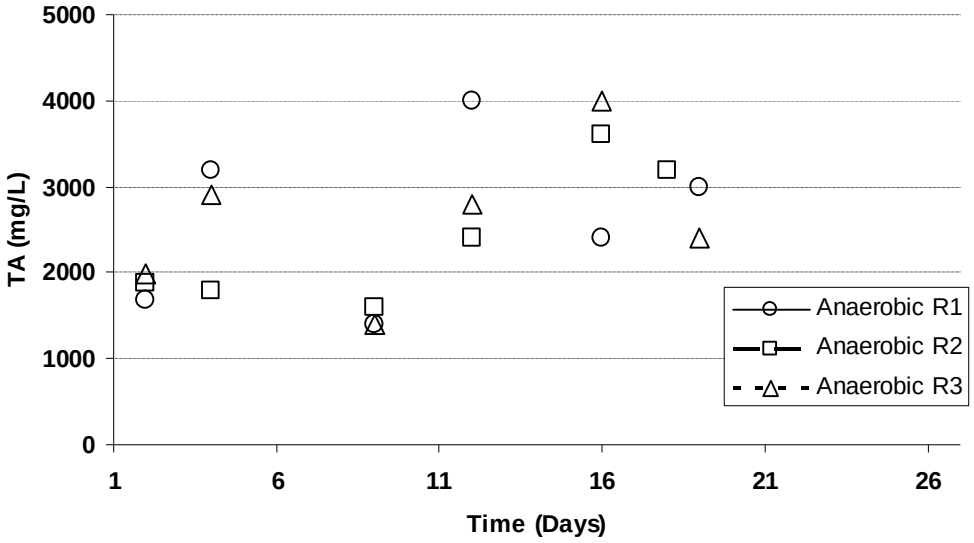


Figure 4.4 Total Alkalinity of reactor on operation time

4.5.5 Dried Solids

Concentration of dried solids was measured between 3% and 5% for the reactors. In first 10 days, lower DS values were obtained for enzyme added reactors than control reactor. DS values decreased and gave a maximum point at first week operation. After the maximal point, DS values gave a plateau. The results are shown in Figure 4.5.

4.5.6 Volatile Solids Reductions

Better volatile solids reductions were observed for enzyme added reactors comparing to control ones for anaerobic unit. Although control reactor performance looks better in the first 10 days, it did not change after this operation time. %27.5, %37.8, and %43.4 VS reductions were obtained for R1, R2, and R3 reactors of anaerobic unit, respectively. Enzyme 2 added reactor (R3) gave lower VS values than Enzyme1 added reactor. The VS results are plotted in Figure 4.6. These results are also supported by measured total gas productions during the operation period as given in the following section.

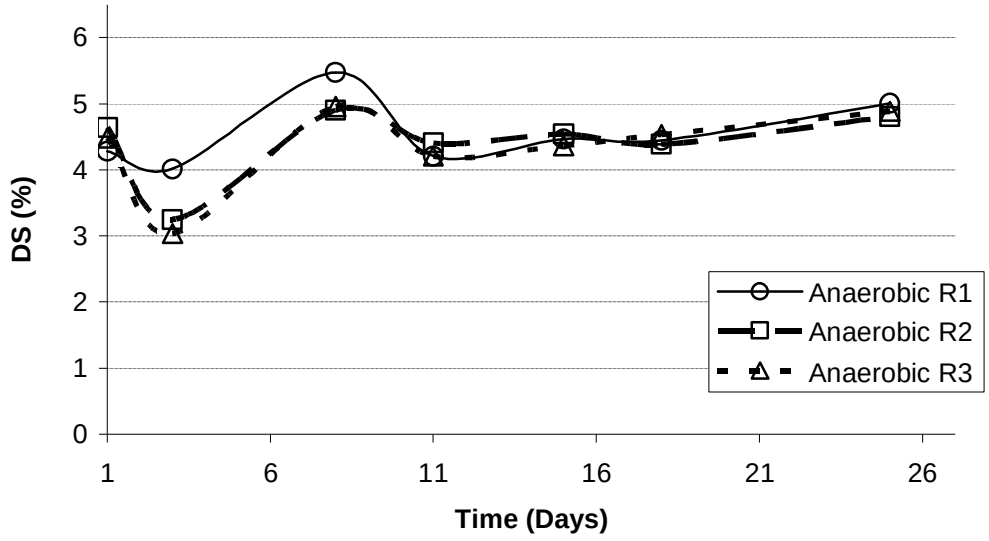


Figure 4.5 DS changes during the operation period- 25 days

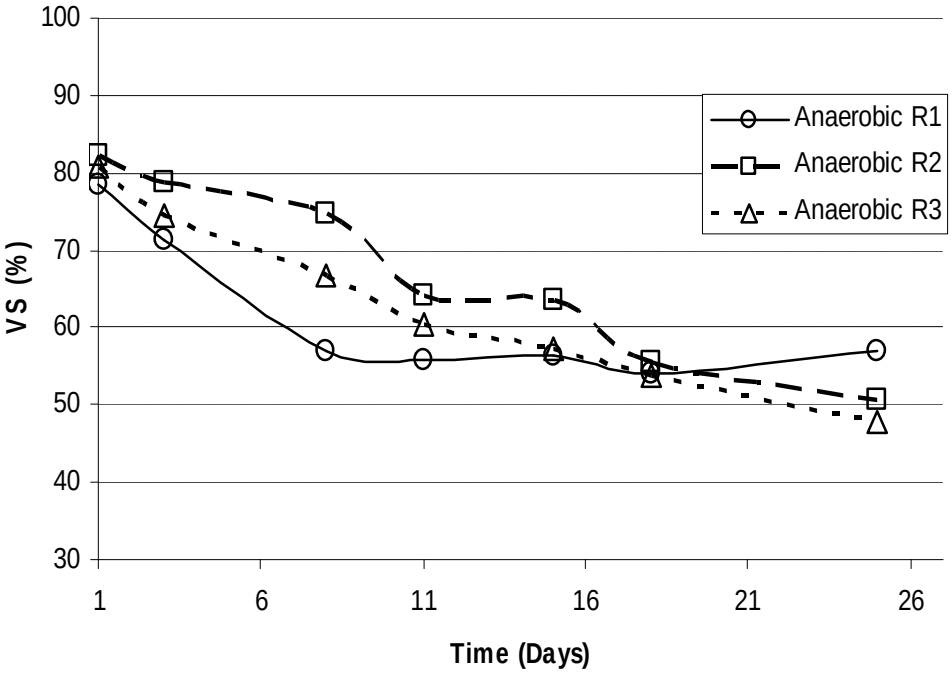


Figure 4.6 VS changes as a function of operation period-25 days

4.5.7 Total Gas and Methane Productions

At the first day, high gas productions were determined in all anaerobic reactors. The enzyme – treated sludge showed an increase in the gas production comparing to the control reactor. Enzyme added reactors gave almost two times higher gas productions. Depending on the reductions of the VS values, it was expected to decreases in total gas production. Although this was not the case, methane percentages in the total gas were getting lower. Total gas production as a function of operation period is given in Figure 4.7.

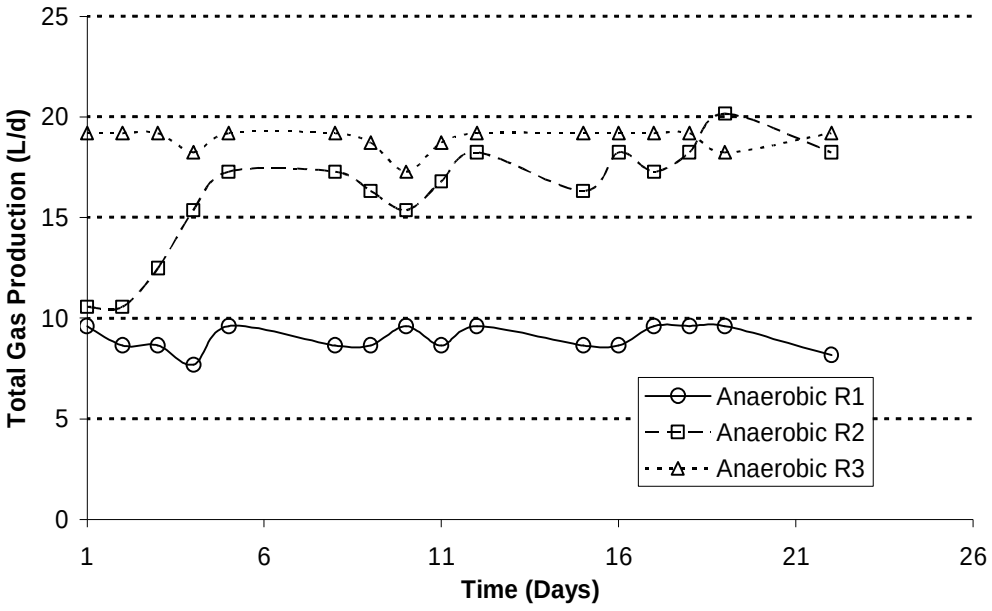


Figure 4.7 Total gas production during the operation period-25 days

Especially after 10 days operation, while methane gas percentage in the total gas was decreasing, higher H_2S concentrations were recorded. The reason might be lessened methanogenic activity and improvement in sulphidogenic conditions. Table 4.2 shows the maximum values of the measured methane and H_2S components.

Table 4.2 Maximum values of measured gas components

Reactors	**Between 1 to 15 days operation**		**Between 20 to 25 days operation**	
	CH_4 (%LEL)	H_2S (ppm)	CH_4 (%LEL)	H_2S (ppm)
AnaerobicR1	35.0	1.0	16.0	68.0
AnaerobicR2	69.0	2.0	23.0	38.0
AnaerobicR3	62.0	2.0	21.0	44.0

4.5.8 Total Organic Carbon Results

Total organic carbon (TOC) provides a speedy and convenient way of determining the degree of organic contamination. A carbon analyzer using an infrared detection system is used to measure total organic carbon. Organic carbon is oxidized to carbon dioxide. By using TOC measurements, the number of carbon-containing compounds in a source can be determined (http://kywater.org/ww/ramp/rmtoc.htm).

TOC measured using aliquots of the centrifugated samples called “soluble TOC”. Table 4.3 shows TOC values measured.

Table 4.3 TOC values of the reactors as a function of operation time.

Reactor – Day	**TOC (mg/L)**
Anaerobic R1 – 1 day	1129.4 mg/L
Anaerobic R2 – 1 day	833.6 mg/L
Anaerobic R3 – 1 day	990.8 mg/L
Anaerobic R1 – 25 day	880.2 mg/L
Anaerobic R2 – 25 day	430.0 mg/L
Anaerobic R3 – 25 day	724.2 mg/L

4.5.9 Volatile Fatty Acids (VFA) Results

The anaerobic digestion process is more stable when the volatile fatty acid (VFA) concentrations approach a minimal level, which can be taken as an indication of a sufficient methanogenic population. The rate – limiting step is the conversion of VFAs by the methanogenic organisms and not the fermentation of soluble substrates by the fermenting bacteria (Metcalf & Eddy, 2003).

Volatile fatty acids (VFA) content was also measured for the determining of the reactor stability. VFA did not exceed 1700 mg HAc/L for all anaerobic reactors except for the start-up phase. VFA values were measured between 2000 and 3600 mg HAc/L in the start – up phase. It is recommended that VFA values should not exceed 1000–1500 mg/L for anaerobic methanogens (Malina & Pohland, 1992).

4.5.10 Capillary Section Time (CST) Results

The time the filtrate requires to travel a fixed distance in the filter paper is referred to as capillary suction time (CST). The whole purpose of Capillary Suction Time Test is to determine dewatering characteristics of a given sludge rapidly and easily. The advantages of this test is to be simple, inexpensive, and easy. A high CST is usually indicator of a poor sludge dewaterability (EPA, 1987, p.50; Eckenfelder et al., 1981, p.77; Vesilind, 1988, p.216).

To optimize the operation of chemical sludge conditioning, CST parameter has been widely used by many researchers (Cristensen et al., 1993; Chitikela et al., 1998; Wu et al., 2000; Lin et al., 2001; Wu et al., 2002). Dewaterability of sludges depends on many factors, such as, particle size, particle charge, pH, solids concentration, organic content, etc (Yukseler et al., 2007).

As commonly accepted index of dewaterability, CST analyses were daily done using samples taken from reactors to evaluate the filterability performances of

enzyme added reactors and the control rector. All measurements were done in triplicate.

Experiments showed that the enzymes improved the dewaterability performances of the sludge. The results are also consisted with the EPS analyses given in the following sections of this chapter. The CST measurements are given in Figure 4.8.

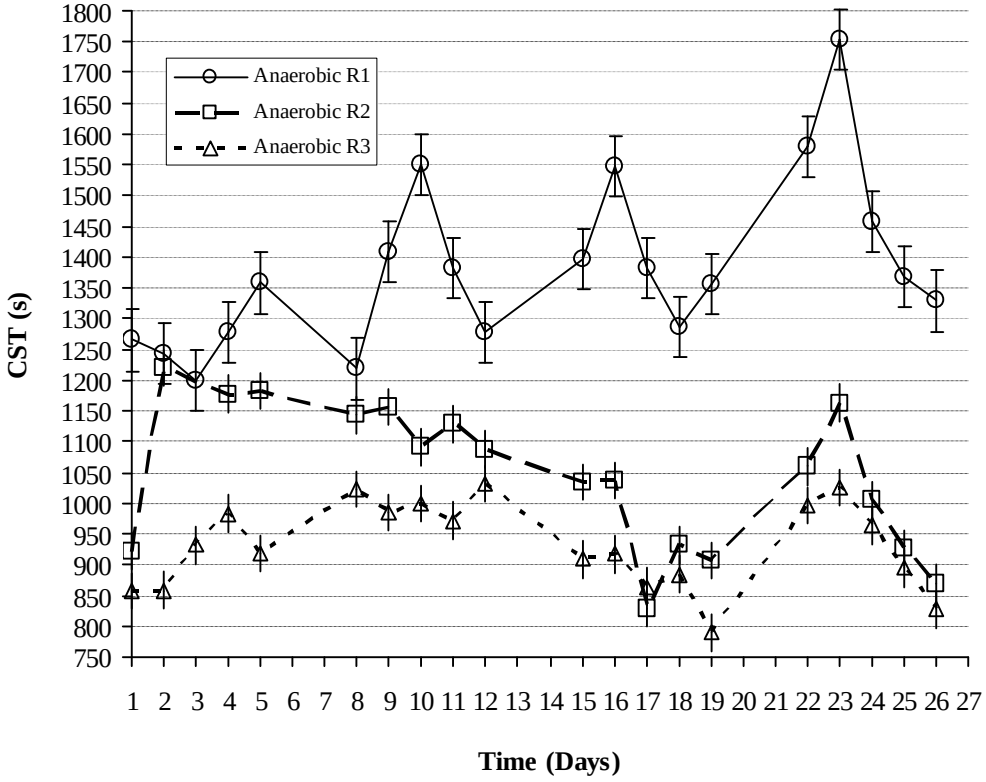


Figure 4.8 CST results as a function of operation time

4.5.11 Particle Size Distribution

Particle size distribution can be used as an indicator of EPS degradation. The changes in the particle size distributions demonstrated that the floc disintegration occurred for the operation period. Reductions in particle size can be clearly seen from Table 4.4. Watson et al. (2004) reported dramatic decreases in floc sizes of enzyme added sludges.

Table 4.4 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)
R1- 1day	62.963	436.314	23.625	273.926	1097.034
R2- 1day	62.863	393.586	23.478	273.330	1018.684
R3- 1day	64.410	436.961	23.876	315.648	1063.157
R1- 22day	66.031	454.996	24.330	344.744	1096.054
R2- 22day	55.271	376.682	21.175	210.890	905.971
R3- 22day	56.412	378.011	21.414	219.139	969.689

4.5.12 EPS – Protein and Polysaccharide Results

Enzyme1 and Enzyme2 enhanced the degradation of extracellular polymeric substances. Protein and polysaccharide concentrations of the samples decreased with the operation time in all reactors, but, the reductions were much higher than control reactors. Higher protein concentrations were measured in the reactors. Protein concentrations were almost two times greater than polysaccharide concentrations in anaerobic reactors. Novak et al. (2003) have reported 3 – 5 times higher protein concentrations when comparing to polysaccharide concentration in anaerobic conditions. The protein and polysaccharide results are depicted in Figures 4.9 and 4.10, respectively.

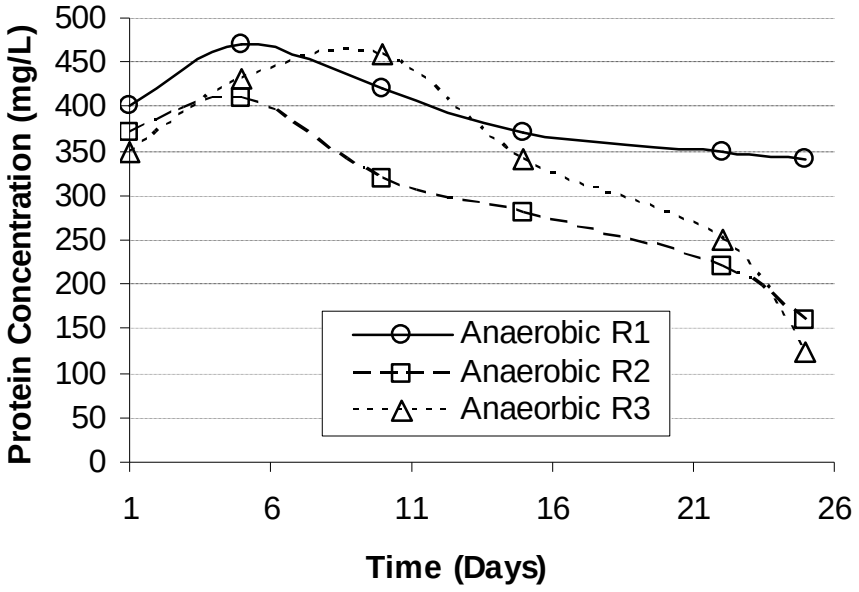


Figure 4.9 Protein concentrations as a function of operation time

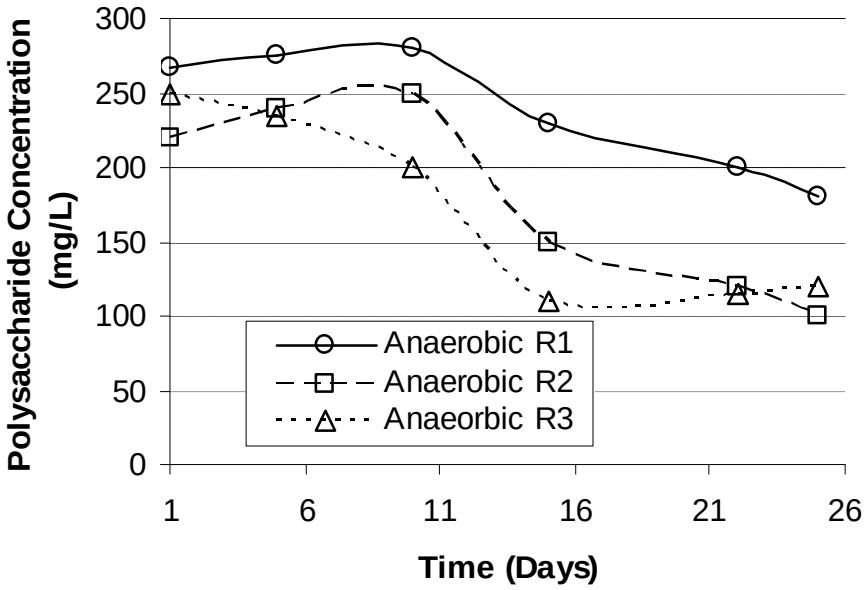


Figure 4.10 Polysaccharide concentrations as a function of operation time

Greater enzyme activity measurements were monitored in enzyme added reactors than control reactor. The activity measurements are given in Figure 4.11.

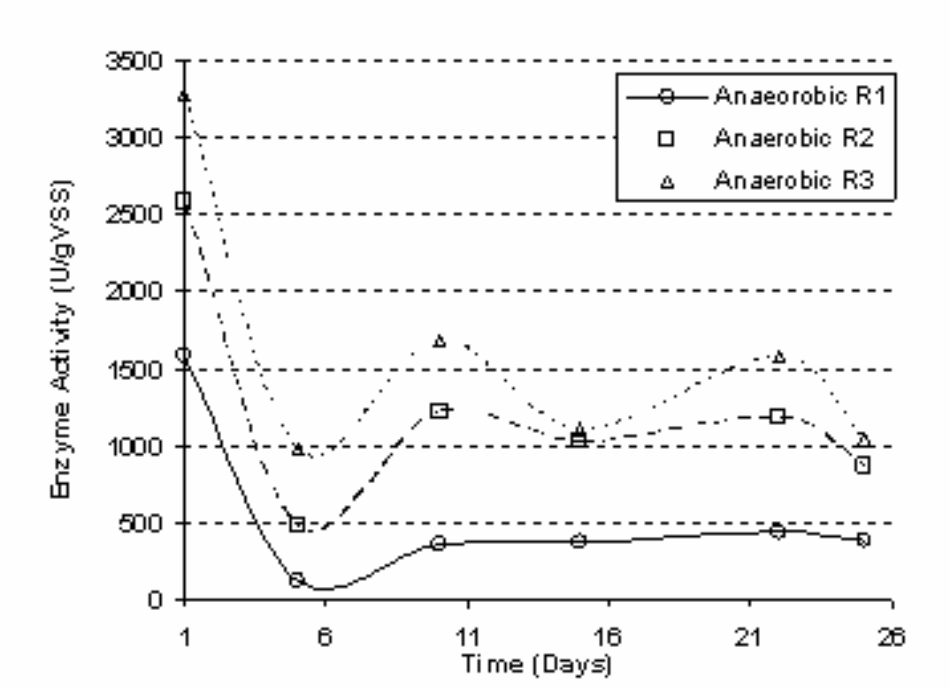


Figure 4.11 Enzyme activity changes as a function of operation time

4.5.13 Sludge Conditioning

Cationic polymers are the predominant form of organic additive used in the conditioning of wastewater sludges, although it may be technically feasible to use either cationic surfactants or anionic polymer some cases (Chitikela et al., 1998).

At the end of the operation time, digested sludges were taken from the reactors for further conditioning analyses. Polymer conditioning was applied to the sludge as explained in Materials and Methods. CST measurements of the conditioned sludge samples at different dosages of cationic organic polymer are shown in Tables 4.5-4.7.

Table 4.5 CST results of the conditioned sludge samples for R1

Polymer addition (mg/L)	Polymer addition (kg/ton)	Average of CST (sec)
0	0	1331.7
25	0.065	1309.7
50	0.129	1047.467
100	0.258	936.667
200	0.517	94.667
250	0.646	18.33

Table 4.6 CST results of the conditioned sludge samples for R2

Polymer addition (mg/L)	Polymer addition (kg/ton)	Average of CST (sec)
0	0	1123.6
50	0.05	1043.6
100	0.231	542.9
200	0.463	550.967
250	0.579	5.467
350	0.810	3.767

Table 4.7 CST results of the conditioned sludge samples for R3

Polymer addition (mg/L)	Polymer addition (kg/ton)	Average of CST (sec)
0	0	834.5
50	0.113	650.4
100	0.227	447.1
200	0.454	21.6
250	0.567	5.73
350	0.794	4.36

Optimal doses as kg/ton were shaded with gray color in the Tables. As can be seen from the tables, polymer dose requirement decreased for the enzyme added sludges.

4.6 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 at first 10 – 12 days of the operation. Therefore, second batch experiments used 15 days of operation time. The results for 15 days operation for the same amount of enzyme addition (0.1%) are given in the following sections.

4.6.1 pH Results

The pH measurements for the operation period demonstrated similar results to first operation period-25 days. The results are shown in Figure 4.12.

4.6.2 Temperature Monitoring

Temperature was monitored for 15 days operation time at 37 – 39 ^{0} C. The results are given Figure 4.13. There were no fluctuations in the temperature during the operation.

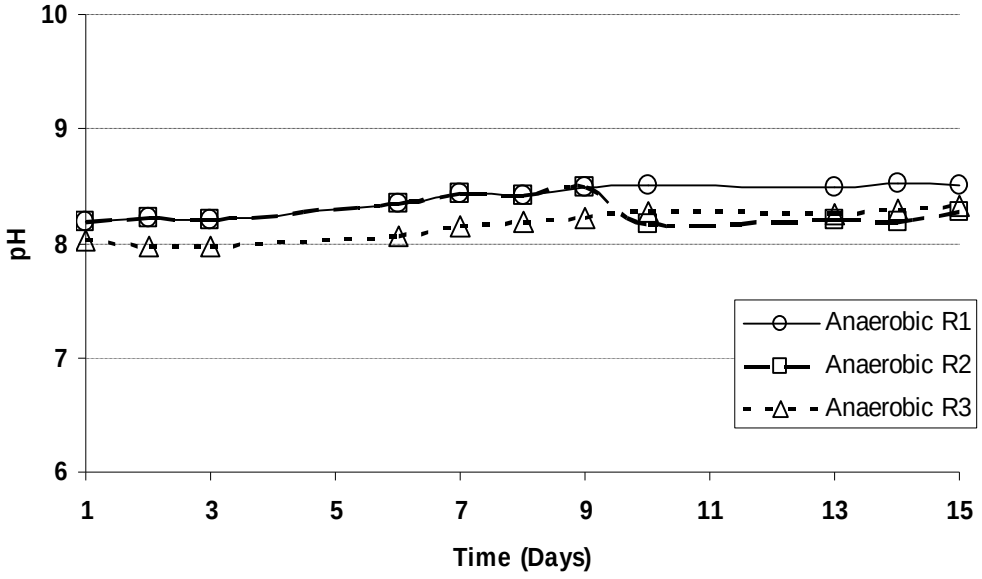


Figure 4.12 pH values during the operation time-15 days.

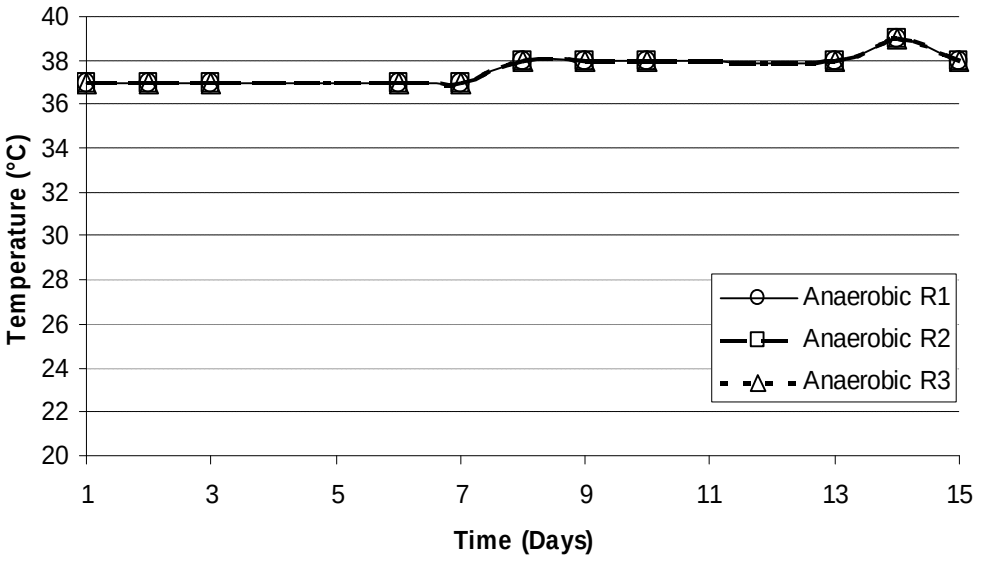


Figure 4.13 Temperature changes during the operation time-15 days.

4.6.3 Oxidation Reduction Potential (ORP) Results

For performance evaluations of the reactors, Redox potential (ORP) was daily measured. ORP values were recorded very negative values less than -280 mV in this operation period. Figure 4.14 shows the ORP measurements.

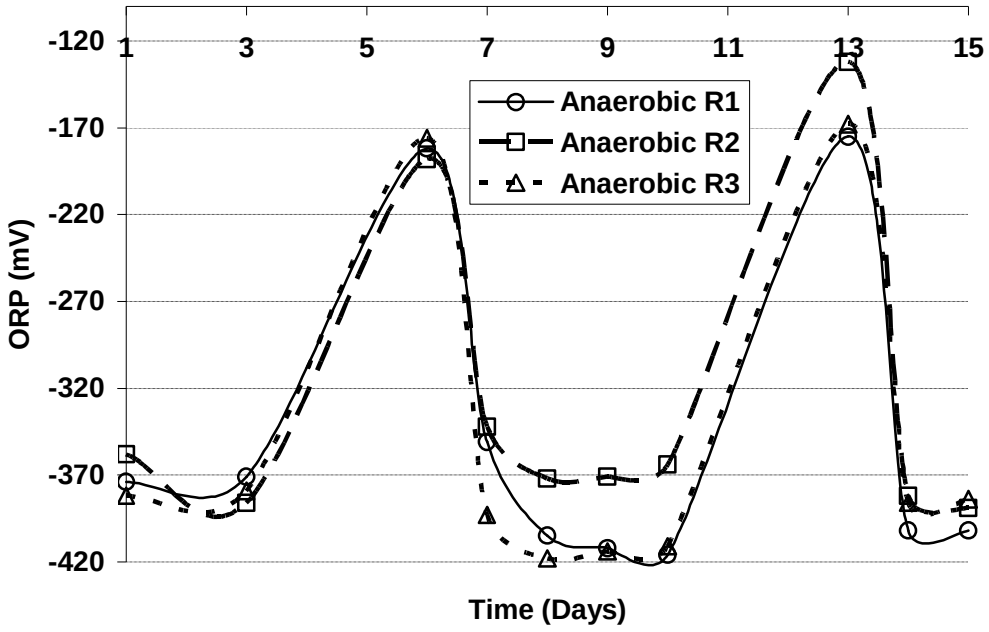


Figure 4.14 ORP results during the operation period-15 days

4.6.4 Alkalinity

Total alkalinity values were measured three times in a week. An alkalinity range of 2000- 4000 mg $CaCO_3$/L was measured for all the reactors.

4.6.5 Dried Solids

Dried solids contents of the sludge taken from the reactors were measured between 2.5% and 5% for this operation time. Enzyme added reactors had lower DS values than control reactor as in the first operation period. At first week, DS values decreased and then a fluctuation was observed for all reactors. The results are given in Figure 4.15.

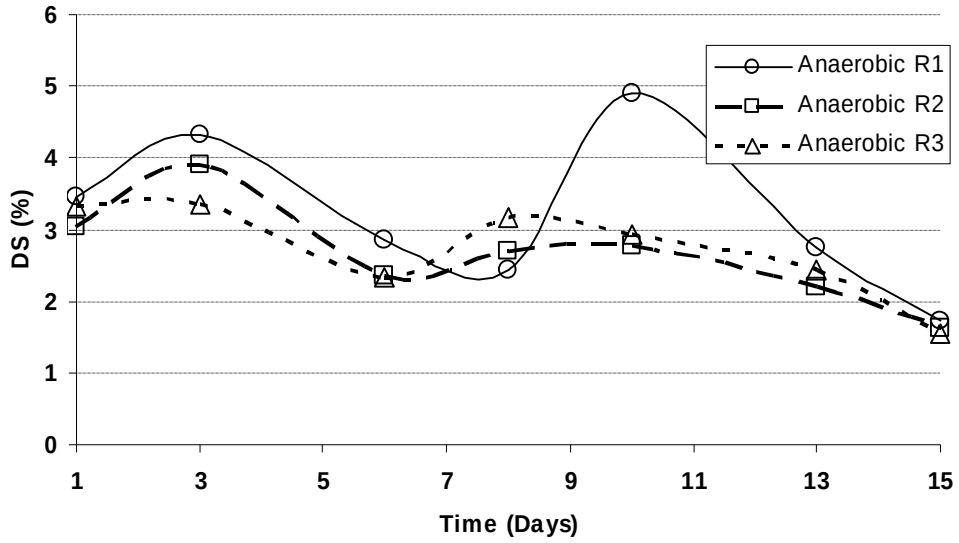


Figure 4.15 DS changes during the operation period- 15 days

4.6.6 Volatile Solids Reduction

The VS removal increases noticeably for enzyme added sludges. Enyzme added reactors (R2 and R3) showed better performance in terms of volatile solids destructions comparing to control one (R1). 18% and 26% higher VS reductions for R2 and R3, respectively, were obtained. VS measurements are shown in Figure 4.16.

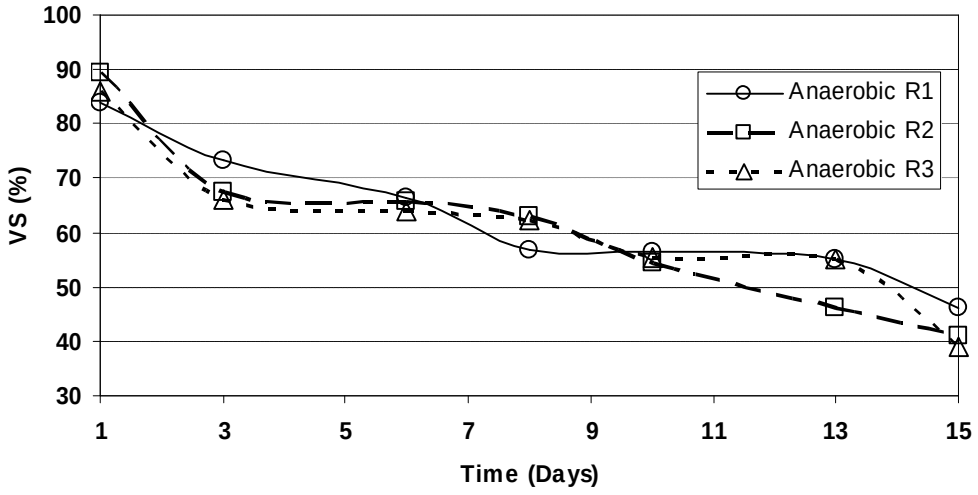


Figure 4.16 VS changes as a function of operation period-15 days

These results were consisted with measured total and methane gas productions during the operation period.

4.6.7 Total Gas and Methane Productions

Anaerobic R2 reactor had high gas production at the first day. The enzyme – treated sludge showed an increase in the gas production comparing to the control reactor. 25% and 32% higher methane gas productions were recorded than as in the control reactor. The results are shown in Figure 4.17.

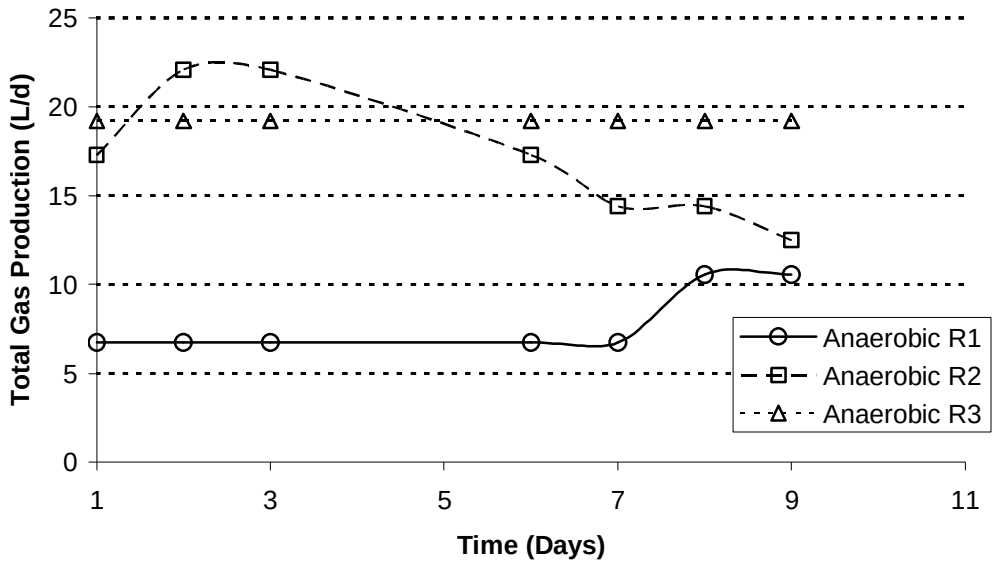


Figure 4.17 Total gas productions during the operation period-25 days

4.6.8 Total Organic Carbon

The enzyme additions improved the hydrolysis of the organic sludge content matter and thus lead to an extensive degradation in the digester content. Table 4.8 shows some TOC results.

Table 4.8 TOC values of reactors during the operation time

Reactor – Day	TOC (mg/L)
Anaerobic R1 – 1 day	479.4
Anaerobic R2 – 1 day	374.0
Anaerobic R3 – 1 day	344.2
Anaerobic R1 – 15 day	523
Anaerobic R2 – 15 day	136.075
Anaerobic R3 – 15 day	193.625

4.6.9 Volatile Fatty Acids (VFA)

Volatile fatty acids (VFA) content did not exceed 1500 mg HAc/L for the reactors except for the start-up phase which VFA values were measured between 2000 and 3600 mg HAc/L in this operation period.

4.6.10 Capillary Suction Time (CST) Results

Depending on the CST results for 15 days of operation period, it is shown that the enzyme additions enhanced the dewaterability of the sludges. It can be attributed to the improved degradations in EPS of the samples indicating better dewaterability properties for enzyme added reactors. The CST results are plotted in Figure 4.18. The enhanced degradations in EPS of the samples will be evaluated in the following sections of this chapter.

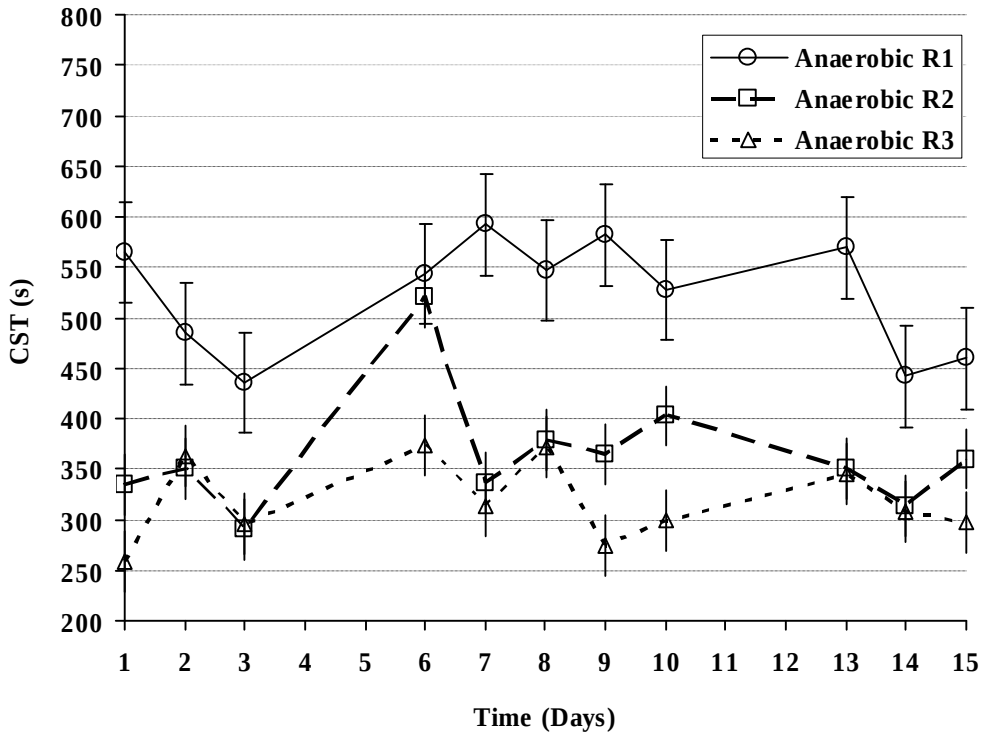


Figure 4.18 CST results as a function of operation time-15 days

4.6.11 Particle Size Distribution

As an indicator for degradation of EPS, particle size distributions were monitored for this operation period as explained in the first operation period results.

Changes in particle size distributions are given in Table 4.9.

Table 4.9 Particle size changes in 1st and 15th operation days for enzyme treated sludges

Sludge ID	Particle Size(mm)				
	Surface weighted mean D[3,2]	Volume weighted mean D[4,3]	d (0.1)	D (0.5)	d (0.9)
R1- 1day	55.764	396.115	22.416	277.825	1079.165
R2- 1day	53.762	368.116	20.114	274.214	1015.147
R3- 1day	52.258	385.256	20.756	274.118	1025.698
R1- 15day	53.716	384.145	20.374	290.316	1084.316
R2- 15day	47.118	325.765	18.716	225.415	934.845
R3- 15day	48.357	335.125	19.325	222.714	956.841

4.6.12 EPS – Protein and Polysaccharide Results

Enzyme1 and Enzyme2 enhanced the degradation of extracellular polymeric substances for this operation period as in the first operation. Protein and polysaccharide concentrations of the samples decreased with the operation time in all reactors, but, the reductions were much higher than control reactors.

Protein concentrations were almost two times greater than polysaccharide concentrations in anaerobic reactors. These results are similar with the results reported by Novak et al. (2003) and Ayol (2005). They stated 3 – 5 times higher protein concentrations when comparing to polysaccharide concentration in anaerobic conditions. The protein and polysaccharide results are given in Figures 4.19 and 4.20, respectively.

The improved degradations in EPS of the samplesled to better dewaterability properties for enzyme added reactors depending on the CST results plotted in Figure 4.18.

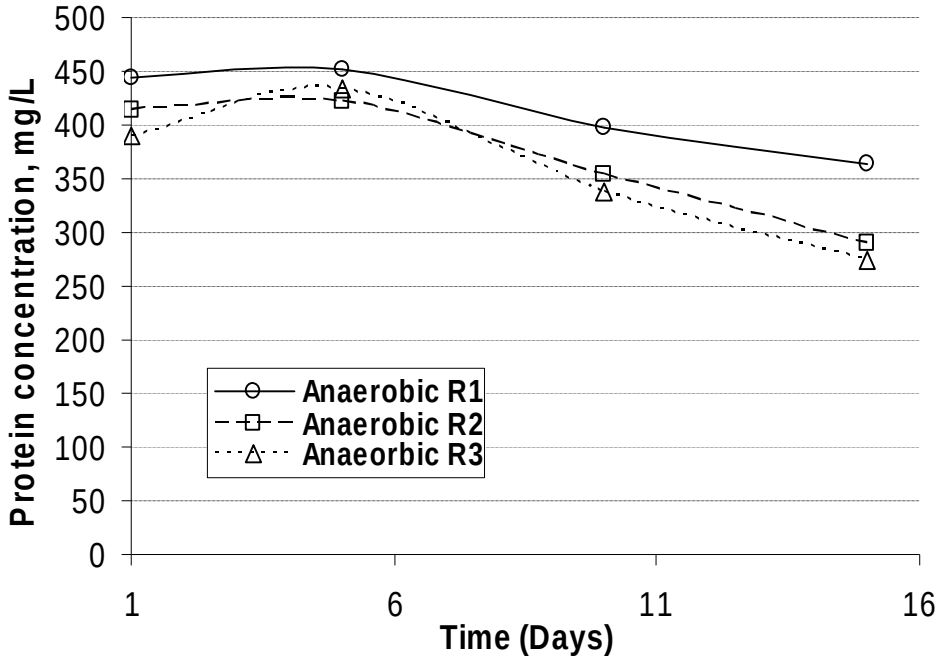


Figure 4.19 Protein concentrations as a function of operation time-15 days

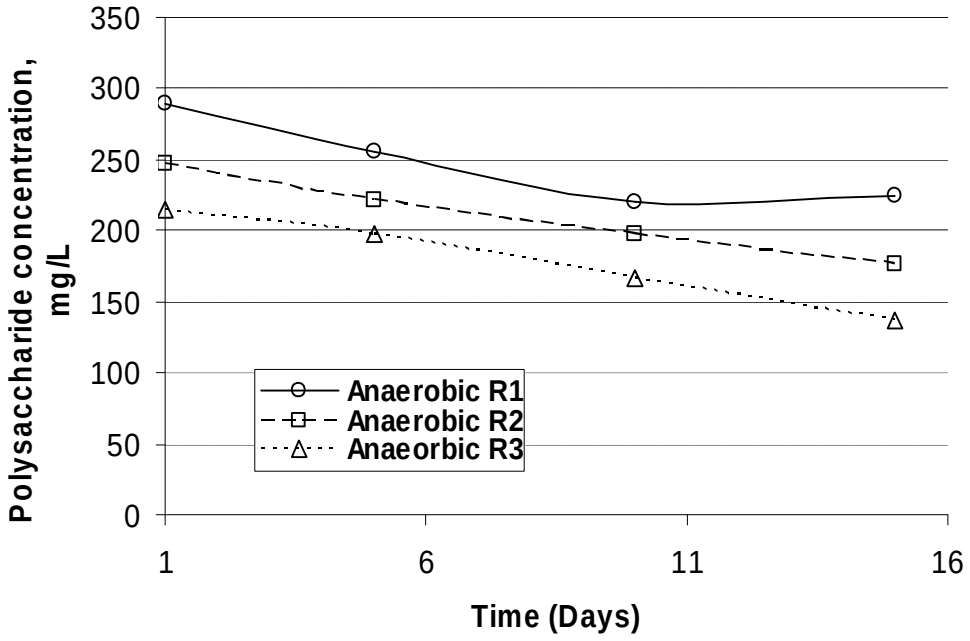


Figure 4.20 Polysaccharide concentrations as a function of operation time-15 days

4.6.13 Sludge Conditioning Results

At the end of the operation time, digested sludges were taken from the reactors for further conditioning analyses as in first operation period. Polymer conditioning was applied to the sludge as explained in Materials and Methods.

Figure 4.21 shows the CST and Zeta potential (ZP) results for conditioned sludge samples. As the polymer doses are increased, the CST tends downward, due to increasingly successful removal of colloidal material. After the minimum CST values, a more gradual increase in CST were obtained. The minimum values indicated the optimum polymer dose for the samples taken from the reactors.

ZP values supported to the data obtained from CST tests in terms of optimum dose range. The negative colloidal surfaces of the sludge transformed to the neutral and/or positive ZP values because of the charge neutralization or reversal by polymer additions. If the charge neutralization accompanies successful destabilization, a zeta potential near zero should indicate good coagulation or conditioning (Ayol et al., 2005).

Flow curves for raw and conditioned samples (not reported herein) showed non-Newtonian behavior, with peak values suggesting polymer-sludge network strength (Abu-Orf & Dentel 1999; Yen et al., 2002). The peak shear stress values for rheological evaluations are depicted in Figure 4.22.

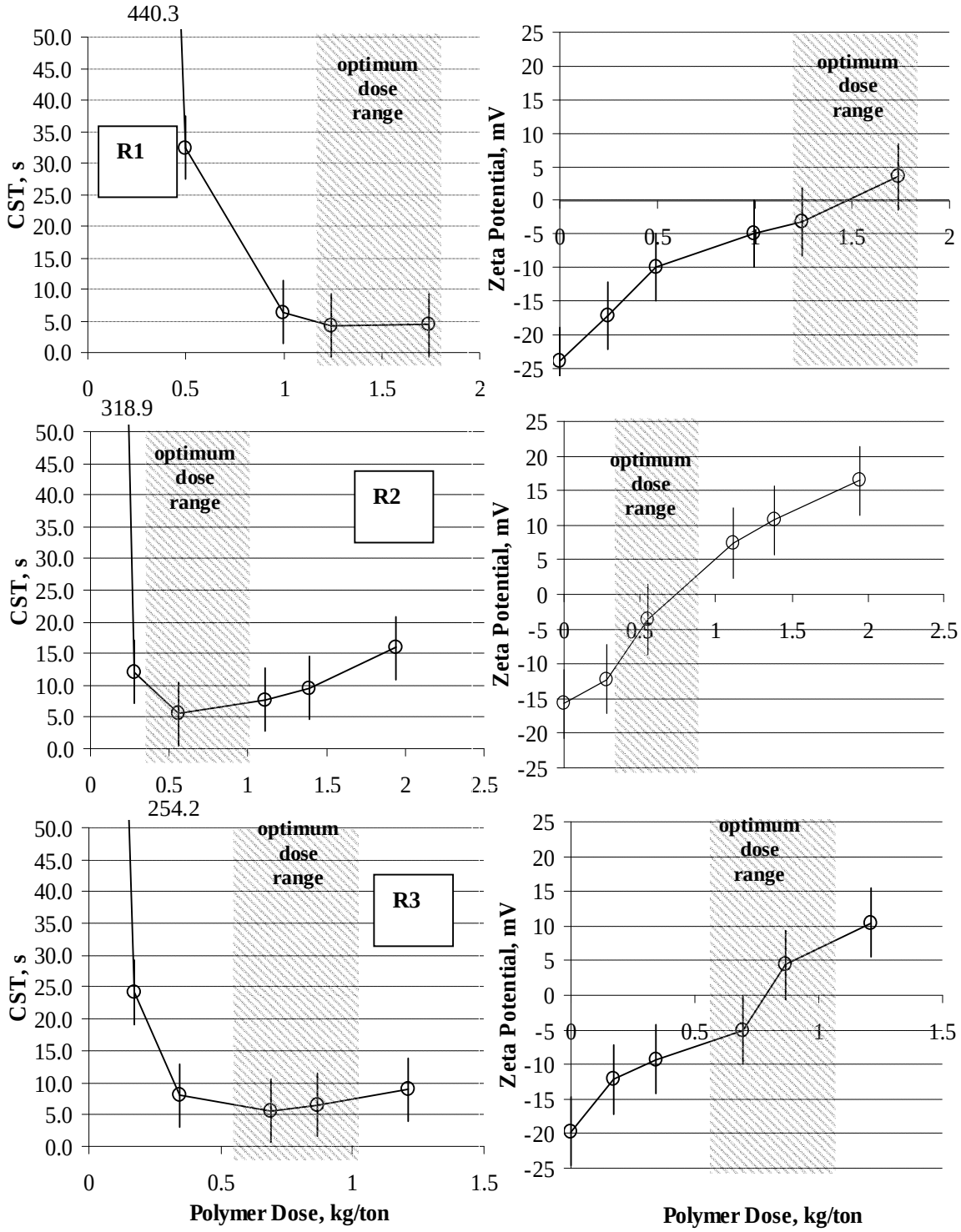


Figure 4.21 CST and Zeta Potential results as a function of polymer additions

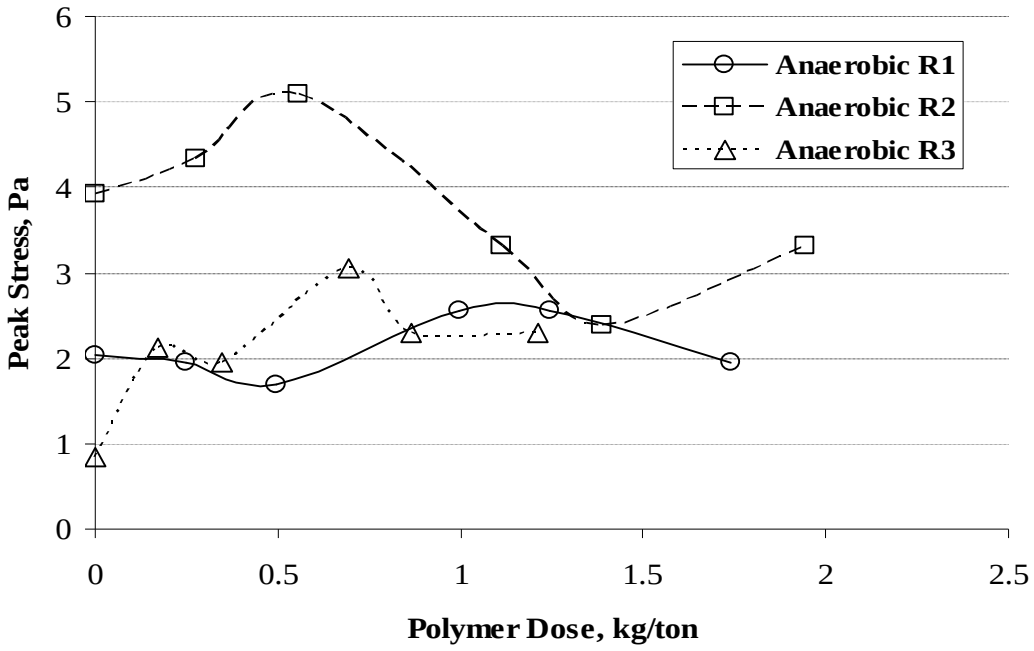


Figure 4.22 Peak stress values as a function of polymer additions

The enzyme added reactor sludges produced higher peak values than did the control reactor sludge. The optimum polymer dose ranges are consisted with the CST and ZP results shown in Figure 4.21. The determination of peak values has been noted to be subject to important analytical inconsistency (Abu-Orf & Ormeci, 2003).

Particle size results for conditioned sludges are given in Figure 4.23. Polymer additions led to increases in the mean floc particle size and the floc volumes increased by up to two orders of magnitude since the important amount of water are built-in in the fractal floc structure.

Zhoa et al. (2004) have indicated that sludge becomes more rigid and compact after polymer conditioning leading to a better solid–liquid separation. Because the sludge contained negative surface charge, cationic polymer conditioning can promote the particle–aggregation by charge neutralization, which induces more compact and better settling sludge (Pan et al., 2007).

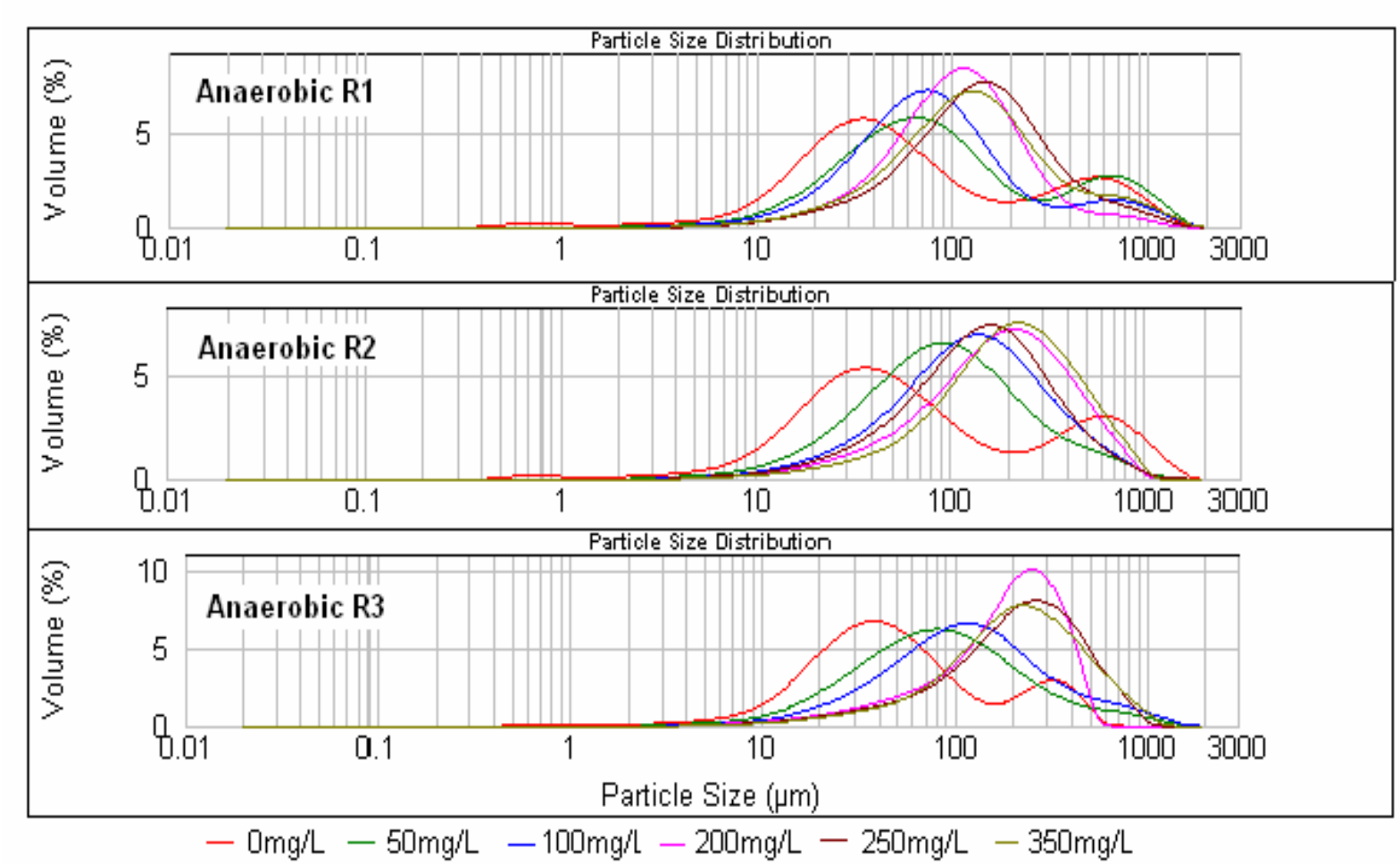


Figure 4.23 Particle size distributions for polymer conditioned sludge samples

The accurate prediction of biosolids dewaterability is important for the selection and optimization of conditioning chemicals, because full-scale trials are laborious and the polymers used for conditioning are expensive. Therefore, dewatering by belt filter press was used in this thesis. In the same manner occurring during cake compression in a full-scale belt filter press, the Crown Press allows controlled periods of mechanical pressure to be applied to drained biosolids between filter cloths as explained in elsewhere (Ayol & Dentel, 2005).

Crown press dewatering results showed that the higher solids concentrations were obtained when comparing to control reactor sludge. Enzyme 2 (R3) produced the highest mass reductions. Because of the better reductions in EPS constituents with the enzyme additions, better dewaterability performances were obtained.

Table 4.10 reported the solids concentration and mass reduction results. Similar observations were obtained by Ayol and Dentel (2005).

The sludge mass decreased by over 20% and 25% for Enzyme 1 and Enzyme 2 additions. Increases in the polymer additions also led to gradual increases in solids levels obtained.

Table 4.10. Dried solid contents (DS) at different polymer doses for samples taken from R1, R2, and R3 and final cake mass reductions after press zone

Anaerobic R1		**Anaerobic R2**				**Anaerobic R3**			
Polymer addition (mg/L-kg/ton)	DS (%)	Polymer addition (mg/L-kg/ton)	DS (%)	ΔDS (R2-R1)	Mass reductions (%)	Polymer addition (mg/L-kg/ton)	DS (%)	ΔDS (R3-R1)	Mass reductions (%)
0-0	10.22	0-0	11.42	1.20	10.51	0-0	12.67	2.45	19.34
200-1.00	16.44	50-0.28	19.44	3.00	15.43	100-0.35	21.78	5.34	24.52
250-1.24	17.04	100-0.56	21.32	4.28	20.08	200-0.69	23.05	6.01	26.07
350-1.74	19.33	200-1.11	23.64	4.31	18.23	250-0.87	23.18	3.85	16.61

CHAPTER FIVE
CONCLUSIONS and RECOMMENDATIONS

5.1 Conclusions

In this thesis, three anaerobic reactors were operated at two different operation periods. The reactors were operated at mesophilic range 37±3 ºC. First reactor was the control reactor with no enzyme addition while the others were operated with the addition of different hydrolytic enzymes.

This thesis investigated the overall fate and effects of hydrolytic enzyme additions whether they can improve the dewaterability of anaerobically digested biosolids or not. Experimental studies showed that the enzymatic treatment remarkably enhanced the dewaterability performance of the digested sludge under anaerobic conditions. The remarkable conclusions from the study can be given as follows:

- Experimental studies showed that the enzyme additions increased the stabilization rate when comparing to the control reactor and performance evaluations indicated that the sludge was stabilized at first 10-12 days of the operation period.
- The improved floc disintegration by enzymes hydrolyzing EPS constituents was obtained at two operation periods. Significant reductions in EPS were observed for enzyme added reactors.
- Enzyme additions exhibit better CST results for R2 and R3 in terms of better filterability.
- As a result of enhancement in the floc disintegration by enzymes , reductions were observed in particle size distributions.
- Higher gas productions and methane percentages in total gas were obtained in enzyme added anaerobic reactors when comparing to that in the control reactor.

- In polymer conditioning studies, polymer requirements of enzymatically treated sludge were almost the half than those for untreated sludge. CST, ZP, and peak stress values results agreed with these findings.
- Higher solids concentrations were obtained when comparing to control reactor sludge and remarkable mass reductions for enzymatically treated samples were reported.

5.2 Recommendations

As promising method, enzymatic treatment of the sludges need to deep research studies to get conclusive results on enzymatic treatment of sludge.

The experimental results should be modeled by commonly used biokinetic models like First order kinetic, Second order kinetic, Monod model to determine the biokinetic constants for full scale application of enzymatic treatment.

On going research of the research project has investigated the effects of protease, lipase, cellulose additions. Therefore, the results should be compared with the results given in this thesis.

Depending on the finding from this thesis, full-scale application in a wastewater treatment plant can be done to determine whether the lab-scale studies simulates the full – scale results or not.

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